



RESEARCH ARTICLE

Exploring the potential of protein-rich moringa and lupin diets on the life cycle, biology and nutritional composition of *Hermetia illucens* and *Tenebrio molitor*

Hafiza Sehrish Bashir¹ , Asif Sajjad^{1*} , Rabia Parveen¹ , Muhammad Sajjad² ,
Rana Muhammad Bilal³ , Muhammad Binyameen²  and Raimondas Mozūraitis^{4,5*} 

¹Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, 63100 Bahawalpur, Punjab, Pakistan; ²Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, 60800 Multan, Pakistan; ³Department of Animal Nutrition, Faculty of Animal Production and Technology, Cholistan University of Veterinary and Animal Sciences, 63100 Bahawalpur, Punjab, Pakistan; ⁴Laboratory of Chemical and Behavioural Ecology, Institute of Ecology, The State Scientific Research Institute Nature Research Centre, Akademijos Street 2, 08412 Vilnius, Lithuania; ⁵Department of Zoology, Stockholm University, Svante Arrhenius väg 18B 10691 Stockholm, Sweden; *asif.sajjad@iub.edu.pk; raimondas.mozuraitis@su.se

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Abstract

The black soldier fly (*Hermetia illucens* L.) and yellow mealworm (*Tenebrio molitor* L.) are commercially produced on bio-wastes for incorporation into poultry diets. This study investigated the impact of food on *H. illucens* and *T. molitor* in terms of larval development, pupation, emergence rates, adult longevity, and egg production. Protein is a crucial component of insect diets, promoting rapid growth and development. However, most bio-wastes, such as fruit and vegetable wastes, are low in protein. Therefore, it is essential to explore efficient and cost-effective protein sources for insect diets. In this study, we evaluated the effects of mixing equal proportions of protein-rich moringa, *Moringa oleifera*, and lupin, *Lupinus albus*, in eight combinations (0, 5, 10, 15, 20, 25, 30 and 35%) with equal proportions of fruit, vegetable, and grain market wastes on several lifecycle and biological parameters of *H. illucens* and *T. molitor*. In both species, the shortest larval and pupal periods, as well as the highest larval and pupal weights and fecundity, were observed in the 35% moringa and lupin mixture. Nutritional composition of *H. illucens* and *T. molitor* larvae significantly improved ($p < 0.05$) when fed 35% moringa and lupin. Therefore, incorporating a blend of 35% moringa and lupin with equal proportions of fruit, vegetable, and grain market wastes can significantly enhance the mass multiplication of *H. illucens* and *T. molitor*. Future studies should evaluate even higher levels of moringa and lupin inclusion in the diet to achieve optimal insect biomass.

Keywords

adult longevity – egg production – emergence rates – larval development – nutritional profile – protein sources – pupation

1 Introduction

Insects are valuable in poultry feed because they convert wastes into nutrient-dense feeds (Khusro *et al.*, 2012). Furthermore, since poultry birds naturally consume insects, incorporating them into the feed formulations could improve poultry well-being (Koutsos *et al.*, 2023). Insects have several advantages, including a short life cycle, the ability to thrive on organic wastes, an efficient conversion rate, minimal land and water requirements, and excellent sustainability (van Huis and Oonincx, 2017). Several insects are raised commercially for incorporation in the poultry diet, such as the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), black soldier fly larvae, *Hermetia illucens* L. (Diptera: Stratiomyidae), and house fly, *Musca domestica* L. (Diptera: Muscidae) (Cortes Ortiz *et al.*, 2016). Most insects used as a potential protein source in poultry feed are naturally consumed organic wastes (Hatab *et al.*, 2020; Sajjad *et al.*, 2024). *H. illucens* larvae devour animal wastes, plant wastes, food wastes, and agricultural by-products (Ilyas *et al.*, 2023; Nguyen *et al.*, 2015; Zheng *et al.*, 2012), and the *T. molitor* larvae consume wheat bran and plant-based water sources such as potatoes to maintain optimal nutrition (Fasce *et al.*, 2022). Insects fed on organic wastes produce biomass rich in protein, minerals, vitamins and fats, which have the potential to replace standard feed ingredients such as soybean meal and fish meal (Franks *et al.*, 2021).

Several factors influence the nutritional composition, growth, and performance of insects, such as their diet, developmental stage, growth conditions, sex, etc. (van Huis and Oonincx, 2017). The nutrients that insects obtain from their diet are crucial for their growth and development and predict how well they can enhance their fitness (Finke, 2013; Gutiérrez *et al.*, 2020; Oonincx *et al.*, 2015). Changes in the diet quality at any developmental stage can significantly impact the life history traits of insects (Barragan-Fonseca *et al.*, 2019; Naya *et al.*, 2007). Proteins and carbohydrates are the two main dietary components that help in insect development (Wang *et al.*, 2018). Protein contains essential amino acids (EAAs) that are vital for viability, and their imbalance has a substantial impact on the development, lifespan, and overall health of insects (Giannetto *et al.*, 2020; Spranghers *et al.*, 2024). Carbohydrate provides energy to support growth and store energy for later use (Cohn *et al.*, 2022). Protein and lipid-rich diets have been shown to improve the biological parameters of insects as compared to carbohydrate-rich diets (Morales-Ramos *et al.*, 2013). The availability of nutri-

ents throughout development influences several characteristics, including growth rate, survival, and various adult traits such as fecundity and body size (Mahmoud *et al.*, 2025; Nijhout, 2003).

Moringa, *Moringa oleifera* Lam. (Brassicales: Moringaceae) and white lupin, *Lupinus albus* L. (Fabales: Fabaceae) are well known for their high nutritional values and potential to improve insect biomass production (Ali *et al.*, 2022; Gresta *et al.*, 2023). Moringa is a highly nutritious plant, high in proteins, vitamins, and minerals (Fahey, 2005), while lupin seeds are rich in protein and essential amino acids (Islam *et al.*, 2021; Sujak *et al.*, 2006). By combining these nutritious plants with bio-wastes, we may be able to provide a more effective and balanced diet for *H. illucens* and *T. molitor*, resulting in increased biomass production.

Keeping in mind the importance of dietary protein in the lifecycle and biology of insects, different ratios of plant protein sources were compared to determine their impacts on the lifecycle and biological traits of *H. illucens* and *T. molitor*. The present study evaluated the effect of mixing moringa and lupin in seven different combinations (i.e. 0, 5, 10, 15, 20, 25, 30 and 35%) with equal proportions of fruit, vegetable and grain market wastes on various lifecycle and biological parameters of *T. molitor* and *H. illucens*.

2 Materials and methods

Insects and diets

The present study was conducted in the Entomology Research Laboratory, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Baghdad-ul-Jadeed Campus. The adults of black soldier fly, *H. illucens*, and yellow mealworms, *T. molitor* were obtained from the stock culture maintained under controlled conditions in the Entomology Research Laboratory. *H. illucens* and *T. molitor* species were maintained on their natural diets under controlled conditions (27 ± 0.5 °C, 60-70% RH, and 12:12 light and dark period). In systematic dietary experiments, eight combinations of the diets were prepared by adding moringa and lupin in eight different ratios with natural diets (fruit waste, vegetable waste, and grain waste) (Table 1). Vegetables and fruits were chopped and oven-dried at 60 °C for 36 h. All treatments (dried fruits, vegetables, grains, moringa seeds, and lupin seeds) were ground into fine particles, thoroughly mixed, and stored in a refrigerator at -20 °C for further use.

TABLE 1 Eight combinations of moringa+lupin with fruits, vegetables, and grains waste

Diet No.	Moringa+Lupin	Fruits	Vegetables	Grains
1	0 + 0 (Control)	33.33%	33.33%	33.33%
2	5 + 5%	30%	30%	30%
3	10 + 10%	26.6%	26.6%	26.6%
4	15 + 15%	23.3%	23.3%	23.3%
5	20 + 20%	20%	20%	20%
6	25 + 25%	16.6%	16.6%	16.6%
7	30 + 30%	13.3%	13.3%	13.3%
8	35 + 35%	10%	10%	10%

Experimental setup

A total of 240 3-5-day-old larvae of *T. molitor* and *H. illucens* were transferred into 24 transparent plastic containers (40 × 20 × 10 cm), each provided with 100 g of diet representing eight different combinations (10 larvae per container; 10 g diet per larva). Each dietary treatment was replicated three times by using a completely randomized design (CRD). The same diets were also provided to the adults to record adult longevity and fecundity. For this purpose, 16 freshly hatched pairs were released into eight plastic containers (2 pairs per container) with eight different diets having a moist oviposition substrate (dietary treatments+water) for *H. illucens* (Ferdousi *et al.*, 2020), while dry substrate (dry diets in powder form+potato as water source) with poultry egg holders for *T. molitor* (Toviho and Bársony, 2022).

Lifecycle and biological parameters

All lifecycle and biological parameters, i.e. larval period, pupal period, larval weight, pupal weight, pupation rate, emergence rate, days to become adult male, days to become adult female, male longevity, female longevity and fecundity, were recorded over one generation. The duration of the larval period was recorded as the time taken to reach the pupal stage after hatching from the egg. The larval weight was recorded when the larvae finished their feeding and reached the wandering stage by using an electronic balance. Then, these pre-pupae were shifted into plastic jars where they pupated and were covered with black cloth to provide a dark environment. The three-day-old pupae were weighed using an electronic balance and then shifted into petri dishes (10 mm in depth, 50 mm in diameter). The pupal period was measured when they reached at resting stage till emergence. The time taken for the emergence of adults was also noted. The pupation and emergence rates were regarded as the percentage of larvae that successfully pupated and the percentage of successfully emerged adults from pupae, respectively. A total number of days

was counted for both males and females as they reached adulthood. The longevity of adult male and female *H. illucens* and *T. molitor* was recorded from their emergence until they died. Five pairs of adults were placed separately in five different transparent containers (7 × 3 × 15 cm) with oviposition substrate. The number of eggs was collected and counted under the microscope with the help of a camel hair brush (Ferdousi *et al.*, 2020).

Proximate analysis of diets

Proximate nutrient compositions i.e. dry matter (DM), crude protein (CP), crude ash, ether extracts (crude fat, EE) and crude fibre (CF) of moringa and lupin-based diets and the larvae of *H. illucens* and *T. molitor* were evaluated. Full-grown larvae were harvested from the leftovers by sieving at the end of the experiments, left starved for 24 h and then killed by freezing at -20 °C and further stored at this temperature until analysis. Diets and larvae were dried to constant weight in a hot air oven at 70 °C for 24 h to determine dry matter and then ground into a fine powder with a blender. The crude protein of the feeding substrates and larvae was determined by Kjeldahl analysis. The ether extract of larvae and feeding substrates was determined by exhaustive Soxhlet extraction using petroleum ether (40-60 °C, BP) and crude fibre was estimated by using a fibre analyser. The Principal component analysis showed the proximate analysis of test diets (Figure 1 and Table 2). These analysis were performed at the Department of Animal Nutrition, University of Veterinary and Animal Sciences (UVAS), Lahore.

Data analysis

The eight dietary combinations were compared in terms of lifecycle (larval period, pupal period, larval weight, pupal weight, pupation rate, emergence rate, days to become adult male and female, adult male and female longevity, and fecundity), biology and nutritional com-

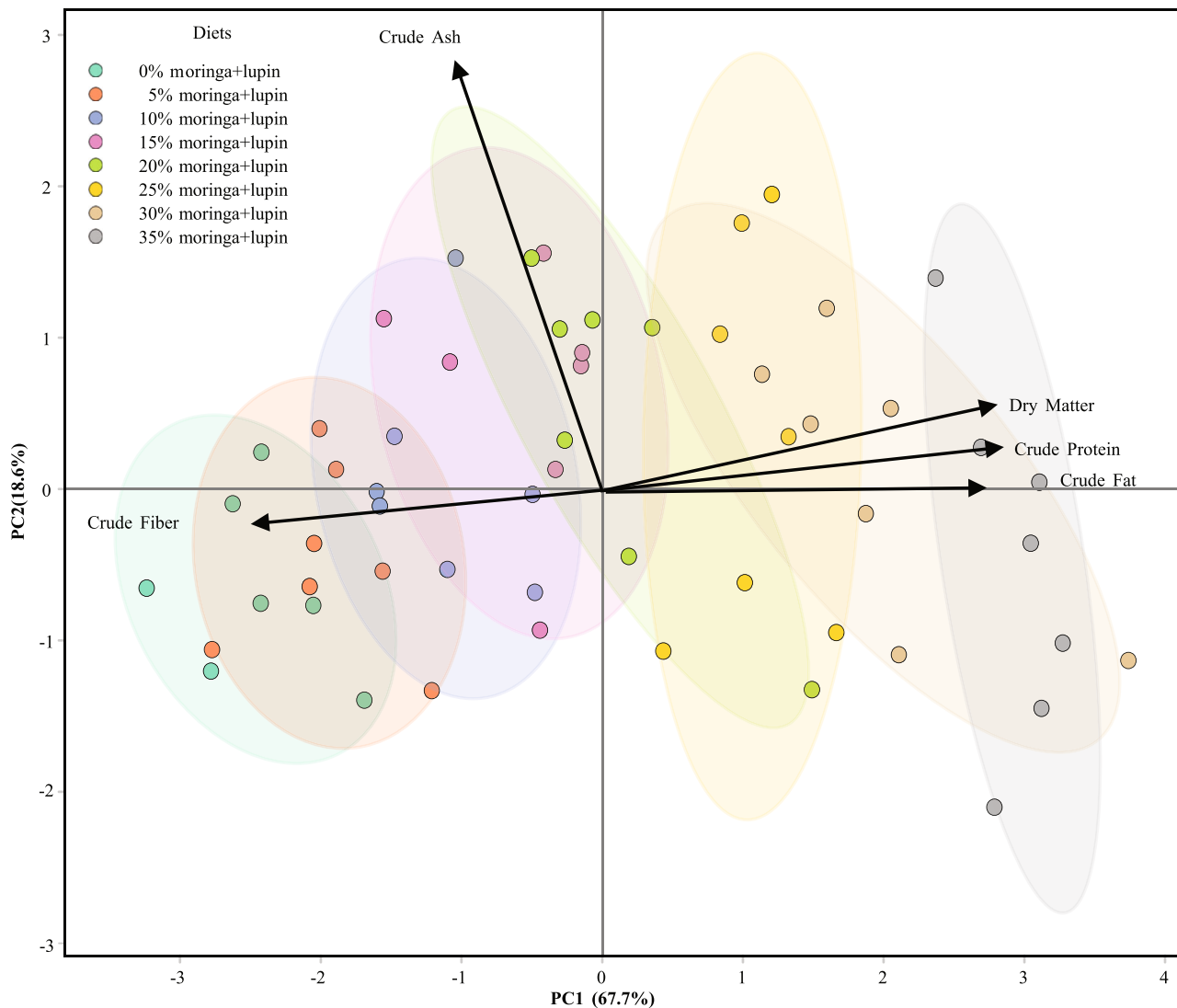


FIGURE 1 PCA scatter plot of the moringa and lupin-based diets analyzed in this study. The explained variance accounted for 86.3% of the total variance, 67.7% accounted for PC1, and PC2 accounted for 18.6%.

TABLE 2 Eigenvectors of each studied variable for PC1 and PC2

	PC1	PC2
Dry matter	0.504	0.216
Crude protein	0.506	0.062
Crude fat	0.478	0.062
Crude ash	-0.239	0.965
Crude fibre	-0.452	-0.075

positions (dry matter, crude protein, ether extract, ash and crude fiber) by using one-way analysis of variance (ANOVA) followed by Duncan Multiple Range (DMR) test at $\alpha = 0.05$. An orthogonal polynomial contrast test was also performed to check the impact of diets on the insect's performance. All the statistical analyses were performed using the Python Programming language using SciPy package (version 3.13).

3 Results

Lifecycle of H. illucens

There was a significant difference ($p < 0.05$) among all the diets in terms of larval period, pupal period, and days to become adult male and female. The maximum larval period was recorded in a 0% moringa+lupin-based diet, while the minimum was in a 35% moringa+lupin-based diet. The maximum pupal period was recorded in 0% moringa+lupin-based diet, while the minimum and statistically similar pupal periods were recorded in 20, 25, 30 and 35% moringa+lupin-based diets. The maximum number of days to become an adult male and female was recorded in a 0% moringa+lupin-based diet, while the minimum was in a 35% moringa+lupin-based diet. The emergence rate and pupation rate remained non-significant, consistently yielding almost

100% results across all eight diets. There was no statistically significant difference among all the diets in terms of adult male and female longevity (Table 3). Orthogonal polynomial contrast showed that there were linear and quadratic responses in the larval period ($p < 0.001$ and $p = 0.003$), pupal period ($p < 0.001$ and $p < 0.001$), days to become adult male ($p < 0.001$ and $p < 0.001$) and female ($p < 0.001$ and $p < 0.001$) of *H. illucens*.

Biology of *H. illucens*

There was a significant difference among all the diets in terms of larval weight, pupal weight and fecundity. The maximum larval weight was recorded in a 35% moringa+lupin-based diet, while the minimum was in a 0% moringa+lupin-based diet. The maximum pupal weight was recorded in a 35% moringa+lupin-based diet, while the minimum was in a 0% moringa+lupin-based diet. The maximum fecundity was recorded in a 35% moringa+lupin-based diet, while the minimum was in a 0% moringa+lupin-based diet (Table 4). Larval weight was affected linearly and quadratically ($p < 0.001$ and $p = 0.03$), while pupal weight ($p < 0.001$) and fecundity ($p < 0.001$) were affected linearly.

Nutritional composition of *H. illucens*

Increasing the inclusion level of moringa and lupin in the diets significantly affected the proximate compositions of *H. illucens* ($p < 0.05$) (Table 5). The dry matter, crude protein, crude fat, ash, and crude fibre contents increased with higher moringa and lupin inclusion. The dry matter and crude fat were the highest in the larvae fed 35% moringa and lupin diet, while the lowest were in larvae fed 0% moringa and lupin diets. Crude protein was the maximum in the larvae fed 35% moringa and lupin diet and the minimum in those fed the 5 and 0% moringa and lupin diets. The highest ash and crude fibre contents were observed in 35% moringa and lupin fed larvae and the lowest was in the 5% moringa and lupin fed larvae. There was a linear response in dry matter ($p < 0.001$), crude fat ($p < 0.001$) and ash ($p < 0.001$), while linear and quadratic responses in crude protein ($p < 0.001$ and $p < 0.001$) and crude fibre ($p < 0.001$ and $p < 0.001$).

Lifecycle of *T. molitor*

There was a significant difference among all the diets in terms of larval period, pupal period, days to become adult male and female, adult male longevity and adult female longevity. The longest and most similar larval period was recorded in a 0% moringa+lupin-based diet, while the shortest was in a 35% moringa+lupin-based

diet. The longest pupal period was observed in a 0% moringa+lupin-based diet, while the shortest was in a 35% moringa+lupin-based diet. The maximum number of days to become an adult male was recorded in a 0% moringa+lupin-based diet, while the minimum was in a 35% moringa+lupin-based diet. The maximum number of days to become an adult female was recorded in 0% moringa+lupin-based diets, while the minimum was in 35% moringa+lupin-based diets. The maximum adult male longevity was recorded in a 35% moringa+lupin-based diet, while the minimum was in a 0% moringa+lupin-based diet. The maximum female adult longevity was recorded in a 35% moringa+lupin-based diet, while the minimum was in a 0% moringa+lupin-based diet. There was no significant difference among all the diets in terms of pupation rate and emergence rate (Table 6). There were linear and quadratic responses in larval period ($p < 0.001$ and $p = 0.001$) and pupal period ($p < 0.001$ and $p < 0.001$), while a linear response in days to become adult male ($p < 0.001$) and female ($p < 0.001$) as well as adult longevity male ($p < 0.001$) and female ($p < 0.001$).

Biology of *T. molitor*

There was a significant difference among all the diets in terms of larval weight, pupal weight, and fecundity. The maximum larval weight was recorded in a 35% moringa+lupin-based diet, while the minimum was in a 0% moringa+lupin-based diet. The maximum pupal weight was recorded in a 35% moringa+lupin-based diet, while the minimum was in a 0% moringa+lupin-based diet. The maximum fecundity was recorded in 30% and 35% moringa+lupin-based diets, while the minimum was in 0% moringa+lupin-based diets (Table 7). There were linear and quadratic responses in larval weight ($p < 0.001$ and $p < 0.001$) and pupal weight ($p < 0.001$ and $p < 0.001$), while a linear response in fecundity ($p < 0.001$) and female ($p < 0.001$) as well as adult longevity male ($p < 0.001$) and female ($p < 0.001$).

Nutritional composition of *T. molitor*

Increasing the inclusion level of moringa and lupin in the diet significantly enhanced the dry matter, crude protein, ether extract, ash, and crude fibre contents in *T. molitor* ($p < 0.05$) (Table 8). The highest dry matter was recorded in larvae fed on 35% moringa and lupin diet, while the lowest and non-significant in larvae fed on 15, 10, 5 and 0% moringa and lupin diets. Crude protein was the maximum in the larvae fed on 35% moringa and lupin diet and the minimum in similar in the larvae

TABLE 3 Impact of eight dietary combinations on lifecycle parameters of *H. illucens*

Diet	Larval period (days)	Pupal period (days)	Pupation rate (%)	Emergence rate (%)	Days to become adult male	Days to become adult female	Adult male longevity (days)	Adult female longevity (days)
0% moringa+lupin	24.95 ± 0.39 ^a	9.1 ± 0.20 ^a	89.33 ± 0.66	90.66 ± 1.20	34.00 ± 0.94 ^a	33.87 ± 0.58 ^a	6.80 ± 0.31	3.70 ± 0.25
5% moringa+lupin	23.60 ± 0.11 ^b	8.20 ± 0.17 ^b	90.66 ± 1.20	90 ± 1.73	31.54 ± 0.20 ^b	31.12 ± 0.29 ^b	7.05 ± 0.26	4.60 ± 0.26
10% moringa+lupin	22.90 ± 0.16 ^c	7.50 ± 0.15 ^{bc}	96.33 ± 4	94.33 ± 1.76	30.27 ± 0.23 ^c	30.88 ± 0.20 ^b	7.05 ± 0.26	5.40 ± 0.28
15% moringa+lupin	21.80 ± 0.15 ^d	7.25 ± 0.16 ^c	96 ± 1	93 ± 3.21	29.09 ± 0.28 ^d	29.00 ± 0.33 ^c	7.35 ± 0.33	5.70 ± 0.33
20% moringa+lupin	20.85 ± 0.20 ^e	7.00 ± 0.15 ^d	96 ± 3.24	92.66 ± 2.40	27.54 ± 0.28 ^e	28.33 ± 0.40 ^c	7.60 ± 0.42	5.70 ± 0.47
25% moringa+lupin	19.85 ± 0.15 ^f	6.95 ± 0.14 ^d	97 ± 1.41	92 ± 1.15	26.81 ± 0.29 ^{ef}	26.77 ± 0.22 ^d	7.80 ± 0.35	5.95 ± 0.36
30% moringa+lupin	19.00 ± 0.16 ^g	6.95 ± 0.14 ^d	98 ± 1.15	90.66 ± 1.20	25.90 ± 0.28 ^{fg}	26.22 ± 0.28 ^{de}	8.00 ± 0.38	6.25 ± 0.45
35% moringa+lupin	19.10 ± 0.18 ^{gh}	6.95 ± 0.14 ^d	95.33 ± 2.60	91.66 ± 1.45	25.90 ± 0.39 ^{fg}	26.00 ± 0.27 ^e	8.20 ± 0.43	6.60 ± 0.47
df	7	7	7	7	7	7	7	7
<i>p</i> -value ANOVA	<0.001	<0.001	0.366	0.574	<0.001	<0.001	0.910	0.920
<i>p</i> -value linear	<0.001	<0.001	0.644	0.970	<0.001	<0.001	0.990	0.990
<i>p</i> -value quadratic	0.003	<0.001	0.898	1.000	<0.001	<0.001	1.000	1.000

Averages followed by the same letters in a column are not significantly different according to DMR at the 5% level (\pm SE).

TABLE 4 Impact of eight dietary combinations on biological parameters of *H. illucens*

Diet	Larval weight (g)	Pupal weight (g)	Fecundity (no. of eggs/female)
0% moringa+lupin	0.18 ± 0.02 ^f	0.13 ± 0.04 ^g	314.75 ± 2.56 ^g
5% moringa+lupin	0.20 ± 0.02 ^e	0.15 ± 0.02 ^f	333.88 ± 3.54 ^f
10% moringa+lupin	0.21 ± 0.02 ^d	0.18 ± 0.03 ^e	343.33 ± 4.52 ^{ef}
15% moringa+lupin	0.21 ± 0.01 ^d	0.20 ± 0.02 ^d	349.33 ± 4.33 ^{ef}
20% moringa+lupin	0.24 ± 0.02 ^c	0.21 ± 0.03 ^d	365.78 ± 3.86 ^{de}
25% moringa+lupin	0.24 ± 0.01 ^{bc}	0.24 ± 0.02 ^c	387.00 ± 4.52 ^{cd}
30% moringa+lupin	0.25 ± 0.02 ^b	0.26 ± 0.02 ^b	390.89 ± 4.54 ^{bc}
35% moringa+lupin	0.29 ± 0.02 ^a	0.30 ± 0.01 ^a	414.56 ± 4.89 ^a
df	7	7	7
<i>p</i> -value ANOVA	<0.001	<0.001	<0.001
<i>p</i> -value linear	<0.001	<0.001	<0.001
<i>p</i> -value quadratic	0.03	0.95	1.00

Averages followed by the same letters in a column are not significantly different according to DMR at 5% level (\pm SE).

fed on 10, 5 and 0% moringa and lupin diets. The maximum crude fat was found in 35% moringa and lupin fed larvae, while the minimum was in 0% moringa and lupin fed larvae. The highest ash content was observed in 35% moringa and lupin and the lowest and statistically non-significant ($p > 0.05$) was in the 25%, 20%, 15%, 10%, 5% and 0% moringa and lupin diets fed larvae. Crude fibre was the highest in 35% moringa and lupin diet, while the lowest and similar was in the 5% and 0% moringa and lupin fed larvae. There was a linear response in dry matter ($p < 0.001$), crude fat ($p < 0.001$), ash ($p < 0.001$) and crude fiber ($p < 0.001$) contents while linear and quadratic responses in crude protein content ($p < 0.001$ and $p < 0.001$).

4 Discussion

Several factors affect the growth and development of insects, such as the nutritional content of a diet, larval density, gut flora, environmental conditions, etc. (Huang *et al.*, 2020; Shin *et al.*, 2011; Storelli *et al.*, 2011).

In the present study, the minimum larval period of both insect species (*H. illucens* and *T. molitor*) was recorded in a 35% moringa+lupin-based diet. The larval developmental period of insects is greatly influenced by the protein contents of the diet (Gobbi *et al.*, 2013; Oonincx *et al.*, 2015; Simon *et al.*, 2011; Van Broekhoven *et al.*, 2015). The protein and fat-rich diet has been shown to fasten the developmental rate of *H. illucens* and *Ceratitis capitata* Wied. (Diptera: Tephritidae) larvae (Kaspi *et al.*, 2002; Nguyen *et al.*, 2015). Morales-Ramos *et al.* (2010) reported the fastest developmental rate and a

lower number of instars in *T. molitor* when fed on an enriched diet.

The minimum pupal period was recorded in a 35% moringa+lupin-based diet. Up to 50% replacement of moringa leaves with wheat bran has shown no negative impact on the development and survival of *T. molitor* (Kotsou *et al.*, 2023). Rumbos *et al.* (2021) observed that the growth of *T. molitor* and lesser mealworm *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) improved when fed on the seed cleaning by-products of cereals and legumes, especially the by-products of lupin. Owing to high protein contents, several other legumes have been evaluated for the growth performance of insects and have shown promising results. Unmole (2009) and Nitta *et al.* (2023) reported higher growth, development, survival, fecundity and less lifespan of pod borer, *Maruca vitrata* Fab. (Lepidoptera: Crambidae) when fed on mung bean and black-grams, respectively.

There was no significant difference among all the diets in terms of adult longevity of *H. illucens*. Cammack and Tomberlin (2017) reported that a balanced larval diet (21% Carbohydrate: 21% protein) decreased the developmental time and increased the body size and survival rate of *H. illucens* while it had no impact on adult emergence and longevity. (Li *et al.*, 2014a) reported that a protein-enriched larval diet does not affect the adult longevity of honey bee *Apis mellifera* L. (Hymenoptera: Apidae). The maximum longevity of adult male and female *T. molitor* was recorded in a 35% moringa+lupin-based diet. A larval diet may or may not affect the adult longevity depending on the species. Runagall-McNaull *et al.* (2015) reported that the inter-

TABLE 5 Nutritional compositions of *H. illucens* larvae fed on different combinations of diets

Diet	Dry matter	Crude protein	Crude fat	Ash	Crude fibre
35% moringa+lupin	91.31 ± 0.63a	50.54 ± 0.39a	12.94 ± .48a	6.82 ± 0.56a	14.51 ± 0.41a
30% moringa+lupin	90.19 ± 0.35a	48.70 ± 0.30b	11.87 ± 0.57a	6.28 ± 0.35ab	13.96 ± 0.42ab
25% moringa+lupin	90.39 ± 0.43a	47.21 ± 0.46bc	10.37 ± 0.45b	5.71 ± 0.28bcd	12.97 ± 0.43bc
20% moringa+lupin	88.13 ± 0.62b	46.18 ± 0.58c	9.61 ± 0.48bc	6.00 ± 0.21abc	12.03 ± 0.40c
15% moringa+lupin	87.52 ± 0.49bc	44.51 ± 0.52d	8.96 ± 0.41cd	5.28 ± 0.28cd	11.68 ± 0.45cd
10% moringa+lupin	87.32 ± 0.77bc	42.61 ± 0.61e	8.07 ± 0.41de	5.28 ± 0.28cd	10.42 ± 0.53de
5% moringa+lupin	86.23 ± 0.73bc	40.72 ± 0.60f	6.69 ± 0.27ef	5.00 ± 0.30d	9.05 ± 0.43f
0% moringa+lupin	87.29 ± 0.44c	39.63 ± 0.73f	7.15 ± 0.28f	5.14 ± 0.26cd	9.21 ± 0.62ef
Df	7	7	7	7	7
p-value	<0.001	<0.001	<0.001	0.0035	<0.001
p-value linear	<0.001	<0.001	<0.001	<0.001	<0.001
p-value quadratic	0.998	<0.001	0.243	0.948	0.002

mediate level of protein in the larval diet of banana stalk fly, *Derocephalus angusticollis* Enderlein (Diptera: Neriidae) could affect the adult lifespan, whereas low or high protein diets have no impact on adult longevity. Li *et al.* (2014b) reported that the protein-rich larval diet had no impact on the adult longevity of *H. illucens*.

The maximum larval and pupal weights were recorded in a 35% moringa+lupin-based diet. High protein diets have been shown to promote life history parameters like larval and pupal weights and the nutritional profile of *T. molitor* (Riaz *et al.*, 2023). Larvae of black dump fly, *Hydrotaea aenescens* Wiedemann (Diptera: Muscidae) reared on a high-protein diet were heavier than those reared on a low-protein diet (Hogsette and Washington, 1995). Protein-enriched diets have been shown to promote the pupal weight of blow fly, *Phormia regina* Meigen (Diptera: Calliphoridae), *M. domestica*, and *T. molitor* (Green *et al.*, 2003; Kökdener and Kiper, 2020).

The maximum fecundity was recorded in a 35% moringa+lupin-based diet. The protein content of the diet has been shown to improve egg production in insects like house cricket, *Acheta domestica* L. (Orthoptera: Gryllidae), carrion beetle, *Necrophila japonica* Kurosawa (Coleoptera: Silphidae), *H. illucens*, and lesser fruit fly, *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Bertinetti *et al.*, 2019; Fanson and Taylor, 2012; Fanson *et al.*, 2009; Lee *et al.*, 2008; Maklakov *et al.*, 2008; Watahiki and Sasakawa, 2019). Kaspi *et al.* (2002) reported that Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) females fed on a protein-rich diet produced more mature eggs. Geister *et al.* (2008) reported that the protein-rich diet had been shown to promote the fecundity, egg composition and egg hatchability of adult squinting bush brown butterfly *Bicyclus anynana* Butler (Lepidoptera: Nymphalidae).

Increasing the inclusion level of moringa and lupin in the diets significantly affected the proximate compositions of *H. illucens* and *T. molitor*. The dry matter, crude protein, crude fat, ash, and crude fiber contents were the maximum in the larvae fed 35% moringa and lupin diet. These results clearly indicate that moringa and lupin supplementation enhance the nutritional quality of insect larvae, likely due to their high content of nutritional and bioactive compounds. The observed increase in crude protein content with higher inclusion levels may be attributed to the excellent protein profile and amino acid composition of moringa leaves and lupin seeds. Both ingredients contain abundant essential amino acids such as lysine, methionine, and thre-

TABLE 6 Impact of eight dietary combinations on lifecycle parameters of *T. molitor*

Diet	Larval period (days)	Pupal period (days)	Pupation rate (%)	Emergence rate (%)	Days to become adult male	Days to become adult female	Adult male longevity	Adult female longevity
0% moringa+lupin	95.30 ± 0.25 ^a	20.90 ± 0.25 ^a	93.33 ± 3.33	91.33 ± 1.85	117.63 ± 0.33 ^a	114.40 ± 0.34 ^a	9.25 ± 0.44 ^d	9.66 ± 0.51 ^d
5 % moringa+lupin	93.35 ± 0.24 ^b	19.45 ± 0.11 ^b	93.33 ± 3.33	90 ± 1.73	113.25 ± 0.31 ^b	112.00 ± 0.36 ^b	11.25 ± 0.59 ^c	11.50 ± 0.42 ^c
10 % moringa+lupin	93.30 ± 0.23 ^b	18.25 ± 0.18 ^c	94.66 ± 1.45	89.66 ± 4.54	112.44 ± 0.24 ^b	110.50 ± 0.22 ^c	12.55 ± 0.37 ^b	11.50 ± 0.42 ^c
15% moringa+lupin	93.35 ± 0.21 ^b	17.30 ± 0.21 ^d	92.66 ± 2.40	89 ± 3.08	111.00 ± 0.22 ^c	109.50 ± 0.18 ^c	12.00 ± 0.27 ^{bc}	12.75 ± 0.36 ^{bc}
20% moringa+lupin	92.65 ± 0.15 ^c	15.50 ± 0.24 ^e	92.33 ± 2.40	89 ± 3.24	108.89 ± 0.30 ^d	106.71 ± 0.28 ^d	12.66 ± 0.33 ^b	13.14 ± 0.40 ^b
25 % moringa+lupin	92.30 ± 0.24 ^c	15.05 ± 0.18 ^{ef}	93.33 ± 2.02	92 ± 1.41	108.27 ± 0.33 ^{de}	106.14 ± 0.26 ^{de}	12.81 ± 0.42 ^b	13.00 ± 0.53 ^b
30 % moringa +lupin	91.35 ± 0.23 ^d	14.80 ± 0.17 ^f	98 ± 1.15	90.66 ± 1.47	107.11 ± 0.38 ^e	105.33 ± 0.23 ^e	13.11 ± 0.42 ^b	14.30 ± 0.47 ^{ab}
35% moringa+lupin	88.35 ± 0.25 ^e	14.00 ± 0.19 ^g	91.66 ± 1.76	88.33 ± 2.33	103.40 ± 0.33 ^f	101.11 ± 0.30 ^f	14.60 ± 0.45 ^a	15.44 ± 0.37 ^a
df	7	7	7	7	7	7	7	7
<i>p</i> -value ANOVA	<0.001	<0.001	0.423	0.145	<0.001	<0.001	<0.001	<0.001
<i>p</i> -value linear	<0.001	<0.001	0.565	0.190	<0.001	<0.001	<0.001	<0.001
<i>p</i> -value quadratic	0.010	<0.001	1.000	0.999	0.996	0.997	0.959	1.000

Averages followed by the same letters in a column are not significantly different according to DMR at 5% level (± SE).

TABLE 7 Impact of eight dietary combinations on biological parameters of *T. molitor*

Diet	Larval weight (g)	Pupal weight (g)	Fecundity
0% moringa+lupin	0.10 ± 0.04 ^h	0.82 ± 0.01 ^g	42.667 ± 2.13 ^d
5 % moringa+lupin	0.10 ± 0.02 ^g	0.89 ± 0.01 ^g	58.00 ± 1.90 ^c
10 % moringa+lupin	0.11 ± 0.03 ^f	0.90 ± 0.01 ^f	63.50 ± 1.26 ^{bc}
15% moringa+lupin	0.12 ± 0.03 ^e	0.90 ± 0.01 ^e	67.66 ± 2.04 ^b
20% moringa+lupin	0.12 ± 0.03 ^{cd}	0.91 ± 0.01 ^d	68.71 ± 2.06 ^b
25 % moringa+lupin	0.12 ± 0.02 ^c	0.93 ± 0.01 ^c	68.44 ± 2.16 ^b
30 % moringa+lupin	0.12 ± 0.03 ^b	0.95 ± 0.01 ^b	71.55 ± 1.68 ^a
35% moringa+lupin	0.13 ± 0.04 ^a	0.97 ± 0.00 ^a	71.71 ± 1.97 ^a
df	7	7	7
<i>p</i> -value ANOVA	<0.001	<0.001	<0.001
<i>p</i> -value linear	<0.001	<0.001	0.036
<i>p</i> -value quadratic	<0.001	<0.001	1.000

Averages followed by the same letters in a column are not significantly different according to DMR at 5% level (\pm SE).

TABLE 8 Nutritional compositions of *T. molitor* larvae fed on different combination of diets

Diet	Dry matter	Crude protein	Crude fat	Ash	Crude fibre
35% moringa+lupin	93.74 ± 0.73 ^a	54.49 ± 0.69 ^a	13.53 ± 0.58 ^a	10.51 ± 0.39 ^a	14.65 ± 0.60 ^a
30% moringa+lupin	93.37 ± 0.67 ^{ab}	51.71 ± 0.96 ^b	12.81 ± 0.30 ^{ab}	9.13 ± 0.40 ^b	12.01 ± 0.44 ^b
25% moringa+lupin	92.18 ± 0.51 ^{ab}	48.71 ± 0.42 ^c	11.75 ± 0.50 ^b	8.00 ± 0.53 ^c	10.637 ± 0.38 ^{cd}
20% moringa+lupin	92.12 ± 0.46 ^b	45.66 ± 1.05 ^d	10.37 ± 0.45 ^c	8.42 ± 0.57 ^c	11.14 ± 0.63 ^{bc}
15% moringa+lupin	90.63 ± 0.46 ^c	44.93 ± 0.98 ^d	9.91 ± 0.48 ^{cd}	8.00 ± 0.53 ^c	10.10 ± 0.32 ^{cd}
10% moringa+lupin	90.66 ± 0.27 ^c	42.07 ± 0.74 ^e	8.96 ± 0.41 ^{de}	8.42 ± 0.57 ^c	10.26 ± 0.57 ^{cd}
5% moringa+lupin	90.21 ± 0.38 ^c	41.25 ± 0.52 ^e	8.07 ± 0.41 ^{de}	8.00 ± 0.48 ^c	9.39 ± 0.31 ^d
0% moringa+lupin	90.01 ± 0.32 ^c	40.07 ± 0.38 ^e	9.01 ± 0.41 ^e	7.92 ± 0.08 ^c	9.63 ± 0.28 ^d
df	7	7	7	7	7
<i>p</i> -value	0.003	<0.001	<0.001	0.0368	<0.001
<i>p</i> -value linear	<0.001	<0.001	<0.001	<0.001	<0.001
<i>p</i> -value quadratic	0.293	<0.001	0.210	0.919	0.942

onine, which support efficient protein synthesis and tissue growth in insects (Chiofalo *et al.*, 2012). Similar findings were reported by Meneguz *et al.* (2018) and Gasco *et al.* (2020), who found that plant-based protein ingredients such as moringa and legumes significantly enhanced the protein content of *H. illucens* larvae. The results are also consistent with those of Piercy *et al.* (2023), who observed that the substitution of conventional substrates with nutrient-rich plant meals improved overall protein deposition in insect biomass.

The increase in crude fat observed in larvae fed higher levels of moringa and lupin might be linked to the elevated lipid content and favorable fatty acid profile of these plants. Moringa leaves and lupin seeds are rich in linoleic and oleic acids, which could stimulate lipid accumulation and energy storage in larvae (Oonincx and Finke, 2021; Spranghers *et al.*, 2017). Higher fat

accumulation in *H. illucens* and *T. molitor* has also been associated with the inclusion of lipid-rich feed ingredients, contributing to their suitability as high-energy feed components for poultry and aquaculture (Copelotti *et al.*, 2025; Rumbos *et al.*, 2020). Tan *et al.* (2018) found the lowest fat content in *T. molitor* larvae fed on watermelon waste, while the highest was on banana peels. A diet rich in carbohydrates and lipids can contribute to producing insects that are lipid-rich (Canavoso *et al.*, 2001; Hoc *et al.*, 2020). The fatty acid profile and fat content of the insects are affected by the nutritional composition of the diet (Riekkinen *et al.*, 2022; Van Broekhoven *et al.*, 2015)

Furthermore, the increased ash content in larvae with higher dietary inclusion of moringa and lupin suggests improved mineral uptake and deposition. Moringa leaves are known to be rich in calcium, phosphorus,

potassium, and trace minerals such as iron and zinc (Biel *et al.*, 2017), which may have enhanced mineral retention in the larvae (Morand-Laffargue *et al.*, 2023). Similarly, the higher crude fibre observed at the 35% inclusion level may be linked to the fiber content of moringa and lupin, which could have positively affected gut motility and microbial balance, thereby improving nutrient absorption efficiency. Li *et al.* (2015), Mancini *et al.* (2019) and Ruschioni *et al.* (2020) reported that plant bio-wastes are rich in dietary fiber; however, *T. molitor* possesses a versatile digestive system that enables efficient utilization of these fibrous substrates, converting them into biomass of high nutritional values.

5 Conclusion

Producing more insect biomass in a shorter time-frame is the most effective strategy for commercial-scale insect farming. The results revealed that a 35% moringa+lupin-based diet (35% moringa, 35% lupin, 10% fruit, 10% vegetables and 10% grains) exhibited the best outcomes in terms of larval period, pupal period, larval weight, pupal weight, and fecundity. The current findings advocate a 35% moringa+lupin-based diet as a potential combination for achieving maximum biomass. Moreover, the inclusion of moringa and lupin up to 35% in larval diets significantly improved the proximate composition of both *H. illucens* and *T. molitor*. These plant ingredients can be used to enrich insect biomass, contributing to the development of nutritionally superior, environmentally sustainable alternative protein sources for livestock and aquaculture feed production. Furthermore, a cost-benefit analysis of using these locally available ingredients as feed sources would provide valuable insights into their feasibility at a commercial scale.

Author contributions

AS, MS, MB, RM, and HSB: conceptualization; AS, MS, HSB, and RT: methodology; AS, MS, RMB, HSB, and RT: data analysis; AS, HSB, RT, and RM: writing (review and editing). All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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