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Utilization of Rearing Residue of The Peach Fruit Fly, *Bactrocera zonata* for Breeding the Yellow Mealworm, *Tenebrio molitor*

Reda S. Hassan; Thanaa M. Sileem and Waheed A.A. Sayed
Biological Applications Department, Nuclear Research Canter, Egyptian Atomic Energy Authority, Cairo, Egypt
***E-mail:** Redahassan28812@yahoo.com

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ABSTRACT

Valorisation of rearing residue of peach fruit flies (PFF) *Bactrocera zonata* into high-value products by yellow mealworm (YMW) *Tenebrio molitor* could consider a sustainable strategy for developing the PFF management using Sterile Insect Technique (SIT). Different ratios of PFF rearing residue (PF) in substantial amounts of hen feed (HF) were used for rearing YMW larvae. The quality control and performance parameters of YMW were assessed. It was noticed that the feeding trials resulted from promising body mass as compared to the control treatment (hen feed). While no progeny of YMW adult was produced when fed on PF 1 diet (100 % rearing residue). Larval and pupal duration of YMW was insignificantly prolonged when reared on PF3 and PF4 diets that contained 50 and 25 % rearing residue, respectively as compared to the HF diet (control). All experimental diets exhibited high rates of pupal production; however, the pupal yields were desirable during larval survival. High percentages of food consumed and food assumed to be assimilated by YMW larvae fed on PF4. There were no significant differences were recorded in the live and dry weight gain of YMW larvae fed on PF4 and HF. Our findings suggest that PFF rearing residue was effective dietary for YMW and could significantly diminish the SIT cost and environmental savings.

INTRODUCTION

The yellow mealworm (YMW), *Tenebrio molitor*, is a species of darkling beetle that has gained attention not only as a potential food source but also for its ability to contribute to waste management (Ramos-Elorduy *et al.*, 2002). The efficient capacity of mealworms to consume and convert various forms of different organic substrates has drawn interest from researchers and industries seeking sustainable side-stream management solutions (Lardé, 1990). Besides, YMW are also known as detritivores, feeding on decaying plant matter, dead insects, and other organic debris in their natural environment. This feeding behaviour can be harnessed to address organic waste management challenges by introducing mealworms into controlled environments where specific waste materials are abundant (Li *et al.*, 2013). While YMW shows promise as waste consumers, several challenges and considerations need to be addressed for their successful integration into waste management systems to produce quality and safety of biomass (Sangiorgio *et al.*, 2021). Understanding the biology and growth rate of YMW is essential for optimizing its production, managing populations, and harnessing its potential in different industries (Toviho and Bársony, 2022). Furthermore, mealworms are being explored as a sustainable and alternative protein source for human consumption. Their high protein content, balanced nutritional profile and potential for large-scale production make them an attractive option to address global food security challenges and reduce reliance on traditional livestock farming (Liu *et al.*, 2020). It is imperative to consider scalability and logistical aspects when implementing waste management systems based on mealworms on a larger scale. Proper facilities, handling procedures, and waste collection networks must be established to effectively utilize mealworms for waste treatment (Madau *et al.*, 2020; Cadinu *et al.*, 2020). Utilization of insect mass-rearing residue of different insect facilities as YMW could establish a novel circular economy system in particular Sterile Insect Technique (SIT) programs. SIT requires a big facility for targeted insect pests that reach millions to billions of sterile insects per week like fruit flies and mosquitos, to induce sterile mating in a natural population that reduces its reproduction to a level below the population maintenance (Dyck *et al.*, 2021). Recently, *Bactrocera spp*. has been the main horticulture pest worldwide, peach fruit fly, *Bactrocera zonata* (PFf) is an invasive pest in Egypt (Sayed *et al.*, 2018), infesting many fruit and vegetable crops, and constantly threatening the food security. SIT against *Bactrocera spp.* has a long history and is considered the most successful control method (Dominiak *et al.*, 2022). Economical and innovative methods are needed for manipulating the rearing waste of produced millions of sterile *Bactrocera* flies. Accordingly, black soldier fly (BSF), *Hermetia illucens* have been used for recycling insect-rearing residue, for instance, medfly *Ceratitis capitata* (Sayed *et al.*, 2023), Se-rich silkworm residuals of *Bombyx mori* (Marco *et al.*, 2021), and locust and cricket rearing waste (Jucker *et al.*, 2020). Moreover, the rearing waste of medfly larvae was fed ruminants, which were quickly degraded and had high nutritional constituents (Mastrangelo *et al.*, 2010). This implies that the residue of PFF mass raring produced from the SIT facilities could also be recycled by YMW. The use of residue as feed has significant economic and environmental ramifications in the context of the circular economy in insect mass rearing, Similar to other facets of animal agriculture (Bordiean *et al.*, 2022). The goal of this research was to investigate the potential for transforming the PFF-rearing residue into high-value products for improving the SIT cost, by exploiting the known role of YMW in organic waste management, taking into account the quality control parameters and growth performance associated with this novel strategy.

MATERIALS AND METHODS

Colony Maintenance:

The yellow mealworm (YMW) *T. molitor* was reared at 26 °C and 50 % relative humidity. in rounded plastic bowls (15 x 70 cm) covered with muslin cloths providing pieces of carton and hen as feed (HF). Approximately 400 adults were placed in oviposition trays with 1 kg of wheat bran as a food source and oviposition substrate. After 1 week, adults were removed and transferred to the new substrate, whereas newly hatched larvae remained in the tray for a period of approximately 11-15 weeks, during which food (hen) was refilled as needed. Adults and larvae were provided with slices of fresh potatoes twice a week.

Feed Substrate:

Five different diets were tested for rearing the YMW, these diets were based on using the peach fruit fly (PFF), *B. Zonata* rearing residue to replace the hen feed (HF). The

HF was purchased from the local market, while the PFF was collected after the larvae left their rearing diet to pupate. The waste was oven-dried at 40 °C for 24 h and kept at room temperature until use. Both PFF and PF were irradiated before use in the experiments with Cobalt 60 gamma cell (1.0 Gy/min dose rate) by a dose of 800 Gy for elimination of unfavorable pests or different pathogens that could infect the waste (Sayed *et al.*, 2023). Five diets differ in the percentages of protein sources used for YMW feed, which were constructed as follows

1) 100 % of PFF rearing residue: 0.0 % Hen feed (PF1 diet);
2) 75 % PFF rearing residue: 25% hen feed (PF2 diet);
3) 50 % PFF rearing residue: 50% hen feed (PF3 diet);
4) 25 % PFF rearing residue: 75% hen feed (PF4 diet) and
5) 100 % hen feed: 0.0 % PFF rearing residue (HF diet) as a control

Bioassay Studies:

For the bioassays, 50 mixed-sex adults of YMW were introduced in plastic boxes (15 x 30 x 10 cm) and left to oviposit for 1 week on 150 g from tested diets. In all treatments, adults were provided with a slice of fresh potato at the beginning of the trial. After 1 week, adults together with the potato slides were removed and adult mortality was determined. For all bioassays, plastic boxes remained until pupation at 26 °C, 50% relative humidity and 12 L: 12 D photoperiod. The removed adult was maintained in other plastic boxes containing the same tested diets and the longevity on different diets was recorded. Also, the Adults were observed every week to estimate their survival on tested diets. Growth performance was determined by comparing the Four-week-old larvae fresh weights on different diets, each group of larvae was weighted at the beginning of the experiment, and their initial weight was recorded. At the end of the experiment near pupation (8 weeks), larvae from each box were separated from the food and weighed as a group. The remaining food and frass were dried in an oven at 50 °C and weighed. The weight gained data and food consumption were used to calculate food utilization parameters (Lienhard *et al.*, 2023). Live weight gain (LWG) was determined by subtracting the initial larval weight from the accumulated weight of live larvae from each dish, while Dry weight gained (DWG) was calculated by multiplying LWG by the dry weight proportion of *T. molitor* (Finke and association, 2002). Food consumption (FC) was calculated by subtracting the weight of the remaining food from the weight of the food provided, and food assimilated (FA) was calculated by subtracting the frass weight from the weight of the food consumed (FA = FC – frass) (Kang *et al.*, 2023). Additionally, the efficiency of ingested food conversion (ECI) was calculated as (ECI= DWG * 100 / FC) and the efficiency of digested food conversion (ECD) was as (ECD = DWG * 100 / FA) (Karapanagiotidis *et al.*, 2023). The boxes were monitored weekly for food consumption to prevent the larvae from running out of food. Food provided per larvae increased every week as larvae food consumption increased with age. After pupation the emerged adults were counted for both diets to compare the ability of insects to reproduce next generation.

Data Analysis:

One-way ANOVA ($P \leq 0.05$) with subsequent Tukey HSD tests was applied to detect the differences from the treatments. The parameters ECD and ECI expressed as percentages, were converted by arcsine tables for analysis. Means of adult longevity, larval and pupal durations, pupal productions, weight gains and food consumption were calculated from the original data.

RESULTS

Yet grouped YMW adults fed on experimental diets had lower longevity than those on PF1(Fig. 1), while no significant difference was recorded when reared on PF2, PF3 and PF4 diets compared with the control diet (HF) ($F_{(3,11)} = 5.05$; $P = 0.173$).

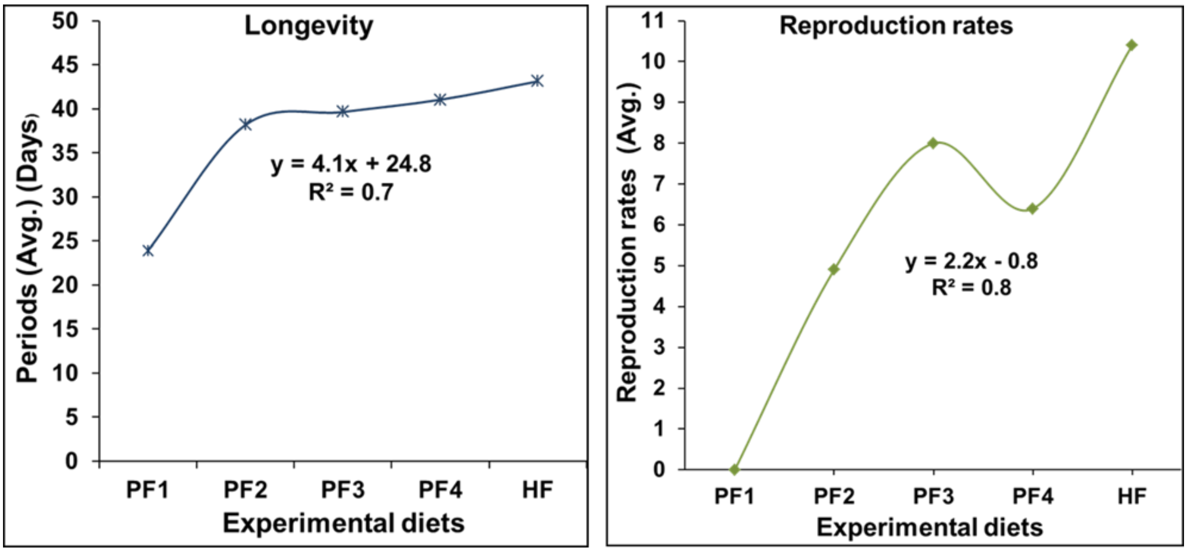

Fig 1. Average adult longevity (days) and average reproduction rates of YNW *Tenebrio molitor* reared on different diets including varying percentages of hen feed (HF) and peach fruit fly rearing residue (PF).

No progeny was obtained by the adults fed on the PF1 diet whereas the reproduction rate was impacted by the type of diet. The adults who developed on the PF1 diet were not able to produce progeny; while the HF diet produced higher significant progeny (10.4) than the other experimental diets. Data revealed that using different ratios of PFF rearing residue as feed had a negative impact on YMW adults to procreate a next generation. The reproduction rates of insects reared on PF2 amounted to 4.9, which was significantly reduced in comparison with the reproduction rate of the insects developed on PF3 (6.4) and PF4 (8.0).

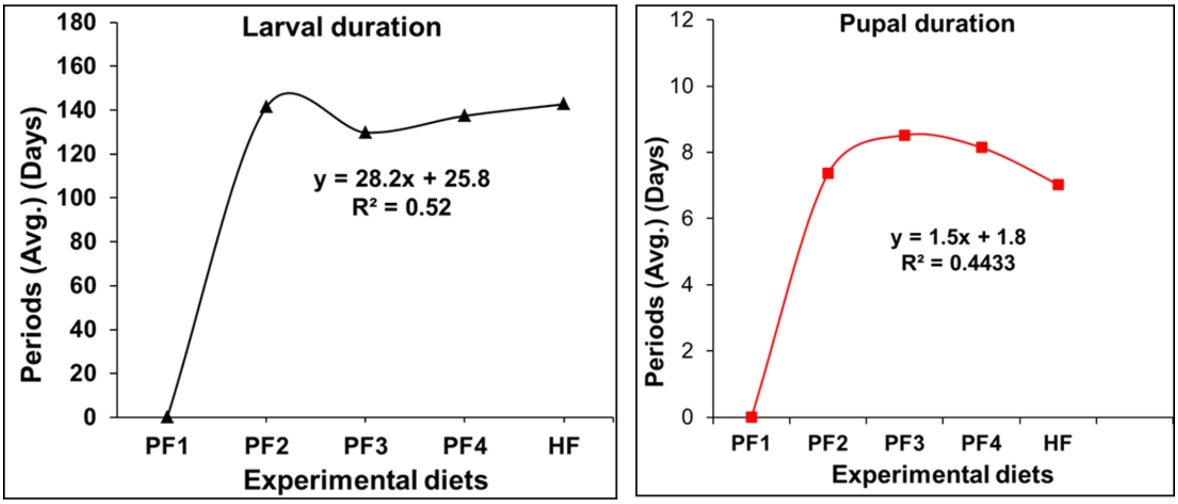

Fig. 2. Averages (days) of larval and pupal durations of YMW *Tenebrio molitor* reared on different respective diets that contained varied percentages of peach fruit fly rearing residue (PF) and hen feed (HF).

The PF3 diet resulted in a significantly shorter larval duration (129.8±3.4 days) compared to the other diets, indicating a significant variation in insect development among the investigated diets. ($F_{(3, 11)}$ = 0.0; P = 0.999). Moreover, the pupal durations developed on PF3 and PF4 diets were significantly higher than those reared on PF2 and PF3 diets ($F_{(311)}$ = 0.01; P = 0.98) (Fig. 2).

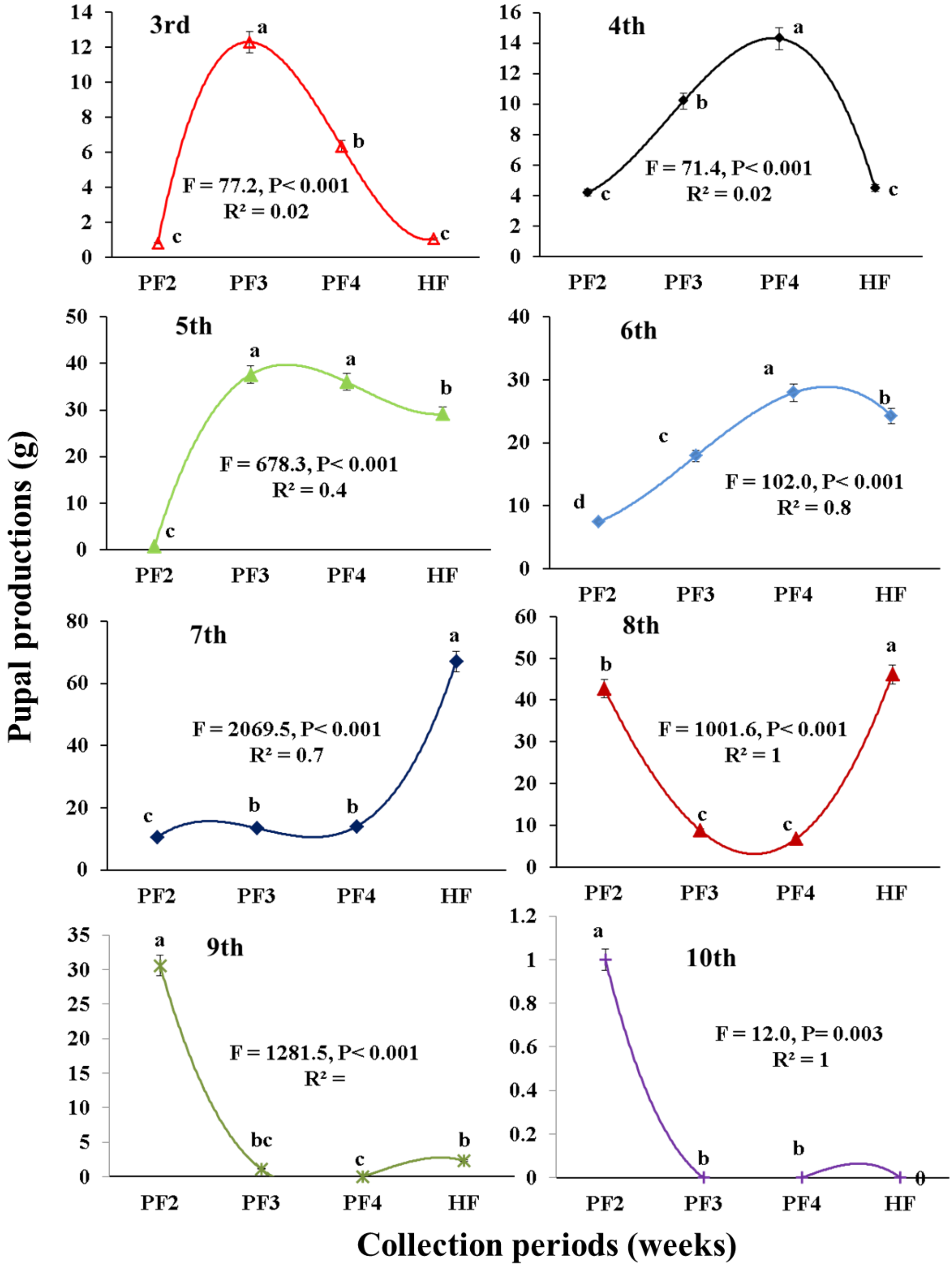

Fig. 3. The average pupal production (g) derived from the egg samples laid by the adult YMW *Tenebrio molitor* reared on different respective diets that contained varied percentages of peach fruit fly rearing residue (PF) and hen feed (HF).

Pupal production from larvae was different during the larvae survival as well as the tested larval diets as shown in **Figure 3.** No pupae were produced in the first and second week of larval survival at all tested diets, while the maximum yield of pupae was produced during 5-9 weeks of larval survival.

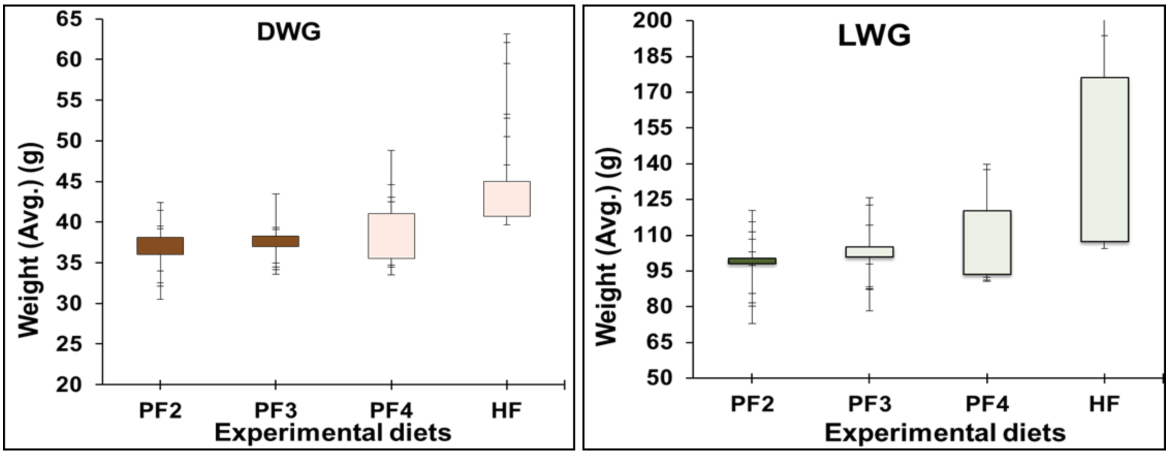

Fig. 4. Average of dry weight gained (DWG) and live weight gain (LWG) (g) of YMW *Tenebrio molitor* reared on different respective diets that contained varied percentages of peach fruit fly rearing residue (PF) and hen feed (HF).

An increase in the weight of individual larvae expressed as larval weight gain (LWG) was monitored and recorded every 7 days for the successful experimental diets. Non-significant differences were in larval weight of the experimental diets with different ratios of PPF rearing residue, while the LWG in the case of the HF diet was significantly higher ($F_{(3,11)} = 3.6$; $P < 0.05$) (Fig. 4).

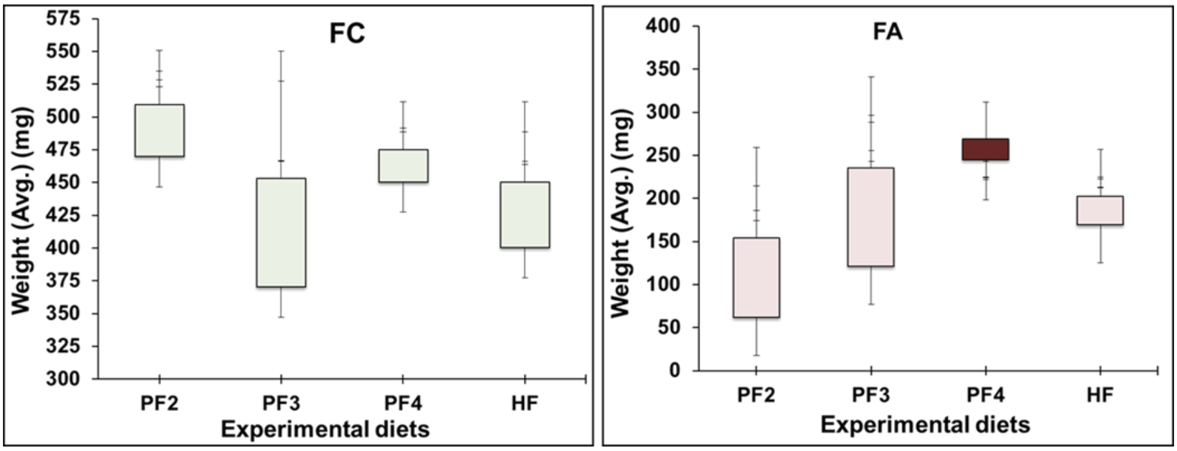

Fig. 5. Averages (mg) of food consumed (FC) and food assimilated (FA) of YMW *Tenebrio molitor* reared on different respective diets that contained varied percentages of peach fruit fly rearing residue (PF) and hen feed (HF).

Considering the increase in larval weight during the 160 days of the experimental period, the larvae exhibit the highest growth rate expressed in daily weight gain (DWG) on the control diet (HF) ($F_{(3,11)} = 3.6$; $P = 0.07$) (Fig. 4).

The food consumption (FC) by YMW larvae developed on PF2 ranged from 469.5 to 539.5 mg with an average of 509.3 mg (Fig. 5) whereas it was significantly higher than the FC averages (425.9, 475.0 and 450.0 mg) of the other experimental diets (PF3, PF4 and HF), respectively ($F_{(3,11)} = 0.77$; $P< 0.05$)= (Fig. 4). In contrary, the larvae fed on PF2 diet had the lowest average food assimilated (FA) (153.95±25.3 mg) that ranged from 61.3 to 237.1 mg □ compared to the averages (234.9± 29.5, 244.4± 6.8 mg and 201.8± 9.4 mg) of PF2, PF3 and PF4 diets, respectively ($F_{(3,11)} = 1.3$; $P = 0.04$).

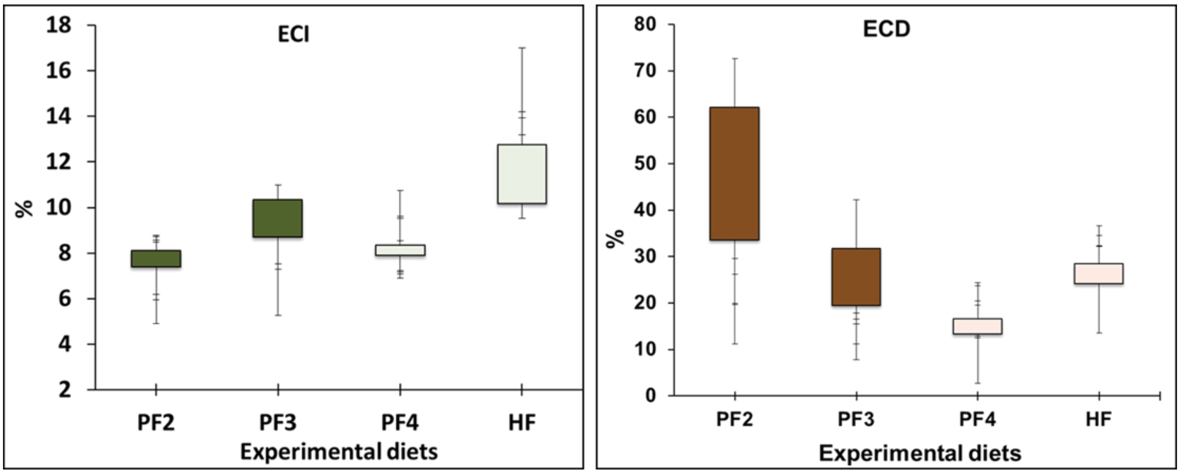

Fig. 6. Percentages Efficiency of ingested food conversion (ICI) and efficiency of digested food conversion (ECD) of YMW *Tenebrio molitor* reared on different respective diets that contained varied percentages of peach fruit fly rearing residue (PF) and hen feed (HF).

Data in (Fig. 6) showed a higher efficiency of ingested food conversation (ECI) by the insect fed on the HF diet than those fed on the diets containing different percentages of PFF rearing residue (PF3, PF4 and HF diets) ($F_{(3,11)} = 7.3$; $P = 0.11$). While significant differences were recorded between the tested diets regarding the efficiency of digested food conversion (ECD) ($F_{(3,11)} = 0.97$; $P = 0.045$).

DISCUSSION

Management of PFF rearing residue remains a challenging and underutilised important issue of the SIT program. Our hypothesis in this study is to extend the efforts for developing the SIT cost by manipulating the mass-rearing residue/waste to a high-value product. The performance and quality control parameters of YMW in response to using PFF-rearing residue as supplementary food were depicted in this current study. Developmental stages of growth, insect reproduction and feed conversion efficiency were assessed. Another possible advantage is the valuation of such residue using YMW in a large production system as a sustainable technology to reduce organic waste, the final larval stage product is a useful supplement to both human and animal meals, creating new global economic opportunities for different enterprise levels, and explored the benefits of this strategy (Finke and association, 2002). The growth curves of fresh and dry weights of mealworm larvae are determined by multiple factors involved in diet composition and type (Li *et al.*, 2013; Shafique *et al.*, 2021). The experimental diets had a substantial effect on adult longevity and reproduction rates of the PF1 diet that contained 100 % PFF rearing residue. Compared to the PF1 diet, increasing in the durations of adult longevity of other experimental diets was recorded. Our results are in contrast with (Riaz et al. 2023) who reported 94 days as a life expectancy in adult beetle. The different reproduction rates in response to the experimental diets are agreeable with (Langston et al. 2023) who found that the fecundity of YMW adults was different among feeding substrates whereas the larval rates were significantly varied. It was reported that the ratio of protein: to carbohydrate intake is important for the fitness and performance of YMW (Kröncke and Benning, 2023).

The proximate composition of fruit fly rearing residue compared to hen feed was previously analysed in our laboratory (Sayed *et al.*, 2023), in which protein: carbohydrate in hen feed was higher. However, the black solder fly larvae were able to transform the residue without negatively impacting feeding efficiencies. Interestingly, the PFF rearing residue exhibited a lower time for larvae to develop into pupae than the HF diet, this reduction of larval duration achieved a decrease in the rearing cost. This hypothesis agrees with (Morales-Ramos *et al.*, 2010) and (Baldacchino *et al.*, 2023) who suggest that the delay of larval development is a potential constraint of diet use for rearing YMW. Given the short pupal life span, the varying times of beetle emergence in the investigated diets may have little impact on the rearing cost. Although the reproduction rate of the diet containing different ratios of both PFF rearing residue and hen feed (PF2, PF3 and PF4), it is probably still commercially viable to rear mealworms using these diets after taking the costs of hen feed (HF). Regardless of the tested rearing diets, its incorporation amounts influenced the pupal productions among the larval life span, the obtained pupae in our trial were high in comparable to the other previous investigations on different substrates (Montalbán *et al.*, 2023), (Yakti *et al.*, 2023) and (Syahrulawal *et al.*, 2023). A high larval weight gained from the control treatment (HF) compared with the PF diets agrees with the hypothesis that larvae may have had a distinct growth rate rhythm because they were fed on various diets, whereas a lower weight gain may be due to feeding on an insufficient diet adapted to their needs (Bordiean *et al.*, 2020). The lower efficiency of ingested feed (ECI) values was lower in PFF diets than in HF diets indicating that the protein content of diets may have a negative impact on food ingestion by YMW larvae. Our results agree with the researchers who confirmed that YMW larvae grown on diets with low protein content showed lower efficiency of conversion of ingested feed (ECI) than larvae grown on high-protein-content diets (Oonincx *et al.*, 2015; Van Broekhoven *et al.*, 2015). Our results of food consumption (FC) indicated that the maximum FC of YMW larvae was on a PF2 diet that contained a high ratio (75%) of PFF-rearing residue. In contrast, the food assimilated (FA) average was highest on HF that contained 100 % hen feed. The larvae reared on the tested diet containing PFF rearing residue consumed more diets than HF. It has been reported that growing and developing YMW on tomato leaf waste is a sole nutritional source, given its ability to consume a wide range of plants. The diet exhibited high digestibility due to higher FA. This could be attributed to the different approximations of tested diets, especially fibre and fat contents (Montalbán *et al.*, 2022).

CONCLUSIONS

This study indicated that the range of peach fruit fly (PFF) rearing residue could be employed in yellow mealworm production (YMW); this strategy can strengthen the fruit flies SIT programs, by eliminating the rearing residue to produce by-products and save the environment. The (YMW) is a species that is well-adapted to a wide range of diet types. Moreover, YMW aligns with the European Union's present strategy by fostering short supply chains and a circular economy. The current study assessed the fitness and quality control parameters of YMW fed on different categories of PFF residue to hen feed (HF). In general, YMW adults and larvae grew adequately in all experimental diets except those that had 100 % PFF rearing residue (PF1diet): 0.0 % hen feed (HF) during the trial. The longevity was significantly lower for beetles reared on the PF1 diet than the other tested diets. Furthermore, the reproduction rate of beetles fed on the PF1 diet was 0.0 whereas no development (egg, larvae and pupae) was produced, while, the maximum rate was recorded in the beetle fed on the HF diet. The periods of larvae and pupae fed on experimental diets were relatively similar, however, the larvae fed on the PF2 diet (50 % rearing residue: 50 % hen feed) had shortened periods, while their pupal periods were prolonged as compared to the other experimental diets. The percentages of pupal production varied during the larval life span depending on the type of diet. The dry larvae' weight gain was the highest of the HF diet, while, the larvae fed on the HF diet consumed a little more food than the other diets that have different percentages of rearing residue. The highest Efficiency of Conversion of Ingested feed (ECI) was found for the larvae fed on HF diet. It can be argued that the diets contained different percentages of PFF-rearing residue tested in this study, except the PF1 diet could successfully be used for mealworm farming. Further researches are required to analyse the nutritional profiles of YMW larvae and also determine the optimum inclusion of PFF-rearing residue in diets of YMW that will complement the current study, whereas this is the first report of the plasticity of YMW in response to PFF-rearing residue as feed.

Declarations:

Ethical Approval: Not applicable.

Competing interests: The authors declare no conflict of interest.

Authors Contributions:I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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