LARVAL FEEDING FROM SOME ARTIFICIAL DIETS AND ITS EFFECTS ON BIOLOGICAL PARAMETERS OF THE MEDITERRANEAN FLOUR MOTH

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ABSTRACT: In this study, we evaluated the effects of five diets including: (1) wheat flour + wheat bran (3:1 w/w) (FB), (2) wheat germ + brewer's yeast + glycerol (10:1:2 w/w) (MYG), (3) wheat germ + brewer's yeast (10:1 w/w) (MY), (4) wheat bran + brewer's yeast + glycerol (20:1:2 w/w) (BYG), and (5) wheat bran + brewer's yeast + glycerol + water (100:5:10:5 w/w) (BYGW) on some biological parameters of the Mediterranean flour moth, *Anagasta kuehniella*. All experiments were done at laboratory conditions of 25 ± 2 ºC temp., 70 ± 8% R.H., and L:D 12:12. Each experiment repeated four times. Some biological properties of larvae and adults investigated. Results showed that rearing diets had no effect on embryonic development and percentage egg hatch. Highest means of larval period, pre-oviposition period, and egg to adult emergence period; and lowest means of weight of male and female larvae, pupae, and adults, female fecundity, and adult males' and females' longevity were observed on BYG diet. In contrast, highest means of female fecundity, percentage of survival from egg to adult, weight of larvae, pupae and adults, and adult longevity, and lowest means of per-oviposition period, larval period, and egg to adult emergence period observed on FB and MYG diets. Based on these parameters, FB and MYG diets (MYG without considering the economic costs) are the best diets for rearing the Mediterranean flour moth.

KEY WORDS: Mediterranean flour moth, artificial diet, biological properties.

The Mediterranean flour moth *Anagasta kuehniella* (Zeller), as a beneficial insect, has its own significance both in mass-rearing programs of parasitoid wasps; and in physiological, toxicological, and ecological investigations. This moth and other stored-product moths and beetles are often used in laboratories with the aim of studying insect life histories and adult mating strategies (Ryne et al., 2004). These insects are cosmopolitan and are able to develop in different commodities and have adapted to live in high densities and on abundant but poor diets (Trematerra, 1997). By literature review, it was revealed that in those laboratory experiments in which this insect and its closely related species, the Almond moth *Ephestia cautella* (Walker), were used for purposes other than mass rearing, larvae were reared on standard artificial diets including wheat germ, glycerol, dried yeast and wheat bran (eg. Cook et al., 1997; Sasaki & Ishikawa, 1999; Ikeda et al., 2003; Ryne et al., 2004) instead of natural diets of wheat flour and bran or flours and kernels of other grains. This is mainly done to eliminate the unrecognizable and variable effects of natural diets (Ryne et al., 2004). In insect artificial dies, glycerol is added as an additional nutrient to prevent mold growth (Norris, 1934; Bell, 1975) and to act as a humectant because first instars rarely survive if dietary moisture is too low (Benson, 1973). Some artificial diets such as an artificial one consisting of a hybrid commercial yellow maize and yeast and a diet containing white maize with very high lysine and

tryptophan contents added to yeast can use for mass rearing of *Trichogramma* spp. on *A. kuehniella* (Magrini et al., 1995). Due to this and because of lacking any comprehensive investigation regarding the effects of these diets on the biology of this insect, the present study carried out to compare selected artificial diets with a natural food containing wheat flour and bran, and also to pave the way for choosing the best and the most appropriate artificial diet in prospective researches with ecological, toxicological, and physiological aims.

MATERIALS AND METHODS

Insect culture

To fulfill the project, newly laid eggs (max. 24 hrs old) of *A. kuehniella* were used. The larvae reared on wheat flour for 5 generations. Experimental conditions were as 25±2 ºC Temp., 70 ± 8% R.H., and L12: D12.

Food treatments

The ingredients of diets in the present study included wheat flour, wheat bran, wheat germ, brewer's yeast, and glycerol. The treatments comprised of: (1) wheat flour + wheat bran (FB) (3:1 w/w) (Yazdanian et al., 2000); (2) wheat germ + brewer's yeast + glycerol (MYG) (10:1:2 w/w) (Ryne et al., 2004); (3) wheat germ + brewer's yeast (MY) (10:1 w/w) (Ryne et al., 2004); (4) wheat bran + brewer's yeast + glycerol + water (BYGW) (100:5:10:5 w/w) (Ikeda et al. 2003); and (5) wheat bran + brewer's yeast + glycerol (BYG) (20:1:2 w/w) (Sasaki & Ishikawa, 1999). The experiments conducted in a one-way ANOVA with four replications.

Biological parameters

In this study, we investigated the following biological parameters: larval period duration (the days between the observations of first instars to emergence of the first pupa); weight of fifth instars, pupae, and adult males and females (weighting of 20 individuals from each developmental stage; in the case of fifth instars, those who were finished their feeding were selected); fecundity (random selection of 25 pairs of males and females with maximum longevity of 12 hrs from each treatment and counting eggs laid every day); Male and female adults' longevity, proportions of eggs hatched, and survival from egg to adult (rearing of 100 eggs in each replication and calculating the percentage of survival using the formula: Survival % = [No. of adults emerged/No. of eggs] × 100).

Data analyses

The data were analyzed by using the MSTAT-C statistical software (ver. 2.10). Means were separated by using the LSD test, at $P = 0.01$.

RESULTS AND DISCUSSION

Larval period

In the present study, the shortest and longest larval periods observed in MYG (14.00 days) and BYG (23.75 days) diets ($F_{4,15} = 57.49$; $P = 0.0000$) (Fig. 1). Yazdanian et al. (2000) reported that in moisten diets (12% moisture content), larval period declined compared to dry diets (8% moisture content). According to our results, in diets containing glycerol, due to provision and retention of moisture by glycerol (Ryne et al., 2004), larvae contribute less energy for feeding and producing metabolic water and their food needs are provided at a higher speed. As a result, larval period decreases (Yazdanian et al., 2000). On the other

hand, in diets containing wheat bran, due to its toughness, the larvae (especially firs instars) feed hardly on them and consequently larval period prolongs (Yazdanian et al., 2000). Adding water to BYG diet (the BYGW diet), because of providing more food moisture, decreased the larval period significantly (Fig. 1).

Weight of fifth instars male and female larvae

The main effects, diet ($F_{4,30}$ = 17.52) and sex ($F_{1,30}$ = 18.17) were highly significant ($P \leq 0.0002$). However, the associated interaction, diet × sex ($F_{4,30}$ = 1.75) was not significant (P = 0.165).

According to the results of this investigation, the highest weights for fifth instars male and female larvae observed in MYG (26.60 and 28.46 mg), MY (26.64 and 27.52 mg), and FB (21.55 and 26.58 mg) diets while the lowest ones observed in BYG (20.40 and 21.45 mg) diet (Fig. 2). In all investigated diets, female larvae weighed more than male ones. Yazdanian et al. (2000) reported that rearing the larvae on diets containing wheat flour + wheat bran (3:1 w/w), wheat flour, wheat flour + wheat bran (1:1 w/w), and wheat flour + wheat bran (1:3 w/w) resulted to the highest weight of fifth instars male and female larvae, respectively. They justified the maximum growth on diet containing 25% bran with the fact that only fifth instars were capable of feeding on bran and this together with the nutrient richness of bran comparing to flour have increased the larval body weight. In diets lacking bran, though larvae fed well on them, they weighed less due to lack of nutrients. In diets containing higher amounts of bran, because of feeding of first, second and third instars on rough particles of bran, and because of tension imposed on them, more body weight loss is depicted that their later effects are shown in pupae and adults. Ziaie Madbooni & Farshbaf Pour Abad (2012) did not observe any significant difference in larval body weight by rearing the *A. kuehniella* on different cultivars of wheat (Rasad, Shiroodi, Tajan, Gowhadasht, Niknejhad, N-80-19, Zagros, Azar 2, Sardari, and Arta), except for Shiroodi and Arta. In these two cultivars, because of insufficient flour available to larvae, their body weight decreased. The kernels of these two cultivars have high moisture contents and roughness, and are not well grind. As a result, larvae especially early instars cannot feed properly. In the present study, feeding of early instars from BYG diet decreased larval body weight due to the presence of bran in the diet and feeding stresses by consuming it. Adding water to this diet (BYGW diet) helped to increase larval body weight (for female larvae significantly). In MYG and MY diets, the presence of brewer's yeast and wheat germ, which are two substances containing vitamins, could lead to higher body weight. In all diets, female larvae weighed more than male ones that this corresponds to Yazdanian et al. (2000) and Eyvazian Kari (2001).

Weight of male and female pupae

As for the previous biological parameter, the main effects, diet ($F_{4,30}$ = 18.15) and sex ($F_{1,30}$ = 11.91) were highly significant ($P \leq 0.0017$) and the associated interaction, diet × sex ($F_{4,30}$ = 1.17) was not significant (P = 0.345).

Highest weights of male and female pupae observed in MYG (24.50 and 27.83 mg) and MY (21.77 and 25.09 mg) diets and the lowest amounts observed in BYG (15.03 and 17.88 mg) diet (Fig. 3). Rodriguez et al. (1988) reared the larval *A. kuehniella* on four seeds (soybean, barley, wheat, and corn) and two vitamin containing foods (wheat germ and bread yeast) and observed that adding wheat germ and bread yeast to diet increased body weight of female pupae. Furthermore, body weight of female pupae was higher than that of male pupae. In our study, this is also true about the male pupae. They suggested that in *A.*

kuehniella, weight of pupae is related to the larval diets. Higher body weight of female pupae comparing to the male ones is in correlation with female and male larval body weight from which they have evolved.

Weight of male and female adults

In this case, not only the main effects, diet ($F_{4,30}$ = 72.30) and sex ($F_{1,30}$ = 103.52) were highly significant (P = 0.0000), but the associated interaction, diet × sex ($F_{4,30}$ = 21.60) was also highly significant (P = 0.0000).

As for the two previous biological parameters, highest body weights for male and female adults observed in MYG (15.16 and 22.49 mg) and MY (12.54 and 20.50 mg) diets, and the least ones observed in BYG (8.57 and 10.31 mg) diet (Fig. 4). Yazdanian et al. (2000) reported that highest means of male and female adults' body weight reared on different diets containing different proportions of wheat flour and bran varied from 9.04 up to 15.13 mg for males, and from 15.39 up to 21.53 mg for females that showed a significant difference. In their study, dry and moisten diets (with 8% and 12% moisture content, respectively) had no effect on body weight. Eyvazian Kari (2001) also reported means from 12.42 up to 17.31 mg and from 17.11 up to 23.96 mg for male and female adults, respectively, reared on diets containing wheat flour and bran. Vieira et al. (1995) reported that the male and female adult weight on three different textures of maize flour (fine, medium, and coarse) were 13.19, 14.77, and 12.38 mg for males and 19.37, 21.81, and 18.30 mg for females. In an experiment with soft wheat flours with the same nutritional value but different particle size, rearing on samples with greatest particle size (250-419 μm) caused the highest mean number of adults and the shortest developmental period (Locatelli et al., 2008). This insect prefers flours (Yazdanian et al., 2000; Eyvazian Kari, 2001) and it is suggested that particle size of flours with the same nutritional value determines the suitability of flour for this species. Like fifth instars and pupae, and in all treatments, female adult weight was more than that of male counterparts. The significance of the interaction of two main effects (i.e. diet × sex) on adults' body weight (but not on the weights of larvae and pupae) reveals the probable different efficiencies of digested food by and hence in the digestive physiology of male and female larvae, which affect the adult stage.

Fecundity

According to results, the higher oviposition rate (eggs/female) observed in FB (325.76 eggs), MYG (295.72 eggs), MY (234.40 eggs), BYGW (205.56 eggs), and BYG (105.56 eggs) diets, respectively ($F_{4,120}$ = 27.08; P = 0.0000) (Fig. 5). These results are correlated with means of females' body weight. Solis et al. (2006) reported that fecundities of *A. kuehniella* on three artificial diets: corn meal, bread crumb, and a mixture of both in equal proportions were equal to 203.82, 154.42, and 226.62 eggs/female, respectively. In a research by Yazdanian et al. (2000), the number of eggs laid by females reared on dry and moisten diets of: wheat flour + wheat bran (3:1), wheat flour, wheat flour + wheat bran (1:1), and wheat flour + wheat bran (1:3) were respectively evaluated as 347.37 & 354, 297.30 & 316.97, 280.00 & 288.00, and 264.00 & 269.00 eggs/female. The number of eggs per female by rearing larvae on different diets containing wheat flour and bran (Eyvazian Kari, 2001) varied from 289 up to 411. Decreasing the fecundity of this species due to the feeding of larvae on rough food particles and weight loss of female adults as e result are in accordance with Yazdanian et al. (2000) and Eyvazian Kari (2001) findings.

Male and female adults' longevity

ANOVA of data showed that the main effect, diet ($F_{4,240}$ = 19.01) and the associated interaction, diet × sex ($F_{4,240}$ = 4.21) were highly significant ($P \leq$ 0.0026). However, the other main effect, sex ($F_{1,240}$ = 3.36) was not significant (P = 0.068).

Male and female adults lived longer in FB (10.44 and 8.92 days) and MYG (10.32 and 8.92 days) diets, respectively, but died sooner in MY (8.80 and 7.48 days), BYG (7.52 and 7.48 days), and BYGW (7.30 and 6.36 days) diets (Fig. 6). In all treatments, males lived longer than females, but the differences were not significant. Kind of diet may have no effect on adult longevity (e.g. Ayvaz & Karabörklü, 2008). In their experiment, Flap et al. (1995) reared four groups of *A. kuehniella* adults including: (1) pairs with free mating, no feeding; (2) pairs with free mating, feeding from honey; (3) pairs allowed to mate once, no feeding; and (4) virgin adults, no feeding. They observed that in all investigated groups, male adults' longevity (about 12 days) was longer than that of females (about 9 days). Ryne et al. (2004) studied the effects of glycerol on fecundity and longevity in the almond moth, *Ephestia cautella* (Walker). Their results showed that larval diets containing glycerol (wet larval diets) significantly increased male and female longevity. It is suggested that glycerol in insect diets is an inert additional nutrient (Bell, 1975; Benson, 1973) and because of its positive effect on moisture retention increases the quality of diets (Benson, 1973). Yazdanian et al. (2000) and Eyvazian Kari (2001) also reported that in this species, male lived longer than females. This phenomenon could possibly be a result of oviposition (one of the most energy-demanding activities) on females.

Proportion of eggs hatched

In all treatments, egg hatchability evaluated equal to 100%. In the research by Solis et al. (2006), proportion of eggs hatched in the *A. kuehniella* reared on three artificial diets: corn meal, breadcrumb, and a mixture of both in equal proportions were equal to 59.30, 85.30, and 90.30 per cent, respectively. Their results differ with our findings and this difference might be due to rearing of larvae on rough and coarse diets by Solis et al. In experiments carried out by Yazdanian et al. (2000) and Eyvazian Kari (2001) on this insect and diets based on wheat flour and bran, proportions of eggs hatched varied respectively from 88.50 up to 90.00 per cent, and from 89.75 up to 91.50 per cent with no significant difference. Proportions of eggs hatched reported by Amaral Filho & Habib (1990), Rodrigeuz Filho et al. (1991), and Jacob & Cox (1977) are 92.54, 98.00, and 97.00 per cent, respectively. Vinuela & Marco (1990) stated that presence of food has the highest effect on egg hatching and emergence of larvae even if there is no direct contact between eggs and food. Thus, we can say that food odor is one of stimulants that encourage larvae to leave the eggs and absence of the odor can decrease proportion of eggs hatched.

Survival from egg to adult

The higher survival rates from egg to adult observed in MYG (98.00%), MY (92.25%), and FB (92.00%) diets, and the lowest means observed in BYG (65.50%) and BYGW (73.75%) ($F_{4,15}$ = 33.94; P = 0.0000) (Fig. 7). According to our results, the presence of bran had a negative effect on survival rate. This finding is in accordance with Yazdanian et al. (2000) and Eyvazian Kari (2001) who stated that this insect prefers flours.

Results obtained from the present study indicated that considering the important biological parameters while rearing the Mediterranean flour moth, and

also considering the economic costs, the natural diet containing wheat flour + wheat bran (3:1) is the best for mass rearing of biocontrol agents on eggs and larvae of this moth. In addition, this diet is prepared fast and easy and its constituents are easily available. In small-scale rearing in research laboratories, using the MYG diet is recommended for aims other than mass rearing of natural enemies such as for carrying out investigations e.g. physiological, ecological, and toxicological studies.

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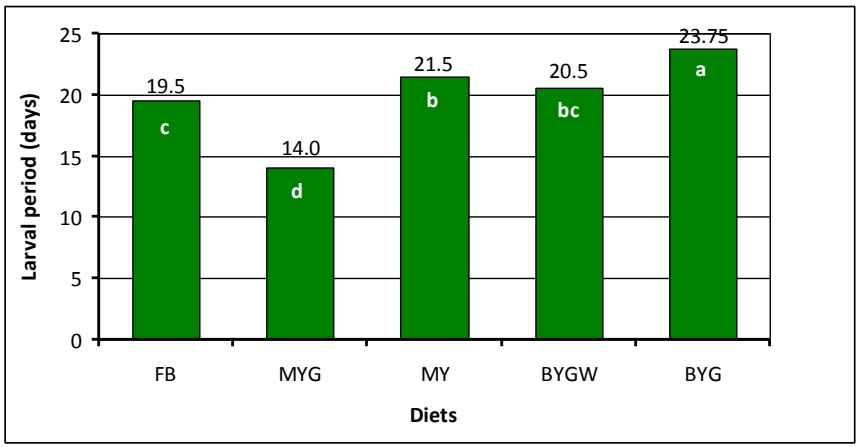

Figure 1. The effect of different larval diets on larval period.

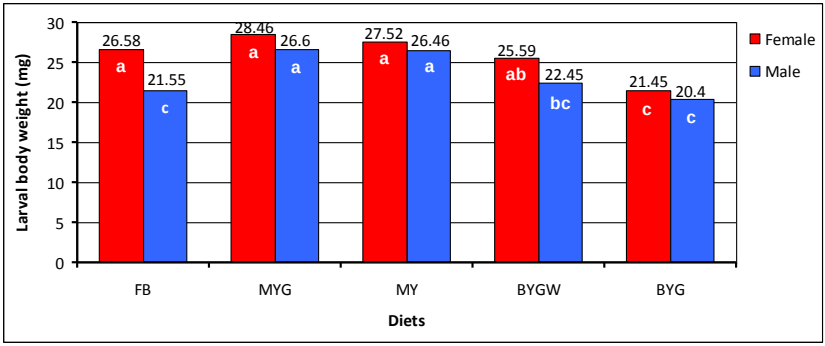

Figure 2. Larval body weight after feeding from different diets by larvae.

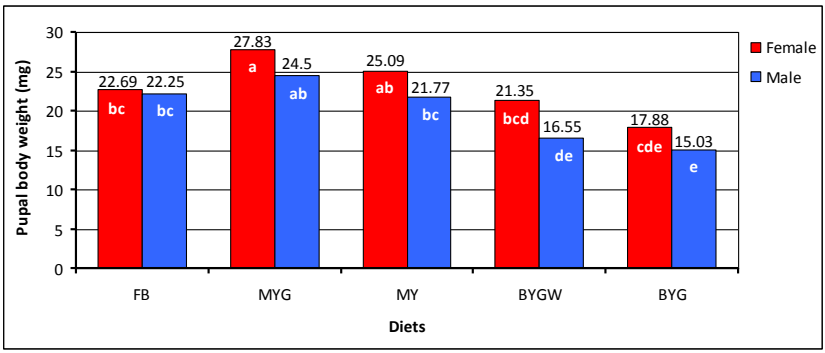

Figure 3. Pupal body weight after feeding from different diets by larvae.

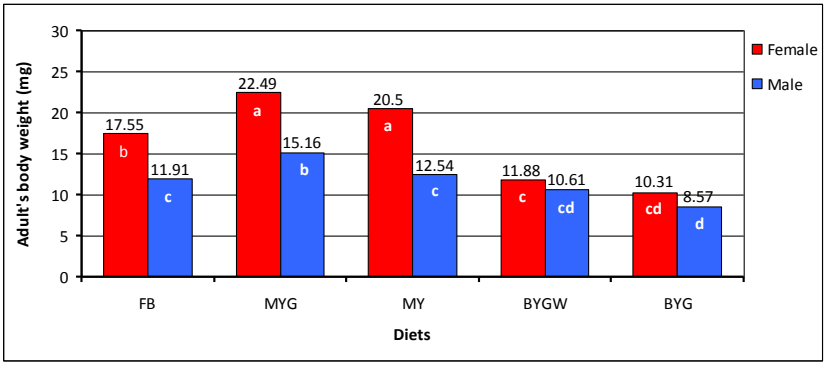

Figure 4. Adult's body weight after feeding from different diets by larvae.

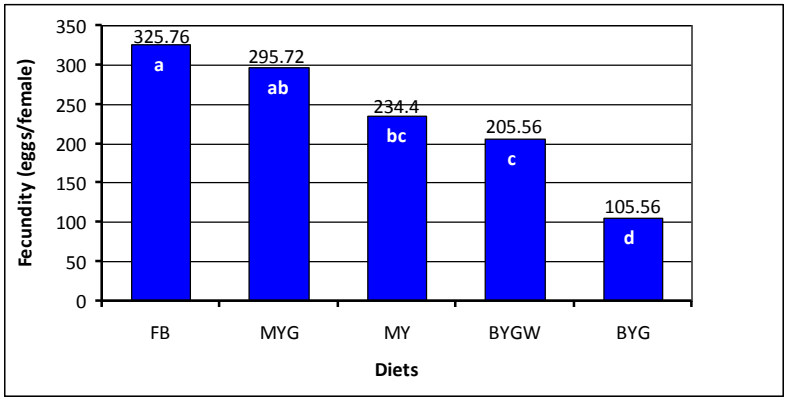

Figure 5. The significant effect of different larval diets on female fecundity.

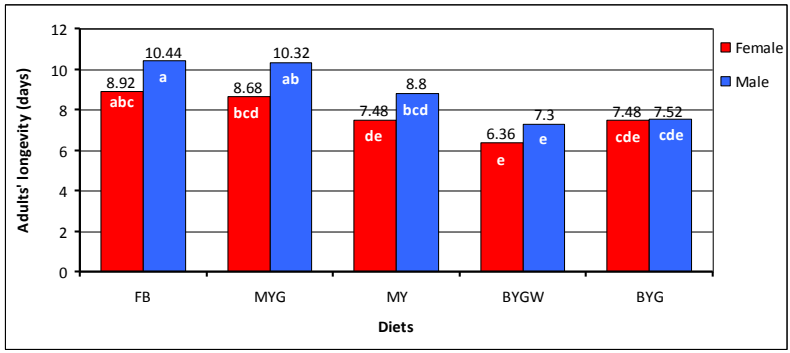

Figure 6. Adult longevity is affected by feeding of larvae from different diets.

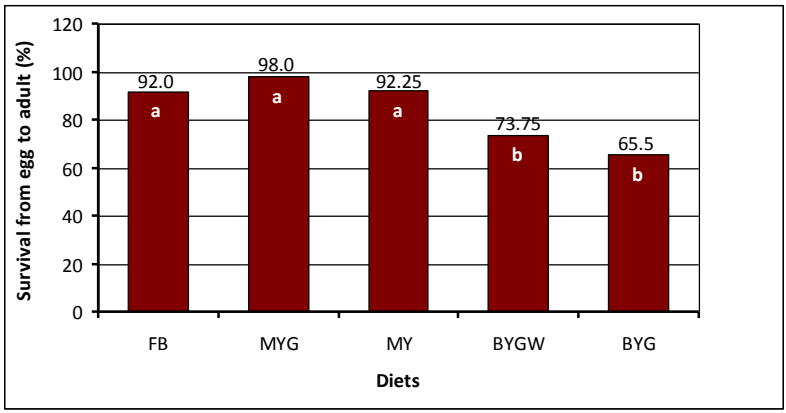

Figure 7. Larval feeding from different diets and its significant effect on survival from egg to adult parameter.