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Araştırma Makalesi

Lahana yaprak güvesi *Plutella xylostella* L.'nın (Lepidoptera:Plutellidae) Laboratuvar Koşullarında Farklı Yapay Diyetler Üzerinde Yetiştirilmesi[&]

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Öz

Lahana yaprak güvesi *Plutella xylostella* L. (Lepidoptera:Plutellidae), Brassicaeous bitkilerin dünya çapında en ciddi zararlılarından biridir. Laboratuvar koşullarında böceklerin toplu olarak yetiştirilmesi için uygun bir yapay diyet formülasyonu gereklidir. Çalışmanın amacı lahana yaprak güvelerin farklı yapay diyetler üzerindeki biyolojik özelliklerini inceleyerek en uygun formülasyonu belirlemek ve yaş-evre ve iki eşeyli yaşam tablolarını ortaya çıkarmaktır. Farklı yapay diyetler ve pozitif kontrol olarak beyaz lahana kullanılarak bazı biyolojik özellikleri belirlemek için iki yönlü hiyerarşik küme analizi kurulmuştur. Hiyerarşik küme analizinde elde edilen sonuçlar göre pupa ağırlığı, ergin ömür uzunluğu gibi bazı biyolojik özellikler için CS diyeti, kontrol ile benzerdi. Yapay diyetlerde GRR, H&H diyetinde 39.92±7.22 yavru/birey olarak en yüksek ve test edilen *Tuta* diyetinde 18.92±5.21 yavru/birey olarak en düşüktü. λ ve r, A diyetinde sırasıyla 1.10±1.01 ve 0.10±9.20 gün ile en yüksek, *Plutella* ve *Tuta* diyetinde en düşük 1.07±1.70 ve 0.07±1.52 gün idi. R_0, CS diyetinde en yüksek (12.08±2.64 yavru/birey) ve en düşük *Tuta* diyetinde (4.58±1.44 yavru/birey) elde edildi. En yüksek günlük yumurta bırakma sayısı (mx) 8.69 yumurta ile H&H diyetinde, en düşük ise 3.06 yumurta ile *Tuta* diyetinde olmuştur. Sonuçlar, bu çalışmada geliştirilen CS diyetinin, lahana yaprak güvesinin laboratuvar koşullarında kitle halinde üretilmesi için alternatif bir diyet formülasyonu olarak sunulabileceğini göstermiştir.

Anahtar kelimeler: *Plutella xylostella, Lahana yaprak güvesi, Yapay Diyet, İki Eşeyli Yaşam Tablosu, Hiyerarşik Kümeleme Analizi*

Rearing of the Diamondback Moth, *Plutella xylostella* L. (Lepidoptera:Plutellidae) on Different Artificial Diets in the Laboratory Conditions[&]

Abstract

The diamondback moth, *Plutella xylostella* L. (Lepidoptera:Plutellidae), is one of the most serious pests of Brassicaeous plants worldwide. The suitable artificial diet formulations are required for mass rearing of insects under laboratory conditions. The aim of the study is to determine the most suitable formulation and to reveal the age-stage and two-sex life tables by examining the biological properties of diamondback moth on different artificial diets. Two-way hierarchical cluster analysis was established to determine some biological traits using different artificial diets and white cabbage as a positive control. In hierarchical cluster analysis, the results were obtained in CS diet was similar to control for some biological characteristics such as pupal weight, longevity etc. On the artificial diets, the GRR was the highest on the H&H diet as 39.92±7.22 offspring/individual and the lowest on the tested *Tuta* diet as 18.92±5.21 offspring/individual. λ and r were the highest in A diet as 1.10±1.01 and 0.10±9.20 days, lowest in *Plutella* and *Tuta* diet 1.07±1.70 and 0.07±1.52 days, respectively. R_0 was the highest on CS diet (12.08±2.64 offspring/individual) and lowest in *Tuta* diet (4.58±1.44 offspring/individual). The highest daily fecundity (mx) was on the H&H diet as 8.69 eggs and the lowest on the *Tuta* diet as 3.06 eggs. The results showed that the CS diet developed in this study may be offered as an alternative diet formulation for mass rearing of the diamondback moth under laboratory conditions.

Key words: *Plutella xylostella, Diamondback Moth, Artificial Diet, Two Sex Life Table, Hierarchical Cluster Analysis*.

Introduction

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is an herbivore and one of the most important pests of Brassicaeous plants worldwide, spreading over a wide area under the influence of climate changes (Talekar and Shelton,1993; Furlong et al., 2013; Machekano et al., 2017). Considering the host-parasitoid-pest relationship, the origin of the diamondback moth is in South Africa (Kfir,1998). It is estimated that the pest causes approximately 4-5 billion dollars of damage annually (Zalucki et al., 2012) and more than 90% yield loss in Brassicaeous plants (Talekar and Shelton,1993; Furlong et al., 2013;Verkerk and Wright, 1996).

Considering the worldwide programs, large numbers of insects are produced each year for sterile insect technique, biological control, food supply, medicinal purposes and most importantly for scientific studies (Cohen, 2015). It is not easy and convenient to use host plant materials as food sources in the laboratory mass rearing. Host plant materials rot over time as the larvae feed and require larval transfer to new plant materials frequently. Rotting plant material makes the larvae susceptible to pathogen infections and adversely effects the survival and development of the larvae. In addition, it may not always be possible to find the required host plant due to seasonal issues (Shen et al., 2006). An artificial diet is defined as an 'unknown food' that has been formulated, synthesized, produced, processed or prepared by humans, on which an insect in laboratory conditions can complete a biological or entire life cycle by feeding on it (Singh, 1977). Developing a successful artificial diet for mass rearing of a pest is essential to facilitate planning an effective IPM programs.

The life chart is an important tool not only for ecological research and pest management, but also for physiological and biochemical research. Understanding the population growth rate, parameters such as survival rates, fecundity, and longevity of insect populations are very important for entomologists. In order to obtain these results, it is necessary to create a life table and use it effectively (Atlıhan et al., 2018). The life chart program (TWOSEX-MSChart) was developed by Chi and Liu (1985) in which age-stage differentiation and two sex life parameters were obtained in detail. The TWO-SEX MSChart program provides the creation of a data set by integrating all the data of the insect with the bootstrap technique (Atlıhan et al., 2018). The TWO-SEX MSChart aims to reveal the age-stage two-sex life table by including the effect of male populations on the traditional female population, so the different biological stages take their places in the life table.

Cluster analysis (CA) is a statistical technique developed for biological classification as well as a part of modern multivariate analysis (Kettenring, 2006). Sneath and Sokal (1973) have demonstrated that cluster analysis can be used to appropriately classify a dataset containing all relevant characters of an organism. Classification of organisms can reveal which characteristics they differ or whether they belong to different species (Gunnarsson, 1999; Saraçlı et al., 2013).

The first attempt to rear diamondback moth larva on artificial diet was accomplished by Biever and Boldt (1971). They were modified a diet developed previously for Heliothis spp. to rear diamondback moth. There are several studies to rear the diamondback moth larvae in the laboratory conditions (Hsiao and Hou, 1978; Guanghong et al., 1996; Carpenter and Bloem, 2002; Htwe et al., 2009; Shelton, 2012). It has been stated that the tested diets were not suitable for the continuous rearing of the diamondback moth (Htwe et al., 2009). The diamondback moth is a severe pest and recently distributed some regions in Turkey. Therefore, studies that will provide sufficient information about its biology, laboratory rearing on its host and/or artificial diet are crucial (Avcı and Ozbek, 1995; Atay et. al., 2019; Saran and Genç, 2021). It is also important to understand life cycle, reproduction and growth potential to provide sufficient management methods. The objective of this study is to test known formulations and modify an artificial diet for the diamondback moth larvae to allow succesfull rearing of immature stages and to investigate the biological stages by using Two sex life table.

Material and Methods

Test insects

The diamondback moth individuals were collected from Brassicae fields in Çanakkale province in 2019-2020. The larvae were allowed to feed until pupal stage on cabbage leaves in Tupperware containers in the laboratory. Pupae were carefully collected with a soft forceps and transferred into a petri dish then placed in the adult cage. The adult cages (45x45x45 cm in dimension) were made of white chiffon, having 10% of sugar: water solution with yellow food coloring and a piece of foil (10x15 cm) was soaked in cabbage juice (20% of cabbage leaves: water) and placed in the adult cages for egg laying. For sterilization of eggs, the foil was kept in 3.8% formaldehyde for 15 minutes and washed with distilled water then kept on blotting paper. The eggs were incubated until hatching and the larvae were transferred on fresh cabbage leaves in plastic containers. The rearing conditions for the

laboratory colony were 25±2ºC, 50% RH and 16:8 (L:D).

Artificial diets

Different diet formulations were tested in this study. White cabbage leaves were used as positive control. To stimulate larvae to feed on artificial diet, cabbage juice was used as phagostimulant and prepared as follows. A hundred gram of fresh cabbage leaves were weighed and mixed with a blender with 500 ml of sterile distilled water. The mixture was filtered to discard the large pieces and autoclaved then transferred into a suitable erlenmeyer flask and stored at +4ºC. Cabbage juice was used in all artificial diet formulations. All ingredients to make artificial diets are shown in Table 1.

Table 1. Artificial larval diet components used for rearing of *Plutella xylostella*

Ingredients	H&H Diet (Hsiao and Hou, 1978)	*Plutella* Diet (Shelton, 2012)	*Tuta* Diet (Bajonero and Parra, 2017)	A Diet (Guanghong et al., 1996)	CS Diet (formulated in this study)
Raw Wheat germ (g)	5.35	8.75	5.35	4	-
Local Wheat germ (g)	-	-	-	-	6
Soybean flour (g)	-	-	-	10	-
Chickpea flour (g)	-	-	-	-	5
Bean flour (g)	-	-	-	-	5
Wheat bran powder (g)	-	-	-	6	-
Sucrose (g)	6.25	6.75	6.23	3.5	4
Casein (g)	6.25	6.3	6.23	-	-
Selulose (g)	0.89	1.25	0.88	-	-
Wesson's salt (g)	1.78	1.8	1.78	-	0.15
Cholesterol (g)	0.3	0.3	0.3	-	0.1
Choline chloride (g)	0.18	-	0.16	0.12	0.01
Brewer's yeast	-	-	3	4	4
Ascorbic acid (g)	0.71	0.7	0.71	0.4	0.4
USDA Vitamin premix (g)	1.78	1.8	1.8	-	1
Streptomycine sulfate (g)	0.27	0.2	0.12	0.05	0.04
Methyl paraben (g)	0.27	0.27	0.35	0.1	0.1
Sorbic acid (g)	-	-	-	0.1	-
Potassium sorbate (g)	-	0.2	-	-	-
Propionic acid (ml)	-	-	-	-	0.3
Olive oil (ml)	1.5	1.5	1.5	1	1
43.6% KOH (ml)	0.45	0.45	0.45	-	-
37% Formaldehyde (ml)	0.15	0.15	0.24	-	-
Agar (g)	4	4.8	2.14	1.6	2
White cabbage juice (ml)	150	150	150	100	100

Adult females usually require a protein source for development of their reproductive systems (Goane et al., 2019). Wheatgerm, chickpea and bean flour were used as protein sources in the tested diets. Vitamins, minerals and lipids are also important for insect growth and development, supplied in diets by adding Brewer's yeast and sucrose which were also source of protein and carbohydrates (Chan et al., 1990; Ling et al., 2000; Moadeli et al., 2018; Song et al., 2010; Hou et al., 2020). Propionic acid, the most effective mold inhibitor, and streptomycin were used to prevent contamination in the diet (Ghosh et al., 1996; Htwe et al., 2009). Polyunsaturated fattyacid are important especially for moths to expand and develop wings succesfully, so olive oil used to fulfill this requirement. Cellulose was also used to solidify the tested diets. Unlike the original formulation of '*Plutella* diet' (Shelton, 2012), streptomycin sulfate and olive oil were used instead of aureomycin and linseed oil. The '*Tuta* absoluta' diet was first developed by Berger (1963) then modified by Bajonero and Parra (2017), we used streptomycin

instead of tetracycline with addition of Brewer's yeast in this diet formulation.

Preparation of artificial diets

Weighed agar was added to the warm cabbage juice then heated until reached 87-90 ºC. The mixture was transferred to a separate container then cooled to 80-85 ºC. Liquid materials were measured and added to the agar mixture. Then dry ingredients were added and mixed thoroughly about a minute. The mixture was cooled to 75 ºC then transferred into a suitable container. After the diet mixture cooled and thickened, it was stored at + 4ºC until used.

Experimental conditions

The 2nd instars were used in all tested diets with 5 replications. The experiments were carried out at 25±2ºC, 50% RH and 16:8 (L:D) in the controlled laboratory conditions. Blotting papers were placed at the bottom and at the top of 0.8 ml polypropylene containers to prevent possible moisture. Approximately 4-5 g of diets were weighed and transferred to each container. Then 25 larvae were carefully transferred into the diet cube in each container. The larvae were checked daily until they reached pupal stage. The dead larvae were removed from the containers. After pupation, they were carefully removed from the diet with a soft forceps then weighed to determine the pupal weights. Development times of prepupa and pupa were determined. Viability of pupae or adult emergence were also reported. Adults were mated (1 ♀ x1 ♂) then pre-oviposition, oviposition and post-oviposition times of females and adult longevity were determined. In the rearing cages, 10% sugar: water mixture with yellow food dye were placed for adult food. A piece of aluminum foil (5x10 cm) dipped in cabbage juice to promote egg laying.

Statistical analysis

The data were evaluated by applying the LSD test according to the PROC GLM procedure using SAS software (Version 9.1.3; SAS Institute, Cary, NC) (1990). The graphs of pupal weight, constellation plot and two-way hierarchical cluster analyzes were obtained using SAS JMP (version 16.1; SAS Institute, Cary, NC) statistical program.

The durations of biological stages, survival rate, adult longevity and fecundity parameters were calculated using the age-stage two-sex theory and the TWOSEX-MSChart program (Chi, 2018). The life table (exj, sxj, vxj, lx, mx) and demographical parameters (r, λ, R0, T and GRR) were calculated by TWOSEX-MSChart computer program (Chi, 2018). To confirm the accuracy of the demographic data, the TWOSEX-MSChart program and a paired bootstrap test with 100,000 replications were used (Akça et al., 2015).

According to Chi and Su (2006), for age-specific life expectancy (exj),

$$exj = \sum_{i=x}^{\infty}\sum_{y=j}^{m} S'iy$$

according to Abbas et al (2014), age stage-specific reproductive value (vxj),

$$\text{Vxj} = \frac{e^{r(x+1)}}{\text{Sxj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^{k} S'iy\ fiy$$

The age-specific survival rate (lx) for each matrix S is calculated by is using the formula,

$$lx = \sum_{j=1}^{m} Sxj$$

for each age stage, the age-specific fecundity (mx);

$$mx = (\sum_{j=1}^{m} Sxj\ fxj)/\sum_{j=1}^{m} Sxj$$

life table parameters, the intrinsic rate of increase (r), the infinite rate of increase (λ) and the mean length of a generation (T) are calculated is using the formula,

$$\sum_{x=1}^{k} e^{-rx}\ \text{lx mx} = \sum_{x=1}^{k} (\ e^{-rx} \sum_{j=1}^{m} \text{fxj sx j}\ )$$

$$= 1$$

The graphs were created with SigmaPlot 14.0 program (Systat Software Inc., Erkrath, Germany) (Chi and Su, 2006).

Results and Discussion

Some biological properties of the diamondback moth were determined on five different artificial diets and its natural host white cabbage as positive control. Larval duration was 10.45±2.79 days on *Tuta* diet, 9.65±1.30 days in control and 13.60±1.50 days in CS diet. Female prepupal duration was 1.15±0.36 days in control and 1.00±0.01 days in H&H diet, *Tuta* diet and A diet. The prepupal durations were recorded as 1.10±0.30 days in control, 1.00±0.01 days in H&H

diet, *Plutella* diet and CS diet respectively. So, there were no statistical difference in male prepupal durations between artificial diets and control. In control diet, pupal duration was 6.75±1.11 days in females and 8.15±0.67 days in males. The shortest pupal duration was in A diet in both sexes (Table 2).

Table 2. Durations of larval, prepupal and pupal stages on artificial diet and control (Mean±SE)*

Artificial diets	Larval duration (day)	Female Prepupa (day)	Male Prepupa (day)	Female Pupa (day)	Male Pupa (day)
H&H Diet	11.05±3.05b	1.00±0.01b	1.00±0.01a	5.95±0.60b	5.85±0.36b
Plutella Diet	10.90±2.51cb	1.05±0.22ba	1.00±0.01a	5.90±1.16b	5.95±0.94b
Tuta Diet	10.45±2.79cb	1.00±0.01b	1.10±0.30a	6.00±1.12b	5.85±0.93b
A Diet	10.60±1.23cb	1.00±0.01b	1.05±0.22a	5.45±0.51b	5.35±0.48c
CS Diet	13.60±1.50a	1.05±0.22ba	1.00±0.01a	5.85±0.93b	5.85±0.58b
Control	9.65±1.30c	1.15±0.36a	1.10±0.30a	6.75±1.11a	8.15±0.67a

*For each parameter, between means within a column followed by the same letter show significant differences ($P < 0.05$,LSD test).

The composition of all tested artificial diets were listed in Table 1. The structure and thickness of four artificial diets were showed in Figure 1. It is important to see larval feeding, feces and larval exiqua in the tested artificial diets. Emergenced adult was also shown in Figure 1C.

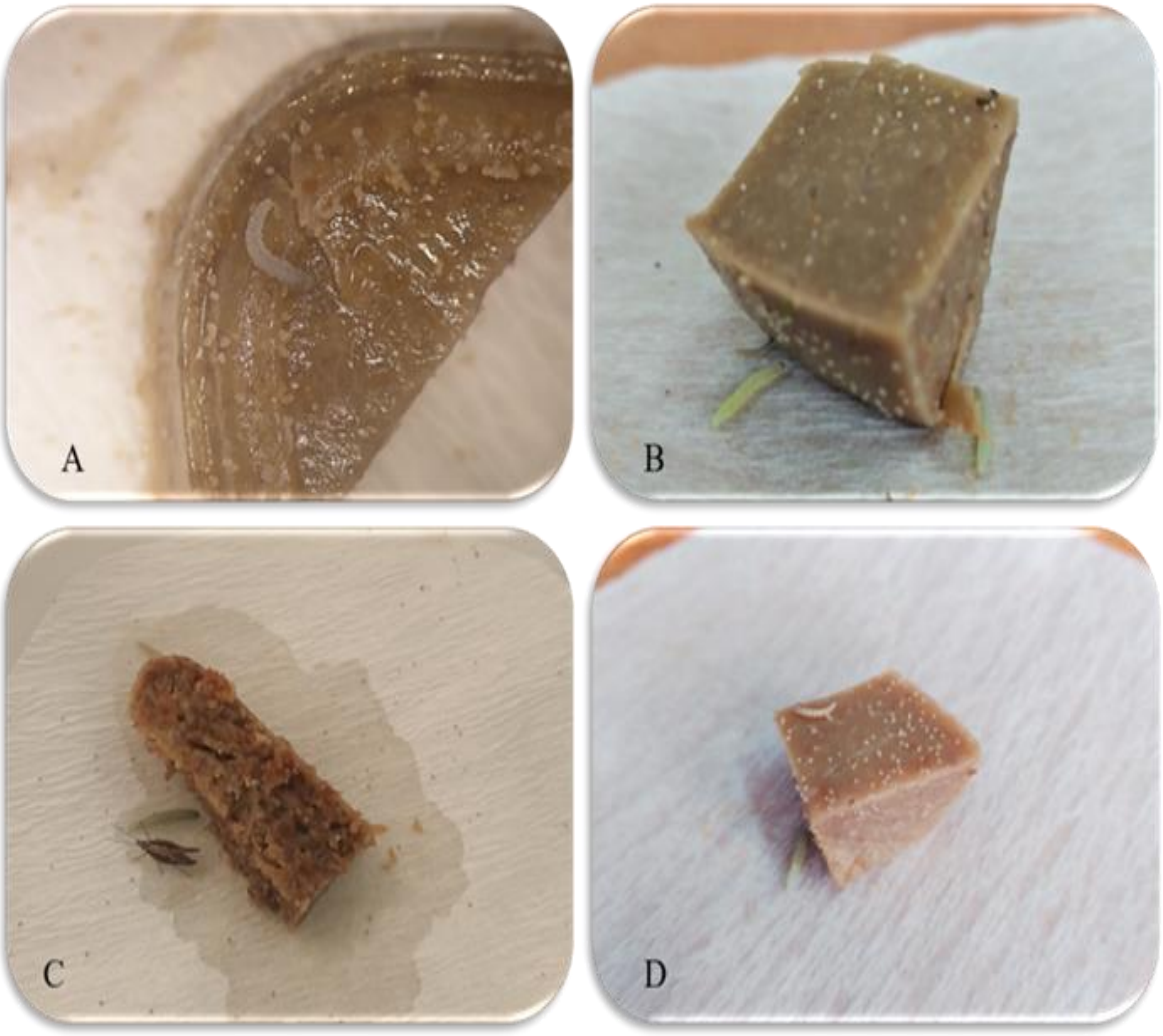

Figure 1. Tested artificial diets to rear diamondback moth larvae, (A) *Plutella xylostella* diet, (B) H&H diet, (C) A diet and (D)*Tuta* diet.

The diet formulated in this study called as CS diet. Even though larval duration was longer than others, we were able to rear the larvae continously on this diet for 5 generations (Figure 2). The structure of diet was suitable for larvae to move in and out during feeding. It was easily seen the head capsules and exiqua on the diet. The diamondback moth larva was seen to pupate succesfully on the diet (Figure 2D).

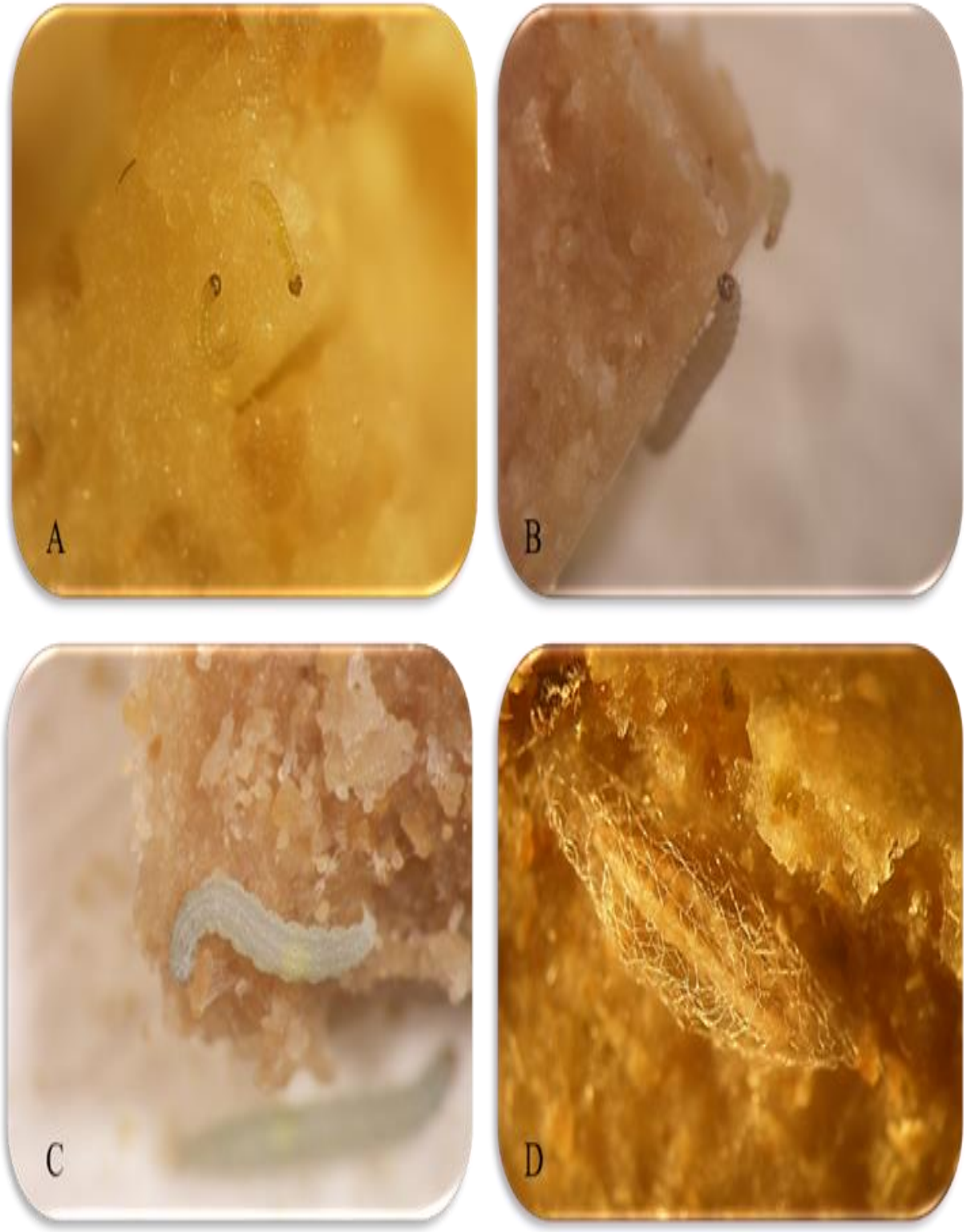

Figure 2. A view of diamondback moth larvae feeding on CS diet. (A) 2nd instar, (B) 3th instar, (C) 4th instar, and (D) pupa.

It is important to have a good larval development to reach succesful pupal stage in the laboratory. In that case, pupal weight are an indicator for larval feeding. Female and male pupal weights were 7.37±0.99 mg and 5.26±0.44 mg in control diet, 5.78±0.58 mg and 5.73±1.06 mg in CS diet and 3.76±0.64 mg and 3.63±0.57 mg in the *Plutella* diet. According to Gilbert (1984), there is a positive correlation between pupal weight and fecundity under constant conditions. Even though it is a good indicator for fecundity and female survival, there are a few cases where there is no relationship at all (Johnson, 1990). Additionally, the longer larval development resulted in the formation of the bigger pupae and there was a positive correlation between them (Roff, 2000). In this study, the diamond backmoth larvae were reared on CS diet, duration of larval development was longer and had heavier pupal weight than other tested diets (Figure 3).

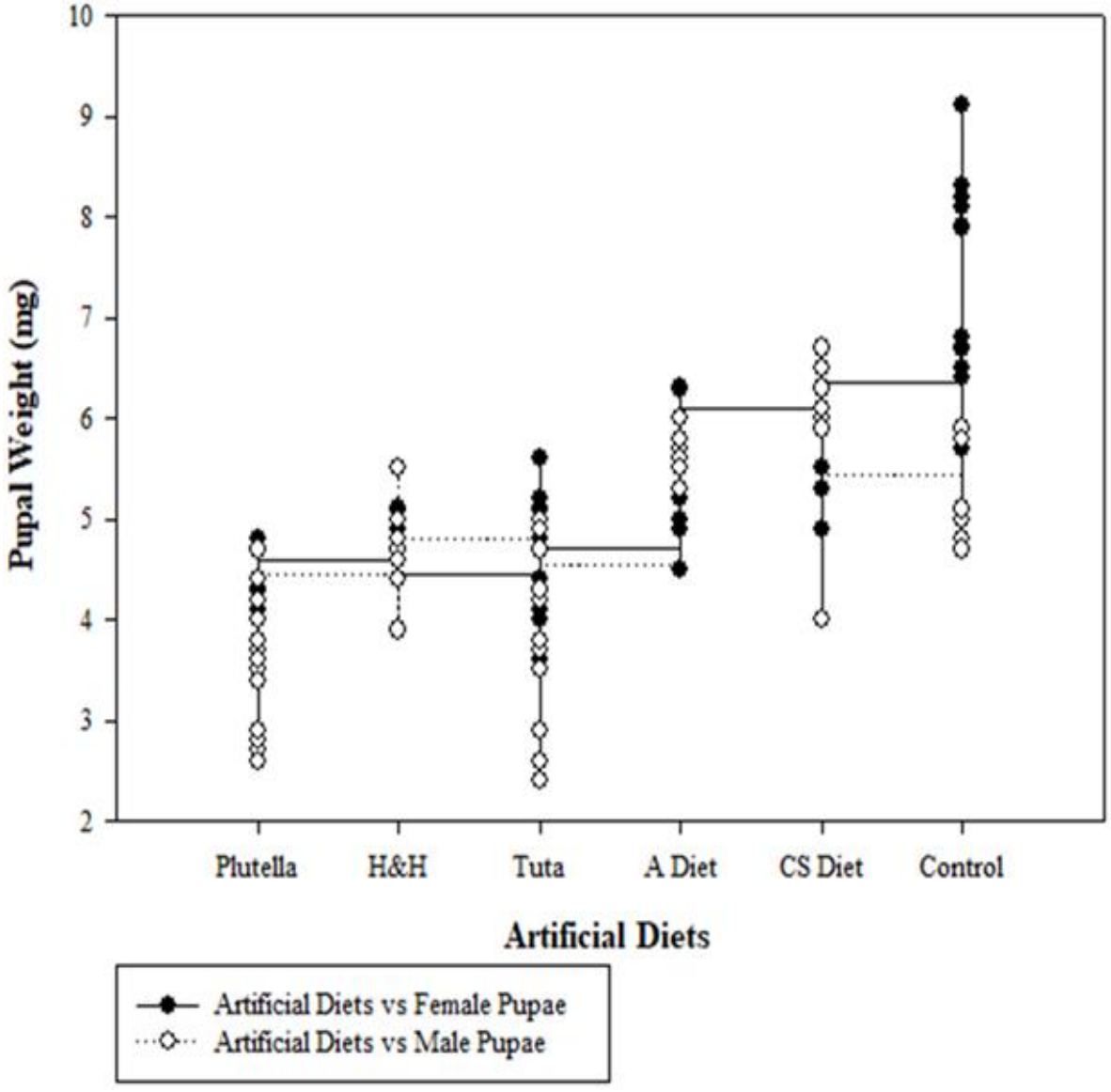

Figure 3. The pupal weights of males and females in all tested artificial diets and control.

The pre-oviposition durations were 4.90±1.48 days in CS diet and 2.30±0.47 days in *Plutella* diet. The longest oviposition duration was 13.95±0.94 days in H&H diet, 11.05±6.93 days in control and 8.10±0.71 days in CS diet. Duration of post-oviposition was 2.85±0.67 days in H&H diet and 9.65±4.36 days in the *Plutella* diet. Females lived longer than males in the laboratory. Female longevity was recorded as 19.20±1.60 days on H&H diet and 13.80±1.64 days on A diet. Male longevity was recorded as 20.25±3.61 days on CS diet and 13.55±4.44 days on the H&H diet (Table 3).

Table 3. Durations of pre-oviposition, oviposition, post-oviposition and longevity of the diamondback moth on artificial diets (Mean±SE)*

Artificial diets	Pre-oviposition (day)	Oviposition (day)	Post-oviposition (day)	Longevity (day)	
				Female	Male
H&H Diet	2.45±0.60c	13.95±0.94a	2.85±0.67c	19.20±1.60a	13.55±4.44c
Plutella Diet	2.30±0.47c	4.65±1.30d	9.65±4.36a	16.50±3.48bc	17.85±1.92a
Tuta Diet	2.40±0.50c	5.05±1.46d	8.50±4.39a	15.90±2.55dc	17.70±2.02a
A Diet	3.30±0.80cb	6.25±0.85dc	4.25±1.29cb	13.80±1.64d	14.90±4.80bc
CS Diet	4.90±1.48a	8.10±0.71c	5.00±2.49b	18.30±4.02ab	20.25±3.61a
Control	3.65±3.42b	11.05±6.93b	4.60±4.04cb	17.30±5.66bac	17.55±7.32ab

*For each parameter, between means within a column followed by the same letter show significant differences ($P < 0.05$, LSD test).

Pupal recovery was recorded as 79% in control, 40% in H&H diet, and 25.6% in the *Tuta* diet. The highest adult emergence was recorded as 95.55% in A diet and the lowest was 74.11% in H&H diet (Table 4).

Table 4. . Fecundity, pupal recovery and adult emergence of diamondback moth on artificial diets

Artificial diets	Pupal recovery (%)	Fecundity (number)	Adult emergence (%)
H&H Diet	40	84.10±6.06b	74.11
Plutella Diet	28	62.05±3.77c	85.95
Tuta Diet	25.6	55.70±10.38c	79.8
A Diet	39.2	56.60±19.77c	95.55
CS Diet	36.8	83.33±5.44b	85.54
Control	76	174.05±44.08a	94.73

*For each parameter, between means within a column followed by the same letter show significant differences ($P < 0.05$,LSD test).

The number of laid eggs and egg survival were important parameters in succesful artificial diet. Fecundity was the highest as 174.05±44.08 eggs in control then in H&H diet as 84.10±6.06 eggs and 83.33±5.44 eggs in CS diet. The lowest fecundity was in *Tuta* diet as 55.70±10.38 eggs. Although, the

highest fecundity and pupal recovery were observed in the H&H diet, adult emergence was the lowest. On the other hand, the lowest fecundity was occurred in the A diet with the highest adult emergence. In CS diet, a more consistent distribution was displayed in terms of tested biological characteristics. Moreover, there is no significant difference between the performance of the CS diet and the performance of the A diet and H&H diets. (Table 4).

The gross reproductive rate (GRR) was 76.14±8.37 in control, 39.92±7.22 in H&H diet, 38.93±7.11 in CS diet and 18.92±5.21 in *Tuta* diet. Infinite rate of increase (λ) was 1.18±8.60 days in control then 1.10±1.01 days in A diet, 1.08±8.86 days in CS diet, 1.07±1.70 days in *Tuta* and *Plutella* diets.

Intrinsic rate of increase (r) value was defined as a determining factor to examine the effects of reproduction, development and survival rate on insect populations (Huang and Chi, 2012). According to life table theory, an insect population can only increase when R0 > 1 and r > 0 (Southwood and Henderson, 2000). Among the artificial diets studied here, the highest R0 ratio was recorded in the CS diet. In addition, it was emphasized that R0 is more important biologically than GRR. Because R0 focuses on pre-adult survival while GRR ignores different points of mx at different ages (Yu et al., 2005). Intrinsic rate of increase (r) was 0.17±7.24 days in control, 0.10±9.20 days in A diet, 0.07±1.52 days in *Plutella* diet and *Tuta* diets. Net reproductive rate (R0) was 54.76±7.01 in control and 12.08±2.64 in CS diet. Mean generation time (T) was 23.19±0.38 days in control and 29.33±0.70 days in CS diet (Table 5).

Table 5. Effects of tested artificial diets on the life table parameters of *Plutella xylostella*

Artificial diets	GRR (offspring/individual)	λ (day)	r (day)	R_0 (offspring/individual)	T (day)
H&H Diet	39.92±7.22b	1.09±1.03b	0.08±9.52b	10.64±2.50b	27.12±0.63b
Plutella Diet	19.54±5.21bc	1.07±1.70b	0.07±1.52b	5.00±1.52c	23.19±0.38c
Tuta Diet	18.92±5.21bc	1.07±1.70b	0.07±1.59b	4.58±1.44d	21.75±0.71cd
A Diet	28.26±4.57b	1.10±1.01b	0.10±9.20b	10.88±2.14b	23.26±0.33c
CS Diet	38.93±7.11b	1.08±8.86b	0.08±8.16b	12.08±2.64b	29.33±0.70a
Control	76.14±8.37a	1.18±8.60a	0.17±7.24a	54.76±7.01a	23.19±0.38c

*For each parameter, between means within a column followed by the same letter show significant differences ($P<0.05$, LSD test), r: intrinsic rate of increase λ: infinite rate of increase GRR: gross reproductive rate R_0: net reproductive rate T: mean generation time.

Age-stage-specific survival rates (sxj) indicate the probability that the second instar will survive to age x and develop to stage j. Age stage survival rate (sxj) was recorded as 0.44 in females and 0.36 in males in control.

In tested artificial diets, the highest rate was as 0.20 in females and 0.18 in males in A diet, the lowest rate was 0.08 in females on *Plutella* and *Tuta* diets, and 0.16 in males on *Tuta* diet (Figure 4).

The reproductive value (vxj) showed the effect of the 2nd instar on the future population at age x and stage j. While the reproductive value (vxj) was 60.41 in control, the highest value was recorded on *Plutella* diet with 54.19, and the lowest value was recorded on the A diet with 37.61. The peak in reproductive value was recorded at 25st day in CS diet, and at 16st day in *Plutella* diet (Figure 5).

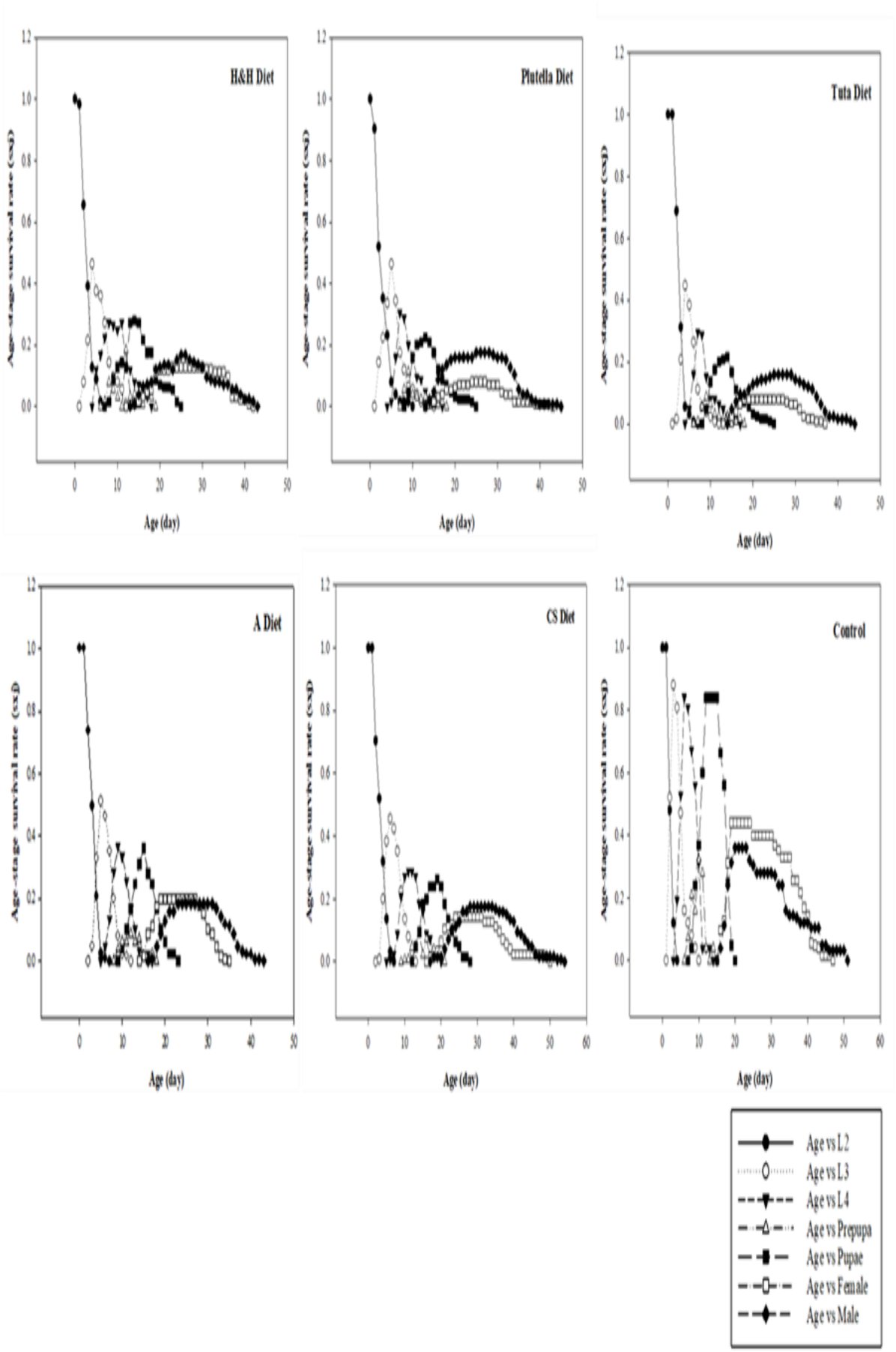

Figure 4. The age-stage specific survival rate of *Plutella xylostella* on artificial diets and control. L2 = 2nd instar, L3 = 3rd instar, L4 = 4th instar.

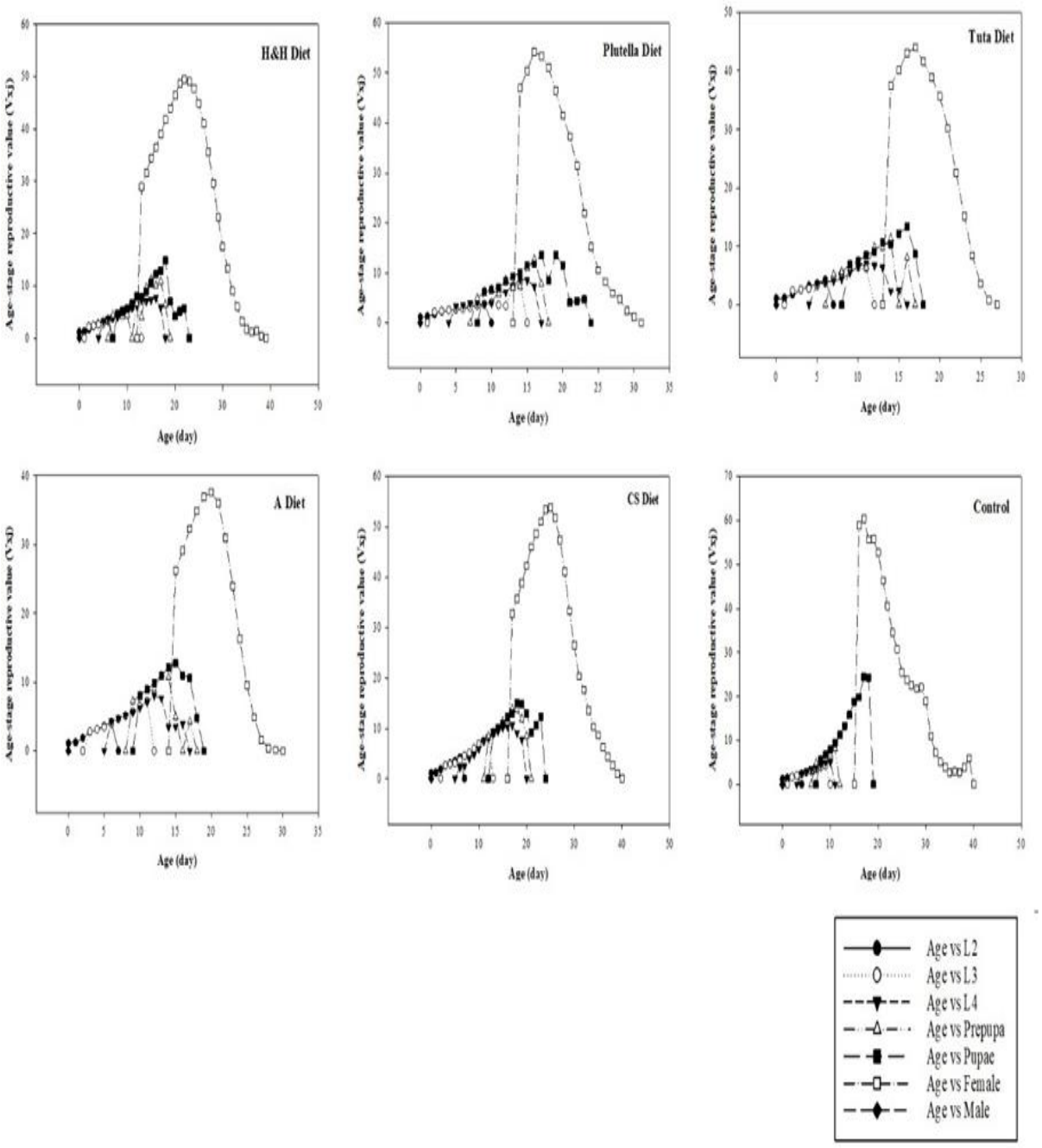

Figure 5. The age-stage specific reproductive value of *Plutella xylostella* on artificial diets and control. L2 = 2nd instar, L3 = 3rd instar, L4 = 4th instar.

Age-stage life expectancy (exj) was shown the estimated life expectancy an insect at age x and stage j. Age-stage life expectancy (exj) was 21.12 in females and 20.51 in males in control. The insects fed on tested artificial diets, the highest value was found in H&H diet with 24.18 in females, and the CS diet with 24.86 in males, while the lowest value was in A diet with 16.00 in both females and 18.01 in males. On the other hand, it was stated that life expectancy can also be evaluated in terms of age-stage (Hou and Weng, 2010). In this case, a female individual is expected to survive for 33 days and a male individual for 36 days on the CS diet. The diamondback moth showed the longest survival performance on CS diet (Figure 6).

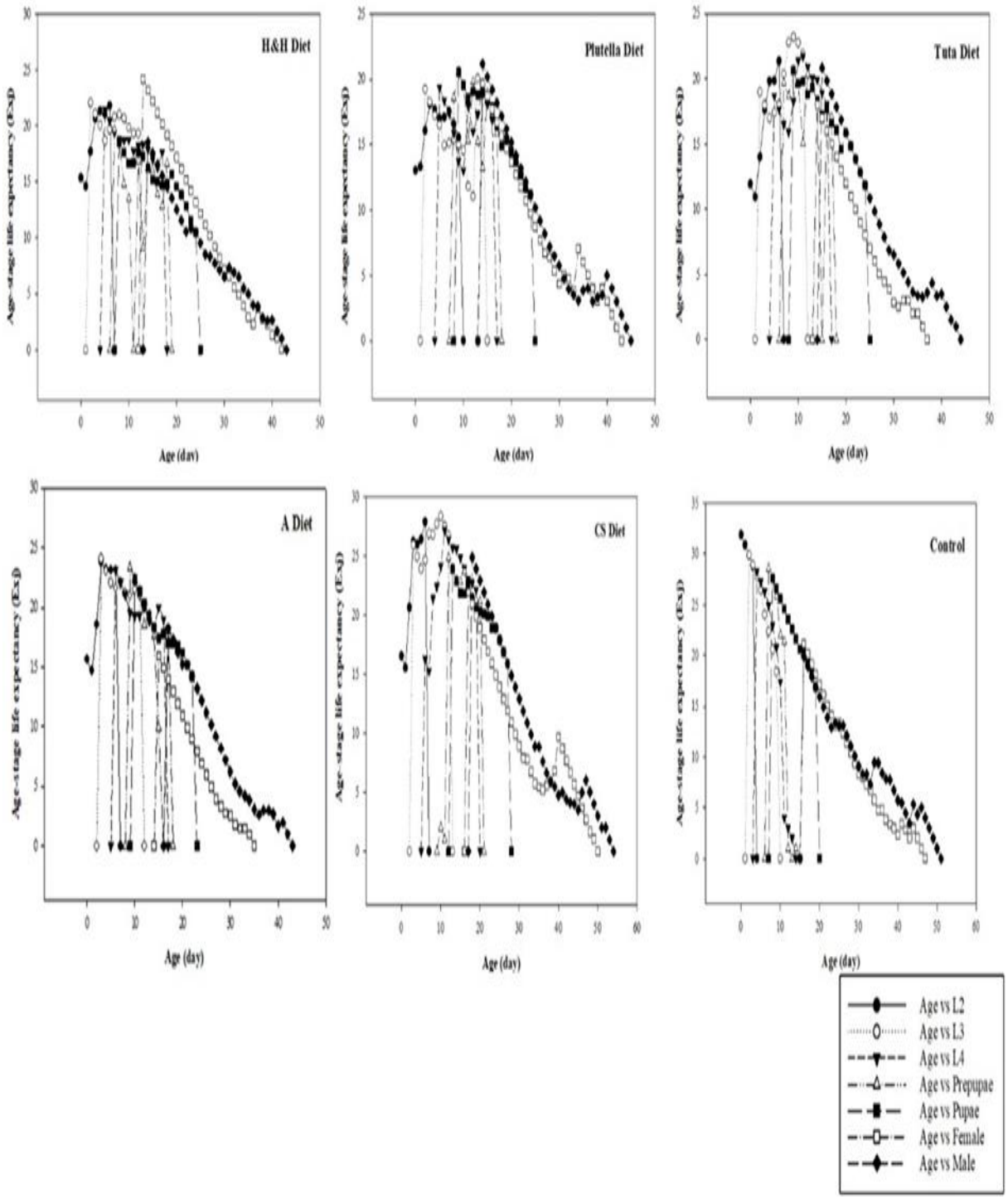

Figure 6. The age-stage specific life expectancy of *Plutella xylostella* on artificial diets and control. L2 = 2nd instar, L3 = 3rd instar, L4 = 4th instar.

Differentiation of biological stages was ignored when calculating the age-stage survival rate (lx), revealed the survival rate of a 2nd instar to age x, focusing on a single age-stage. A rapid decline was occurred in the early stages of all tested diets. However, A diet and CS diet showed a constant ratio in the following days. Eventhough, this period is longer in CS diet, the mortality rate was lower than other tested diets. Age stage survival rate (lx), the highest value was observed in CS diet as 54 days while the lowest value was recorded in A and H&H diets as 43 days (Figure 7).

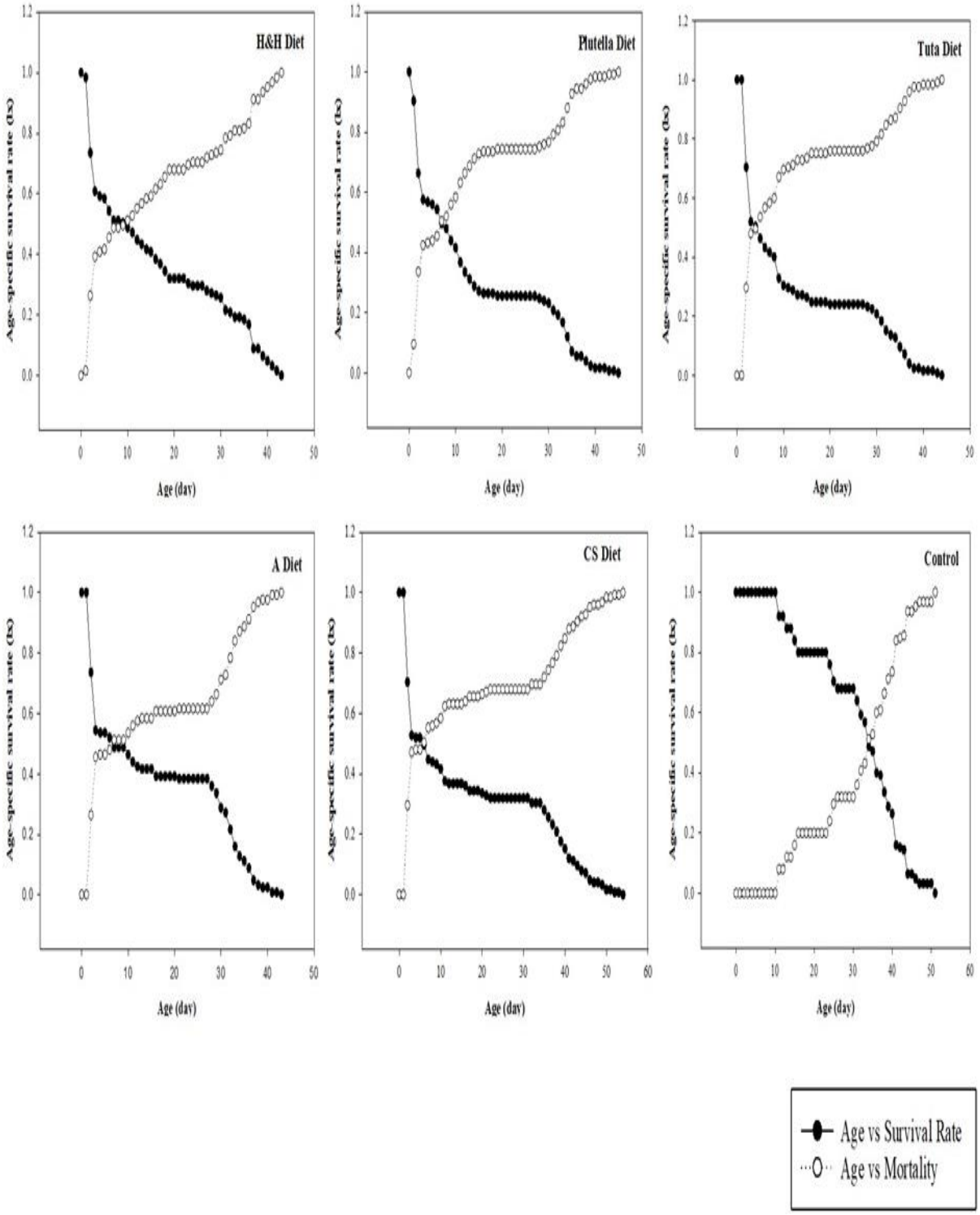

Figure 7. The age-specific survival rate of *Plutella xylostella* on artificial diets and control. L2 = 2nd instar, L3 = 3rd instar, L4 = 4th instar.

The maximum average daily fecundity (mx) of females decreased from 8.69 eggs per female on day 17 on H&H diet, the lowest was *Tuta* diet as 3.06 eggs per female daily on day 21. The fecundity chart of the CS diet had consistently increased and decreased. Females reared on CS diet had the highest fecundity along with H&H diet, which was also suitable for rearing of the diamondback moth larvae and may serve as an alternative artificial diet (Figure 8).

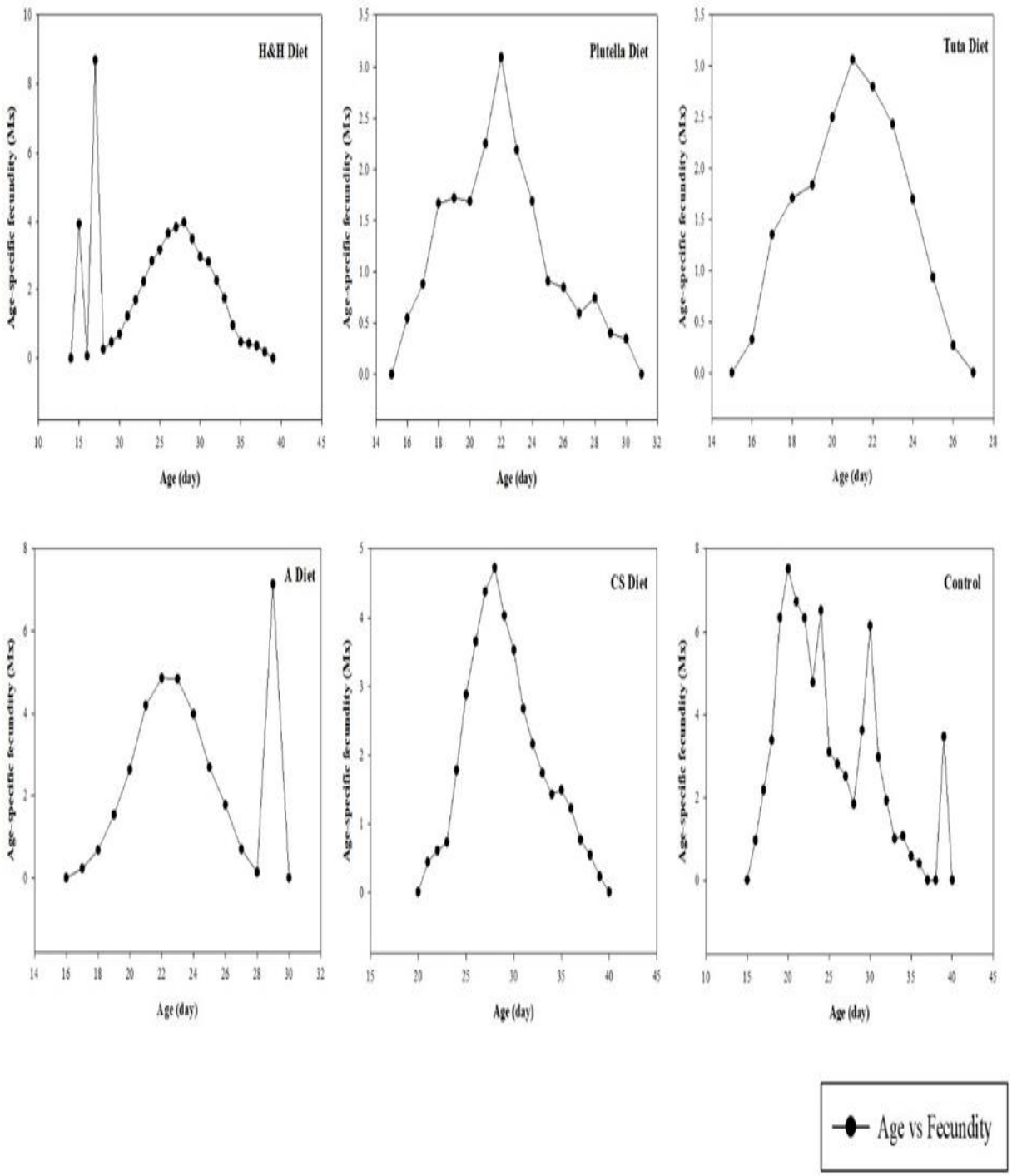

Figure 8. The age-specific fecundity of *Plutella xylostella* on artificial diets and control.

The purpose of cluster analysis is to show the similarities of the units based on the certain characteristics of the organisms and to categorize the units into the correct groups in line with these homogeneity (Çokluk et al., 2010).

In the first way of hierarchical clustering, artificial diets and control were distributed in a cluster. The first group was consisted of two clusters separated under one cluster, included as *Plutella* and *Tuta*, the second cluster was seperated as A diet and H&H (Figure 9).

In the second way of the clusters was based on the different biological properties, distributed into two clusters. According to the examined biological characteristics, similarities were revealed between the *Plutella* and *Tuta* diets then A diet and H&H diet. Likely, when some biological characteristics are taken into consideration, similarities have been observed between CS diet and Control (Figure 9).

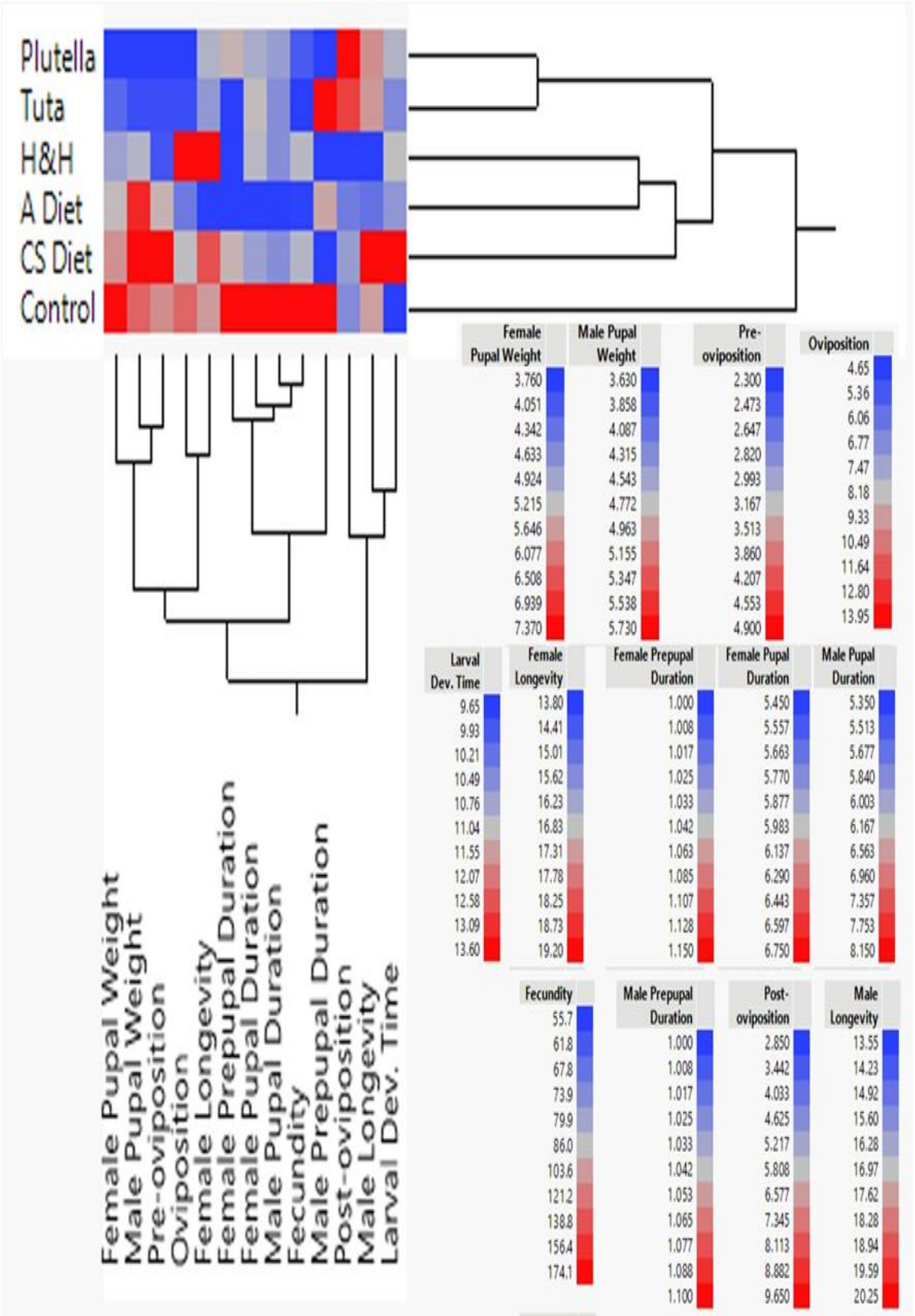

Figure 9. Two-way hierarchical cluster analysis of artificial diet, control and their different biological properties.

Constellation plot or an agglomeration method, was organized the results from artificial diet and control as endpoints, and each cluster formed converges at a new point with the lines in which the member with similar characteristics was expressed. Mediums expressed with longer lines indicated the size of the distance between clusters. The plot was divided by the artificial diets and control as 4 clusters. It was observed that *Plutella* diet and *Tuta* diet formed a cluster, while H&H diet and A diet formed another cluster while CS diet and control were expressed as a separate cluster (Figure 10).

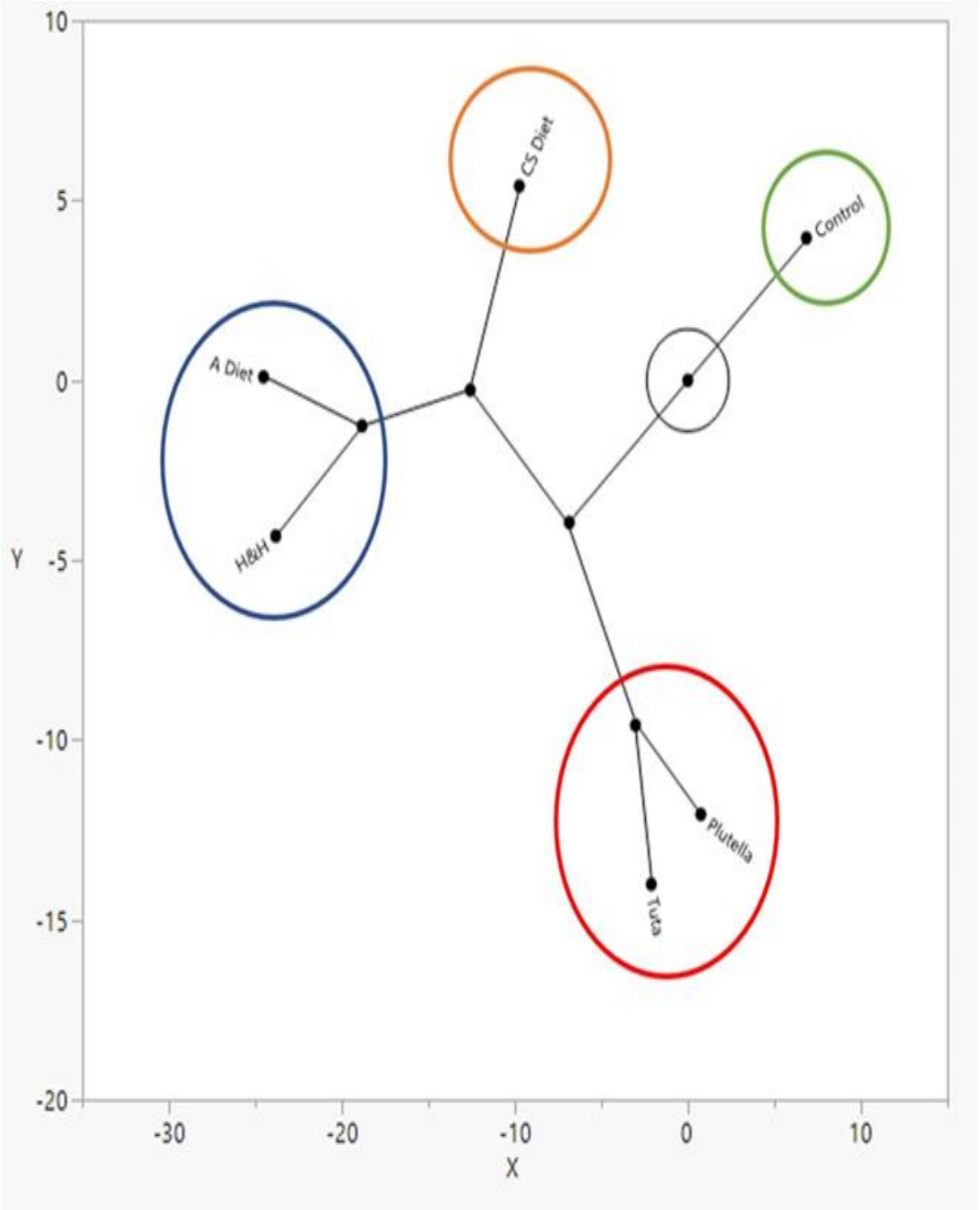

Figure 10. Cluster analysis depicted constellation plot of artificial diets and control.

Larval artificial diets are important to study pest insects without the need for host plant in the laboratory. It is important to rear insects for several generations continuously in the laboratory conditions to study their dietry requirements (Genc, 2006). The different biological stages of a species may have different and unique requirements (Kraus et al., 2019).

In this study, several artificial larval diets were tested to rear P. xylostella in the laboratory conditions. The durations of biological stages on artifical diet, completion of each biological stages and fecundity are crucial (Genc, 2006). Here, the differences in the durations of the biological stages of P. xylostella on each tested diets can be attributed either nutrient compositions, formulations and physiological stress.

Pupal weight, fecundity, development time and longevity are directly proportional to the nutritional quality of the insect (Saran and Genç, 2021). In this study, pupal weights of those fed on the CS diet (5.78±0.58 mg), unlike other diets, were very close to the control (7.37±0.99 mg).

Likewise, when the adult longevity was examined, the highest values were obtained in the CS diet (18.30±4.02 days in female, 20.25±3.61 days in male), resulted longer survivorship in the laboratory. Another important parameter for mass rearing of insects is the fecundity. The females laid a good amount of eggs (83.33±5.44 eggs) when they reared on CS diet larval diet compared with the control (174.05±44.08 eggs).

The comparation of R0 and r values provide important knowledge beyond analyzing individual life table parameters (Zhang et al., 2007). The intrinsic rate of increase (r) is a parameter that explains the survival, development and reproduction dynamics of the insect (Qin et al., 2017). In the study, the intrinsic rate of increase (r) was reported and there were no statistical difference between the artificial diets, unlike the control. The infinite rate of increase (λ) is also an important parameter, which is closely related to the net reproduction rate and defined as the rate of increase per individual per unit time. There was no statistical difference between the tested artificial diets in the infinite rate of increase (λ) as well as in the intrinsic rate of increase (r). To estimate the potential population growth rate of a pest, it is important to know the net reproduction rate (R0) (Ullah et al., 2020).

In this study, the net reproductive rate (R0) repoted as the highest value in the CS diet (12.08±2.64) compared to the control and other tested diet formulations. It was emphasized that R0 is biologically more important parameter than gross reproduction rate (GRR). Accordingly, it was stated that survival in the immature stages was emphasized in R0 (Zhang et al., 2015). When the GRR parameter was examined, it was observed that the CS diet (38.93±7.11) had one of the closest values to the control (76.14±8.37) in this study.

The lx value gives the survival rate of the 2nd instar to age x. We have calculated all survivors from individuals at different biological stages, the highest value was 54 days when they were reared on CS diet (54 days). Additionally, age stage life expectancy (exj) estimates the expected life expectancy of individuals at age x and stage j, higher values were obtained on H&H diet and CS diet for both adult individuals than the control. The exj value was the highest in the H&H diet (24.18) in females and in the CS diet (24.86) in males. Two-way hierarchical clustering analysis showed the effect of artificial diets on each biological development stages. Accordingly, *Plutella* diet and *Tuta* diet generally formed a cluster among themselves and revealed values that were quite far from the values obtained in the control. A diet and H&H diet were provided values close to control although they were mostly clustered among themselves. CS diet, was closed to the cluster formed by A diet and H&H diet, however when some biological characteristics were considered, it has revealed the closest observations to control.

Artificial diet components and textures are crucial for rearing insects in the laboratory conditions. In conclusion, we have tested a new diet formulation to rear larvae of diamondback moth in the laboratory. CS diet was suitable for larval feeding and growth of the diamondback moth contiounsly based on the examined biological parameters.

Conclusion

Life table study of the diamondback moth on different diets is important for mass breeding potential and integrated pest management under laboratory conditions. In our study, hierarchical clustering analysis was used to compare the demographic characteristics of the diamondback moth reared on five different diets, age-stage, two-sex life table, and to examine the differences between different biological stages. According to the results obtained under laboratory conditions, the results in the CS diet in the examined biological properties are similar to the results obtained in the natural host. The population characteristics of the diamondback moth in different artificial diets and in its natural host suggest that in-depth research is required for it to perform similar to natural populations when grown in mass.

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