

## Full Length Research Paper

# A meridic diet for laboratory rearing of Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)

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The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) was fully developed and reared on a meridic diet consisting of agar, distilled water, commercial yeast as well as laboratory produced amino and fatty acid rich brewer's yeast (*Saccharomyces cerevisiae*), wheat meal, corn flour, benzoic acid, ascorbic acid, sorbic acid, vitamin mix and tetracycline hydrochloride. A group of weevils reared on date palm trunk under the same laboratory conditions was used as control. Diet reared female weevils laid fertile eggs which successfully hatched into healthy larvae in 3.6 days. Between the first and last (eighth) larval instars there was a 2311 fold increase in the larval body biomass before pupation. Linear regression analysis involving log of head capsule width of each instar and instar number resulted in a straight line ( $R^2 = 0.978$ ) indicating that Dyar's rule is applicable in the case of RPW. Larval development was completed in 43 to 47 days while the pupa reached the adult stage in 31 to 38 days with an average of 35 day. The whole life cycle of the weevil from egg to adult, was completed in 78 to 85 days. The average adult longevity for male and female weevils was 83.3 and 74.8 days, respectively. Besides the above, important biological parameters including pre-oviposition period, incubation period, per cent egg hatchability, fecundity and larval weight gain were recorded. A food-fiber pupation technique was developed with 100% pupation efficiency.

**Key words:** *Rhynchophorus ferrugineus*, meridic diet, biological parameters, food-fiber pupation technique, larval instar, Dyar's rule.

## INTRODUCTION

The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), is an internal tissue borer reported to have infested 26 palm species worldwide in diverse agro-ecosystems (Malumphy and Moran 2009 <http://www.defra.gov.uk/fera/plants/plant>). RPW is originally from South and Southeast Asia, where it is a key pest of coconut, *Cocos nucifera*, besides infesting other palm species (Nirula, 1956; Wattanapongsiri, 1966; Li et al., 2009; Matsuura, 1993). Since the mid 1980s the spread of RPW has been rapid mainly through infested

planting material shipped for farming and landscape gardening. In the Middle-East RPW was first reported from Rass-El-Khaima in the UAE in 1985 (Zaid et al., 2002) and reached the Eastern region of the Kingdom of Saudi Arabia in 1987 from where it spread to other areas of the country (Abozuhairah et al., 1996; Al-Abdulmohsin, 1987). In 1990s it was recorded in several Middle-Eastern countries including Iran, Israel, Jordan, the Palestinian Authority Territories, Kuwait and Bahrain (Faghieh, 1996; Kehat, 1999). The first report of RPW from

**Table 1.** Different feeding substrates reported by different authors as constituents of artificial diet for red palm weevil, *R. ferrugineus*

Feeding substrates	Reference
Sugarcane, bagasse, fresh coconut cake, brewer's yeast, sugarcane, potassium hydroxide, methyl parahydroxyl benzoate, and sorbic acid solutions	Rahalkar et al. (1978)
Agar, distilled water, brewer's yeast, wheat germ, corn meal, M-nipagine, benzoic acid, ascorbic acid, chloramphenicol, coconut fiber, vitamins and amino acids additive and crude protein	Barranco et al. (1997)
Oats, coconut cakes, coconut fruit pieces, vegetable oil, potatoes, soybean flours, date palm leaves, palm fiber sheath, sugarcane fibers, bacto-agar, multi-vitamins, preservatives and water.	Kaakeh et al. (2001)
Potato, carrot, casein, agar, cereals, and vitamin B and D	El-Sebey et al. (2003)
Oats, potato, pineapple, and palm fiber sheath	Kaakeh (2005)
Agar, brewer's yeast, wheat germ, corn flour, ascorbic acid, benzoic acid, amino acid-vitamin mix, chloramphenicol and nipagin	Martin and Cabello (2006)

Africa came from Egypt in 1992 (Cox, 1993) while in Europe it was detected in southern Spain in 1995 (Barranco et al., 1996). The first report of RPW in the American continent is from the Caribbean Island of Curacao (Dutch Antilles) in 2008 where the pest arrived through date palms imported from Egypt for ornamental purposes [http://www.redpalmweevil.com/newlook/RPW\\_Report/Caribbean.htm](http://www.redpalmweevil.com/newlook/RPW_Report/Caribbean.htm). In USA RPW invasion occurred in late 2010 in Laguna Beach, California, USA ([http://www.cdfa.ca.gov/egov/Press\\_Release](http://www.cdfa.ca.gov/egov/Press_Release); NAPPO, 2010), where it was found infesting *Phoenix canariensis* hort. ex. Chabaud. However, morphological parameters may suggest that this invasion could be the related Asian palm weevil, *Rhynchophorus vulneratus* and not *R. ferrugineus*. These *Rhynchophorus* species of weevils were synonymised as colour morphs by Hallett et al. (2004). RPW is now present in almost all countries of the Gulf region of the Middle East where it has been designated as a category-I pest of date palm, *Phoenix dactylifera* L., by the Food and Agriculture Organization of the United Nations. Economic losses between 1 and 5% due to eradication of severely infested palms are estimated to range from \$5.18 to \$25.92 million, respectively (El-Sabea et al., 2009). In the Mediterranean basin RPW has become a key pest of *P. canariensis* which is extremely sensitive to its attack (Dembilio et al., 2009). Damage to date palm is mainly caused by the larval stage feeding within the trunk of palms. Adult females of RPW lay eggs in protected parts of the date palm tree, including wounds on the trunk of established trees, at the base of fronds, the crown of the tree and adjacent to offshoots. Early stages of attack are difficult to detect in palms but can be cured with insecticide (stem injection). Palms in the later stages of attack often harbor several overlapping life stages of the pest with extensive tissue damage due to feeding by grubs, and have to be

eradicated (Faleiro, 2006). Pheromone (Ferrugineol) traps have been used to monitor and mass trap RPW in area-wide integrated pest management (IPM) programs. However, these conventional food baited pheromone traps (FBPTs) have to be periodically serviced (change food bait and insecticide solution) which is labor intensive. Therefore, a bait-free method to 'attract and kill' RPW adults, has recently been developed for weevil control in date palm (ElShafie et al., 2011).

The term artificial diet means a diet that does not include the insect's natural food except in minute traces and it is often conveniently divided into meridic and holidic diets. In the holidic diet all the ingredients, with the common exception of agar, are more or less chemically defined. In the meridic diet, one or more of the ingredients cannot be defined chemically for example, yeast or wheat germ (Mckinley, 1971). Ideal diet for insect mass rearing programs should supply all nutrients needed to produce acceptable insects, and should be easy to prepare and store for long period, inexpensive and should produce an average yield of adults of at least 75% from initial viable eggs (Singh, 1983). Various feeding substrates were used by many investigators to rear RPW in the laboratory for experimental purposes (Table 1). In many cases, the laboratory rearing and mass production of RPW is very important to maintain purity, age, physiological stage homogeneity and sex-based selection to carry out laboratory, semi-field and field experiments. This improves understanding the life-history parameters of RPW including development, survivorship and reproduction which will eventually lead to improvement of control strategies. The ability to produce quality insects in large quantity is critical for many research projects. Rearing of RPW on meridic diet could be an alternative to rearing on date palm tissues which is expensive and also difficult to follow the

**Table 2.** The different ingredients of the meridic diet used in rearing the red palm weevil, *Rhynchophorus ferrugineus*

Component	Amount
Distilled water	875 ml
Agar	18.5 g
Brewer's yeast	45 g
Wheat grains	45 g
Corn flour	45 g
Sorbic acid	1.6 g
Ascorbic acid	4 g
Aminobenzoic acid	1.6 g
Pharmaton® capsule (1.55g/capsule)	2 capsules
Tetracycline (250 mg)	2 capsules
DPRC produced yeast ( <i>Saccharomyces cerevisiae</i> )	25 ml

**Table 3.** Percentage of amino and fatty acids in the DPRC yeast, *Saccharomyces cerevisiae* used in the meridic diet for rearing of *Rhynchophorus ferrugineus*.

Amino acid	%	Fatty acid	%
Aspartic	0.40	Caprylic	0.010
Threonine	0.47	Capric	0.025
Serine	0.26	Lauric	0.175
Glutamic	0.79	Myristic	0.640
Glycine	0.26	Myristoleic	0.140
Alanine	0.58	Pentadecanoic	0.065
Valine	0.57	Palmitic	11.100
Methionine	0.27	Palmitoleic	35.095
Isoleucine	0.26	Margaric	0.110
Leucine	0.49	Stearic	5.560
Tyrosine	0.72	Elaidic	0.140
Phenylalanine	0.42	Oleic	43.240
Histidine	0.36	Linoleic	0.850
Lysine	1.02	Eicosanoic	0.080
Arginine	0.52	Linolenic	0.135
Tryptophan	0.09	Docosanoic	0.150
Cysteine	0.07	Tetracosanoic	0.095
		Pentacosanoic	0.140
		Hexacosanoic	0.745
		Heptacosanoic	0.070
		Octacosanoic	0.050

development of the larvae, since they are concealed inside the palm tissues. The present study was thus carried out to formulate a meridic diet for mass production of RPW with the following objectives:

1. Develop a process and quality control system to assure availability of high quality research insects including production of different developmental stages of the weevil of comparable physiological status for

bioassay experiments.

2. Assurance of continuous supply of experimental insects throughout the year.

## MATERIALS AND METHODS

### The meridic diet

The larval diet used for mass rearing of RPW was modified from that developed by Martin and Cabello (2006). The amino acid mixture used was replaced in our diet with yeast (*Saccharomyces cerevisiae* Meyen ex. E.C. Hansen) produced from date syrup at DPRC. The main ingredients of our diet included agar, commercially available baker's yeast as well as produced from date syrup at DPRC, wheat meal, corn flour, in addition to other basic ingredients (Table 2). The DPRC produced yeast is rich in amino and fatty acids and substituted for the commercially available amino acid mixture as recommended by Martin and Cabello (2006). The amino acids and fatty acids profiles of the DPRC produced yeast are shown in Table 3. Pharmaton™ capsules were added to diet to provide vitamins and minerals (Table 4). Pharmaton™ is manufactured by Ginsana SA, Bioggio, Switzerland on behalf of Boehringer Ingelheim international GmbH Germany. Tetracycline 250 mg, produced by Julphar, Gulf pharmaceutical industries, Ras Al Khaimah, U.A.E. was also incorporated as an antimicrobial agent.

### Baker's yeasts

The Baker's yeasts used in this study were a commercial Saf-instant active dry Baker's yeast (S. I. Lesaffre 59703 Marcq, France) and a strain from Specialized Laboratory: A *Saccharomyces cerevisiae* strain NCYC 1530 (laboratories of the National Center Britain). The strain NCYC 1530 was provided in a freeze-dried form and was kept in its original form until use. This yeast was produced using date syrup as described by Al-Eid et al. (2009) and Al-jasass et al. (2010) and was used in the meridic diet. Fats and fatty acid profile in the yeast reported here were determined according to the A.O.A.C. (1995) official method No. 996.01, amino acid composition of the proteins according to the method No. 994.12 and Tryptophan according to the method No. 988.15.

### Diet preparation

The basic diet was prepared based on the protocol of Martin and Cabello, 2006. Thirty seven gram of agar was mixed in 875 ml distilled water in glass beaker. The mixture was put in a microwave oven at maximum potency for 8 min and stirred using magnetic stirrer at 2, 4 and 6 min to dissolve the agar. The mixture was removed from the oven to facilitate each stirring and finally removed at 8 min. The main ingredients were mixed with agar jelly using a kitchen blender which was previously washed with distilled water and surface sterilized with 0.5% sodium hypochlorite. The mixture was homogenized at high speed for 2 to 3 min and left to cool till 60°C. As the diet cooled, two multi-vitamins (Pharmaton™) capsules and one tetracycline tablet were added while stirring the media. The diet was then poured into surface sterilized plastic containers while still warm and left to solidify and kept in the refrigerator for further use. Before being used, the cups containing diets were kept at room temperature to warm up. Small hole was

**Table 4.** Vitamins complex, minerals and trace elements used in the preparation of 1000 g of diet for rearing *Rhynchophorus ferrugineus*

Vitamins	Amount	Minerals and trace elements	Amount
Vitamin A	5334 IU	Copper	4.0 mg
Vitamin D3	400 IU	Selenium	100.0 mg
Vitamin E	20.0 mg	Manganese	5.0 mg
Vitamin B1	2.8 mg	Iron	20.0 mg
Vitamin B2	3.2 mg	Zinc	2.0 mg
Vitamin B6	4.0 mg	Calcium	200 mg
Vitamin B12	2.0 mg	lecithin	200 mg
Biotin	300 mg	Magnesium	20.0 mg
Nicotinamide	36.0 mg		
Vitamin C	120.0 mg		
Folic acid	0.2 mg		

made on the diet surface to facilitate the feeding and burrowing of the neonate larvae. Diet cups were covered with a lid vented by several small holes.

#### Test insects

Adult RPW females and males were captured using insecticide-free pheromone (Ferrolure™) traps deployed by Directorate of Agriculture in Al-Ahsa, Saudi Arabia. Active weevils were carefully selected from freshly captured collections, placed in perforated plastic containers and brought to the entomology laboratory of the Date Palm Research Center (DPRC). Weevils were sexed based on the presence of a tuft of fine bristles on the dorsal end of the rostrum in males and which were absent in females.

#### Collection and incubation of eggs

The collected insects were paired and kept in small plastic containers for mating. They were provided with a piece of sugarcane and left for at least 24 h. The males were removed thereafter, and the females were observed for egg laying. For production of maximum number of eggs, the females were put in an incubator at 27°C for 3 to 4 days.

The pieces of sugarcane in each container were carefully inspected and peeled to search for eggs which were collected and transferred into petri dishes using a fine camel hair brush. Only clean ivory colored and shining eggs were collected. The petri dishes contained cotton pads soaked in 10% sugar solution to provide nutrients for the newly hatched larvae before being transferred to the artificial diet. A 90 mm diameter Watman paper was then put on top of the cotton pad to retain the moisture. The eggs were then kept for three days in an incubator at 27°C and 50% RH for hatching. The newly hatched larvae were then carefully transferred to cups containing artificial diet. At the early stage of larval development, only one larva was placed into each cup to avoid cannibalism. From the 4<sup>th</sup> instar onwards, 3 to 5 larvae were put together in one cup. Cups of three different sizes (30, 50 and 200 ml) were used for small (neonate: 1 to 3<sup>rd</sup> instar larvae), medium (4 to 5<sup>th</sup> instar larvae) and large (6 to 8<sup>th</sup> instar larvae) specimens respectively.

#### Development of immature stages

Immediately after hatching, one neonate was carefully transferred

to each 30 ml plastic cup containing 5 g diet with a fine camel hair brush (n=12). Diet cups with the larvae were checked daily for signs of moult (cast skins and head capsules). As the larvae grew, they were transferred to larger diet cups as mentioned previously. The larval instars were determined by the number of moults. Visible exuviae including presence of head capsules in the diet were used as evidence of moulting. The surviving larvae were transferred daily into new diet cups after inspection. The development of Larvae was followed until they reached pupation. The time periods between instars as well as the number of moults were recorded.

#### Increase in larval biomass

In order to follow the increase in larval biomass, the mean weight of larvae (n=24) was recorded every three days starting from the first day of hatching till the last instar. The absolute increase in body biomass (weight/time) was determined using a sensitive four-digit electric balance, AL 204 Mettler Toledo manufactured by Mettler-Toledo Group.

#### Width of larval head capsule

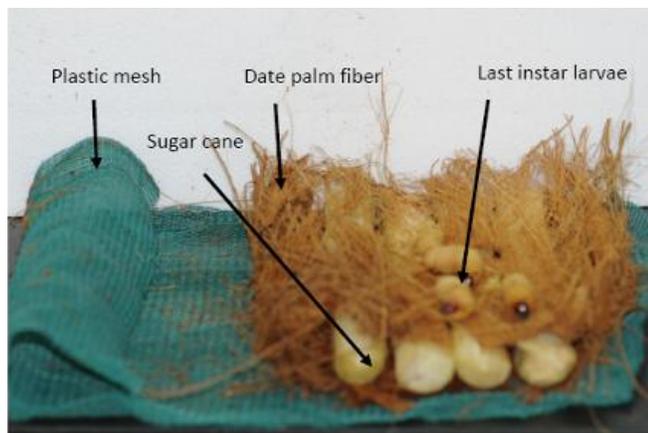
A digital vernier caliper was used to measure the width of larval head capsules. The head capsules of the first and second instars were measured by using microscopy digital USB Camera (Optikam™) programmed with Optika vision pro 4.1 software as these larval stages were too small to handle with a vernier caliper. Permanent microscopic slides of the first and second instars head capsules were prepared for the measurements using Canada balsam as mounting medium. The postmoult/premoult ratios of head capsule width in successive larval ecdyses were tested using Dyar's rule (Klingenberg and Zimmermann, 1992).

#### Food-fiber pupation technique

One of the challenges in the successful rearing of RPW on a meridic diet is to get the final instar larvae to pupate. Entomologists have extensively used sugarcane stem for this purpose. However, the diameter of stem is a determining factor in the success of this process. Sugarcane stem with narrow diameter usually leads to small size adults in addition to its low efficiency (adult emergence). The food-fiber pupation technique as the name implies, consisted of two layers of date palm fiber and a layer of sugarcane pieces (15 cm long), split longitudinally and stacked side by side to make a



**Figure 1.** RPW larvae growing on the meridic diet.



**Figure 2.** Food-fiber pupation technique developed to facilitate pupation by *Rhynchophorus ferrugineus* reared on meridic diet

mat 20 cm wide. The sugarcane pieces were sandwiched between the layers of date palm fiber and wrapped with fine plastic mesh. The sugarcane-date palm fiber mat was rolled into a cylindrical bundle resembling date palm offshoot (Figures 1 and 2). The date palm fiber and the sugarcane there by simultaneously provided material for the construction of the cocoon for fully matured larvae that wanted to pupate and nutrients for those larvae that wanted to feed and require additional time to complete their development. The sugarcane stem also provide a solid support that is required by the larvae during the cocoon spinning process. The food-fiber bundle was placed in 30 × 20 × 14 cm plastic container specially fabricated with 20 holes (5 mm diameter; 8 on the lid, 4 at the bottom and 2 on each side below the container rim) to provide aeration and to conserve the required humidity. The plastic container was kept in a ventilated glass cupboard and was checked after three weeks for cocoons and thereafter, regular inspection was carried every three days. The inspection process was done carefully to avoid disturbing the pupating larvae. The efficiency of the technique was calculated and expressed as percentage of mature larvae that pupated.

#### Percentage of adult emergence

Cocoons were carefully collected by hand. Whenever needed,

scissor was used to separate those cocoons which were firmly attached to the sugarcane pieces. The collected cocoons were put each in a plastic cup with four holes on the lid. The cups with cocoons were kept in an incubator at  $27 \pm 2$  °C and  $75 \pm 5\%$  R.H. in complete darkness until adult emergence. The percentage of adult emergence was then calculated.

#### Oviposition, egg hatchability, and adult longevity

Upon adult emergence individuals were weighed, sexed and 1 male and 1 female paired (10 pairs) were kept in 400 ml plastic containers provided with sugarcane pieces. These insects were used to study oviposition and longevity of RPW reared on the test diet. Sugarcane pieces were replaced every three days after being thoroughly examined for eggs which were collected and kept in petri dishes to determine the incubation period, fecundity and hatchability. To determine the pre-oviposition period, females were observed every day after emergence from the pupae until the first egg was laid. The fecundity was evaluated by the number of eggs laid during the life span of the female after the first egg laying. Adult longevity was also recorded. The experiment was continued until the weevil died.

#### Rearing of red palm weevil (RPW) on date palm trunk

Concurrently, individuals of RPW were reared on date palm (natural host) bolts in the laboratory to allow for comparison with those reared on the test meridic diet. The weevils were reared on the trunk of 3 to 4 year old Khalas cultivar which is predominant in Al-Ahsa region of Saudi Arabia. The rearing of RPW on date palm trunk followed the protocol described by Al-Ayedh (2008).

#### Data analysis

Results of biological parameters are given as mean  $\pm$  SD. Regression and correlation analysis, Chi-square, and t-test were performed whenever necessary using MINITAB statistical software, version 13.30, Copyright 2000; Minitab Inc.

## RESULTS

Table 5 shows different biological parameters of RPW reared on the meridic diet. The Pre-oviposition period lapsed about 4.8 day while the incubation period was 3.66 day. The total number of eggs laid per female (fecundity) was on average 198 eggs with an egg hatchability or viability of 88.10%. The larval weight of the first instar was 1.7 mg compared with 3.93 g for the last instar (Figure 3). Thus, the biomass increase of the last instar relative to the first one was 2311 times. Pupal period was 33.66 day with a pupation per cent of more than 97% and adult emergence of 93%. The total life cycle (egg to adult) was completed in about 82.4 day. The average larval period was 45 day while the average weight of last larval instar was 3.42 g. The mean longevity of the female was 74.8 day while that of the male was 83.3 day. The mean adult life weight was 0.54 g for the male and 0.57 g for the female. There was no significant difference between the sexes with respect to life weight ( $t = 1.049$ ,  $df = 1$ ,  $p = 0.05$ ).

**Table 5.** Important biological parameters of *Rhynchophorus ferrugineus* reared on meridic diet and date palm trunk under laboratory conditions.

Sl. No.	Biological parameter	Mean ± SD	
		Meridic diet	Date palm trunk
1	Pre-oviposition period (days)	4.80 ± 1.03 (10)	3.80 ± 0.78 (10)
2	Incubation period (days)	3.66 ± 0.88 (12)	3.08 ± 0.79 (12)
3	Fecundity (number of total eggs/female)	198 ± 24.00 (10)	206.30 ± 14.64 (10)
4	Hatchability or viability of eggs (%)	88.10 ± 5.17 (10)	93.10 ± 2.72 (10)
5	Total larval period (days)	45.09 ± 1.44 (22)	36.06 ± 3.24 (20)
6	Number of larval instars	8 ± 0.00 (25)	—
7	Total pupal period (days)	33.66 ± 2.30 (12)	22.5 0 ± 1.78 (12)
8	Total life cycle from egg to adult (days)	82.41 ± 1.54 (10)	61.64 ± 1.93 (10)
9	Weight of last larval instar (g)	3.42 ± 0.21 (10)	5.31 ± 0.32 (10)
10	Head capsule width of last instar (mm)	5.43 ± 0.27	7.42 ± 0.12 (10)
11	Adult male longevity (days)	83.30 ± 1.63 (10)	91.58 ± 2.23 (10)
12	Adult female longevity (days)	74.80 ± 2.20 (10)	81.80 ± 3.55 (10)
13	Adult male life weight (g)	0.54 ± 0.08 (16)	1.02 ± 0.08 (16)
14	Adult female life weight (g)	0.57 ± 0.11 (16)	1.16 ± 0.04 (16)

Figures in parentheses are number of individuals tested.

**Table 6.** Chi-square comparison (observed and expected) of larval head capsule width in *Rhynchophorus ferrugineus* reared on meridic diet

Instar	Head capsule width (mm)							
	1	2	3	4	5	6	7	8
Observed	0.68	1.05	1.5	2.0	2.98	3.2	3.9	5.4
Predicted	0.82	1.07	1.40	1.82	2.36	3.0	4.0	5.4

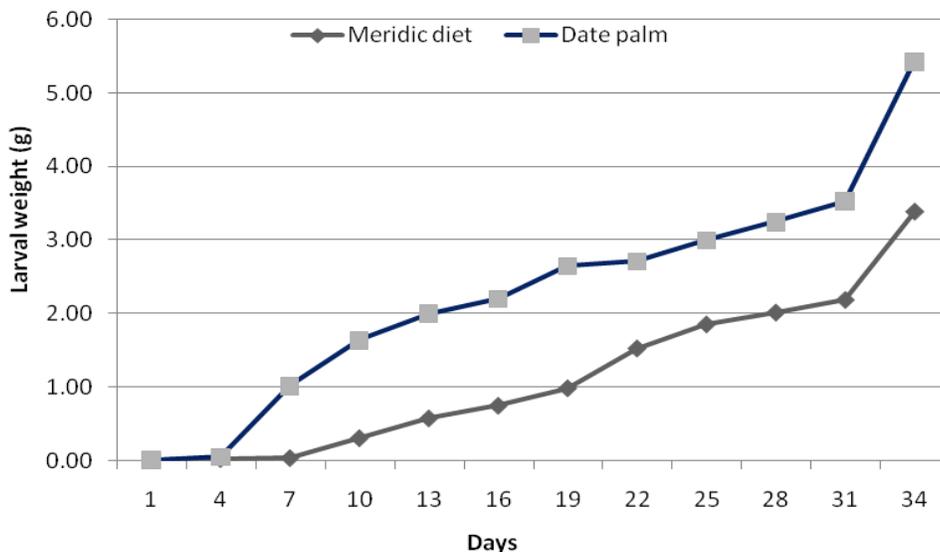
Dyar's ratio = 1.3. Chi-square statistic was not significant ( $\chi = 0.08$ ,  $df = 1$ ,  $p = 0.05$ ) indicating that there was no significant difference between the observed and predicted head capsule measurements.

This study revealed that in all there were 8 larval instars (Table 6). Linear regression analysis showed significant relationship between larval instars and head capsule width ( $R^2 = 0.978$ ; Figure 4). Plotting the logarithm of the head capsule width measurements against the respective instar generated a straight line indicating the conformity to the law. Therefore, head capsule width could be used for estimation of larval instar in the laboratory population of the RPW. A food-fiber pupation technique was developed in this study with 100% pupation efficiency (Figure 1).

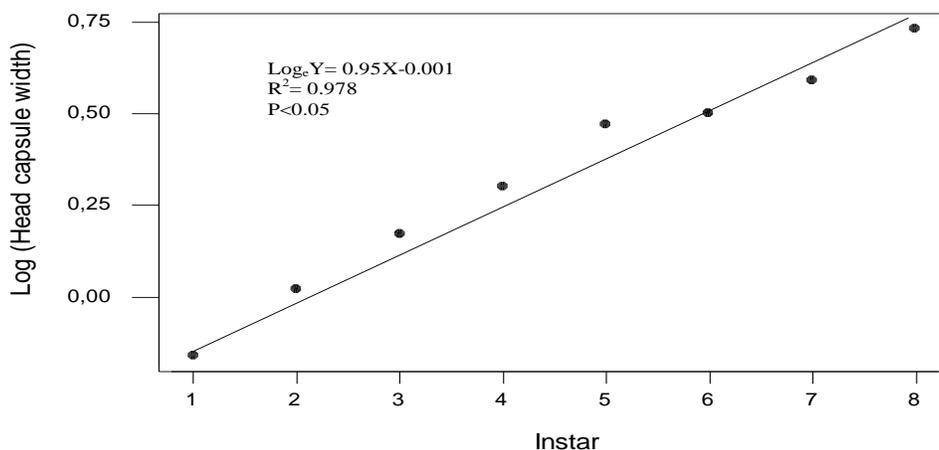
**DISCUSSION**

The suitability of an artificial diet can be compared with respect to development, survival, longevity and fecundity (Ki-Sang and Lee, 2004). Artificial diets for rearing RPW previously reported had the amino acid mixture as a vital component of the diet (Barranco et al., 1997). In this

investigation, the amino acids were incorporated in the diet through the yeast (*Saccharomyces cerevisiae*) produced from date at DPRC. This yeast was mainly considered as a protein source, it also contains carbohydrates, lipids, minerals and vitamin B-complex (Robinson, 1986; Hamad, 1986). The yeast is essential component in any artificial diet and can make it either successful or non-successful (Cohen, 2003). Eight larval instars were recorded on the meridic during our study and it was technically difficult to trace the number in the date palm trunk. However, the life cycle of the weevil was completed in about 62 d on date palm trunk compared with 82 day on the meridic diet. The shorter life cycle on date palm could indicate less number of instars. This is in line with the findings of Ju et al. (2010) who stated that on suitable host plants, larvae may have fewer instars and thus its development can be shortened. The number and nature of molts can be frequently altered by external factors, mainly temperature, diet and their interaction (Wigglesworth, 1954; Stamp, 1990) and accordingly the number of instars will also be changed. Large discrepancies occur in the literature concerning the number of larval instars of RPW reared on different diets and under different laboratory conditions. Ju et al. (2010) reported eight instars on Canary Island (*Phoenix canariensis*) and Washington palms (*Washingtonia* sp). They also reported nine instars on Chusan palm (*Trachycarpus fortune*), Pindo palm (*Butia capitata*), and silver date palm (*Phoenix sylvestris*). Seven instars on sugarcane and nine instars on coconut palm (*Cocos nucifera*) were recorded in Philippines by Jaya et al. (2000) and Viado and Bigornia (1949) respectively. Nilura (1956) reported only three instars on coconut in India



**Figure 3.** Increase in biomass during larval development of *Rhynchophorus ferrugineus* reared on meridic diet and date palm trunk



**Figure 4.** Relationship between head capsule width and instar of *Rhynchophorus ferrugineus* reared on meridic diet.

while thirteen instars in Canary Island palm (*Phoenix canariensis*) were recently estimated by Dembilio and Jacas (2011). This variation in the number of instars could be attributed to the rearing conditions and diet/rearing media used by previous workers. Dyar's law has been reported to be applicable to other insects by several workers where head capsule width was used to determine the number of instars (Klingenberg and Zimmermann, 1992). Differences in estimates of life parameters of RPW reared on meridic diet and date palm as natural host may have been due to food-type, and rearing conditions (Joem and Behmer, 1997). Although there is some success in efforts to rear successive

generations of economically important insects entirely on an artificial diet, in many cases there is loss of both fitness and reproductive potential which cause longer development times and lower fecundity (Coudron et al., 2002). In the present study, RPW was successfully reared on the meridic diet for one generation without any apparent deformity or difficulty in rearing as compared with individual insects reared on date palm as natural host. Thus, the meridic diet employed in this work can be a suitable substrate for mass rearing of RPW in the laboratory. However, further evaluation is required to determine the effect of the test diet on the weevil survival and biological parameters over more than one generation

as compare to individual insects reared on date palm as a natural host.

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