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Successful Laboratory Culture for the Red Palm Weevil *Rhynchophorus ferrugineus* (Colioptera: Curculionidae) Reared on Semi-artificial Diet.

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ABSTRACT

The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier.) is one of the most important pests of palm trees. It can cause the death and falling down the trees. The RPW reared on semi-artificial diet in the laboratory for several generations from the adult emergence and compared with other two natural diets (leaf base petioles of date palm and sugarcane stem). All larval stages that reached to 15 stages within mean 96.7 day were fed on the artificially diet till pupation, the adult weevil also were fed and oviposeted on it. Duration and all the developmental stages were recorded. Duration of Pupal stage was 21.1 day, high percentage of adults emergence were obtained, the life cycle (from egg to adult stage) nearly reached 119.4 days. The average egg production per female were 250.2±52.1 egg and the greatest number of eggs were laid during the first three week , then decreased with increasing weevil age. The percentage of egg hatchability was 82.7, the size of adults that reared on the artificial diet were smaller and lighter in their color from that reared on the natural host (palm leaf base). Red palm weevils were successfully reared on this semi-synthetic diet and these ingredients were found capable of supporting larval development. The main purpose of our study was to evaluate the effectiveness of semi-synthetic diet developed from locally available ingredients for rearing the RPW and comparable with that reared on natural diet (Base leaves of Date palm petioles and Sugarcane stem pieces.) under laboratory condition. The development of suitable artificial diets for maintaining laboratory colonies of insects became of great importance for facilitating different investigations of the biology, control, physiology and behavior of insect, especially, if the green natural plants were not available or for difficult and expensiveness rearing on the natural host like in case of the red palm weevil *Rhynchophorus ferrugineus* .

KEY WORDS: Red Palm Weevil, *Rhynchophorus ferrugineus*,, Semi-artificial diet, Laboratory culture, Biological parameters..

INTRODUCTION

The red palm weevil (RPW) *Rhynchophorus ferrugineus* Oliver. (Coleoptera: Curculionidae), is an economically important , tissue- boring pest of date palm in many parts of the world. The insect is a major pest of date palm in some of Arabian Gulf States including Saudi Arabia, United Arab Emirates, Sultanate of Oman and Egypt [1]& [2]. Red palm weevil is a concealed borer and spends all of its life stages inside the palm tree. Damage symptoms can be categorized by one or more of the following [2] , presence of the tunnels on the trunk and base of leaf petioles made by the feeding grubs, oozing out of thick yellow to brown fluid from the tree, appearance of chewed up plant tissue in and around opening in the trunk, presence of a fermented odor from the fluid inside the infested tunnels in the trunk, presence of adults and pupal cocoons in the leaf axils, chewed up frass on the ground around the palm, fallen empty breaking of trunk or toppling of the crown when the palm is severely infested. Researches on different experimental investigations of RPW required large number of insect individuals of various stages. For this we needed to develop successful diet for continues laboratory maintenance of the RPW. In the laboratory, the RPW can be successfully reared on both natural (Sugarcane stem and banana fruit) and synthetic diet (Semi-artificial) diet [3]. In India [4] devised a new method for mass rearing of RPW using coconut petiole. [5] can be cultured the RPW using cut petiole or stem tissue from date palm and from Coconut plants [6] . The method was improved by incorporating Sugarcane in nutrient agar for young larvae and whole Sugarcane stem pieces for older larvae [6]. Rearing methods of this and several species were reported [7], [8], [9 [10] and[11]. [12] pointed out several reasons for including host plant material in an artificial diet, as natural host plants have complex chemicals that may serve as token stimuli, provide cryptic nutrients and provide

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nutrients in suitable properties needed for insects specialized feeding habit as well as attractant insects towards a particular plant tissue.

The objectives of this study was to develop successful semi-artificial diet from the available locally ingredients for continues laboratory culture of the RPW.

MATERIALS AND METHODS

Various stages (larvae, pupae and adults) of *R. ferrugineus* were collected from infested date palm trees in. Each developmental stage was placed individually in covered vented plastic container Fig.4. in a controlled room at 30± 2 °c and 30 – 50% RH. The photoperiod was approximately 12: 12 L.D [13] . Insect adults kept as individual pairs of males and females, provided with 50 g. piece of artificial diet or piece of leaf base petiole of date palm tree or pieces of Sugarcane stem as a control Fig 5 A&B. for feeding and egg laying. The ingredients of the synthetic diet were indicated in(Table 1) . The deposited eggs were transferred with a fine hair brush from the oviposition site and placed on wet filter papers inside a Petri dishes Fig. 4. for further studies. After 2 to 5 days, larvae from hatched eggs were removed to separate containers and provided with 5 g. piece of artificial diet or leaf base petioles of the palm tree or pieces of Sugarcane (natural host) for feeding, another food quantity were introduced when consumed or dry. One week after feeding, larger larvae transferred to large fresh pieces of diets in larger jars. Larvae were reared individually to avoid cannibalism habit and left for pupation. Cocoons were separated and placed in plastic containers, wet with water as needed. The cocoons were checked daily after two weeks for adult emergence. The emerged adults were collected and placed individually in plastic containers then sexed, paired males and females (two males with one female) . The deposited eggs by females were counted (fecundity) and the percentage of egg hatchability (fertility) was estimated. Different biological parameters were recorded (pre oviposition period, oviposition period, number of larval instars, larval duration, pupal duration, percentage of pupation, percentage of adult emergence , longevity and life cycle).

STATISTICAL ANALYSIS

The obtained data were subjected to Analysis of Variance (ANOVA). When F Values were significant ($P < 0.05$), means were compared using the Least Significance Difference test (LSD) for all parameters using [14] Multiple Range Test($P<0.05$) and SD was also calculated.

Preparation of the artificial diet:.

Semi artificial diet were developed from the locally available ingredients as recorded in (Table 1) comparing with the rearing on the natural host (leaf base petioles of the palm tree and pieces of sugarcane stem). The diet were evaluated in preliminary test for larval and adult biomass gain, survival and the rate of development. All ingredients except agar were blended with one liter of water, agar was dissolved in another 500ml. warm water and added to all other ingredients. The mixture of the diet were boiled for 2 minutes, then poured in cups while still warm and after cooling it placed in the refrigerator till required for the experiments.

RESULTS &DISCUSSION

Data in(Table2) indicated that there were no significant differences in the Adult longevity of both males or females that resulted from larvae reared on the artificial diet or the other natural diets (leaf base petioles of date palm tree and Sugarcane stem), also no differences in Pre- oviposition , oviposition and egg incubation periods. The adult longevity of females was 90.7, 92.6 and 99.3 day, while for males 62.7, 70.3 and 64.2 day on palm base leaf, sugarcane and the artificial diet respectively. Pre-oviposition period on all diets ranged from(3- 5 days) , oviposition period from (4-7 day) and the incubation from (4-6 day). These results agree with that estimated by [15], [16] and [17]. There were a significant differences for the larval duration which reared on natural hosts(leaf of the date palm or sugarcane pieces) comparing with that read on the artificial diet ,the shortest time for the larvae that reared on the palm base leaf petioles lasted mean 79.4 day with range (65- 92 day) , sugarcane diet 82.8 day by range (70-98 day) compared by longest duration 96.7 day (73- 98 day) on the artificial diet. [18[, [19], and [20] mentioned that host plant quality has a direct impact on food consumption, survival and development of larvae [21] found that larval growth was influenced by the quality of the host plant. Understanding the chemical reactions of the semi-synthetic

diet ingredients in the larvae midgut might help in improving the diet. From our observations we noticed that the larvae molt 13 (14 instars) times on the synthetic diet comparing with 12 moult (13 instars) for the two natural hosts as observed in Table (2), synthetic diet was able to achieve growth and development of larvae of the RPW., the ingredients used in the synthetic diet were found to be capable of supporting the larval development . No significant differences in the developmental time of pupae occurred when larvae reared on the synthetic diet (25.1 day), palm base leaf (25.3 day) and sugarcane stem (26.8 day) Table (2). The results agree with the results estimated by [15] where the pupal period lasted (range 19- 26 day), and [15] mentioned that pupal measurements were not significantly affected by diet differences, pupae that obtained from the natural diet were longer and wider than pupae developed on the semi-synthetic diet. The percentages of pupation were 87.5 % on palm base leaf followed by 72 % on semi-synthetic diet and finally 69% on the sugarcane. The highest percentage of adult emergence was obtained from rearing on the semi-synthetic diet (87%) comparing with the other natural hosts (75.5%) on palm base leaf and 72.5 on sugarcane pieces . The emerged adults were very important for giving the new generation from the insect. There were 5% adults malformation (range: 2-5%) through all the tested diets, this may be due to the suboptimal condition (food type , rearing methodology , and the presence of insects confined in small jars may have interfered with damage to larvae and pupae that reflected on appearance of adult malformation) than in the nature habitat. Table (2) recorded the average number of egg production per female was 270.4 eggs (range: 125- 200), 250.2 eggs (range: 105- 290) and 230.7 eggs (range: 135- 250) for females resulted from pupae their larvae reared on sugarcane pieces, semi-synthetic diet and palm base leaf respectively. These numbers are comparable to the previous estimates of [22] (77- 283 eggs) and [15] (55- 412 eggs). The percentage of egg hatchability (viability of eggs) was 95.6 , 87 , 82.7 % when females resulted on palm base leaf , sugarcane pieces and semi-synthetic diet respectively (Table2). Our results agree with that mentioned by [17]. [6] reported that *R. ferrugineus* laid less eggs when confined with males than without. Significant differences in the life cycle (a period from egg stage to adult emergence) were noticed in (Table 2) . The mean longer time of life cycle 119.4 day (range: 97-129) for the synthetic diet and 116.6 day (range: 99- 132) for the sugarcane, then decreased to 108. 8 day (range: 88 – 132) in case of the palm base leaf . The generation span reported by [22] was 95- 210 day and [15] recorded 223 day .

We could be concluded that Red Palm Weevil *R.ferrugineug* can be successfully reared on Semi-synthetic diet described herein. The ingredients used in the diet were found to be capable of supporting the larval development of the insect. All biological parameters were comparable to the other previously successful natural hosts (Pieces of Date palm tree and Sugarcane) as a control .

Fig. 1: Rearing boxes.:

Fig:2 A. RPW reared on leaf base petiole of the palm tree.

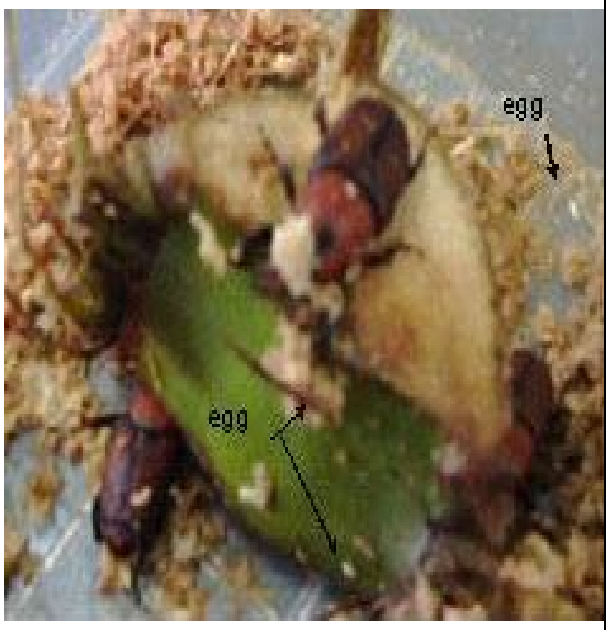

Fig: 2 B. Adults laid eggs on the natural host.

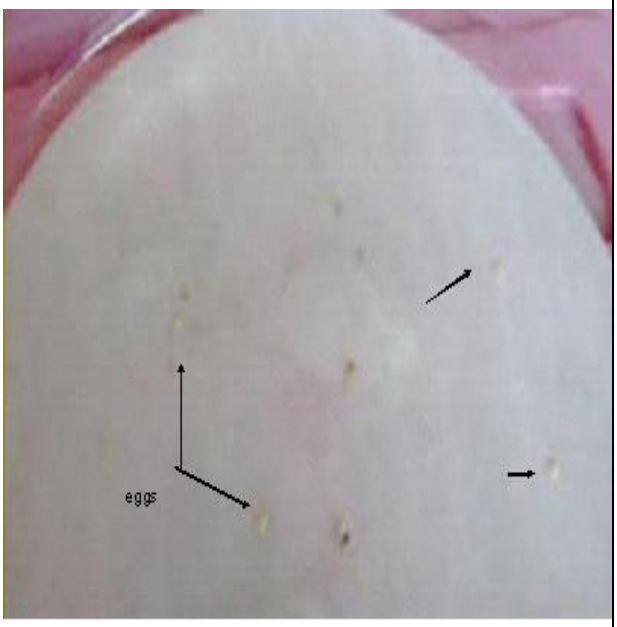

Fig.3: Eggs on moistened filter paper inside Petri dish.

Fig 4 : Larva fed on the artificial diet

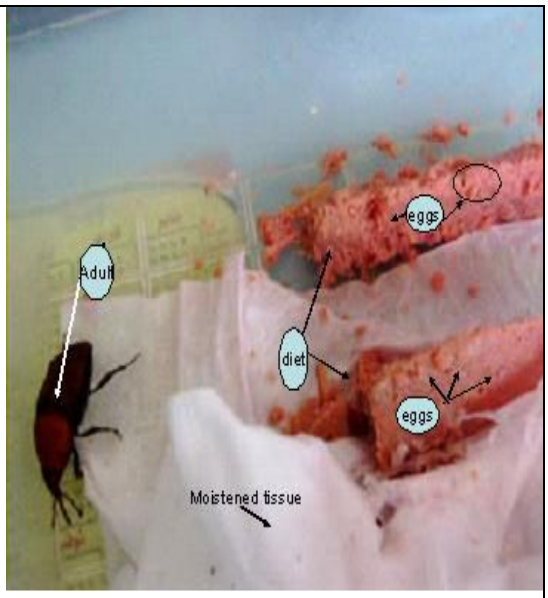

Fig.5: Adult weevil fed on the artificial diet and laid their eggs on it

Table 1 : Ingredients of the Semi-artificial diet used for rearing the *RPW Rhynchophorus ferrugineus.*

Ingredient	**Weigh**
Fruits of Carrot plant *(Dacus carota).*	25 g.
Sugar beet (*Beta vulgaris*).	25 g.
Soya bean powder .	50 g.
Powder of dry beans.	50 g.
Palm tree fibers.	10 g.
Egg yolk.	One .
Dry milk (Nido milk و , NESSLA).	10 g.
Brewers yeast.	15 g.
Agar.	20 g.
Molasses.	5 ml.
Coconut oil .	1 ml.
Vitamin B complex (ALEX, Faizer Company.).	1 tablet.
Folic acid.	2 g.
Benzoic acid .	2 g.
Sorbic acid .	2 g.
Ascorbic acid .	2 g.
Water .	1000 c c.

Table(2): Biological aspects of the RPW *Rhynchophorus ferrugineus* reared on semi-synthetic diet and two natural host plants.

Biological parameters	Rearing media Mean ± SD (Range)		
	Palm base leaf petioles	Sugarcane stem pieces	Semi-synthetic diet
Larval duration In days.	79 ± 4.0 a (65- 92)	82.8 ± 21.3 a (70- 98)	96.6 ± 26.2 b (73- 98)
No. of larval instars.	13	13	14
Pupal duration in days.	25.3 ± 3.9 a (22- 29)	26.8 ± 0.13 a (25- 30)	25.1 ± 5.6 a (23- 28)
% Pupation.	87.5	69	72
% Adult emergence.	75.5	72.5	87
Adult longevity in days. For ♀.	90.7 ± 31.9 a (45- 105)	92.6 ± 17.5 a (80- 117)	99.3 ± 20.5 a (70- 115)
Adult longevity in days for ♂ .	62.7 ± 1.02 a (36- 80)	70.3 ± 5.6 a (42- 90)	64.2 ± 10.5 a (44- 77)
% Adult malformation.	2	5	5
Pre-oviposition period in days.	3.6 ± 3.01 a (3- 5)	3.1± 0.11 a (3- 5)	3.5 ± 1.01 a (3- 5)
Oviposition period in days.	6.4 ± 10.02 a (4- 7)	6.2± 0.29 a (4- 7)	6.8 ± 11.01 a (4- 7)
No. egg production /♀.	230.7 ± 13.7 a (135- 250)	270.4± 29.2 b (125- 200)	250.2 ± 52.1 a b (105- 290)
Incubation period in days.	4..5 ± 0.65 a (4- 6)	4..0 ± 0 a (4- 4)	4..2 ± 0.87 a (4- 5)
% Egg hatching.	95.6	87	82.7
Life cycle in days.	108.8 ± 0.45 a (88- 132)	116.6 ± 9.02 b (99- 132)	119.4 ± 12.2 b (97- 129)

Note: Means with the same letter had no significantly difference in horizontal columns.

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