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Original article

Semi-artificial diet developed for the successful rearing of red palm weevil: *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) in the laboratory

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ABSTRACT

Objectives: The red palm weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae) is the most destructive insect pest of several palm species, worldwide. The maintaining of RPW laboratory colonies for experimentation has been very challenging. We synthesized a standardized semi-artificial diet which is easy to prepare and economical for the rearing of RPW.
Methods: The effects of semi-artificial diet on biological traits related to RPW development were studied at the Economic Entomology Research Unit (EERU), Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia. The diet was synthesized by combining shredded date palm petiole tissues, corn flour, wheat flour, ascorbic acid, sodium benzoic, sorbic acid, agar, and distilled water. Three replicates, each containing fifty newly hatched larvae, were reared on the semi-artificial diet. The developmental time of RPWs fed on the newly synthesized diet was compared to the growth rate of conspecifics fed on date palm and previously published artificial diets.
Results: Fully-grown larval weight ranged from 4 to 6 g (5 ± 0.19 g), and larval duration ranged from 46 to 57 days (53 ± 0.3 days). Average adult emergence was 58–98 % (78 ± 0.6), with an average weight of 0.90 g for males and 0.97 g for females. Complete life span was between 48 and 98 days (77 ± 0.9 days). On present semi artificial diet, several larvae had 11 larval instars. The diet has a shelf life of at least seven days and can last up to two weeks if properly kept and saved.
Conclusions: In conclusion, the present study proved that semi artificial diet is quite capable for the successful rearing of RPW. The growth and development of RPW larvae reared in this experiment were comparable to those fed on a natural diet.

1. Introduction

Red Palm Weevil (RPW) has been the most destructive insect pest of date palm in the Middle East since the mid-1980's (El Ezaby et al., 1998, Ferry and Gomez, 2002). Recently, RPW has become an invasive insect pest in several countries around the globe (Murphy and Briscoe, 1999; Malumphy and Moran 2007, Bozbuga and Hazir, 2008). Due to its economic importance, many studies have attempted to construct control strategies using biological control agents (Llacer, 2008; Dembilio et al., 2010; Güerri-Agulló et al., 2011), baited trap systems (Abbas et al., 2006, Al-Saoud et al., 2010, Faleiro et al., 2011), sterile male techniques (Al-Ayedh and Rasool, 2010), microwave heating (Massa et al., 2011), chemical insecticides (Abraham et al., 1975), and integrated pest management (Tapia et al., 2011). Although many techniques have been proposed to control the pest, none are satisfactory due to its unique biological behavior, which includes the concealed

Abbreviations: RPW, Red Palm Weevil; EERU, Economic Entomology Research Unit; CRD, Completely Randomized Design; DMRT, Duncan'S Multiple Range Test.
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nature RPW. Larvae, pupae and even adults remains inside the tree trunk and larvae feed on the soft tissues.

Several techniques for the detection of RPW infestation on palms have been established, such as using acoustic sensors (Gutiérrez et al., 2010, Siriwardena et al., 2010), sniffing dogs (Nakash et al., 1999), and X-ray CT (computed tomography) scans (Ma et al., 2012), but they are not sufficiently accurate. Determining RPW presence in tree trunks usually encounters difficulties that affect the accuracy of results. From above mentioned evidences regarding huge numbers of experiments, it is obvious that, mass rearing of the RPW is very important to provide RPW different developmental stages to be used in experimentation/bioassays. In lab-based biological assays, the insects used must be homogeneous and available in large numbers. In this regard, the present study objective was to develop a cost effective and nutritious, artificial diet with enhanced shelf life for mass rearing of the RPW for the successful experimentation.

Artificial diets should contain enough nutrition for the growth and development of the insects, and have both physical and chemical properties that are palatable, digestible and absorbable by the insect (Coudron et al., 2004). Attempts to mass rear RPWs have been reported. Rearing of the weevil can be achieved using date palms trunk (Al-Ayedh, 2008, Aldawood and Rasool, 2011), or sugarcane as host plants (Prabhu and Patil, 2009). Additionally, vegetables-based diets (Alfazairy, 2011) and date palm frond semi-synthetic diets (Al-Ayedh, 2011) can be used. Using natural diets increases the risk of uncontrolled contamination and has a larger space requirement. On the other hand, the use of artificial diets is more practical, as the risk of contamination can be controlled and less space is needed (Situmorang, 1997). This study aimed to develop an artificial diet with a longer shelf life that satisfies the nutritional requirements of RPW larval stages, and evaluate this through its effects on various RPW development stages.

2. Materials and methods

The experiment was conducted at the economic entomology research unit (EERU) Plant Protection Department, College of Food and Agriculture Sciences, King Saud University. The parent generation of RPWs was collected from date palm fields in the Riyadh Region (24.4164N, 46.5765E) and comprised both adults and larvae. Adults were kept in plastic boxes (size, 1 Kg) and were provided with cotton saturated with 10 % (w/v) bee pollen solution. The laid eggs were collected and kept in petri dishes (d: 8 cm; h: 2.5 cm) containing wet filter paper. Newly hatched larvae were then maintained on the artificial diet. Therefore, in the present experiments, second-generation individuals were reared on the present semi artificial diet.

The present semi artificial diet synthesized was modified from Al-Ayedh (2011) by adding preservatives, antioxidants, and agar in order to improve the texture and to enhance the shelf life of the diet. The artificial diet was mainly composed of shredded date palm petioles. To prepare the ground petioles, date palm petioles were cut into pieces (3 cm × 3.5 cm) and air dried for three days to reach 35 – 40 % water content. Then, 500 g of ground petiole, 250 g wheat flour, 250 g corn flour, and two g ascorbic acid were homogenized (mix 1.). 1.6 g potassium benzoate, 1.6 g sorbic acid, 20 g agar, and two litters distilled water were boiled, then cooled until reaching 60 °C. After this, mix. 1 was homogenized with agar solution.

In this experiment, three replicates with 50 larvae per replicate were used. Newly hatched larvae were maintained in a plastic cup (d: 5 cm; h: 3 cm) and provided with 60 g of the diet until the 5th instar. After 5th instar, they were transferred into a plastic jar (d: 6 cm; h: 8 cm) with 250 g of the diet until full grown. During the last instar, the larvae starts wandering to find fiber for pupation, these full grown wandering larvae were transferred into a box (l: 17 cm; w: 11 cm; h: 7 cm) and provided with 10 cm long sugar cane piece/stick for pupation. Emerged adults had a 1:1 sex ratio. Then, laid eggs were observed to measure fecundity. Diets were changed every-seven to 10 days. As a control, 10 larvae maintained on the previously published artificial diet and 10 larvae reared on date palm bolts (one-meter-long date palm trunk) were used, with three replicates for each. All colonies were maintained in a growth chamber set at 70 % RH and 25 °C temperature.

Larval growth and development were monitored daily by recording the exuviae or head capsules, while weight was measured two days after molting. Measured parameters were: larval instar development, larval weight, adult weight, fecundity, and egg hatchability. These parameters are common in published papers within the field, and within the literature.

2.1. Experimental design and Analysis

A completely randomized design (CRD) was used in this experiment. Analysis of variance ($\alpha = 0.05$) was conducted to compare the developmental stages of RPWs reared on the diets, and this was followed by Duncan's multiple range test (DMRT) to separate means. Statistical analyzes were completed using SPSS version 13.0 (SPSS Inc 2005).

3. Results

3.1. Larval development

On newly synthesized semi artificial diet, RPW larvae completed ten to eleven instars (Table 1). Results indicated significant

Table 1
Growth and development of red palm weevil larval stages fed on semi synthetic diet (Al-Ayedh, 2011), date palm, and present semi artificial diet.

Parameters	Semi-Artificial Diet			Date Palm			Al-Ayedh, 2011			F-test	p-value	
	N	Range	Mean ± SEM	N	Range	Mean ± SEM	N	Range	Mean ± SEM			
Instar duration (d):												
1st instar	102	3 – 4	3.42 ± 0.05 a	30	2–5	3.47 ± 0.16 a	30	1–4	2.73 ± 0.12b	16.353	3.48E-07	P < 0.0001
2nd instar	102	3 – 6	4.41 ± 0.05 a	30	2–5	3.23 ± 0.13b	30	2–5	3.30 ± 0.16b	57.675	2.20E-16	P < 0.0001
3rd instar	102	4 – 6	4.67 ± 0.05 a	30	2–5	3.66 ± 0.12b	30	2–5	3.36 ± 0.15b	64.844	2.20E-16	P < 0.0001
4th instar	102	4 – 6	4.51 ± 0.05 a	30	3–6	3.86 ± 0.17b	30	2–8	3.66 ± 0.21b	17.478	1.38E-07	P < 0.0001
5th instar	102	5 – 8	6.16 ± 0.07 a	30	3–6	4.30 ± 0.12b	30	2–5	3.66 ± 0.11c	182.35	2.20E-16	P < 0.0001
6th instar	102	4 – 7	4.96 ± 0.07 a	30	3–7	4.86 ± 0.23 a	30	2–8	4.16 ± 0.28b	6.7058	0.0016	P < 0.01
7th instar	102	4 –6	5.05 ± 0.06b	30	3–8	4.60 ± 0.21c	30	3–8	5.53 ± 0.22 a	7.8524	0.0005595	P < 0.001
8th instar	102	4 – 8	5.27 ± 0.09b	30	2–13	5.43 ± 0.39b	30	2–11	7.26 ± 0.42 a	19.61	2.44E-08	P < 0.0001
9th instar	102	4 – 8	5.09 ± 0.08b	30	4–26	8.10 ± 0.78 a	30	5–10	7.16 ± 0.24 a	30.278	7.22E-12	P < 0.0001
10th instar	95	4 – 13	8.99 ± 0.26b	30	12–39	23.16 ± 1.37 a	30	5–19	9.86 ± 0.69b	137.24	2.20E-16	P < 0.0001
11th instar	7	0–5	0.49 ± 0.14b	–	–	–	30	10–28	17.86 ± 0.94 a	591.83	2.20E-16	P < 0.0001
Larval duration (d)	102	46–57	53.05 ± 0.27c	30	48–89	64.7 ± 1.95b	30	47–83	68.6 ± 1.43 a	97.085	2.20E-16	P < 0.0001

Table 2
Comparison of Red palm weevil performance on different diets.

Parameters	Nirula (1956)	Alsuhaibani et al. (2001)	Prabhu and Patil (2009)	Abd El -Fattah et al. (2009)			Al-Ayedh (2011)		Salama and Abdel-Razek (2002)	Aldawood et al. semi-artificial diet
	Coconut	Date palm	Sugarcane	Sugarcane	Date palm	Artificial diet	Artificial diet	Date palm		
Egg										
Fecundity	76–355 (204)	55–412 (227)	211–380 (278)	275 ± 24	259 ± 26	222 ± 22.74	35.2 ± 7	32 ± 8	184 ± 19	48–193 (96) ± 15
Oviposition period	25–63 (45)	54–137 (96)	–	–	–	–	–	–	–	35–61 (48) ± 3
Hatchability %	–	–	70–90 (83)	84 ± 5	87 ± 5	829 ± 5	21–71 (40) ± 5	0–61 (30) ± 5	94–100	43–100 (73) ± 8
Incubation period	2–5 (3)	2–5 (4)	2–4 (3)	3.16 ± 0.17	3.16 ± 0.16	3.09 ± 0.17	–	–	–	2–6 (4.5)
Larva										
# instars	–	–	8	–	–	–	11	10	–	10–11
Duration	35–71 (55)	120–241 (182)	32–65 (56)	68 ± 4	37 ± 2	45 ± 3	45–81 (66) ± 1	48–89 (65) ± 2	45	46–57 (53) ± 0.3
Head Capsule width	–	–	–	–	–	–	5.16	5.69	–	–
Weight	–	−2.73	4–6.4 (4.95)	–	–	–	3–6 (5) ± 0.1	0.1–5 (2) ± 0.2	–	4–6 (5) ± 0.2
Pupa										
Duration	12–21 (16)	21–26 (23)	10–12 (16)	–	–	22–64	–	–	–	10–49 (25) ± 0.7
Weight	–		1–3 (2)	–	–	–	1–3 (2) ± 0.1	1–3 (2) ± 0.2	–	–
Adult										
Emergence %	–	–	85–90 (87)	–	–	–	–	–	–	58–98 (78) ± 0.6
Male longevity	48–113 (84)	76–257 (161)	62–78 (71)	92.4 ± 6.84	93.4 ± 5.37	98 ± 5.96	–	–	95.2	33–72 (52) ± 3
Female longevity	43–76 (60)	70–335 (112)	59–75 (69)	82 ± 6	91 ± 6	95 ± 7	–	–	98	15–72 (49) ± 3
Sex ratio	1.3:1	0.8:1	1:1	0.53:0.47	–	–	051: 0.49	–	0.53: 0.47	1:0.79
Male weight	–	–	0.53–1 (0.9)	–	–	–	0.5–1.4 (1) ± 0	0.65-0.96 (1) ± 0.1	–	0.6–1.21 (0.90) ± 0.1
Female weight	–	–	0.6–1.43 (1)	–	–	–	0.6–1.4(1) ± 0	0.6–1 (1) ± 0.1	–	0.7–1.24 (0.97) ± 0.1
Total Life cycle development										
Egg to Adult	54–120 (81)	208–223 (216)	58–97 (82)	165–195	–	–	–	–	–	48–98 (77 ± 0.9)

differences (F = 48.9; df = 2, 74; $P < 0.001$) in larval weight at the full-grown stage. Larval weights developed from 0.001 g for the 1st instar, to an average of 5.5 g (range: 4–7 g) for the tenth instar, to an average of 5 g (range: 4 to 6 g) for the eleventh instar. The total RPW larval duration was 53 days (range: 46–57) days. Mortality during larval stage was nil. Only 7 % larvae completed eleven instars while 93 % larvae pupated after 10th instar (Table 1).

3.2. Pupal development

After larvae were fully grown and stopped feeding, they were transferred onto sugar cane for pupation. Mortality during the pupation stage was very low, at roughly 2 %. The pupal period varied from 10 to 49 days, with an average of 25 days (Table 2).

3.3. Adult development

Red palm weevil larval stages fed on newly synthesized semi artificial diet successfully developed into adults. An average of 78 % successfully emerged, with a range of 58 to 98 %. More males emerged than females, resulting in a sex ratio of 1.00 males to 0.79 females (Table 2).

RPW adults provided with pollen solution varied in longevity. Longevity of males ranged from 33 to 72 days, while for females the range was 15 to 72 days. There was a difference in adult longevity between females and males, with females having shorter life spans than males. On average, females survived 49 days post-emergence, while males survived 52 days on average.

Adult male and female weights ranged from 0.6 to 1.21 g, and 0.7 to 1.24 g respectively, with an average of 0.90 g and 0.97 g respectively. Additionally, we observed that females could lay between 48 and 193 eggs, with an average of 96 eggs, in a 35 to 61-day period (Table 2).

The egg laying period for RPWs fed on newly synthesized semi artificial diet ranged from 35 to 61 days, with an average of 48 days. Total development time for RPWs from egg to full size ranged from 48 to 98 days, with an average of 77 days on present semi artificial diet (Table 2).

4. Discussion

In the present research, we studied RPW larval growth and development on our newly synthesized semi artificial diet and compared its results with other diets claimed to be suitable for red palm weevil rearing. The diet succeeded in supporting RPW larval growth and development.

RPW larvae completed ten to eleven instars on newly synthesized semi artificial diet. Around 93 % of the population fed on the newly synthesized semi artificial diet had ten instars, while the remaining had eleven instars. This result was similar to that observed when larvae are fed on chopped date palm fronds (Al-Ayedh, 2011). In our study, most larvae had a tenth instar for 4–13 days (mean = 8.9 days), and about 7 % of the population needed an additional five days to develop an eleventh instar.

Larval weights developed from 0.001 g for the 1st instar, to an average of 5.5 g (range: 4–7 g) for the tenth instar and an average of 5 g (range: 4–6 g) for the eleventh instar. In comparison to larvae fed on sukkary date palm (Al-Ayedh, 2008), for which larval weight averaged 2.73 g, larvae fed on this artificial diet were heavier. There were significant differences in larval weight at the full-grown stage. If we compare it with other diets, larval weight on date palm bolt was significantly lower than on other diets. On the Al-Ayedh, (2011) diet, larval instar development was faster compared to other treatments until the 6th instar, but was slower than other diets in the advanced stages of larval development.

RPW average larval duration was 53 days (range: 46–57) days. RPW larval duration was shorter than observed by Al-Ayedh (2011), Alsuhaibani et al. (2001), Nirula (1956), and Prabhu and Patil (2009), who reported 66, 182, 55, and 56 days, respectively, but longer than observed by Ghosh (1912), Ghosh (1912), Watanapongsiri (1966), and Abd El-Fattah et al. (2009), which reported 35, 35 to 38, and 45 days, respectively.

The use of an artificial diet for RPW rearing during larval stages is more suitable for developing insects for use in experimentation. Larval growth and development can be observed more easily than in larvae fed on a natural diet. This is because the texture of the diet makes collection of the desired larval stage easier, reducing mishandling effects. Additionally, less space is required for rearing. It is easy to control contamination by adding preservatives and anti-fungal agents. The percentage of RPWs reaching full-grown development ranged from 58 to 98 %, with an average of 78 %.

The pupal period varied from 10 to 49 days, with an average of 25 days. When compared to observations made by Prabhu and Patil (2009), Nirula (1956) and Alsuhaibani et al. (2001), in which pupal duration averaged 16, 16, and 23 days respectively, pupal duration in this study was shorter. Abd El-Fattah et al. (2009) developed an artificial diet, and larvae fed on it required 22.64 days to develop into adults from the pre-pupae stage.

Through continuously rearing RPWs on the diet, it was found that rough chopped petioles could be used as a medium for cocoon spinning, as they provide fibers for cocooning. Fresh rough ground date palm petioles should be combined with 1 % ascorbic acid solution (w/v) as an antioxidant to overcome oxidation and provide humidity for the fibers.

Red palm weevil larval stages fed on present semi artificial diet were successfully developed into adults. An average of 78 % successfully emerged, with a range of 58 to 98 %. On a diet of sukkary date palm trunk, almost 72 % of the population developed into adults (Al-Ayedh, 2008), while on sugarcane this figure rose to 87 % (Prabhu and Patil, 2009) (Table 1). More males emerged than females, resulting in a sex ratio of 1.00 males to 0.79 females. Al-Ayedh (2008) and Nirula (1956) also observed the same phenomenon, with ratios of 0.51: 0.49 and 1.3: 1, respectively.

RPW adults provided with pollen solution varied in longevity. Longevity of males ranged from 33 to 72 days, while for females the range was 15 to 72 days. There was a difference in adult longevity between females and males, with males having shorter life spans than females. On average, males survived 49 days post-emergence, while females survived 52 days on average. These life span averages were shorter than for larvae fed on sugar cane (Prabhu and Patil, 2009), date palm (Alsuhaibani et al. 2001), artificial diet (Abd El-Fattah et al. 2009), and coconut (Nirula, 1956). Male life spans for these diets averaged 71, 161, 98 and 84 days, respectively, whilst females survived for an average of 69, 112, 95 and 60 days, respectively. Deprived of food and humidity, the weevil can survive for no more than six days, while if only humidity is provided it may survive for three to six weeks (Alsuhaibani et al. 2001). When fed on date palm bolts, females survived up to 172 days, while males were capable of surviving for up to 176 days (Al-Ayedh, 2008).

There were significant effects of diet on adult performance. Male weevils' weight differed significantly between diets, with males fed on date palm bolt being lighter than those reared on artificial diets. On the contrary, weight of females reared on the present semi artificial diet was lighter than on the Al-Ayedh (2011) diet. The present semi artificial diet improved fecundity, which was significantly higher than on the Al-Ayedh (2011) diet and date palm bolt treatments, with higher hatchability percentage.

Adult male and female weights ranged from 0.6 to 1.21 g, and 0.7 to 1.24 g respectively, with an average of 0.90 g and 0.97 g respectively. Prabhu and Patil (2009) observed that RPW weight ranged from 0.53 to 1.09 g and 0.57 to 1.43 g for males and females

respectively when fed on sugarcane. We also observed that females could lay between 48 and 193 eggs, with an average of 96 eggs, in a 35 to 61-day period. This result was similar to Faghih (1996), which recorded that female RPWs could lay between 127 and 276 eggs. On sugar cane, adult females laid 278 eggs. Each female laid up to 412 eggs when reared on date palm trunk. The egg laying period for females living on this tree ranges from 54 to 137 days (Alsuhaibani et al., 2001). On coconut trees, RPW females lay eggs for between 25 and 63 days (Nirula, 1956). Weevils fed on an artificial diet by Abd El-Fattah et al. (2009) and Salama and Abdel-Razek, (2002), produced 275 and 184 eggs, respectively. Fertile laid eggs required between 2 and 6 days (mean: 4.5 days) to hatch. This range was similar to those observed by Ghosh (1912), Nirula (1956), Watanapongsiri (1966), and Faghih (1996), Alsuhaibani et al. (2001), and Prabhu and Patil (2009).

The oviposition period for RPWs fed on present semi artificial diet ranged from 35 to 61 days, with an average of 48 days. This duration was shorter than in those fed on date palm. On date palm trees, egg laying duration varied from 54 to 137 days, and the average was 96 days (Alsuhaibani et al. 2001). RPWs fed on coconut laid eggs for between 25 and 63 days (Nirula, 1956). Total development time for RPWs from egg to full size ranged from 48 to 98 days, with an average of 77 days on present semi artificial diet. This complete life cycle period was shorter than for those fed on sugar cane (Prabhu and Patil, 2009), on date palm (Alsuhaibani et al., 2001), and on coconut (Nirula, 1956); average life cycle periods on those diets were found to be 82, 216, and 81 days, respectively.

5. Conclusions

In conclusion, the present semi artificial diet was successful in supporting growth and development during RPW mass rearing in the laboratory. The growth and development of RPW larvae reared in this experiment were comparable to those fed on a natural diet. This study also observed that roughly grinded date palm fronds provide satisfactory results for pupation with no need for the addition of sugarcane during laboratory rearing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Disclosure of any conflict of interest

The authors declare that they don't have any type of cconflict of interest which might affect the present work.

Appendix A. Supplementary data

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