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Assessment of selected biological traits of the red palm weevil *Rhynchophorus ferrugineus* (Olivier, 1790) reread on apple and efficacy evaluation of thiamethoxam and emamectine benzoate for its control

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Abstract
Laboratory insect pests' readings are essential to better understand their biological traits, assess their host plant and assess the efficacy of insecticides. This work aims at studying the selected biological traits of the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) 1790 reared on semi-artificial died based on apple slices and maintained in natural conditions. Furthermore, the adulticide and larvicide efficacy of two insecticides recommended for the control of this pest which thiamethoxam and emamectine benzoate was assessed in the laboratory. Results showed that, on apples slices and maintained in natural conditions the Average daily fecundity is 2.5 eggs/female. The hatching rate of eggs ranged between maximum of 76% in October and a minimum of 5% in the end of November. The shortest egg incubation period is 3 days. However, starting from the first week of November, this duration exceeded 10 days. The RPW develops 7 larval stages requiring 63 days. Fourth instar larvae were 80 larger than first instar ones.
The assessment of the efficacy of two insecticides: thiamethoxam and emamectine benzoate showed that the 2 products are efficient against larvae and adults ensuring 100% of larval mortality 24 hours after their application. However, ememectine benzoate is more efficient against adults than thiamethoxam. In the case of emamectine benzoate it ensured 100% adult mortality 96 hours after its application with a choc effect preventing food intake starting from the first hours of application.

Keywords: Red palm weevil, rearing, morphology, biology, chemical treatment, thiamethoxam, emamectin benzoate

Introduction
The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) 1790 is considered as one of the most destructive insect pests in the world. It is a Coleoptera, Curculionoidea, from the Dryphtorine subgroup, sometimes considered as a family [1]. It has been described for the first time as a pest of coconut palm trees [2]. Furthermore, Arecacea and dicots are reported as natural host plants of the pest [3, 1]. The preferred host plants are *Phoenix dactylifera* L. [4, 5]. In addition, *Phoenix dactylifera* and *Phoenix canariensis* are highly susceptible hosts in the new invasion area such as Tunisia [6]. However, *Areca catechu, Arenga pinnata, Broassus flabellifer, Cibotium cumingii, Caryota maxima, Cocos nucifera, Corypha umbraculifera, Elaies guineesis, Livistona chinensis, L. saribus, Metroxylon sagu, Nypa fruticans, Oncosperma horridum, O. tigillarium and Roystonea regia* are the original hosts [1].
The symptoms of attacks of *R. ferrugineus* on palm trees are distinctive but their early detection can be difficult [7, 8]. The primary symptoms are generally brown viscous liquid seeps at the trunk of the date palm (case of *P. dactylifera*) with specific smell [9, 10]. Larvae are endophytic and are the most damaging stage [1]. On palm trees, late infestation often causes weakening of the host plant followed by sudden drought sometimes preceded by a marked tilt of the canopy [9, 12].
The red palm weevil is a cryptic insect as larvae are concealed in host tissues [9]. In several studies, the RPW was laboratory reared in order to better understand its biological traits [13- 18]. Early studies proved that *R. ferrugineus* is able to carry out its entire cycle under artificial and semi-artificial conditions under different climatic parameters [19].

In this context, rearing in semi-artificial conditions is considered as key procedure to assess suitability of host plants for the pest [19, 20]. In addition, laboratory rearing of the red palm weevil allows to maintain age and sexing selection in order to perform insecticides efficacy tests and improve control strategies [21]. For instance, several researches were conducted to evaluate the efficacy of biological agents such as entomopathogenic nematodes and fungi [22-26], plant extracts [27] and chemical products. Nevertheless, the rearing of *R. ferrugineus* in semi-artificial environments showed that it survived with different life parameters depending the rearing diet and conditions [15].

The objectives of this work are to study selected biological parameters of *R. ferrugineus* in semi-artificial and natural climatic conditions of Catania, Italy and to evaluate the adulticide and larvicide efficacy of two insecticides recommended for its control.

Material and methods

Laboratory rearing of the RPW

The colony of the RPW was initiated in October 2016 with adults collected from 5 Picusan® traps (Sansa Prodesing SL, Náquera, Valencia, Spain) baited with the aggregation pheromone type Pheromosa Rhynchophorus containing 1000 mg of the aggregation pheromone, and ethyl acetate diffusers, installed in the University of Catania, Italy. The rearing was conducted under natural climatic conditions in the laboratory of Entomology, University of Catania, Italy. Adults were maintained in cages of metal proof insect laying on a plate containing apple slices as food and for egg laying. Immediately after hatching, first instar larvae were placed individually, in a Petri dish containing a slice of apple and reared until adult emergence.

Insecticides

Two insecticides were assessed for their efficacy against adults and larvae of the RPW. The first one is Renova® containing 2.5g/l of thiamethoxam. It is systemic product acting by ingestion and contact on the target insects. According to the manufacturer, it has a systemic activity and a rapid penetration into plant tissues either through leaves or roots. It interferes with the nicotine acetylcholine receptors of the insects' nervous system. This insecticide is used to control the RPW by endotherapy [28].

The second product is Proact® 50 EC (Tasmid, Tunisia) composed of 50g/l of emamectin benzoate. Although it has been specially marketed for the control of the South American tomato leafminer *Tuta absoluta* (Meyrick), it is used as a systemic product to control the RPW.

Study of selected biological parameters of the RPW

In order to study selected biological traits of the RPW on semi-artificial diet based on apple under natural climatic conditions, the number of eggs produced by females (fecundity) was counted daily. Furthermore, the number of hatched eggs (fertility) was registered during the period of the study. To determine the number and the duration of larval instars, each larva was daily inspected from hatching to pupation while checking the presence or not of the exuvia indicating the changing of larval instar. Besides, for each larval stage, the weight of the larva and the width of it cephalic capsule were assessed. Measurements were performed under a stereomicroscope related to the software Leica Application Suite version 3.7.0 (Built: 681) (Switzerland).

Laboratory assessment of two insecticides against the RPW

The efficacy of tested insecticides was evaluated on larvae and adults of the RPW. To assess the adulticide effects, pupae of RPW were individually placed in plastic boxes having 16 compartments allowing to check adult emergence and to facilitate to select insects having same age. 24 plastic boxes (18 x 8 x 6 cm) covered with insect proof mesh (1x1mm) were used to test each insecticide. In each container, 8 females and 2 males having the same age were introduced after a starving period of 24 hours. Slices of 45 g of apple were treated with 2 ml of each insecticide and left for two hours before being offered to the RPW adults. For each insecticide, 8 boxes served as controls where slices of apples were treated with tap water. Apple slices were weighed daily to determine the quantity consumed by adults.

The larvicide test was carried out on 150 larvae from different stages which were put in 15 plastic containers similar to those used for the adults at the basis of 15 larvae/container. Containers were lined with filter paper and 15g of treated apple slices. The control consists of 5 boxes containing apples slices treated with water.

Statistical analyses

Linear regressions were performed between larval stage and the weight of the larvae and the width of the larval cephalic capsule. Regarding insecticide efficacy, mortality rates of larvae and adults were subjected to the analysis of variance ANOVA followed by the post hoc test of Duncan at the level of 5%.

Results

Study of selected biological parameters of the RPW

The biological traits of the RPW were assessed on 74 females reared on apple slices. The Average daily fecundity was 2.5 eggs/female (Figure 1). From 1455 eggs, only 506 larvae emerged which corresponds to an average fertility of 34.77% (Figure 2).

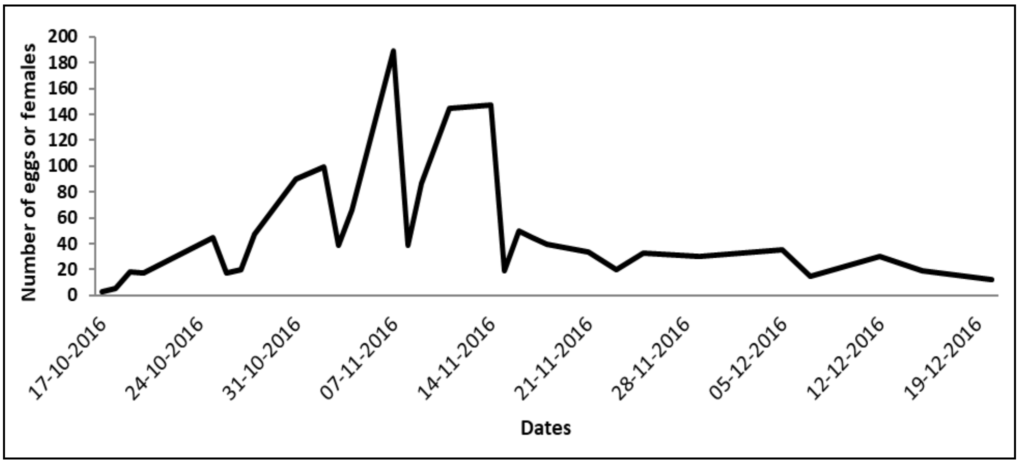


Fig 1: Fecundity of females of *R. ferrugineus* when reared on apple under natural climatic conditions in Catania, Italy in 2016.

Besides, the hatching rate of eggs ranged between maximum of 76% in October and a minimum of 5% in the end of November.

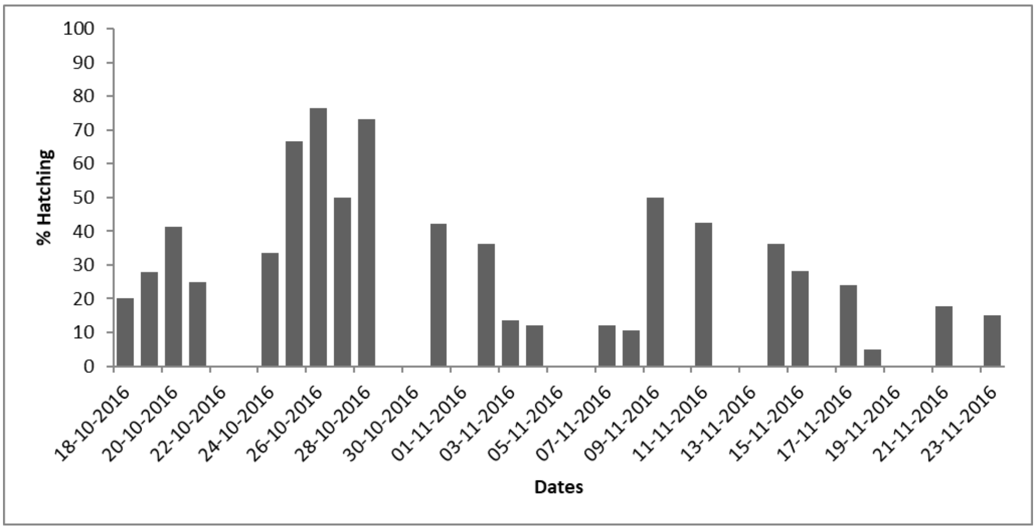


Fig 2: Egg hatching rate of *R. ferrugineus* when reared on apple under natural climatic conditions in Catania, Italy in 2016.

The shortest egg incubation period is 3 days. However, starting from the first week of November, this duration exceeded 10 days. In fact, an average of 5 days is required under natural conditions for egg incubation (Figure 3).

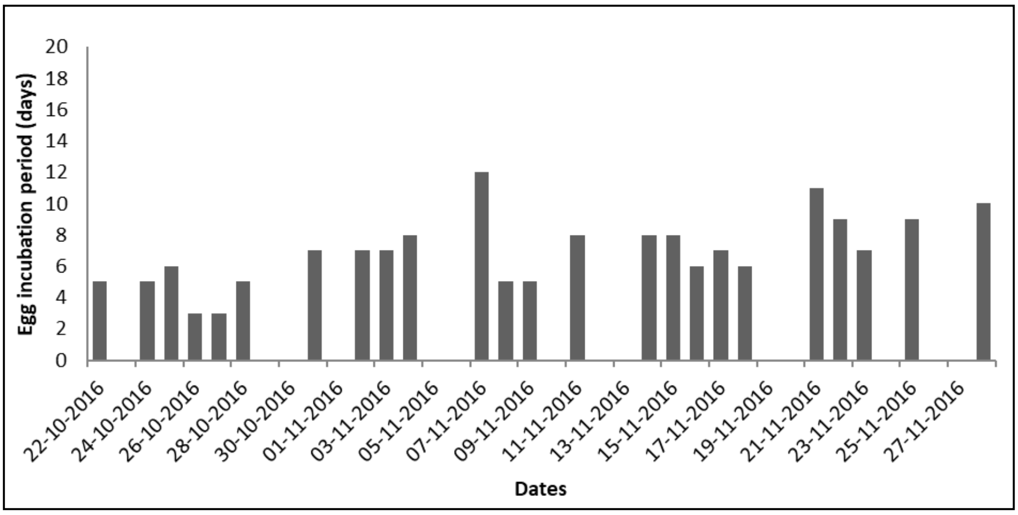


Fig 3: Egg **i**ncubation period of *R. ferrugineus* when reared on apple under natural climatic conditions in Catania, Italy in 2016.

Newly emerged larvae immediately tunnel into of the apple slices to hide and feed. As for the weight of the larvae, it can reach for the fourth larva (L4) up to 80 times that of the first larva (L1) and 7th stage larvae (L7) weigh 333 times more than first stage larvae (L1) (Figure 4). Measurements of larval length and cephalic capsule width showed the existence of seven larval stages when *R. ferrugineus* is reared on apple slices under ambient conditions. A positive correlation was found between the width of the cephalic capsule and the corresponding larval stage. Linear regression showed a significant relationship between larval stage and cephalic capsule width (R^2=0.9436) (Figure 5). The width of the cephalic capsule of larvae varied from 2.6 to 8.72 mm respectively in larvae of the first and seventh stages. The length of the larva ranged from 0.817 to 2.65cm. A period of 63 days, from 18 June 2016 until 20\12\2016 and under the ambient conditions was necessary for the larvae to achieve complete development. Passing from a larval stage to another required on average a duration of 4 days for the first four stages and up to 9 days for 3 last stages.

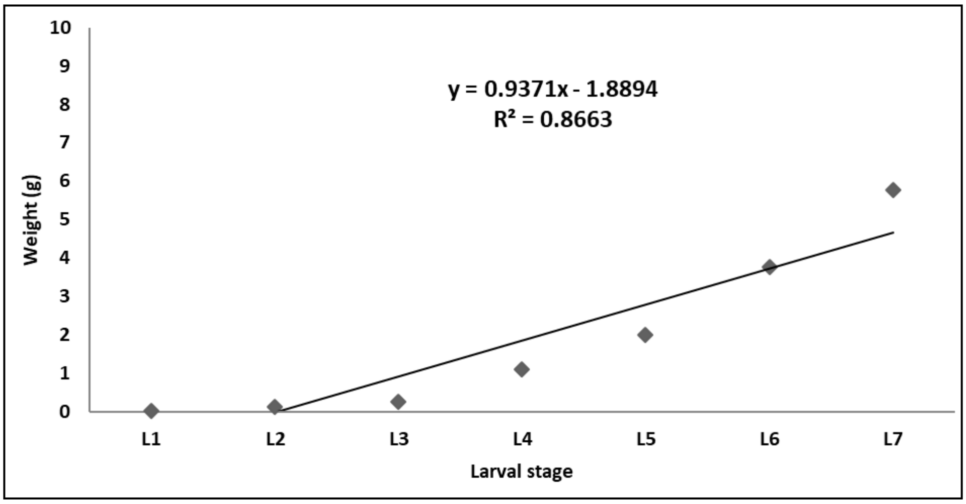


Fig 4: Linear regression between larval stages and larval weight of *R. ferrugineus* when reared on apple under natural climatic conditions in Catania, Italy in 2016.

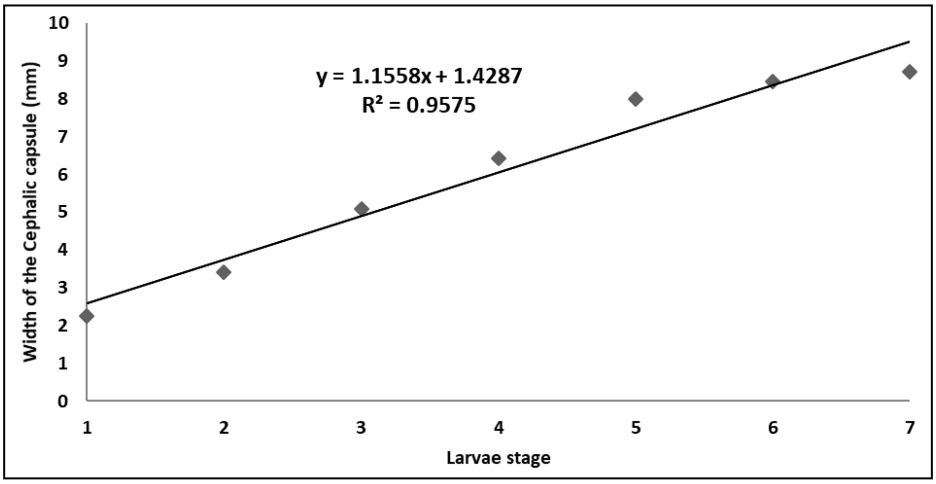


Fig 5: Linear regression between larval stages and cephalic width of *R. ferrugineus* when reared on apple under natural climatic conditions in Catania, Italy in 2016.

In laboratory assessment of two insecticides against the RPW

Analysis showed a significant difference (p<0.05) between the control and the two active substances thiamethoxam and emamectin Benzoate. In fact, the larval population was reduced by 42, 78 and 100% respectively after 7, 12 and 24 hours after treatment with Renova® (thiamethoxam). A similar reduction was obtained after treatment with Proact® (emamectin benzoate) with 40, 86, and 100% respectively after 7, 12 and 24 hours (Figure 6). The same active substances were tested on adults and showed significant efficacy against the insect compared to the control. The rate of reduction in Renova® treated adult was just 5% at the end of the first 24 hours and then the mortality gradually increased to 100% after 9 days of treatment (Figure 7). Procat® caused a 6% reduction in the first 24 hours of treatment and then 100% mortality after 96 hours. Since the first hours of treatment, the treated adults stopped food intake. The quantity of apple consumed by adults in control treatment was significantly higher (p<0.05) compared to that consumed by treated adults (Figure 8 and 9).

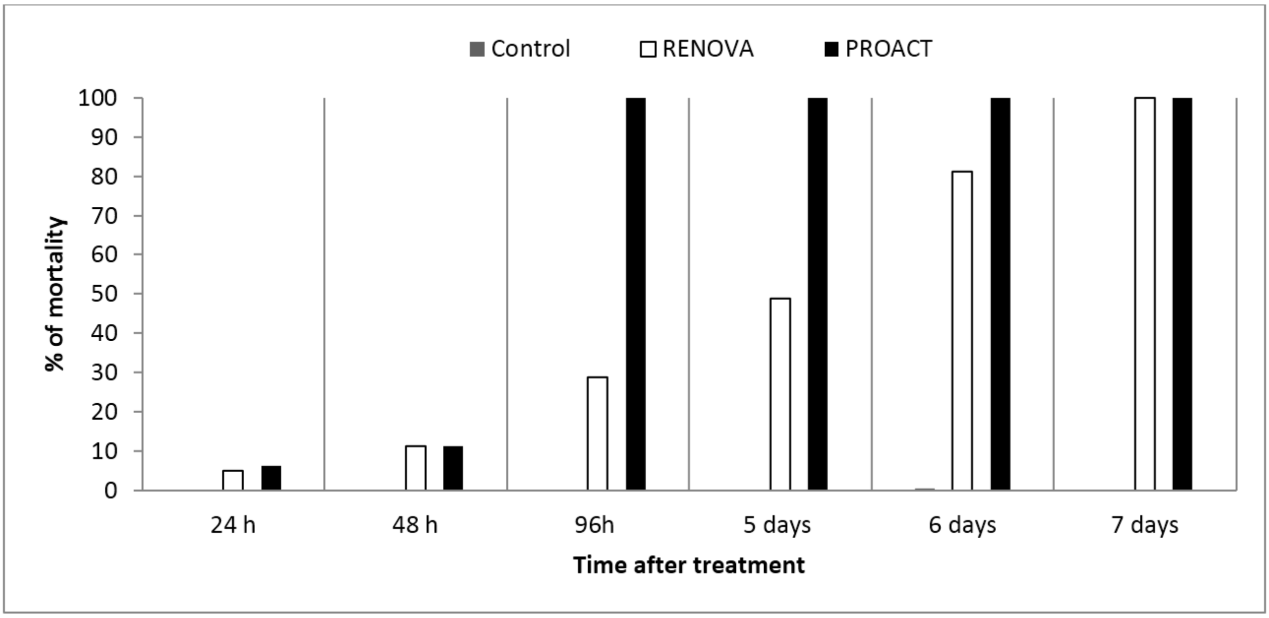


Fig 6: Mortality rate of *Rhynchophorus ferrugineus* adults fed on treated apple slices with Renova® and Proact® under laboratory conditions.

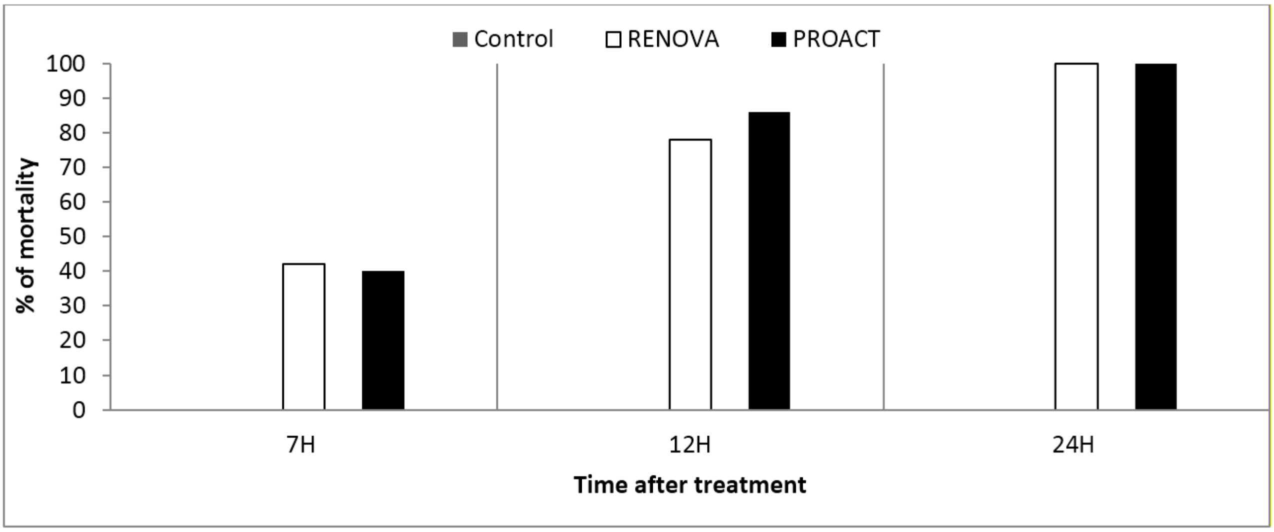


Fig 7: Mortality rate of *R. ferrugineus* larvae fed on treated apple slices with Renova® and Proact® under laboratory conditions.

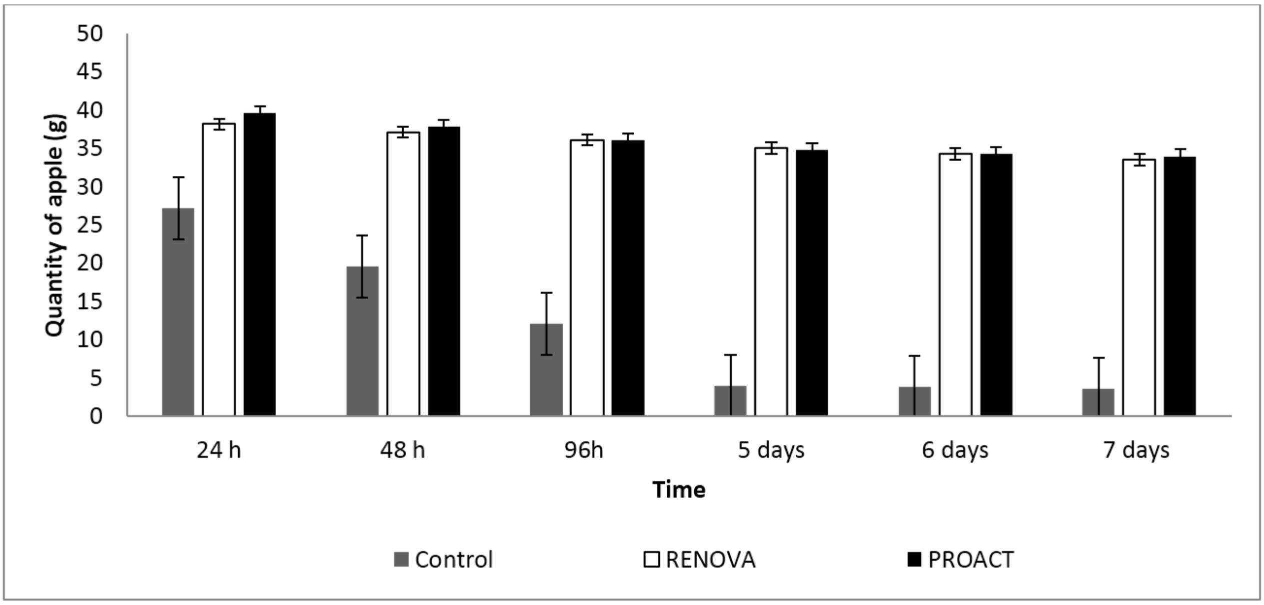
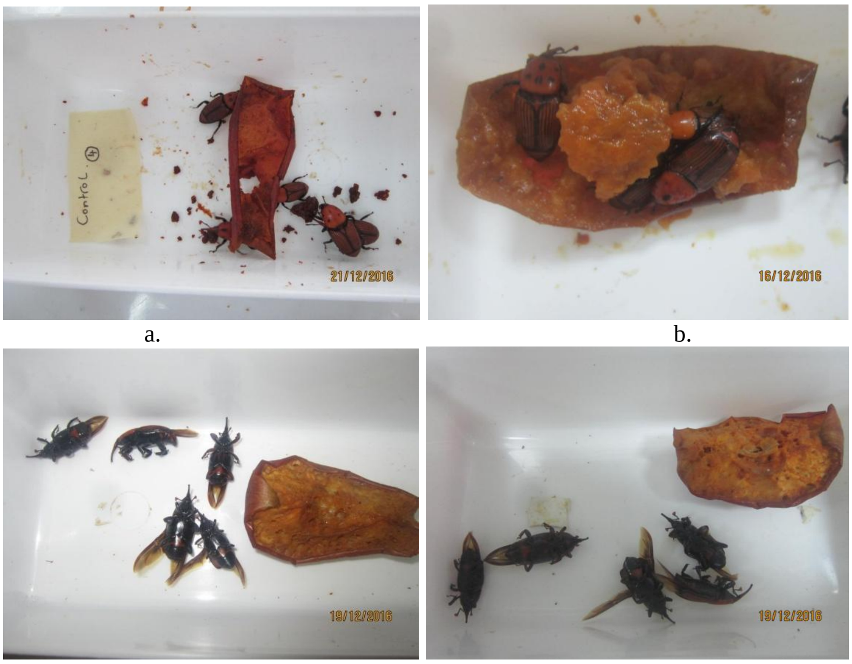


Fig 8: Food intake of *R. ferrugineus* adults fed on treated and control apple slices.



a. b. c. d.

Fig 9: Food intake of *R. ferrugineus* adults fed on control (a and b) and treated (c and d) apple slices.

Discussion

The rearing of the red palm weevil on apple slices was carried out under ambient natural climatic conditions in Catania, Italy between September and November 2016. During our study, the preoviposition period was 2 days. Generally, it varies from 3.2 to 3.8 days depending on the diet [16]. Some other studies reported that the preoviposition period of the red weevil varies between 3 and 5 days independently of the type of the diet [29, 14]. Some other studies have shown that this period depends on the substrate used and that it varies from 1 to 6 days [30, 17]. Similarly, a preoviposition period of 5-day when red palm weevil is reared on apple was reported [21].

In our study, the larval development required 63 days wears, in previous works it took 103 days with only 5 larval stages when using apple as diet [17] which could be explained by the climatic conditions (temperature, relative humidity and photoperiod) that can lengthen the biological cycle. In addition, mortality of larvae in the early stages was recorded. Indeed, the mortality of artificially fed larvae exceeds 58% for those weighing less than 0.25g, compared with only 9% for those with a higher weight [31].

The study of the different stages of development of the red weevil showed that larvae of the 7th instar are 333 times larger than those in the first stage. In this context, it was found that the weight of the late-stage (8th instar) larva fed on dates is 2311 greater than that of first instar larvae [18]. This difference can be explained by the composition of the rearing diet. Moreover, larvae gain more weight when fed on sugar-reach diet [14].

The assessment of the efficacy of two insecticides under laboratory conditions showed the efficacy of thiamethoxam and emamectin benzoate. However, emamectin benzoate exhibited a rapid action. In case of thiamethoxam, 100% of larval mortality was recorded after 24 hours however the mortality of all treated adults required 9 days using the same product and dose. In the same context, it has been shown that mortality of adults treated with thiamethoxam started 96 hours after treatment [32]. After application of emamectin benzoate, 100% of larval mortality was obtained 24 hours after treatment and total mortality of required 96 hours. In the same context, the dose of 600 (µg/ml) of abamectin caused 60% mortality of treated adults after 24 h and a dose of 200 (µg/ml) of the same product is sufficient to cause 100% mortality of larvae after 96 hours [33]. Similarly, few days were sufficient to reach 100% adult mortality, although insects stopped feeding 24 hours after treatment. These insecticides should be tested in the field to confirm their efficacy. Furthermore, their impacts on human and environment should be taken in consideration.

Conclusion

The rearing of the RPW *R. ferrugineus* on apples slices under natural conditions in Catania, Italy, showed that the pest is able to develop 7 larval instars and to achieve larval development. Females were able to lay viable eggs with maximum fecundity in the month of November. Furthermore, the assessment of two frequently used insecticides against the RPW under laboratory conditions revealed that emamectin benzoate and thiamethoxam are efficient against larvae and adults. The efficiency of both insecticides should be evaluated in field especially when used in endotherapy.

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