# INFESTATION RATES OF DATE PALM BY THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* OLIVER AND ITS ASSOCIATED NATURAL ENEMIES AT BALTEEM, KAFR EL-SHEIKH, EGYPT.

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## INTRODUCTION

The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* Olivier is an economically important tissue-boring pest of palms in many parts of the world (Abraham *et al.*, 1998 and Faleiro *et al.*, 2002). It is the most destructive pest of coconut in South and Southeast Asia (Sadakathulla, 1991). In addition, it has caused severe damage to date palm, *Phoenix dactylifera* L., in several Middle Eastern countries (Abozuhairah *et al.*, 1996). This weevil has bean advancing westwards very rapidly since the mid 1980 (Gomez and Ferry, 1999). It reached the kingdom of Saudi Arabia, United Arab Emirates and Oman in 1985 (Abozuhairah *et al.*, 1996; El-Ezaby, 1997), Savaran region of Iran in 1996 (Faghih, 1996), Sharkia Governorate of Egypt in 1992 (Cox, 1993), Israel, Jordan, Palestine and the occupied territories in 1999 (Kehat, 1999). It even has been accidentally introduced into south east of Spain through imported palms (Barranco *et al.*, 1996). The RPW damage symptoms are recognized by the presence of tunnels in the trunk, oozing of thick yellow to brown fluid from the tree and the appearance of chewed-up plant tissue in and around openings in the trunk (Kaakeh *et al.*, 2001).

The RPW in date palm has been managed with an Integrated Pest Management (IPM) approach comprising several tactics including the biopesticides based on insects, mites, nematodes and microorganisms to control the RPW (FAO, 1996; El-Bishry *et al.*, 2000 and Deadman *et al.*, 2001).

In this study, the susceptibility of different date palm cultivars to the RPW infestation was determined and a survey of the natural enemies of the RPW was conducted. In addition, the natural and artificial incidence of *Beauveria bassiana* mycosis disease on RPW was verified.

## MATERIAL AND METHODS

An area of 5000 feddans covered with different date palm cultivars (Haiany, Araby, Samany, Zaghlol, and Benteasha) at Balteem region was chosen to survey the incidence of the Red Palm Weevil *Rhynchophorus ferrugineus* (RPW) and its associated natural enemies for one year (October 2005 - September 2006). Monthly, orchards of the five palm cultivars were visited. In each visit, other fifty trees of each cultivar were randomly chosen as a sample to be examined for RPW infestation. Each cultivar was represented by three samples. The visited orchards included different date palm ages. As a concealed tissue borer and its life stages are found inside the palm trees, trees were examined for the RPW tunnels or oozes on trunks. Percentages of infested trees were calculated.

Each visit, the highly infested trees were shopped and all stages of the RPW, except eggs, were carefully gathered using alcohol-flaming surface sterilized metallic forceps. Every sample consisted of 100 cocooned stages (adult, pupa) and 100 larvae. The collected specimens were directly put in separated sterilized jars and transferred to the laboratory. Under aseptic conditions, the RPW individuals were maintained and examined for viability. The natural mortality percentages were estimated and the noticed insect and mite natural enemies were observed, separated under a binocular stereoscopic microscope and identified at both Department of Economic Entomology and Department of Agricltural Microbiology at Kafr Elsheikh. To full estimate of *Beauveria bassiana* natural infection, further incubation of the naturally dead individuals was performed in damp chambers as described by Müller-Kögler (1965). The RPW individuals covered with flat white mycelial growth bearing mealy white conidia were considered, and microscopic preparations were used to assure the specific characteristics of *Beauveria bassiana* (Balsamo) Vuilemin recorded by Domsch *et al.* (1980). Percentages of the natural infection by *Beauveria bassiana* on the dead individuals were calculated.

**Artificial fungal infectivity:**

Fungal conidia produced on natural mycosed weevils were used to prepare a suspension containing $1.5 \times 10^8$ conidia/ml of sterilized 0.1% Tween 20 water solution. The suspension was used to contaminate the 3rd, 4th and 5th instars' larvae and adults of the RPW (50 active alive individuals of each) by dipping them individually for 3 seconds in the suspension. Individuals dipped in sterilized 0.1% Tween 20 water solution served as check treatment. Each treatment was replicated 3 times. The treated individuals were kept in sterilized glass containers with fresh pieces of sugar cane recommended by Rahalkar and Rananavare (1972) for RPW

rearing. The sugar cane pieces previously were surface sterilized by alcohol-flaming for 5 times. The sugar cane pieces were renewed when needed. Containers were kept at 27+1°C and examined daily. Dead individuals were isolated and counted. The dead ones were subjected to *Beauveria bassiana* mycosis test as mentioned above. Cadavers of either RPW stages showed external growth of the fungus were considered killed by this bioagent.

The obtained experimental data were statistically analyzed according to Duncan (1955).

## RESULTS AND DISCUSSION

### 1. Susceptibility of date palm cultivars to RPW infestation

Data in Table (1) revealed that the highest infestation by the RPW was recorded on Haiany date palm trees during the whole year (Oct. 2005-Sept. 2006) reaching 29.39%, followed by Araby (25.10%), Zaghlol (21.43%), Samany (14.08%), and the lowest was on Bentaesha (10.00%). As shown in Table (1), the highest infestation by the RPW was recorded during September, October and November when the number of infested date palm trees reached 10.2, 11.43 and 12.86 %, respectively. On the other hand, the lowest infestation was recorded during January and February when the recorded infestation was only 5.3 and 5.51 %, respectively. The statistical analysis showed high significant infestation of the RPW during the autumn months. The minimum infestation was recorded in the cold months, especially January. That may be due to the relationship between the infection of trees by the RPW and some weather factors especially temperature and relative humidity as previously recorded by Faleiro *et al.* (2002).

### 2. Survey of insect and mite enemies of RPW

As shown in Table (2), two natural enemy groups of RPW belonging to two arthropod classes, Insecta and Acarina, were recorded during 2005/2006. Class Insecta was represented by three families each had one species. Two of the three species were parasites on the RPW (*Scolia erratica* and *Sarcophaga fuscicouda*) and the third (*Chelisoches morio*) was a predator. On the other hand, the class Acarina was represented by three species. Two of them were predators (*Hypoaspsis* sp. and *Tetrapolypus rhynchophori*). The third mite species *Uropodina phoritic* found on the RPW was a parasite, which was sorted under Gamasida group. The mite *U. phoritic* was recorded on the RPW at Balteem, Egypt for the first time. This mite

was observed aggregating under the elytron and/or the external lower surface between segment membrane of thorax in the RPW adults especially in January, February and March months. These habitations are suggested to offer protection for this mite from low temperature in this period and to enable it to feed on the blood of adults (Fig 1 A&B).

**3. The entomopathogenic fungus** *Beauveria bassiana*

3.1. The pathogen and symptoms of disease:

Among the gathered stages of RPW, dead individuals covered with flat white fungal mycelium were observed (Fig 2). Cadavers were rigid and mummified. When cadavers were maintained at 100% R.H., the mycelium covered the entire body surface bearing mealy white conidia. The observed symptoms were the same of the white muscardine disease described in Bell (1974). The preliminary microscopic examination revealed the morphological characteristics specific to the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuilemin recorded by Domsch *et al.* (1980). Koch's postulates for the pathogenic fungus were performed through an artificial inoculation experiment to verify the fungus infectivity.

**TABLE (I)**

Susceptibility of different date palm cultivars to *Rhynchophorus ferrugineus* infestation at Balteem region.

| Months | Mean numbers of date palm trees infested by RPW*. | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | Haiany | Araby | Zaghlol | Samany | Bentaesha | | |
| **Oct. 2005** | 15 | 13 | 13 | 9 | 6 | 56** | 11.43 |
| **Nov.** | 17 | 15 | 14 | 10 | 7 | 63** | 12.86 |
| **Dec.** | 11 | 10 | 8 | 6 | 5 | 40 | 8.16 |
| **Jan. 2006** | 9 | 7 | 5 | 3 | 2 | 26 | 5.30 |
| **Feb.** | 8 | 7 | 6 | 3 | 3 | 27 | 5.51 |
| **Mar.** | 10 | 8 | 7 | 4 | 4 | 33 | 6.73 |
| **Aril.** | 11 | 9 | 7 | 4 | 3 | 34 | 6.94 |
| **May.** | 12 | 10 | 7 | 4 | 3 | 36 | 7.35 |
| **Jun.** | 12 | 11 | 8 | 5 | 3 | 39 | 7.96 |
| **July.** | 12 | 10 | 9 | 6 | 4 | 41 | 8.37 |
| **August.** | 13 | 11 | 10 | 7 | 4 | 45 | 9.18 |
| **Sept.** | 14 | 12 | 11 | 8 | 5 | 50** | 10.20 |
| **Total** | 144** | 123** | 105 | 69 | 49 | 490 | |
| % | 29.39 | 25.10 | 21.43 | 14.08 | 10.00 | | |

*= Mean numbers of three samples each contained 50 trees.

** = Significant at $P$= 1 % level.

TABLE (II)

Insects and mites naturally associated with *Rhynchophorus ferrugineus* on different date palm cultivars at Balteem.

| Classes | Families | Natural enemies | Relation to RPW |
|---|---|---|---|
| **Insecta** | | | |
| | Scoliidae | *Scolia erratica* | Parasite |
| | Sarcophagidae | *Sarcophaga fuscicouda* | Parasite |
| | Forficulidae | *Chelisoches morio* (F.) | Predators |
| **Acarina** | | | |
| | Pyemotidae | *Tetrapolypus rhynchophori* | Predators |
| | Laelapidae | *Hypoaspsis sp.* | Predators |
| | Uropodidae | *Uropodina phoritic* | Parasite |

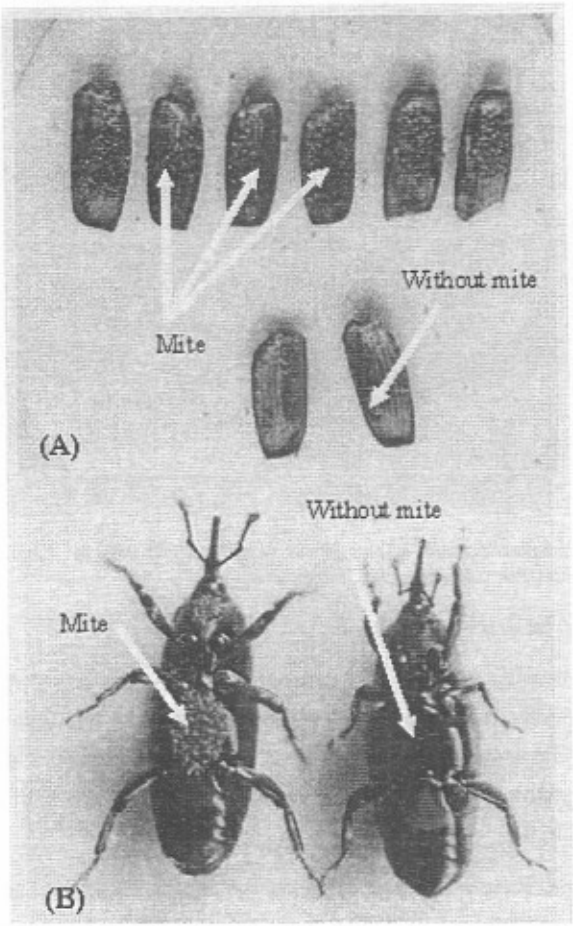


Fig. (1): Sites of aggregation of the mite *Uropodina phoritic* on the red palm weevil:
A- Under the elytrons, B- On the thoracic sternites.

3.2. Fungus infectivity:

Figure (3) shows the mortality percentages of *R. ferrugineus* larvae and adults artificially inoculated with a suspension of *B. bassiana* containing $1.5\times10^8$ conidia/ml taken from natural mycosed weevils. The fungus was infective and killed all larvae and adult weevils during 25 days. The first dead larvae were recorded 4 days after treatment. Data indicate that the 3rd instars' larvae were the most sensitive stage, then, the 4th and the 5th instars, respectively. They required < 10, 12 and 16 days, respectively for 50% mortality incidence, meanwhile, adults required ~18 days. In the control treatment, neither larvae of the same instars tested nor adults died till the last treated individual died.

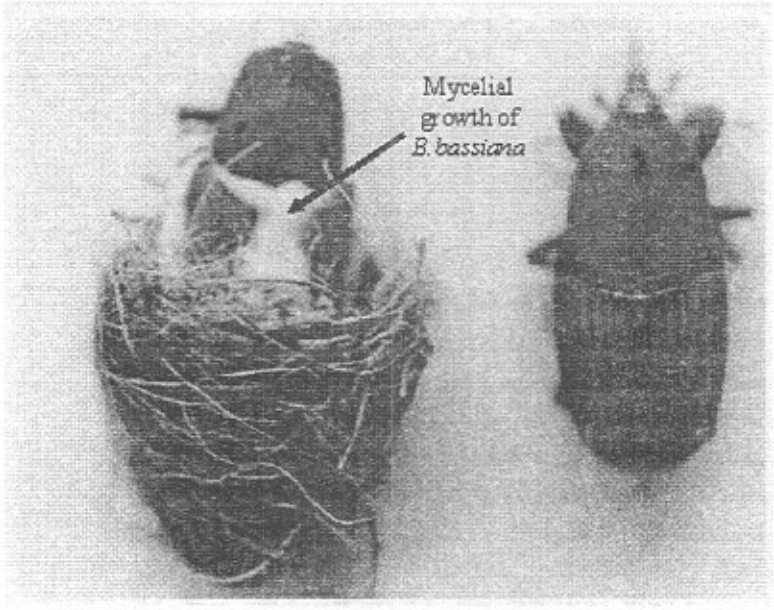


Fig. (2): White muscardine diseased *R. ferrugineus* weevil covered with the fungus *B. bassiana* collected from date palm orchards (C= uninfected weevil).

3.3. The natural incidence of *B. bassiana*

The incidence of *B. bassiana* on the naturally dead individuals of *R. ferrugineus* was assessed during the whole year. Figure (4) demonstrates the total natural mortality of RPW individuals, and among these naturally dead individuals, percentages of *B. bassiana* infection was calculated. *B. bassiana* infection ranged from 11% to 68 % of the naturally dead RPW individuals.

The fungus, *Beauveria bassiana* (Balsamo) Vuilemin is known to be an effective microbial natural agent infecting and killing numerous insect species belonging to different

orders in the field (Müller-Kogler, 1965; Jaques and Maclellan, 1965; Walstad and Anderson, 1971; El-Sufty and Boraei, 1987 and Ibrahim, 1996).

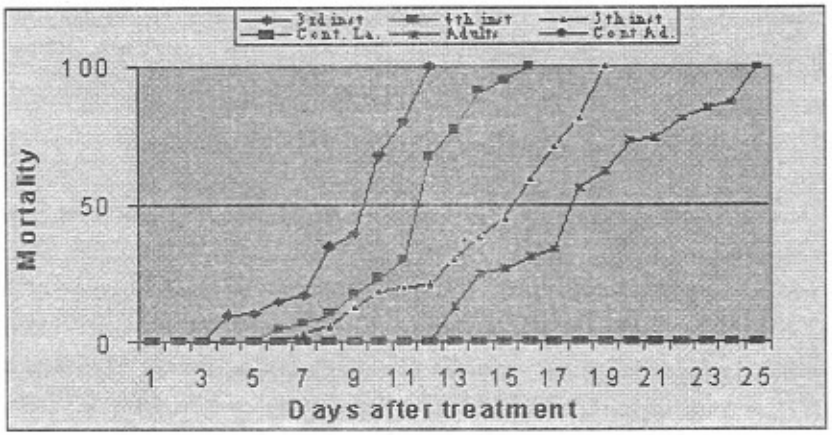


Fig. (3): Mortality percentages of R. *ferrugineus* larvae and adults treated with a suspension of *B. bassiana* containing $1.5\times10^8$ conidia/ml.

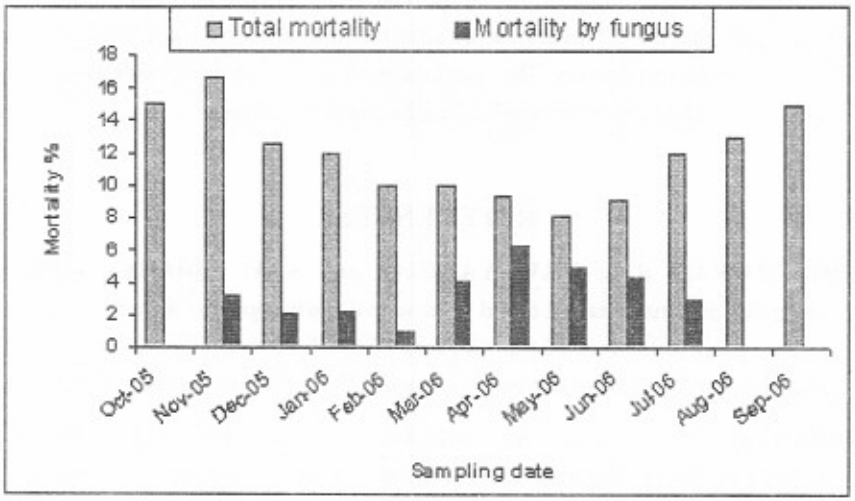


Fig. (4): Percentages of total natural mortality and *B. bassiana* natural infection of *R. ferrugineus* in date palm orchards.

Although the entomopathogenic *Beauveria bassiana* was previously isolated from Egyptian sources (El-Sufty and Boraei, 1987; El-Sufty *et al.*, 1993 and Ibrahim, 1996), this is the first time to isolate *Beauveria bassiana* from RPW in Egypt. Abroad, preliminary investigations on the biological control of RPW using *Beauveria bassiana* were performed (Deadman *et al.*, 2001 and Gindin *et al.*, 2006). So, the obtained local isolate is a promising isolate to perform such purpose in Egypt.

During our investigation, this local isolate was observed establishing and colonizing the internal chewed-up trunk tissues of some date palm trees (data not shown). However, preparation of the obtained *Beauveria bassiana* isolate for field applications needs more investigations.

## SUMMARY

In this investigation, 5000 feddans covered with different palm trees cultivars (Haiany, Araby, Samany Zaghlol, and Benteasha) at Balteem region were tested for one year (October 2005 - September 2006) to survey the incidence of the Red Palm Weevil *Rhynchophorus ferrugineus* (RPW) and its associated natural enemies. The obtained results indicated that the highest infestation percentage by RPW was recorded on Haiany (29.39%), while the lowest was on Bentaesha (10%). Results revealed the occurrence of three acarina species, three entomophagous insect species and the entomopathogenic fungus *Beauveria bassiana* as natural enemies of RPW on the date palm varieties. Among the naturally dead RPW individuals, the infection by *B. bassiana* ranged from 11% to 68%. The *in vitro* artificial inoculation tests indicated that the 3$^{rd}$ instars' larvae were the most sensitive stage, then, the 4$^{th}$ and the 5$^{th}$ instars, respectively. They required < 10, 12 and 16 days, respectively for 50% mortality achievement, meanwhile, adults required ~18 days.

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