

Comparison of the Efficacy of Bioshell (Essential Oils) and Entomopathogenic fungi (*Beauveria bassiana*) with Imidacloprid against the Red Palm Weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)

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Abstract – The red palm weevil (RPW, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is one of the most severe pests of various palm. Entomopathogenic fungi and biological pesticides are being put forward as biological control agents in Integrated Pest Management (IPM) to control RPW. The aim of this study is to evaluate the efficacy of two biological products (the entomopathogenic fungi: *Beauveria bassiana*) and the biological insecticide (Bioshell) derived from essential oils against red palm weevil compared to the confidor (Imidacloprid) as a reference product. In various study sites, the treatment of the palms trees was carried out by the three products to be tested. To verify the effectiveness of each product, the evolution of infestation of treated palms was checked. Controlling the number of infested palms, the results obtained in this study show no significant difference between palms treated with biological products and those treated with Imidacloprid. These studies can prove the effectiveness of biological control against red palm weevil. Moreover, the use of biological products should be included in the integrated pest management against RPW.

Keywords – RPW, Entomopathogenic fungi, *Beauveria bassiana*, Essential Oils, Bioshell (Bioweevil), Imidacloprid, Effectiveness.

I. INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) (RPW) is an economically important pest of palm trees in the different growing area in the world ([1]; [2]; [3]; [4]).

RPW is originally from South Asia where it is a major pest of coconut. In the Mediterranean basin RPW has become a key pest of *P.canariensis* which is extremely sensitive to its attack [5].

By the mid-1980's, multiple introductions of *R.ferrugineus* to the Middle East from Pakistan and India have occurred and the Asian palm weevil (APW) is now a serious pest of date palms, in the Arab Gulf States.

The rest of the Mediterranean countries were totally infested by 1994, eventually to report the pest attack in North America in 2009 [5].

The RPW reportedly attacks over 26 species of palm belonging to 16 genera globally in several regions of the world.

The latest report of an RPW invasion occurred in late 2011 in Tunisia where it was found infesting Phoenix canariensis.

Infested palms are hard to detect since the larvae feed on the internal tissues of the palm. Under careful observation, surveyors may be able to detect infested plants with holes in the crown or trunk. Additionally, distorted or “clipped” fronds may be seen. Leaves may droop because of loss of support by bored axils and a collapsed canopy [6] a very typical sign of infestation is the distorted growing point at the top of the palm. The growth at the top of the canopy can become deformed and offset. This distortion is a very common symptom and is more easily seen than other symptoms of infestation.

As the eggs of RPW are deposited inside concealed places of the stem, larvae hatch and start destructing reaching generally the apical growth area.

Life cycle is than wholly sealed within the stem upon emergence of the adults from the cocoon which fly out and infest new palms or remain in the same palm and cause re-infestations at a new site [7].

The existing methods of RPW management largely rely on the IPM strategies, which include: phytosanitation, use of conventional insecticide, pheromone traps, attract and kill technology and bio-control agents.

The chemical control method against RPW, include spraying and injecting of synthetic insecticides into infested palms [8].

Neonicotinoids, comparatively new group of synthetic insecticides, agonists the nicotinic acetylcholine receptors (nAChR).

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine] possess both systemic and contact mode of action and is compatible with different application methods for example foliar application, seed treatment [9], soil drench and stem application in different crops and trees [10].

Imidacloprid causes irreversible blockage of post synaptic nicotinic acetylcholine receptor of the central nervous system [11].

In laboratory and semi-field conditions, imidacloprid SL formulation successfully controlled RPW [12].

Preventive and curative methods were often based on chemical pesticides, until an extended alternative has been introduced involving the use of biological products and natural enemies ([13]; [14]).

For the sake of date palms, we have to take into consideration the accumulation of insecticides in the fruit. Amongst the variety of natural products available, essential oils (EOs) are being investigated since a decade

to provide environmentally-safe alternatives to pesticides ([15], [16]). They can be obtained from various plant parts such as flowers, leaves, stem, fruits, seeds, roots, etc. EO is volatile and lipid-soluble natural mixtures of terpenes, terpenoids, polyphenols, fatty acid esters, etc. They have been reported to possess several types of bioactivities including antibacterial, antiviral, antifungal, antifeedant, insecticidal and medicinal (analgesic, sedative, anti-inflammatory, spasmolytic, locally anesthetic) ([17], [18], [19]).

Among microorganisms, entomopathogenic fungi are considered promising agents for red palm weevil biocontrol because there are numerous strains, relatively inexpensive, convenient to use, safe to apply, transmitted horizontally, and safe to non-target organisms and the environment [18]. Upon contact with the host, entomopathogenic fungi penetrate the cuticle of the host, germinate the spores, and overcome the host immune system. The blast pores proliferate producing conidia in cadavers of the host [20], hence the host can be infected either by direct contact, horizontal transmission from infected cadavers to healthy host or through the germination of appressoria from dead cadavers ([21], [22]). The aim of this study is to evaluate the repercussions of two biological products against red palm weevil, the entomopathogenic fungi *Beauveria bassiana* and the product Bioshell (Bioweevil) based on essential oils. The efficacy of these two products were compared with the reference product 'confidor' whose active substance is imidaclopride.

II. MATERIALS AND METHODS

1. Study Sites

In this article we treated 3 experimental fields included within the regions concerned by the pest control campaign. Each site was marked by its population of stems and the density of plantation.

1.1 National Guard of Tunisia (L'aouina)

The palms were treated in this site starting on August the 11th 2016 and their survey was carried on until December the 10th 2016. In that period the infestation level was high.

In this site the efficacy of the bioweevil product was tested against the confidor (Imidacloprid).

65 palms were sprayed by the imidaclopride 5% at 100g of product per tree (Figure 1). 2 palms were initially infested.



Fig.1. Treatment of the palm by imidaclopride 200 using 7 cc active ingredient/ tree. Mixed with water, applying 30l/tree

Are 58 palms sprayed by the bioweevil product (Figure 2)? The dose used is 1 l of the product / 100l of water, each palm of which is treated with 30 l of prepared solution.

Among these 58 palms, 2 were initially infested.



Fig. 2. Bioshell product (Bioweevil)

The state of the palm trees was checked on the following two dates 22/09/2016 and 10/12/2016.

1.2 Bouchammaoui Site

This trial was conducted in Bouchammaoui house on April the 26th 2016. This treatment was repeated on October the 14th 2016.

In this site the efficacy of the bio-weevil product was compared with the confidor (Imidacloprid 5%). There are 5 palms sprayed by the bio-weevil product with the dose used in Pastor Institute.

For the second product tested (Confidor) 2 tested: 10 palms were sprayed by the imidaclopride 5% at 100 g of commercial product per tree and 8 palms were sprayed by the imidaclopride 5% at 50 g of commercial product per tree.

1.3 Pastor Institute (Ariana)

This trial was conducted in Pastor Institute on April the 12th 2016. This treatment was repeated every month until May the 2nd 2017 (more than one year).

In this site, 5 palms were sprayed by the Imidaclopride 200. Two palms were initially infested.

The second product to be tested in this site is *Beauveria bassiana* in powder formulation (Figure 3).



Fig. 3. *Beauveria bassiana* product

There are 9 palms treated with the product *Beauveria bassiana* who's required a dose is 200 g of product per palm. The treatment with this product was carried out with a special powdery apparatus (Figure 4).



Fig. 5. Infested prepupae with *B. bassiana* in The cocoon



Fig. 4. Special powdery for *Beauveria bassiana* product. The powdery machine used by nacelle

III- RESULTS

A logistic model was used to analyze the effect of the product, the effect of the date and the date /product interaction in 3 sites.

In the 3 sites, no product effect, date effect or date /product interaction effect was observed (Tab 1, Tab2, and Tab 3).

1. Efficacy of Beauvoir bassiana Compared to Imidacloprid200

For all sampling dates, for analyzing the deviance of the logistic model, the mean number of the infested palms using Imidacloprid and *Bauveria bassiana* are as follows $0,07425 \pm 0,42, 0,07535 \pm 0,5 (\pm SD)$.

Infested palms before application become healthy after one month and remain healthy, even healthy palms remain unassigned.

No significant difference between the two products used (Biological and chemical) for treatment against red palm weevil at this site (Tab1).

Table 1: Deviance analysis table in Pastor Institute

Wald Statistics For Type 3 Analysis			
Source	DF	Chi-Square	Pr>ChiSq
Date	8	2,03	0,9799
Product	1	0	0,9799
Date*Product	7	0,07	1

The treatment with *B.bassiana* showed the same efficacy as the confidor (Imidacloprid 200) (Figure 5).

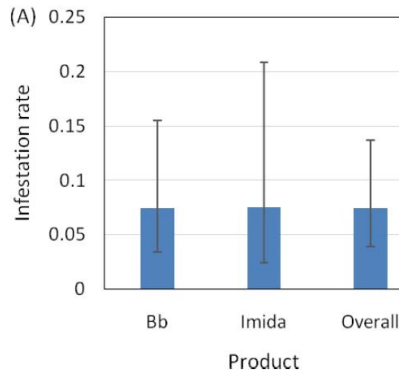


Fig. 5. Mean of infested palms using B.b (*Beauveria bassiana*) and Imidacloprid 200 in Pastor Institute (Mean \pm SD)

Adults caught at the traps show the presence of CRP throughout the trial but with marked decrease due to treatment (Fig 6).

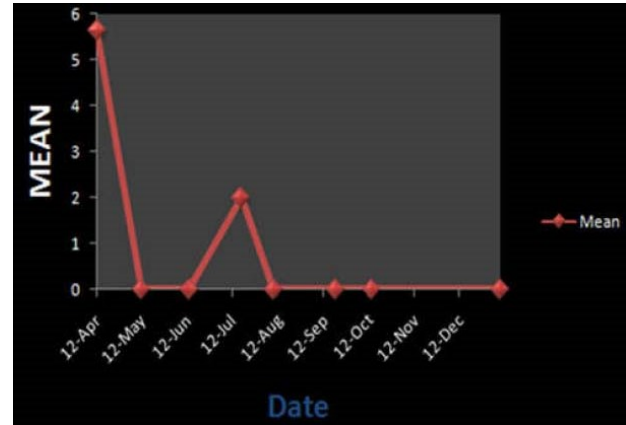


Fig. 6. Mean captures of traps in Pastor Institute

1. Efficacy of Bioweevil compared to Imidacloprid

1.1 Bouchemmaoui's Site

For all sampling dates, for analyzing the deviance of the logistic model, the mean number of the infested palms using Imidacloprid with both doses (50g/Palm and 100g/Palm) and Bioweevil are as follows $0,0668 \pm 0,04558, 0,0835 \pm 0,05647 (\pm SD)$ and $0,2668 \pm 0,1142$.

As in the site of Bouchemmaoui's home, neither date effect nor effect of the product was observed in this site too. There is no significant difference between the two products used (Biological and chemical) for treatment against red palm weevil at this site (Tab 3).

Table 2: Deviance analysis table in Bouchemmaoui's house

Wald Statistics For Type 3 Analysis			
Source	DF	Chi-Square	Pr>ChiSq
Date	2	0,52	0,7717
Product	2	0,09	0,9566
Date*Product	4	0,2	0,9952

The treatment with Bioweevil showed the same efficacy as the imidaclopride 5% at 100 g of product per tree (Figure 7).

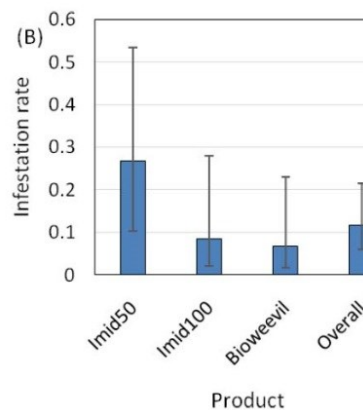


Fig. 7. Mean of infested palms using Bioshell (Bioweevil), Imid 50 (Imidacloprid: 50g/palm) and Imid

100(Imidacloprid: 100g/palm) in Bouchammaoui's house (Mean ± SD)

Adults caught at the traps show the presence of CRP throughout the trial but with marked decrease due to treatment (Fig 8).

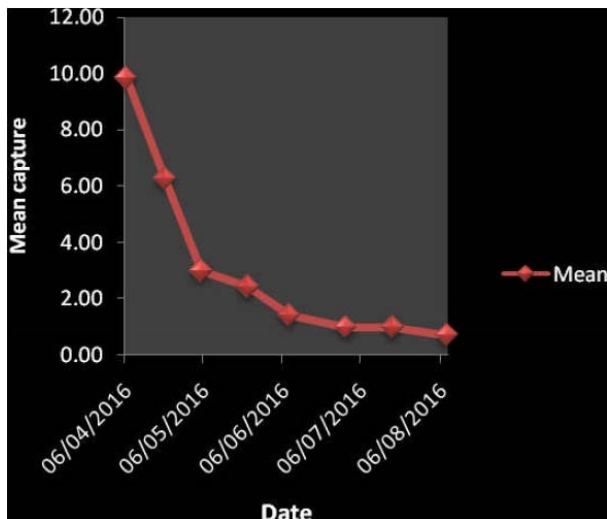


Fig. 8. Mean captures of traps in Bouchammaoui site

1.2 National Guard of Tunisia (L'auina)

For all sampling dates, for analyzing the deviance of the logistic model, the mean number of the infested palms using imidaclopride 5% at 100 g of product per tree and Bioweevil are as follows $0,05747 \pm 0,01764$ and $0,03077 \pm 0,01515$ (\pm SD).

In this site neither date effect nor effect of the product was observed. There is no significant difference between the two products used (Biological and chemical) for treatment against red palm weevil at this site (Tab 3).

Table 3: Deviance analysis table in National Guard of Tunisia (L'auina)

Wald Statistics For Type 3 Analysis			
Source	DF	Chi-Square	Pr>ChiSq
Date	2	0,51	0,775
Product	1	0,51	0,4742
Date*Product	1	0,29	0,5885

The treatment with Bioweevil showed the same efficacy as the confidor (Imidacloprid) (Figure 9).

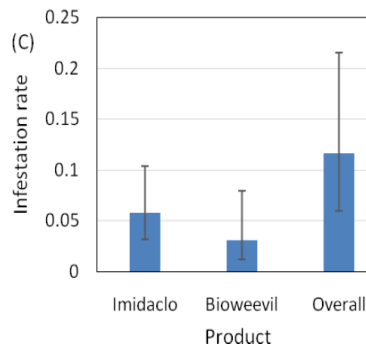


Fig. 9. Mean of infested palms using Bioshell (Bioweevil) and Imidaclo (Imidacloprid) in National Guard of Tunisia (L'auina) (Mean ±SD)

Adults caught at the traps show the presence of CRP throughout the trial. The increase in adult catches at this site is due to the installation of traps in an untreated plot. These results demonstrate the need to integrate the two methods of control: trapping and treatment with pesticides

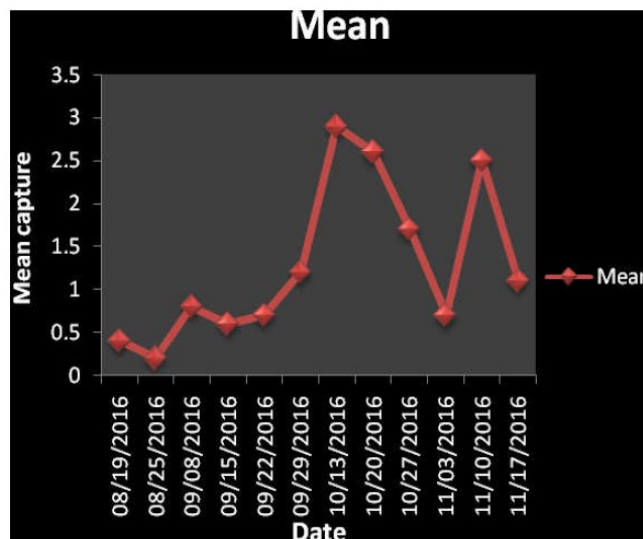


Fig. 10. Mean captures of traps in National Guard of Tunisia (Fig 10).

IV. DISCUSSION

Since many species of Coleoptera are serious agricultural and palm pests specially the Red Palm Weevil (*Rhynchophorus ferrugineus*) regarding the difficulties and obscurities in field study of these pests, effects have been made to use some of pathogens as bio pesticides ([21];[22]).

The use of enthomopathogenic fungi and essential oils in monitoring and controlling RPW populations has become an important tool for managing this pest ([15], [16]; [18]).

Since its report in Canary Island date palm in Spain, Italy and other European countries, imidacloprid is recommended for the control of this alien pest [9].

The present study showed that fungal treatment reduced palm infestation. Thus, the main contribution of the fungus

to RPW control was found to be through the premature death of the infected adults.

The treatment with *B.bassiana* showed the same efficacy as the confidor (Imidacloprid).

Antifeedant activity of EOs can be useful in preventing new infestations of RPW. Though it has been investigated against a few stored-grain coleopteran pests including Curculionidae weevils ([17], [18], and [19]).

Our study showed that Bioweevil based on essential oils reduced palm infestation with the same efficacy as the confidor (Imidacloprid).

The results obtained in this work could improve the efficacy of biological products against Red Palm weevil populations.

V. CONCLUSION

Biological products have been successfully used to control rpw in several countries. Our studies compare the efficacy of entomopathogenic fungi, biological pesticide (bioshell) with the chemical insecticide (imidacloprid).

Present results demonstrated that there is no significant difference between the two types of products used (Biological and chemical) for treatment against red palm weevil.

It should be mentioned that treatment with the entomopathogenic fungi *B.bassiana* must be repeated every month. Based on the results presented above, we propose that using the biological products tested can give good results in the control of RPW.

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