Journal of Agriculture and Crops
ISSN(e): 2412-6381, ISSN(p): 2413-886X
Vol. 2, No. 6, pp: 57-61, 2016
URL: http://arpgweb.com/?ic=journal&journal=14&info=aims

First Records of Endogenous Bio-Agent of the Red Palm Weevil *Rhynchophorus Ferrugineus* (Olivier) (Coleoptera: Curculionidae) in Palestine

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Abstract: Red palm weevils *Rhynchophorus ferrugineus* is becoming a serious insect pest on date palm in the Mediterranean region and in Palestinian territories. Naturally occurring enemies collected from several localities could have a great potential in controlling invasive insect species. An indigenous strain of *Beauveria bassiana* (Ascomycota: Clavicipitaceae) isolated from naturally infected *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) larvae, pupae and adults were collected from several sites from the northern part of the West Bank. Identification and pathogenicity test were evaluated under laboratory and field conditions on module insect pests reared in the laboratories of Kadoorie Agriculture Research Center (KARC)/ PTUK, West-bank/ Palestinian territories. Laboratory results showed that indigenous strains of *B. bassiana* can infect target insect pest tested (LC_{50} was 120-132 conidia per ml). Field preventive bioassays on apple trees infected with aphid, confirmed the potential of this strain as a biological control agent under certain environmental conditions.

Keywords: *Rhynchophorus ferrugineus*; *Beauveria bassiana*; Lethal concentration 50; Conidial germination.

1. Introduction

The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), is a major tissue boring insect pest attacking date palm *Phoenix dactylifera* (L.), it was recorded for the first time in the Arabian peninsula in the 80s [1], ever since; RPW had spread all over the world [2]. Larvae fed within the apical growing point of the palms and damage extensively the palm tissues, boring tunnels into the palm trunk; heavy viscous yellow to brown fluids oozing from these tunnels [3], a strong and distinctive fermented odor, insect frass, and remarkable reduction in date production [4].

Early detection of RPW infection is difficult because of larval cryptic as palm trees don't show any visual evidence of infection until it's too late to recover from their damage [5]. Chemicals were the most commonly used method to control RPW [6], but development of insect resistance against most insecticides used [7], and the general public concerns related to environmental pollution, and potential chemical residues; many integrated pest managements systems are considering an alternative approaches to the chemical control with biological, semichemicals, or sterile insect techniques [8]. Entomopathogenic fungi could be potential biological agents because of their wide hosts range of insect species, it can infect different stages of insect hosts, can cause natural epizootics, and often have minimal effects on non-target organisms [9].

The potential of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Clavicipitaceae) as biocontrol agents against RPW has been investigated by few researcher [10-12]. However, none of the strains tested were isolated from infected RPW [13]. The aim of the current study was to identify the endogenous fungal pathogens *B. bassiana* collected from different sites in West Bank from RPW and to estimate the virulence of the three isolates of the entomopathogenic fungus.

2. Materials and Methods

2.1. Insect Collection and Rearing

Insects used in the laboratory and field assessments were collected from plants non-treated with any pesticides from the campus of Palestine Technical University –Kadoorie (PTUK). Insects such as oleander aphid *Aphis nerii* (Boyer de Fonscolombe) were collected from Oleander plants *Nerium oleander* (L.), rose aphids *Macrosiphum rosae* (L.) were collected from rose plants *Rosa* spp. (L.), blue alfalfa aphids *Acyrthosiphon kondoi* (Shinji) were collected from milk thistle *Silybum marianum* (L.), oleander scale insects *Aspidiotus nerii* (Bouch) were collected from *N. oleander* (L.). Mediterranean flour moth *Ephestia kuehniella* (Zeller) were maintained under laboratory condition on a diet of a 8:2: 2 mixture of oat bran: what germ: wheat flour in plastic containers (15 cm length x 20 cm width x 8 cm height. Non-target natural enemies such as Mealybug ladybug *Cryptolaemus montrouzieri*

(Mulsant), *Aphidius* spp. wasp, parasitic wasp *Anagyrus pseudococcianagrus* wasp were maintained under laboratories.

2.2. Isolation of the Pathogens

Adults and larvae of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) collected from three locations Palestine Technical University (strain BBK) (32° 19′ 8.34″ N, 35° 1′ 43.3″ E), Atil nursery (BBA); (32° 22' 14.4583" N; 35° 4' 13.5095" E), Al-Jarushiyaa (BBJ) (N 32° 20' 48.5866" N; 35° 2' 58.2703" E), were used for the isolation of entomopathogenic fungi. Larvae were first examined under the microscopic for the presence of any fungal infection. Whole infected adult or larvae that already showed hyphal growth on their bodies were placed on potato dextrose agar (PDA) containing dextrose as a carbohydrate source to stimulate fungus growth, and potato infusion that provides a nutrient base for most fungi. Agar is added as the solidifying agent. Larvae were incubated at 23±2 °C until adequate growth of fungus is observed, and then the fungus was transferred to fresh PDA medium and incubated for at least 7 days under the same conditions. After sporulation, microscopic examinations of the fungus were accomplished.

2.3. Preparation and Application of Conidial Suspensions

Fungal conidia were harvested and suspended by flooding the spores from culture plates with sterile distilled water containing 0.1% Tween- 80, mixing for 2 min on a vortexer (Scientific, Bohemia, NY), The clumping of the fungal spores was removed gently by scrubbing the concentrated suspension with a sterile spatula to produce a homogenous suspension. Then the suspension was filtered through several layers of cheesecloth into 500 ml flask to remove mycelia and debris, then shaking for 30 min. Under microscope, the spore concentration was then determined [14]. Serial dilutions of *B. bassiana* spores were prepared evenly over half-strength plates in triplicate. The concentration giving 30 to 300 colonies per plate was noted and used in all further testing, as concentration varied for each isolate [15].

2.4. Virulence Bioassays

Comparative virulence of the novel isolate strains of *B. bassiana* was determined in a series of five-dose bioassays against several insects. The bioassay protocol was essentially the same as described previously for single-dose assays [16]. Insects were exposed to the fungus by the either spraying or dipping technique. Treated and for control insects were incubated at room temperature (25 ± 5 °C). Mortality percentages were recorded 2, 4, 6 and 8 days post-exposure or up to 100 % mortality. Abbott's formula was used to correct bioassay data for control response.

2.5. Data Analysis

Control mortality in bioassays higher than 10% were discarded and repeated. All Aphid natural mortality was corrected using Abbott's formula [17] for each treatment. Then slope, intercept and LC_{50} values were calculated from Probit regressions analysis.

3. Results and Discussion

The entomopathogenic fungus s one of the most common bio-agent to insect pests; it has been naturally found and isolated from soil, and dead insects [18]. In the current study, *B. bassiana* was recovered from infected RPW larvae and adults collected from several location in Palestine, then it was grown on nutrition medium (PDA). Endogenous *B. bassiana* isolates induced significant mortality to treated insect pest tested, depending on the isolate and the inoculum concentration used. Similar results were obtained by Castrillo, *et al.* [16] and Qazzaz, *et al.* [19].

Mortality rates of *A. nerii* treated with *B. bassiana* increased with increasing conidial concentration and time of exposure (Table 1). The LC_{50} value of fungus strains (BBK; BBA; BBJ) 7 day after treatment (DAT) on the aphid *A. nerii* were 121,122,132 ppm; respectively) (Table 1). The percentage of cumulative mortality aphid *A. nerii* population treated with *B. bassiana* strains (BBK; BBA; BBJ) increased proportionately with time and reached to 80-90% (figure 2). Dead insects showed typical symptoms of infection and were covered with fungus especially when kept in sterile petri dishes were the humidity is high. Infected aphids turned hard and dry, and then the fungus outgrowth and sporulation on treated aphids were observed few days after application.

The conidial germination of BBA strain applied to *C. montrouzieri*, *E. kuehniella*, *A. nerii* aphid, and *A. nerii* scale was 5, 7, 11 and 21 days after inoculation; respectively (figure 2).

Results from bioassays on aphids, scale, moth and bugs showed that the *B. bassiana* isolates were virulent against these pests at the concentration of 2.27 x 10^7 conidia mg^{-1}. However, a relatively high concentration of 9.8 × 10^5 conidia mg^{-1} diet is needed to cause 50% mortality during 7 d [20]. These results stand somewhat in agreement with that of Buda and Peciulyte [21]. Dose-mortality respond for different developmental stages have been reported in many other pest species [22].

Conidial germination of *B.bassiana* strain applied at 250, 500 ppm appeared after 4 day. During per-experiment; the geminating conidia didn't appear because of unsuitable growing conditions, under greenhouse condition (data are not shown). No result at treated crops the temperature was over 40°C and the humidity was over 85%, and this prevent growth sporulation (data not shown), this agreed with Doberski [23].

One strain *B. bassiana* caused infection even at 20 ^{o}C, infection of adult beetle by *B. bassiana* was tested at 15, 20 ,25 ^{o}C, fungal infection occurred at all three temperature, but at 25^{o}C beetle tended to succumb to bacterial infection. The effect of relative humidity on infection of larvae by *B. bassiana* was tested at 51, 74, 86, 90, 95, 97.5 and 100% relative humidity, *B. bassiana* caused some infection at all humidity. Mortality due to infection was most rapid at the highest humidity. Sublethal effects of entomopathogenic fungi on aphid seem to depend on aphid species and/or the fungal species [22].

The current study is the first report about finding and isolation of endogenous pure cultures of endogenous Palestinian isolate of the fungal pathogens *B. bassiana* from natural populations of RPW. Bioassays assessment of the entomopathogenic fungi *B. bassiana* is only the first step to

further studies but they are a hopeful sign as well. They show that search for a proper bioinsecticide with potential for practical utilization in agriculture for RPW control could be directed to development of new mycopesticide or to selection of proper one among commercially available formulations of *B. bassiana* isolate as an active substance with coleopteran pests in its host range. Both directions are attended with a hard investigation work in the laboratory and under field conditions to compare *B. bassiana* with conventional chemical control as well as combination with other endogenous entomopathogenic bio-agent.

Table-1. Bioassay assessment of *B. bassiana* strains (BBK; BBA; BBJ) to aphid *A. nerii* treated with different concentration, mean mortality percentage, standard deviation under laboratory conditions. Slope, intercept and LC_{50} values were calculated with the Probit regression analysis

Strain	concentration (ppm)	Mean Mortality	StD		
BBK	50	22.43	29.84	**slope:**	0.72
	100	34.46	24.56	**intercept :**	-1.00
	250	34.04	12.65	**Test value**	0.50
	500	25.38	13.59	**log (c%)**	2.08
	750	20.75	17.49	**LC50**	**120.69**
BBA	50	0.00	0.00	**slope**	0.49
	100	7.10	5.15	**intercept**	3.98
	250	11.43	1.16	**Test value**	5.00
	500	7.48	6.54	**log (c%)**	2.09
	750	0.00	0.00	**LC50**	**122.23**
BBJ	50	12	4.359	**slope**	0.28
	100	17	4.704	**intercept**	4.40
	250	10	0.577	**Test value**	5.00
	500	19	2.517	**log (c%)**	2.12
	750	13	3.055	**LC50**	**132.53**

Figure-1. Abbott-corrected cumulative mortality (%) of *B. bassiana* strains (BBK; BBA; BBJ) isolated form local sites. Bioassay test were carried on 30 *A. nerii* aphids under laboratory conditions for 12 days.

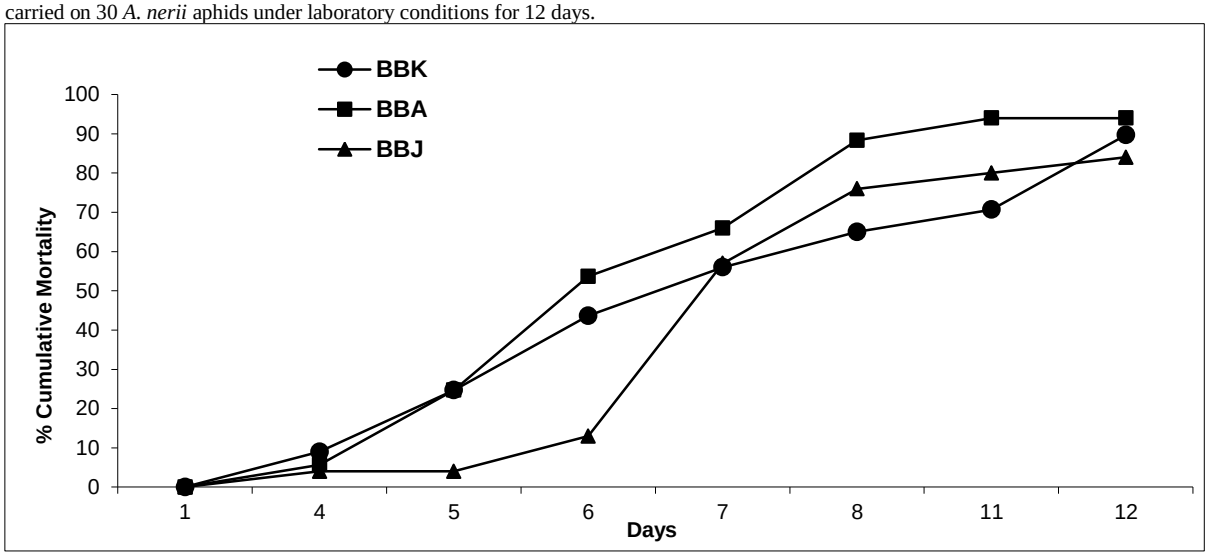
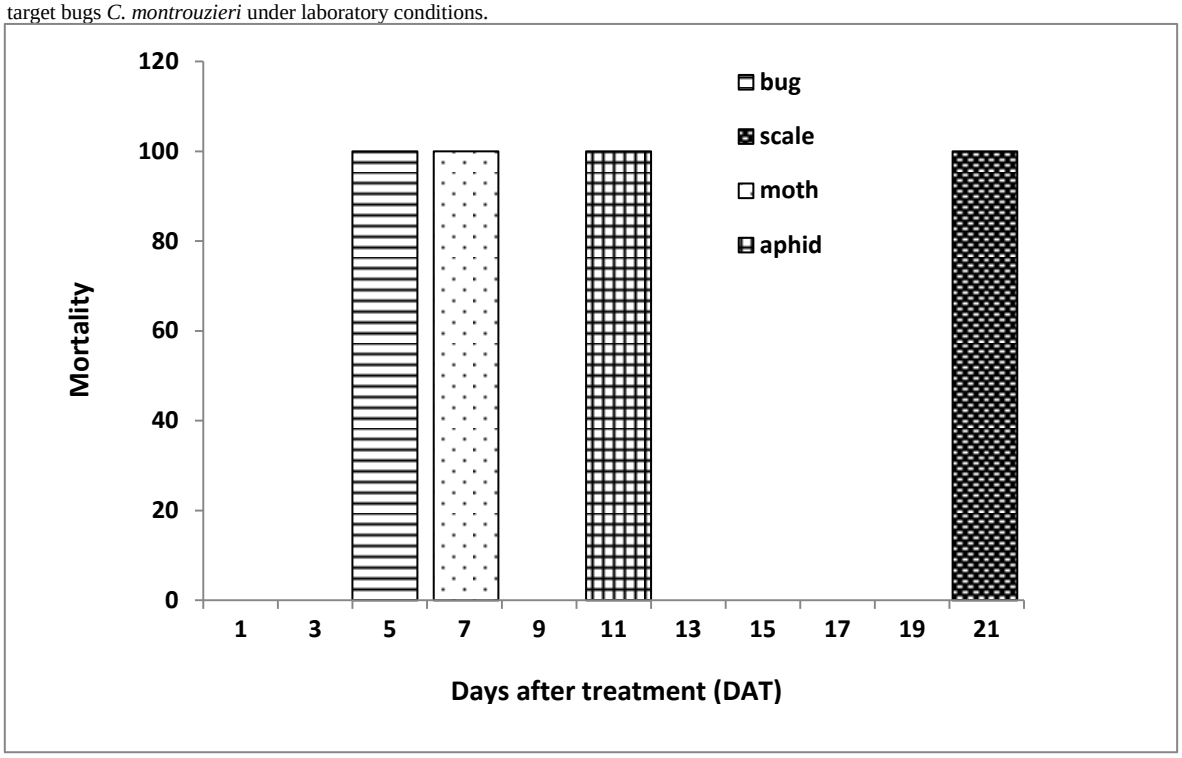


Figure-2. The conidial germination for *B. bassiana* (strain BBK) to target insects (*A. nerii* aphid, *A. nerii* scale, *E. kuehniella*), and non-target bugs *C. montrouzieri* under laboratory conditions.



Acknowledgements

The author thanks many capable technicians and undergraduate students for their technical assistance and laboratory and field work.

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