Pakistan Entomologist

Journal homepage: www.pakentomol.com

BIOLOGICAL CONTROL OF THE DATE PALM TREE BORERS, *ORYCTES AGAMEMNON ARABICUS* (COLEOPTERA: SCARABAIDAE: DYNASTINAE)

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ARTICLE INFORMATION

Received: February 10, 2018
Received in revised form: May 18, 2018
Accepted: June 02, 2018

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ABSTRACT

The efficacies of the entomopathogenic nematodes (EPN), *Rhabdits blumi* and the entomopathogenic fungi (EPF), *Beauveria bassiana* has been studied as biocontrol agents against the date palm tree borers (Arabian Rhinoceros Beetle), *Oryctes agamemnon arabicus* (Coleoptera: Scarabaidae: Dynastinae) in laboratory and field trials, during 2015. Laboratory results demonstrated that direct spray of 1000 infective juveniles (IJs) per ml of *R. blumi* on Arabian Rhinoceros Beetle, *Oryctes agamemnon arabicus* (ARB) larvae caused 71.67% mortality less than 72 hours and 15% in the adults. While, treating the food source of the larvae (pieces of fresh tissue of the frond bases) with the same dose and period resulted in 48.33% mortality in larvae and 10% in the adults. Laboratory results also showed that using concentration 1×10^{9} conidia/ml^{-1} of *B. bassiana* as direct spray of the *O. a. arabicus* larvae caused 66.7% mortality and 60% as treatment of the food source. Field experiments results showed that injection of 50 ml per palm tree with a concentration of 1000 IJs/ml of *R. blumi* inflected about 42% mortality in ARB larvae infested the tree. Meanwhile, injection of 50 ml having concentration of 1×10^{9} conidia/ml^{-1} of *B. bassiana* imposed 50% mortality in larvae. Results of this investigation illustrate the possibility of using *R. blumi* and, *B. bassiana* as a bicontrol agents against palm borers in IPM programs.

Keywords: Entomopathogenic nematode, *Rhabdits blumi,* Entomopathogenic fungi, *Beauveria bassiana* , biocontrol, endophyta, Palm borers, *Oryctes* spp.

INTRODUCTION

Palm borers, especially *Oryctes* spp. are considered economically important insect pests of most of the date palm species in Iraq and most adapted to climatic conditions of the region (Khalaf *et al.*, 2011; Khalaf *et al.*, 2014). Arabian Rhinoceros Beetle*, Oryctes agamemnon arabicus* caused severe damages to the bases of fronds and bunches making long tunnels inside tissue, which acting as weakening and breaking factors for these parts (Khalaf and Al-Taweel, 2015). Entomopathogenic nematode (EPN) and fungi (EPF) have been used to enhance biological control measure of many insect pests (Ehier, 1990; Hazirs *et al.*, 2003). France *et al.* (2015) reported that the farmers usually based their pest management practices on chemicals pesticides, but the growing restrictions and concerns, encourage farmers to microbial control, like EPF and EPN. Khudhair *et al.* (2015) illustrated that the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* were tested against Arabian Rhinoceros Beetle, *Oryctes agamemnon arabicus* larvae, and both were found to be effective. Krell *et al.* (2015) mentioned that the endophytic EPF like *Beauveria* spp. or *Metarhizium* spp were used in biological crop protection to many different insects. Eidt and Thurston (1995) and Simoes and Rosa (1996) reported the applications of EPN were used as a biocontrol agents against many insect pests especially soil insects. In Iraq, EPN *Steinernema* sp. isolated for the first time from the long horn date palm stem borer, *Jebusaea hammeschmidtii* and fruit stalk borer, *Oryctes elegans* in the date palm cultivation areas (Al-Jboory, 2007). The aims of this study were to investigate the efficacy of entomopathogenic nematode (EPN), *Rhabdits blumi*, and fugi EPF, *Beauveria bassiana* as endophytic biocontrol agents against palm borers, *Oryctes* spp. especially Arabian Rhinoceros Beetle (ARB), *Oryctes agamemnon arabicus* under laboratory and field conditions in date palm orchards.

Cite this article as: Khalaf, M.Z., A.M.Tareq, F.H. Naher, A.H. Salman and H.S. Khalaf, 2018. Biological control of the date palm tree borers, *Oryctes agamemnon arabicus* (Coleoptera: Scarabaidae: Dynastinae). Pak. Entomol., 40(1):1-6.

MATERIALS AND METHODS

Collection of samples

Samples of ARB, EPN *R. blumi* and EPF *B. bassiana* were collected from severely infested trees in date palm orchards at South of Baghdad with palm borers showing the symptoms of damages. Borers' larvae were collected by using hand collection from tissues of the frond bases. While, adults were collected using light traps that distributed in the orchards during June to August, hence the high peak of adult activity. *R. blumi* was isolated from the collected of ARB larvae and reared on wax moth *Galleria mellonella* larvae under laboratory conditions (fig.1). While, *B. bassiana* was isolated from both contaminated tree tissues (trunk, leaves and shoots) and adults borer.

Fig 1.
Entomopathoginic nematode, *Rhbditis blumi* rearing on wax moth, *Galleria mellonella*

Nematode and fungal identification

EPN identified by Dr. Brian Darby, Nematology Department, University of North Dakota, USA using molecular biology of high quality sequence, and blast-matched them to the NCBI database, which classified the EPN as *R. blumi* (Personal communication). EPF isolates were identified by pathologist in the Directorate of Agricultural Research using microscopic characteristics which showed that the EPF isolated was *B.bassiana.*

Oryctes borer culture

Oryctes agamemnon arabicus borer larvae (last instar) were kept in special plastic containers 30×20×22.5 cm supplied with small pieces of frond base tissue. Larvae were reared at 25±2°C, complete darkness and 65% RH. fresh frond bases pieces were added regularly every two weeks to keep enough fresh food to the larvae. Larvae were kept until used for different experiments.

Experimental design

The laboratory trials were conducted in Biological Control Dept. of IPM Center. Field trials were conducted in date palm orchard Global positioning system, GPS: latitude 33.12740, longitude 44.82124 located in Almadain district (30km South of Baghdad), planted with mature date palm trees of 25-30 years old with 6-7 m length and 50-55 cm diameter.

Laboratory experiments include using four concentrations (0, 250, 500 and 1000 IJs per ml) of *R. blumi* and one concentration 1×10^{9} conidia/ml^{-1} of *B. bassiana* by direct spray on the larvae or their food (pieces of frond bases tissue). Fresh food pieces were added regularly every two weeks to keep enough fresh food to the larvae. Three replicates, 5 larvae each were used for each treatment as laboratory test. Larval mortality was counted in all treatments daily through 4 weeks of experiment.

Fifty ml solution of EPN 1000 IJs/ml or 1×10^{9} conidia/ml^{-1} of EPF were injected through tree trunk using syringes 50 ml in size after drilling holes with a brad point drill- bit (diameter, 20mm and length, 200mm) 1m above the ground level (Fig. 2). Three replicates (trees) were used for each treatment as field trials.

Larvae of ARB in trees crowns were collected after fourth weeks of injection EPN or EPF (Fig.2), dead and alive larvae were counted in each treatment and kept in plastic containers. In addition, fresh frond bases tissues were collected and healthy larvae of *G. mellonella* were added to test if it contains EPN, *R. blumi* in each treatment. Observations were taken pieces frond bases as a test of transfer EPN by plant vascular bundles in each treatment. Observations were taken after 48, 72, 96 and 120 hr. of treatment. Larval mortalities that infected by EPN and EPF in each treatment were counted (Fig.3). The experimental designs were complete randomized design and complete randomized block design, and the results were analyzed according to the least significant differences (LSD) using GenStat 3 program.

RESULTS

Mortality and infection percentages of ARB larvae after direct spray under laboratory conditions with EPN *R. blumi* revealed that the highest concentration used (1000 IJs/ml) inflect the highest mortality among larvae reaching 71.67% after 120 hr., while lower concentrations of 500 and 250 IJs/ml recorded mortality of 50.0% and 16.67% respectively (table 1). Meanwhile, mortality reached 38.33%, 46.33% and 48.33% at concentrations of 250, 500 and 1000 IJS/ml respectively in experiments of using treated larval food (table 1). While, the adults mortality for direct spray and treated food were: 0%, 6.67%, 15.0% and 0%, 5.0%, 10.0% at concentrations 250, 500 and 1000 IJS/ml respectively (table 2). It is obvious from the results above that larvae of ARB were more sensitive to infection by EPN than the adults, and this could be due to fact that larvae have soft wall compared to the hard cuticle of adults (Kaya and Gaugler, 1997). Moreover, direct spray treatment was more effective than treated food trail

Table 1
Effect of entomopathogenic nematode *Rhbditis blumi* as biocontrol agent against palm borer, *Oryctes agamemnon arabicus* larvae under laboratory conditions.

Treatment method	Concentration of *R. blumi* (IJs/ml)	Total of larvae treated	%Corrected mortality(accumulation) After(hr)				
			48	72	96	120	Mean
Direct spray	Control (water)	15	0.0	0.0	0.0	0.0	0.0
	250	15	13.33	١٣.٣٣	20.0	20.0	16.67
	500	15	40.0	53.33	53.33	53.33	50.0
	1000	15	66.67	73.33	73.33	73.33	71.67
	Mean	15	40.00	46.7	48.89	48.89	46.11
Treated diet (frond bases)	Control (water)	15	0.0	0.0	0.0	0.0	0.0
	250	15	33.33	40.0	40.0	40.0	38.33
	500	15	46.67	46.67	46.67	46.67	46.67
	1000	15	40.0	46.67	53.33	53.33	48.33
	Mean		40.0	44.44	46.67	46.67	44.44

L.S.D. at 5% for Time: 5.27, for concentration: 13.01, for treatment method: 14.13, for concentration × Treatment method: 19.0, for time × Treatment method: 14.69, for concentration × time: 13.45, for

Table 2
Effect of entomopathogenic nematode Rhbditis blumi as biocontrol agents against palm borer, Oryctes agamemnon arabicus adults under laboratory conditions.

Treatment method	Concentration of *R. blumi* (IJs/ml)	Total of larvae treated	%Corrected mortality(accumulation)				
			48	72	96	120	Mean
Direct spray	Control (water)	15	0.0	0.0	0.0	0.0	0.0
	250	15	0.0	0.0	0.0	0.0	0.0
	500	15	0.0	0.0	13.33	13.33	6.67
	1000	15	6.67	13.33	20.00	20.00	15.0
	Mean	15	2.22	4.44	11.11	11.11	7.22
Treated diet (frond bases) method.	Control (water)	15	0.0	0.0	0.0	0.0	0.0
	250	15	0.0	0.0	0.0	0.0	0.0
	500	15	0.0	6.67	6.67	6.67	5.0
	1000	15	6.67	6.67	13.33	13.33	10.0
	Mean	15	2.22	4.44	6.67	6.67	5.0

L.S.D. at 5% for Time: 3.68, for concentration: 11.49, for treatment method: 4.30, for concentration × Treatment method: 11.35, for time × Treatment method:5.80, for concentration × time:11.41, for time × concentration × Treatment method:12.81

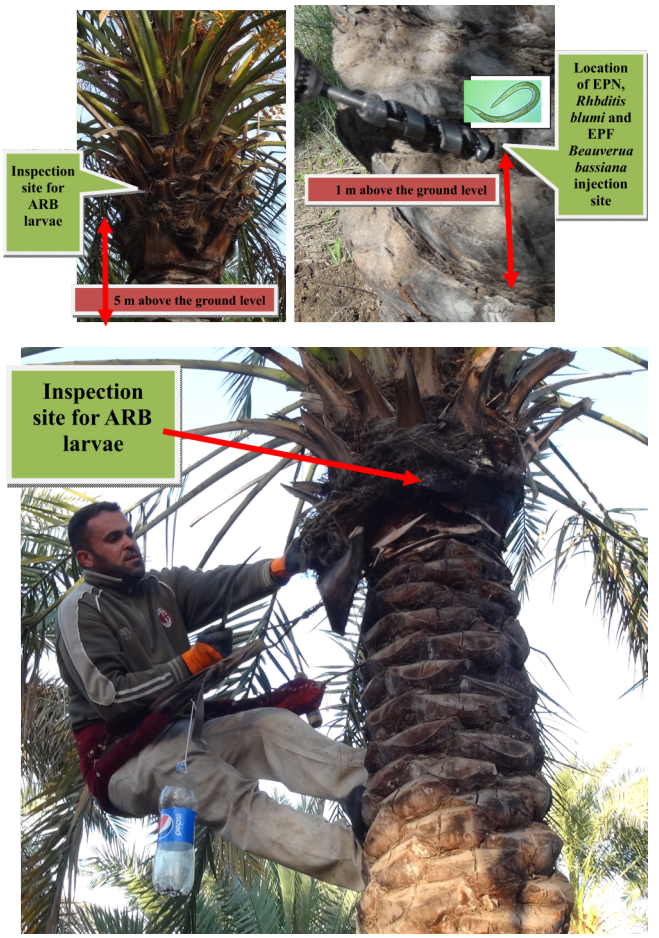

Fig. 2
Injection of bioagents and inspection of palm tree crown for collecting ARB larvae

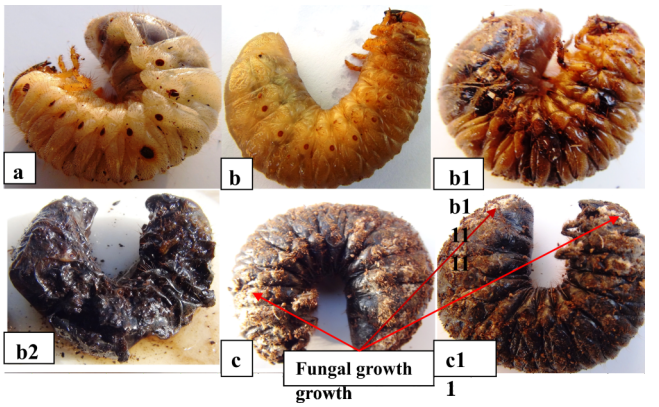

Fig. 3
Larvae of *Oryctes agamemnon arabicus*: healthy (a), infected by EPN *Rhbditis blumi* (b after 6 days, b1 after 8 days, b2 after 12 days) and infected by EPF *Beauveria bassiana* (c after 21 days, c1 after 28 days)

Field efficacy results (Table 3) indicated that the mortality percentage of ARB larvae after direct spray of the tree grown with 1×10^{9} conidia/ml^{-1} of *B. bassiana* spore suspensions reached 66.7 in direct spray and 60.0% in treated food source after 4 weeks.

The present results revealed that there was an acceptable efficacy of applying either of the two biocontrol agents, isolated from Iraqi date palm orchards environment, EPN *R. blumi* and EPF *B. bassiana* reflected by the reducing survival percentage of ARB, *O. agamemnon arabicus* larvae and adults in laboratory and field trials. In addition, results indicated that the EPN and EPF suspension could translocated through date palm vascular bundles after injection. Ricaño *et al.* (2013) found that using more than one formula of *B.*

Table 3
Effect of direct spray and food treatment of entomopathogenic fungi (EPF) *Beauveria bassiana* as biocontrol agents against palm borer, *Oryctes agamemnon arabicus* larvae under laboratory conditions.

Treatment	Total of larvae treated	Number of larvae after 28 day		% corrected Mortality
		life	dead	
Control	15	15	0	0.0
Direct spray (concentration 1×10^{9} conidia/ml^{-1}) of EPF, *B. bassiana*	15	5	10	66.7
Food Treatment (concentration 1×10^{9} conidia/ml^{-1}) of EPF, *B. bassiana*	15	6	9	60.0

L.S.D. at 5% for concentration 11.33
Results in table 4 indicated that the field trials of EPN, *R. blumi* and EPF, *B. bassiana* revealed that trunk the injection of 50ml of each biocontrol agents inflict moderate mortality percentage among larvae of ARB reaching 41.7% and 50% after 4 weeks in EPN and EPF treatments, respectively.

Table 4
Effect of entomopathogenic nematode (EPN) *Rhbditis blumi* and entomopathogenic fungi (EPF) *Beauveria bassiana* as biocontrol agents against on palm borer, *Oryctes agamemnon arabicus* larvae under field conditions.

Treatment (Trunk injection)	Number of larvae per three trees (in tree crown only)			
	Before treatment	After 4 weeks of treatment		% corrected mortality
		Live	dead	
control	20	20	0	0
50 ml per tree from solution with concentration 1000 IJs/ml of EPN, *R. blumi*	12	7	5	41.7
50 ml per tree from solution with concentration 1×10^{9} conidia/ml^{-1} of EPF, *B. bassiana*	10	5	5	50.0

L.S.D. at 5% for treatment 8.01

bassiana can remarkably reduce survival and increase mortality rate among red palm weevil, *Rhynchophorus ferrugineus* larvae and adults. Abbas and Mahmoud (2009) reported that IJs of EPN, *Steinernema riobrave* caused 44-100% mortality in third larvae of *O. agamemnon* under laboratory conditions, and 33 – 78% mortality when applied to soil of fig orchard. Yang *et al.* (1993) have been successfully controlled banana weevil by injecting EPN suspensions directly into borer holes. In addition to the most common application method for EPN is to use the same type of equipments used for spraying chemical pesticides, nematodes could be applied to target site with most commercially available spray equipment such as hand or ground sprayers, mist blowers and aerial sprayers on helicopters (Georgis *et al*., 1995).

In conclusion, the results of this investigation depicted the possibility of using EPN, *R. blumi* and EPF, *B. bassiana* as effective biocontrol agents against ARB, *O. agamemnon arabicus* and probably to other palm borers species in date palm orchards either by direct spray or trunk injection through IPM program.

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