Plant Archives Vol. 19, Supplement 1, 2019 pp. 1139-1143 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF ENTOMOPATHOGENIC NEMATODE, RHABDITIS BLUMI SUDHAUS (NEMATODA: RHABDITID AS A BIOCONTROL AGENT ON THE PALM BORER, ARABIAN RHINOCEROS BEETLE (*ORYCTES AGAMEMNON ARABICUS*)

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Abstract

Insect borers are serious pests of date palms in Iraq and many other date-palm growing countries. These pests severely affected the palms trees, decrease the quantity and quality of date fruits and weaken the trunks which lead to fall and death. The pathogenicity assessment of entomopathogenic nematode (EPN) *Rhabditis blumi* Sudhaus (Nematoda: Rhabditida) against palm borer Arabian Rhinoceros Beetle (ARB), *Oryctes agamemnon arabicus* was provided in the lab and date palm orchards between 2015 and 2017. In the laboratory test, EPN was used against the larvae and adults as a direct spray and as treated food (pieces of fresh tissue of the frond bases)at a rate of 0, 500, 1000, 1500 Infective Juveniles (IJs) per mL of *R. blumi*. The obtained results demonstrated that EPN resulted in 89%, 61%, 25% and 20% mortality when used as a direct spray and as treated food on larvae and adults of ARB, respectively. The *in vivo* experiments detected that injection of 50 mL for each palm tree with a concentration of 1500 IJs/mL of *R. blumi* achieved 45.5% mortality in ARB larvae infesting the tree. Meanwhile, population density of ARB larvae reduced to 45.8%, 59.6% during first and second year of treated date palm trees by injection method respectively. Results of the current study revealed the possibility of using EPN *R. blumi* as a bio control agent for controlling borers in date palm orchards under natural conditions.

Keywords: Bio-control, Entomopathogenic nematode, Palm borers, *Oryctes agamemnon arabicus*, *Rhabditis blumi*

Introduction

Palm borers, especially *Oryctes* spp. are considered as an economically important insect pest of date palm trees in Iraq and most adapted to climatic conditions of the region (Khalaf *et al*., 2011). Arabian Rhinoceros Beetle (ARB), *Oryctes agamemnon arabicus* causes severe damages to the bases of fronds and bunches making long tunnels inside the tissue, which are acting as weakening and breaking factors for these parts (Khalaf *et al*., 2015). Many control methods have been used through different application methods: spray, drench and injection (Al-Jboory *et al*., 2001; Felsot, 2002; Timmeren *et al*., 2012) against to date palm pests. Khalaf *et al*. (2016) reported that the application of thiamethoxam and imidacloprid against ARB larvae resulted in 85.8%, 100% mortality in injection method compared with 75%, 80% in drench method respectively. Khudhair *et al*. (2015) tested locally isolated entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against ARB larvae and reported high mortality rate among larvae reaching up to100% after 29 days of treatment under lab conditions. Entomopathogenic nematodes will only be widely used as pest control products when they become available on demand by the different clients, commercial growers and small farmers (Mwaniki *et al*., 2013). Rhabditid nematodes are generally recognized as bacteriovores and often associated with invertebrates, their relationship with invertebrates is known as necromancy, which means waiting for the death host (Park *et al*., 2011; Schulte, 1989; Wilson *et al*., 1993; Blaxter *et al*., 1998; Stock *et al*., 2005). Khalaf *et al*. (2016) tested entomopatogenic nematodes (EPN), *Rhabditis blumi*, and the entomopatogenic fungi (EPF), *Beauveria bassiana* as biocontrol agents against larvae and adults of ARB, *O. agamemnon arabicus*. Biological control potentials of *R. blumi* against 5 coleopteran species and 5 lepidopteran species was evaluated by (Park *et al*., 2012). The aims of this study were to investigate the efficacy of EPN, *Rhabditis blumi* as entophytic biocontrol agents against palm borers, *Oryctes* spp. especially ARB, *O. agamemnon arabicus* under laboratory and field conditions. Another objective was to investigate the feasibility of application and persistent in date palm orchards.

Materials and Methods

Borers and Nematodes cultures

Samples of ARB *O. agamemnon arabicus* were obtained from the lab. colony reared on natural foods

(palm tissues, fresh frond bases pieces) at 25±2°C, complete darkness and 65% relative humidity in the IPM Center, Directorate of Agricultural Research, Ministry of Science & Technology (Khalaf *et al*., 2016). *R. blumi* was obtained from the lab. colony reared on larvae of wax moth *Galleria mellonella* in labs of IPM center (Khalaf *et al*., 2016).

Laboratory Experimental Treatments

The laboratory trials were conducted in Biological Control Dept. of IPM Center. Laboratory experiments included using four concentrations (0, 500, 1000 and 1500 IJs per mL) of *R. blumi*as direct spray on larvae and adults of ARB or mixed with their food (pieces of frond bases tissue). Fresh food pieces were added regularly through the period of treatment to keep enough fresh food to the larvae. Five replicates, 6 larvae/rep as larval treatment and four replicates, 5 adults/rep as adult's treatment were used for each treatment as for laboratory experiments. Larval and adults mortalities were counted in all treatments after 24, 72, 96 and 120 hours of treatment.

Field Experimental Treatments

For field experiments, injection of 50 mL solution of EPN 1500 IJs/mL was done through tree trunk using 50 mL syringes after drilling holes with a brad point drill- bit (diameter, 20mm and length, 200 mm) 1m above the ground level (Fig. 1-A ,B). A same number of trees were left as control treatment in each orchard. Five replicates (trees) were used for each treatment. Larvae of ARB in trees crown were collected after four weeks of injection EPN, dead and live larvae were counted in each treatment and kept in plastic containers (Fig.1-C). 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. Samples of tissue were taken after 4 weeks of treatment for examination and to explore the presence and movement of EPN through plant tissue. Larval mortalities that infected by EPN in each treatment were counted.

Efficacy and Persistent of Entomopathogenic Nematode *Rhabditis blumi* in Date Palm Orchards

Efficacy and persistent of EPN *R. blumi* were studied in two date palm orchards, the first one was untreated (control) orchard, the second orchard was treated with EPN mixed in 50 mL ata concentration of 1500 IJs by used injection methods in ten tree trunk. EPN distribution and efficacy in reduction of ARB Larvae population density were counted by calculating larvae in ten palm tree Brem variety in each orchard before treatment and during the first and second year after treatment.

Experimental Design and Data Analysis

The experimental designs implemented were complete randomized design and complete randomized block design. Genstat program was implied in statistical analysis and determine the significances efficacies. Henderson and Tilton (1955) was used to calculate corrected mortality efficacies% on larval and adults ARB treated with EPN in lab treatments, while Schneider-Orelli's formula (1981) was applied for measuring efficacies of the EPN tested on ARB in field treatment.

Results

Results in Table 1 indicated that the mortality percentage of ARB larvae after direct spray under laboratory conditions with EPN *R. blumi* revealed that highest concentration (1500 IJs/mL) caused the highest percentage of mortality 89% after 120 hr., while the lower concentrations of 1000 and 500 IJs/mL recorded mortality of 79% and 57% respectively. Meanwhile, mortalities reached 50%, 54% and 61% at concentrations of 500, 1000 and 1500 IJs/mL respectively in experiments when EPN was mixed with larval food (Table1).

Adult's mortality for direct spray and treated food were 0%, 15%, 20%, 25% and 0%, 10%, 15%, 20% at concentration 0, 500, 1000 and 1500 IJs per mL respectively (Table2). Field efficacy results indicated that the mortality percentage of ARB larvae after trunk injection with 50 mL in concentration 1500 IJs per mL reached 45.5% (calculated in the crown tree only) after 4 weeks of treatment (Table 3). The use of EPN *R. blumi* in date palm orchards as a biocontrol agent against Oryctes larvae caused a reduction in population density reached 45.8% and 59.6% after one month and during the second year of treatment respectively (Table 4).

Discussion

Results of field studies revealed that there was an acceptable efficacy of applying local isolate of EPN *R. blumi*(isolated locally from Iraqi date palm orchards ecosystem) as biocontrol agents. The EPN *R. blumi* can persist habitat causing more reduction in the population density of ARB, *O. agamemnon arabicus* larvae. In addition, results indicated that the EPN, *R. blumi* solution could translocated through date palm tissue after injection in the trunk. Park, *et al*. (2012) reported that EPN *R. blumi* against major cruciferous insect pests and evaluated pathogenicity in lab and greenhouse and showed that EPN caused high mortality rate in larvae. Entomopathogenic nematodes have certain advantages over chemical as control agents; it's non-polluting agent and thus environmentally safe and acceptable (Georgis,

1990). There are many reports for rhabditid nematodes causing mortality of various invertebrates species: beetles, termite, millipede and rice yellow stem borer (Schulte, 1989; Smart and Nguyen 1994; Carta and Osbrink, 2005; Richter, 1993; Padmakumari *et al*., 2007). Schulte, (1989); Sudhaus and Schulte (1989) found that the Infective Juveniles of rhabditid nematodes enter an invertebrate, remain until it is dead, and complete their development by feeding on bacteria growing inside the cadaver of insect, and it usually possesses some attributes of a potential biological control agents, such as short life cycle, easy culture condition with bacteria, high fecundity and virulence, and good association with invertebrate pathogenic bacteria.

Conclusion

EPN *Rhabditis blumi* showed significant mortality against Arabian rhinoceros beetle *Oryctes agamemnon arabicus*, but the mortality rate of larvae was higher than that of adults in lab trails at direct spray or treated food. When EPN was mixed with 50 ml of water at a concentration of 1500 IJs/ml and injected in the trunk, a moderate mortality rate was reported among ARB larvae .However, dispersal and efficacy were increased in treated orchards after one year of treatment.

Therefore, this nematode can be considered as a good and safe alternative to managing borer's population on date palm trees and other insect pests.

Conflict of Interest

None of the authors have any conflicts of interest to declare**.**

Source of Funding

The research was performed independently, there is no funding, influence over study design, analyses, manuscript preparation, or scientific publication**.**

Ethical clearance

The project was approved by the local ethical committee in University of Middle Technical, Baghdad, Iraq.

Table 1: Effect of entomopathogenic nematode *Rhabditis blumi* as a 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		
			Larva dead	% mortality	% Efficacy	Larva dead	% mortality	% Efficacy	Larva dead	% mortality	% Efficacy	Larva dead	% mortality	% Efficacy
Direct spray	Control (water)	30	0	---	---	0	---	---	1	---	---	2	---	---
	500	30	13	43	43	17	57	57	17	57	55	18	60	57
	1000	30	21	70	70	23	77	77	23	77	76	24	80	79
	1500	30	25	83	83	26	87	87	27	90	89	27	90	89
Treated diet (frond bases)	Control (water)	30	0	---	---	0	---	---	1	---	---	1	---	----
	500	30	15	50	50	15	50	50	16	53	52	16	53	50
	1000	30	14	47	47	15	50	50	17	57	55	17	57	54
	1500	30	16	53	53	16	53	53	19	63	62	19	63	61

Table 2 : Effect of entomopathogenic nematode *Rhabditis blumi* as a biocontrol agent 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) After(hr)											
			48			72			96			120		
			Adult dead	% mortality	% Efficacy	Adult dead	% mortality	% Efficacy	Adult dead	% mortality	% Efficacy	Adult dead	% mortality	% Efficacy
Direct spray	Control (water)	20	0	---	---	0	---	---	0	---	---	0	---	---
	500	20	0	0	0	0	0	0	3	15	15	3	15	15
	1000	20	1	5	5	3	15	15	4	20	20	4	20	20
	1500	20	3	15	15	5	25	25	5	25	25	5	25	25
Treated diet (frond bases)	Control (water)	20	0	---	---	0	---	---	0	---	----	0	---	----
	500	20	0	0	0	2	10	10	2	10	10	2	10	10
	1000	20	2	10	10	2	10	10	3	15	15	3	15	15
	1500	20	3	15	15	3	15	15	4	20	20	4	20	20

Table 3 : Effect of entomopathogenic nematode, *Rhabditis blumi* as biocontrol agents against on palm borer, *Oryctes agamemnon arabicus* larvae under field conditions.

Treatment (Trunk injection)	Number of larvae per five trees (in tree crown only)			
	Before treatment	After 4 weeks of treatment		% corrected mortality
		Life	Dead	
Control	Unknown	35	0	–
50 mL (1500 IJs per mL) per tree	Unknown	12	10 (dead and new place of larva)	45.5

Table 4 : Distribution and efficacy of entomopathogenic nematode *Rhabditis blumi* in date palm orchards at Middle of Iraq.

Treatment (orchard)	Number of larvae per ten tree in crown tree only (Brem variety)				
	Before treatment	During first year after treatment		During second year after treatment	
	Life larva	Life larva	% Reduction in population	Life larva	% Reduction in population
Control Orchard No 1 Without Nematode	120	131	---	115	----
Orchard No.2 Nematode 1500 IJs	93	55	45.8	36	59.6

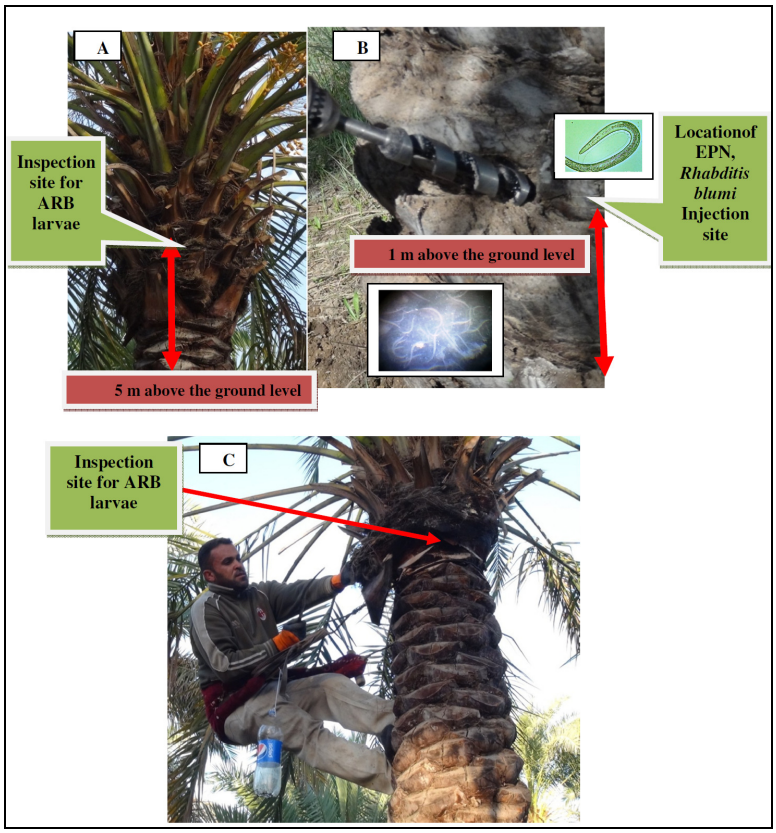

Fig 1. : (A) Inspection site for ARB larvae, **(B)** location of EPN Injection site, **(C)** Inspection of palm tree crown for collecting ARB larvae.

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