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THE PATHOGENICITY OF STORED POLYHEDROSIS VIRUS OF THE RED PALM WEEVIL, *Rhynchophorus ferrugineus* (OLIVIER) (COLEOPTERA : CURCULIONIDAE)

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ABSTRACT

The laboratory bioassays showed the frozen stored *Rhynchophorus* polyhedrosis virus (FSRPV) and newly extracted one from died red palm weevil, Rhychophorus ferrugineus (oliv.) (RPW) collected from the field. The FSRPV was stored for 18 months under -4^{o}C. The newly extracted *Rhynchophorus* polyhedrosis virus (NERPV) was used for contamination of laboratory reared larvae of RPW at different ages in comparison of the pathogenicity between FSRPV and NERPV. The infected larvae were bioassayed and LC_{50} and LT_{50} values were determined. The range of LC_{50} for NERPV was from $2.6x10^{7}$ to $4.0x10^{7}$ Polyhedra Inclusion Bodies (PIBs) /100 g diet, while the LC_{50} for FSRPV ranged between 3.3 to $3.8x10^{7}$ PIBs/100 g diet. The range of LT_{50} for NERPV was from 2.4 to 24.3 days while it was from 3.2 to 25.9 days for FSRPS.
Keywords: Rhynchophorus ferrugineus, polyhedrosis virus.

INTRODUCTION

The red palm weeil (RPW), *Rhynchophorus ferrugineus* (Olivier) is one of the most destructive pests attacking date palm trees (*Phoenix dactylifera* L.) in the Middle East, North Africa, and Gulf States. It is likely that the weevil could invade other areas and countries where the date palms are grown (Van Der Lean, 1981). The RPW was first recorded in Egypt in 1992 (Cox, 1993), and has now become established as a devastating pest of date palms in Egypt. Larvae of RPW were reared on a cooked diet based on carrot sweet potato and peeled pieces of sugarcane internodes (Alfazairy *et al*. 2003a).

Polyhedrosis virus was isolated for the first time in Egypt(Alfaziry *et al.* 2003b) from dead larvae collected from destroyed palm trees as recommended by the Ministery of Agriculture. In fact, the polyhedrosis virus of the RPW was first recorded by Gopinadhan *et al.,* 1990, in India. The laboratory bioassays carried out by Alfazairy *et al.*, (2003c) revealed that the noctuid *Spodoptera littoralis* (Boisd.) was considerably susceptible to the polyhedrosis virus originally isolated from the *curculionid R.ferrugineus* . Also, the cross-infectivity studies revealed that *S. littoralis* polyhedrosis virus did not cross-infect the early or late larval stages of *R. ferrugineus*. The RPW polyhedrosis virus can therefore be mass-propagated in the cotton leaf worm *S. littoralis* which is easily and cheaply mass reared.

The aim of the present work is to evaluate the efficacy of frozen stored *Rhynchophorus* polyhedrosis virus of RPW, (stored for thirtysix months at -

4^{o}C) on RPW larvae of different ages. Also, the efficiency of fresh extracted polyhedrosis virus from diseased larvae of the RPW was propagated in RPW larvae in the laboratory and evaluated on reared ones.

MATERIALS AND METHODS

1. Rearing the RPW:

The method of Alfazairy *et al*. (2003a) for rearing RPW was followed in this work. The stock colony was established from field-collected larvae, adults and pupae. The larvae were easily reared on the carrot-sweet potato mash. The diet is composed of 0.5 kg carrot , 0.5 kg sweet potato and 3.5 g methyl-p-hydroxybenzoate.

For larval feeding, a teaspoonful of the diet was dispensed in the plastic rearing cups (6.5 cm diam and 4.5 cm height) where a larva was transfered by means of a fine brush (for newly hatched larvae) or by a forceps (for larger larvae). RPW larvae were introduced individually into these rearing cups and then tightly covered with a piece of soft aluminium foil and a rubber band. At the same time, RPW adults were reared on sugarcane peeled internodes. Clean and sterilized glass jars (5.5cm diam and 11.5 cm hight) were used as oviposition sites . Each jar contains a couple and a small piece of peeled sugarcane internode (ca. 3 cm x 1 cm) , covered with a piece of soft aluminium foil and tied with rubber band.

All rearing cups and jars were covered with black cloth sheets and kept at 21.8±0.2^{o}C, 78.6±0.3% RH. The cups and jars were checked daily for food and bioassay data.

2. Bioassay trails:

The polyhedrosis virus was originally isolated from naturally dead RPW larvae in 2001 and was stored at -4^{o}C for 36 months (Hendi, 2003) to be used in this work. The FSRPV was bioassayed against larvae of different ages. The NERPV was isolated from natural diseased larvae of RPW collected from the field, propagated and kept as a suspension in the refrigerator. The two suspensions were quantifed by counting of the polyhedral inclusion bodies (PIBs) with a haemacytometer. Larval mortality was recorded by daily inspection of all treatments and controls. The LC_{50} and LT_{50} values (median lethal concentration, time, respectively) were estimated from regression lines (Finney,1971).

RESULTS AND DISCUSSION

The pathogenicity of frozen stored *Rhynchophorus* polyhedrosis virus (FSRPV) and newly extracted *Rhynchophorus* polyhedrosis virus (NERPV) on RPW larvae, was established at different ages inoculated with median concentrations as shown in Fig.1, and Table 1. The median lethal concentration (LC_{50}) at 11,13,16 and 18 days post-treatment ranged from 2.6 to 4.0x10^{7} PIBs/100g diet (Table 2). The narrow confidence limits of the

LC_{50s} estimated in this bioassay reflected adequate selection of the dosages used in the assay. The influence of the age of the red palm weevil larvae, i.e., older versus younger or middle aged larvae, used in this bioassay was obvious when using the NERPV.

Table 1: RPW larvae treated with different concentrations of both NERPV and FSRPV indicating first and last days of dead larvae after treatments.

Ages(days)	Concentration of (PIBs/100g diet)	NERPV		FSRPV	
		First day of death	Last day of death	First day of death	Last day of death
0-14	0.0 Control	-	-	-	-
	$2.8x10^7$	5	18	4	21
	$4.2x10^7$	2	12	3	17
	$5.6x10^7$	1	4	3	6
15-45	0.0 Control	-	-	-	-
	$2.8x10^7$	6	22	7	26
	$4.2x10^7$	4	15	5	21
	$5.6x10^7$	1	8	1	10
46-85	0.0 Control	-	-	-	-
	$2.8x10^7$	8	31	9	33
	$4.2x10^7$	3	26	7	26
	$5.6x10^7$	2	18	2	18

The laboratory bioassay conducted here showed that the younger larvae (0-14 days old) were susceptible to the FSRPV isolated from RPW larvae since 36 month . The LC_{50} of NERP at 11,13 days post-treatment for young larvae of 0-14 and 15-45 day-old were significantly lower (2.9 and $2.6x10^7$ PIBs/100 g rearing diet) than the corresponding values of $4.0x10^7$ PIBs/100 g diet for older larvae of 46-85 day-old, respectively (Table 2). In fact, no significant differences in LC_{50} occurred between FSRPV and NERPV (Table 2). Meanwhile, the increase in post-treatment period by two days have slightly, but not significantly, reduced the LC_{50} of NERPV for larvae of 46-85 day-old from 4.0 to $3.3x10^7$ PIBSs/ 100 g diet (Table 2). The LC_{50} of stored solution of polyhedrosis virus, at 11,13, 16, and 18 days post-treatment has no significant differences between young, middle age and older larvae for concentrations of 3.5, 3.8, 3.4 and 3.3 $x10^7$, respectively.

The time to attain 50 % mortality was affected by both the age of tested larvae and the dose used (Table3). The decrease in virus concentrations for some treated larvae by FSRPV and NERPV prolonged the survival time of lethally infected larvae at 50% LT value. The lowest concentration of the virus, 2.8×10^7 PIBs/100 g diet, used on older larvae of 46-85 day-old had lethally responded to the viral infection and significantly differed with longer period of time (11.5-days)for NERPV and (9.7 days) for

FSRPV compared to the corresponding LT_{50} for the younger larvae of 0-14 day-old. In addition, the LT_{50} for *Rhynchophorus* older larvae of 15-45-day-old was shorter by about 1.2 days for NERPV and 4.3 days for FSRPV than the younger larvae of 0-14 day-old at the lowest virus concentration (2.8 x 10^7) as indicated in Table 3.

Table 2: Probit analysis data for mortality of *Rhynchophorus ferrugine*us larvae infected individually with NERPV and FSRPV suspension of polyhedrosis virus.

Polyhed-rosis virus	Days post-treatment of different ages	Regression equation	LC50 (PIBs/100g diet)	95% confidence limits	Slope	Fit of the drawn-L-c-p line Chi2 P Degrees of freedom
NERPV	11(0-14)	Y=60.6831+9.3020x	2.9 x10^7	2.8 x10^7to3.2 x10^7	9.3	0.49
	13(15-45)	Y=36.0651+5.4785x	2.6x10^7	2.3 x10^7to2.9 x10^7	5.4	8.329
	16(46-85)	Y=35.3689+5.5330x	4.0 x10^7	3.8 x10^7to4.3 x10^7	5.5	0.780
	18(46-85)	Y=5.7473+7.9927x	3.3 x10^7	3.1 x10^7to3.5 x10^7	7.9	8.301
FSRPV	11(0-14)	Y=44.7645+6.9454x	3.5 x10^7	3.3 x10^7to3.7 x10^7	6.9	5.739
	13(15-45)	Y=51.5166+8.3063x	3.8 x10^7	3.7 x10^7to4.0 x10^7	8.0	33.881
	16(46-85)	Y=38.7357+5.9915x	3.4 x10^7	3.2 x10^7to3.6 x10^7	5.9	1.910
	18(46-85)	Y=51.7473+7.9927x	3.3 x10^7	3.1 x10^7to3.5 x10^7	7.9	1.555

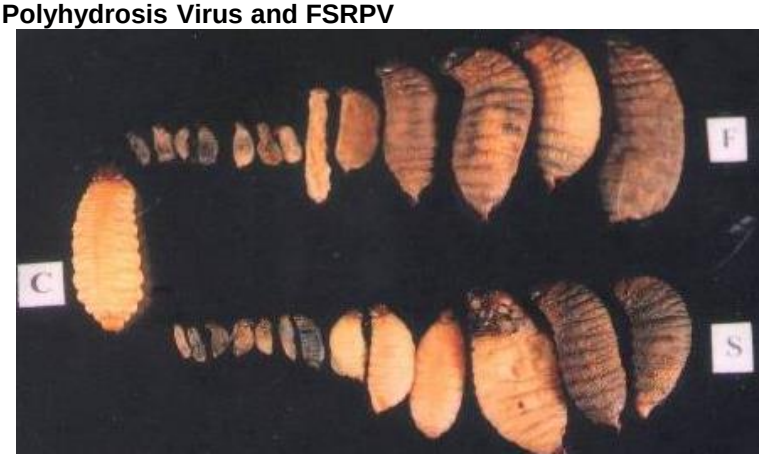
Table 3: LT_{50} values, correlation and Regression determination for *R. ferrugineus* larvae of different ages treated individually with different concentrations of newly extracted and frozen stored *Rhynchophorus* polyhedrosis virus

Concentration of PIBs/100 g diet	Newly extracted suspension					Frozen stored suspension				
	Age of tested larvae ( )-day-old	LT50 (days	Correlation	Regression Determinati -on	Slope	Age of tested larvae ( )-day-old	LT50 (days)	Correlation	Regression Determinati -on	Slope
2.8x10^7	(0-14)	12.8	0.92	0.86	5.8	(0-14)	(0-14)	12.8	0.92	0.86
	(15-45)	14.0	0.84	0.71	3.9	(15-45)	16.2	0.91	0.84	4.1
	(46-85)	24.3	0.99	0.98	4.0	(46-85)	20.5	0.96	0.93	4.4
4.2 x10^7	(0-14)	7.9	0.93	0.88	3.3	(0-14)	25.9	0.94	0.90	0.1
	(15-45)	9.5	0.98	0.97	5.8	(15-45)	11.4	0.92	0.85	5.3
	(46-85)	16.2	0.97	0.94	4.0	(46-85)	14.8	0.97	0.94	5.3
5.6 x10^7	(0-14)	2.4	0.97	0.95	0.2	(0-14)	15.1	0.92	0.85	4.1
	(15-45)	5.6	0.97	0.95	0.1	(15-45)	3.2	0.77	0.60	0.1
	(46-85)	12.0	0.97	0.95	0.0	(46-85)	6.1	0.95	0.90	0.1

The latter findings could probably be a consequence of greater and faster intake of virus-contaminated feeding by the older larvae compared to that of the younger ones. However, this phenomenon was not clear at virus doses

above 2.8x10^7 PIBs, but as it might be generally expected that susceptibility level would be decreased with larval age, and also, taking into consideration that increasing the virus dose might initiate the severe pathogenic effect on the treated younger larvae faster than the older ones.

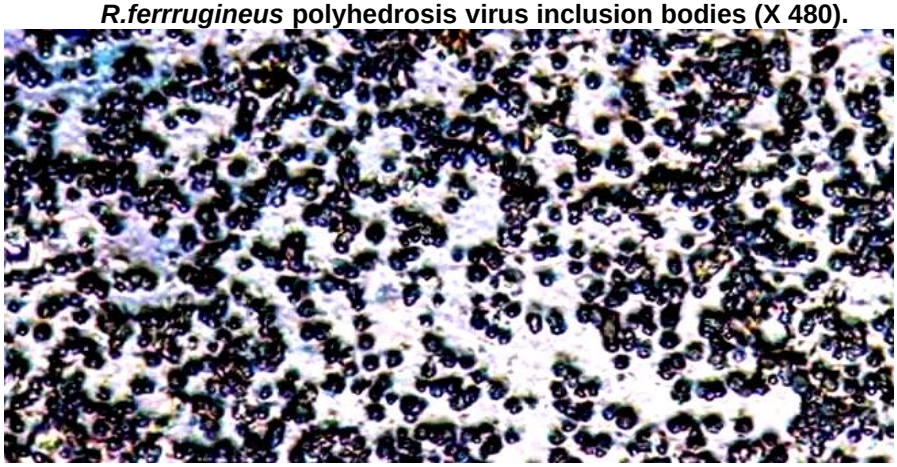
Fig.(1): Symptoms of diseased larvae of red palm Weevel (RPW) contaminated with the newly extracted Rhynchophorus Polyhydrosis Virus and FSRPV



C: Control F: NERPV S: FSRPV

NERPV FSRPV

Fig.(2): Naturally occurring entomopathogen of the red palm weevil *R.ferrrugineus* polyhedrosis virus inclusion bodies (X 480).



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القدرة المرضية للفيروس المخزن المتعدد الأوجه على سوسة النخيل الحمراء

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معهد بحوث وقاية النباتات – مركز البحوث الزراعية

يهدف البحث إلى دراسة فاعلية الفيروس متعدد الأوجه المعزول من سوسة النخيل الحمراء والمحفوظ تحت درجات حرارة منخفضة ومقارنته بمستخلص الفيروس المعزول حديثا من حشرات مريضه جمعت من الحقل. خزن المعلق الفيروسي لمدة ٣٦شهرا على درجة -٤ ° م . وقد تم مقارنة القدرة المرضية لكل من المستخلص الفيروسي المخزن تحت درجات حرارة منخفضة والآخر المعزول حديثا من حشرات ميتة وذلك بخلطه بالغذاء المقدم ليرقات سوسة النخيل الحمراء .

تم إجراء التقييم الحيوي على اليرقات المصابة وكذلك استخراج قيم LC_{50} (التركيز اللازم لقتل ٥٠ % من اليرقات) و LT_{50} (الوقت اللازم لقتل ٥٠ % من اليرقات) باستخدام الكمبيوتر. وتراوحت قيم LC_{50}للمستخلص الفيروسي الحديث من ٢,٦ × ١٠ ^{٧} إلى ٤ × ١٠ ^{٧} أجسام حاوية للفيروس/١٠٠ جم غذاء بينما تراوحت قيم LC_{50} للمستخلص الفيروسي المخزن من ٣,٣ – ٣,٨×١٠ ^{٧} أجسام حاوية للفيروس/ ١٠٠ جم غذاء. وكان مدى LT_{50} للمستخلص الحديث من ٢,٤ – ٢٤,٣ يوما بينما للمستخلص المخزن ٣,٢- ٢٥,٩ يوما.