Biological Control Against Four-Stored Product Beetles Pests by Using Cytoplasmic Polyhedrosis Virus (Cypovirus1)

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ABSTRACT

The rice weevils, tobacco beetle, drug beetle, and Darkling beetle were collected from different locations in Mecca regions, KSA. The four different larvae attack stored products cause economic losses by incredible damage and post-harvest. This investigation was Carrie out to identify characters these insects include. In addition, Cypovirus1 was isolated and collected from living and dead stem and cob borer larvae and pupae from Maize in farmers' fields at Mecca regions, KSA. Samples have chronic diseases were tested by indirect ELISA (The enzyme-linked immunosorbent assay) and confirmed by TEM (Transmission electron microscopy) showing inclusion bodies demonstrated that the occlusion bodies were of irregular shape and ranged from 2.2 to 4.9 µm in diameter. On the other hand, we are studying the effects of different concentrations of a new Bio-component of Cypovirus1 infection on the rice weevil adults, tobacco beetle, bread beetle, and darkling beetle larvae study their impact on the rates and severity of the infection. Also, the SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis) test for showing various distinguishable sole bands in infected adults comparing with non-infected.

Key Words: *Biological Control, Cypovirus1, Rice weevils, Tobacco beetle, Drug beetle, Darkling beetl, KSA*.

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INTRODUCTION

There are about 30 species of insects that invade many of the grain and products stored. Most insects that invade stored grains of small sizes are less than 0.5 mm in length but cause very serious damage. The rice weevil (Sitophilus oryzae) is one of the most important insects to infect whole grain rice and other stored grains. The color of the chitin is of a dark color to a light color and is characterized by the presence of four large patches of orange-red color on the cover of the pavilion size ranging from 2.5 to 4.5 ml in length. There are some stored grain insects in the secondary injury where the infection of the grain after the initial infection of primary insect infection. The darkling beetle (Tribolium castaneum) of the secondary stored grain insects is a type of beetle belonging to the family Tenebrionidae. It is one of the most important global insects that attack stored products, especially stored food grains. Were the attack grain cereals, nuts, beans, and biscuits, causing loss and damage [1-3]. The drug beetle (Stegobium paniceum) crosses insects that have widespread worldwide and are also multi-existent in different environments [4]. The drug beetle is named paniceum by its very high ability to feed on a very wide range of grains, pulses, nuts, flour, stored products, and spices, meaning it can feed on all kinds of stored materials [5].

The tobacco beetle (Lasioderma serricorne), also known as cigar beetle, is very similar to the drug beetle, belongs to the family Ptinidae. 3 mm long, brown in color, can fly, has a life cycle of up to 6 weeks, and can starve hunger [6-8].

For 68 years, fumigants such as phosphine or methyl

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bromide have been used to control stored grain pests in warehouses and silos [9]. There are many reasons why researchers are replacing methods of controlling stored grain pests in other biological ways, mainly because of the ability of these pests to resist chemical insecticides. As well as its dangerous impact on human health. This project will be covered, evaluation of Cypovirus1 infection on larvae of rice weevils, darkling beetle, drugstore beetle, and cigarette beetle were studded.

Cypovirus1 was collected from living and dead stem and cob borer larvae and pupae and examined by electron microscopy showed that particles of Cypovirus1 are shaped icosahedral, which belong to Reoviridae. Cyoovirus1 has a single-shelled capsid [10-13].

The Cypovirus1 infects the insect larvae of stored grain pests four days after inoculation with the Cypovirus1, causing chronic larvae disease and less-lethal chemical insecticides. However, sudden destruction of the central gastric tissue leads to larvae abstaining from feeding, causing a series of physiological changes accompanied by symptoms of vomiting and diarrhea. To the death of the larva after 10 days of inoculation [14-16].

This work was carried out to viral identity was confirmed by host range studies. Electron microscopy examination of fresh cells of healthy and infected by Cypovirus1 revealed occlusion bodies in the infected cells. RT-PCR (Reverse transcription-polymerase chain reaction) test was used for the identification of the virus was isolated from nucleic acid extracts of an infected cell. SDS-polyacrylamide gel electrophoresis test showed sole bands become variously distinguishable between infected cells by Cypovirus1 comparing with healthy cells in these larvae under study.

MATERIALS AND METHODS

Survey of rice weevils, darkling beetle, drugstore beetle, and cigarette beetle in Mecca regions, KSA

756 samples of stored products infected with rice weevils, darkling beetle, drugstore beetle, and cigarette beetle were collected from different 7 locations in Mecca regions. Per location was collected 108 samples were from 4 areas. Nine samples per one area and 3 replicates for all samples were collected.

Identification and the variation of morphological characters the rice weevils, darkling beetle, drugstore beetle, and cigarette beetle

Rice weevils and red flour beetle can be easily separated, and we can distinguish between cigarette and drugstore beetles by morphological and physical characters.

Effect of (Cypovirus1) infection on rice weevils, red flour beetle, drugstore beetle, and cigarette beetle larvae

Collection of infected samples

Samples of living and dead stem and cob borer larvae and pupae were collected from Maize and sorghum in farmers ‘fields in Ismailia governorates.

Maize fields were selected based on plant growth stage and farmer's consent at intervals of approximately 5 Km along major roads within the field, plant will inspect at random for symptoms of stem borer damage. Further samples were collected by soil baiting [17] with stem borer larvae Sesamia calamistis was placed with the soil in plastic basins and held at ambient temperature after 1 week, live, dead and mycoses larvae were recovering and treated according to the following procedures.

Identification of pathogen

Samples containing suspected virus isolates (Cypovirus1) following examination of larval smears, were examined by symptoms appearance and Cypovirus1 particles were purified report to protocols of [18, 19] and showing inclusion bodies and viral particles by TEM and confirmed by Indirect- ELISA, according to Cherry *et al.* (1984).

Virus production

Suspensions of a virus isolate were prepared by triturating the original host larva in sterile distilled water to a volume of 1 ml and then filtering. Concentrations were defined as 1 larval equivalent (l.e.) ml−1. Suspensions were fed to 10 3rd instars larvae per isolate by applying 1 ml droplets via micropipette to small discs of artificial diet (15–20 mm3). Larvae were held in small glass tubes plugged with cotton wool. After 24 hours all larvae which had consumed the treated diet were supplied with a 0.5 cm3 plug of a clean diet and held until death or palpation.

Isolation of Total Genomic RNA

According to the manufacturer’s instructions, Genomic RNA was extracted from purified polyhedral by a standard guanidium isothiocyanate method after dissolving the purified polyhedral in alkali with triazole reagent (Gibco, BRL). RNA was then separated in 1% agarose gel in Tris-phosphate buffer. RNA segments were visualized on the ethidium bromide-stained (final concentration of 0.5 mg/ml) gel. Size markers (Bio Basic Inc. Canada) of 1,000 bp were used to determine segment sizes.

Preliminary assays with the propagated virus

Bio-component of (Cypovirus1) was prepared by mixing 1mg of powder infected larvae with 999mg inactivated integrate containing Carborandum and Talcum. Add 1gm from this component to 10 g, 100 g,1000 g, and 10000 g from stored products and mixture, put 60 larvae per each replicate and 180 larvae per each concentration, mortality

was recorded daily until death or pupation, and all assays were replicated 3 times.

Effect of (Cypovirus1) on protein pattern of all larva stages

Protein extraction

The three larvae stage 3 stage, 6stage and 8 stages were collected from infected and healthy rice weevils, darkling beetle, drugstore beetle, and cigarette beetle larvae separately and ground (0.5g) to flour in a mortar by using liquid nitrogen in a mortar then proteins were extracted with 1ml extraction buffer. The sample will then transfer to a precool Eppendorf tube, vortex for 3 min, then store at 4°C for 30-60 min. The samples will centrifuge for 10 min at 500xg at 4°C and was transferred to a new Eppendorf and store at -20°C.

SDS-PAGE

SDS-PAGE was performed with a separating gel 12% (w/v) (pH8.8) and stacking gel 5% (w/v) (pH6.8) as described by [20]. Protein samples were mixed with an equal volume of protein sample buffer, denature at 80-90°C for 3-5 min. and immediately cool on ice and 25ml of each protein sample will load on each lane. Electrophoresis at 100 volts and 80 ma until the tracking dye reached the bottom of the gel. The gel was stain overnight in a protein staining solution and then wash twice with water. The gel was discolored in distaining solution several times. The protein molecular marker used was from Sigma (St. Louis, Mo, USA).

Statistical analyses

We were used ANOVA-type one-way to calculate the noteworthy diversity in the averages of the experimental treatments. A probability at a level of 0.05 or less will measure considerably [21].

RESULTS AND DISCUSSION

Survey of rice weevils, darkling beetle, drugstore beetle, and cigarette beetle in Mecca regions, KSA

From a total of 756 stored products, 608 (80.42%) Have been infested with at least one stored product beetles pests. Drugstore beetle was the most common stored product beetles with an infection rate of 67.20% **(Table 1)** and the incidence was particularly high in stored products (Ground anise, Ground cumin, all type of pasta, Nigella sativa, and Wheat flour these results were illustrated with **(Figure 1)**, [4, 5] grown in Khulais (85.19%) and Taif, Rabigh, Jeddah, Bahra, Makkah, and Aljummum (74.10%, 71.30%, 71.29%, 67%, 54.63%, and 46.30%, respectively. Rice weevils ranked second (54.37%) and were mostly similarly distributed in all the regions (Khulais, Rabigh, Taif, Makkah, Jeddah, Bahra and Aljummum, 91.67%, 67.6%, 56.48%, 52.78%, 48.15&, 39.82% and 38.89%, respectively [1, 2]. In addition, the Darkling beetle recorded a high infection rate (50.93%) in the Khulais region, while, recorded a low infection rate in Aljummum (29.63%) [1]. On the other hand, the Cigarette beetle has recorded a low infection rate in all regions (36.38%), **(Figure 1 and Table 1)**.

a) **b)** **c)** **d)**

e) **f)** **g)** **h)**

i) j) k) l)

Figure 1. (a), (b) Dried watermelon seeds infested with bread beetle, (c) Ground anise infested with drugstore beetle, (d), (j) Ground cumin infested with drugstore beetle, (e) Damage by rice weevil leaving only intact pericap shell of wheat grains, (f) Presence of profuse powdery substance revealing damage by drugstore beetle, (g) *Ceratonia siliqua* infested with *Lasioderma serricorne*, (h),(k) Pasts infested with drugstore beetle, (i) *Nigella sativa* infected with *Lasioderma serricorne* and drugstore beetle, and (l) Wheat flour infested with and red flour beetles and with drugstore beetle.

Table 1. Incidence of Rice weevils, Darkling beetle, Drugstore beetle, and Cigarette beetle infestation stored products samples from seven provinces in Mecca regions, KSA.

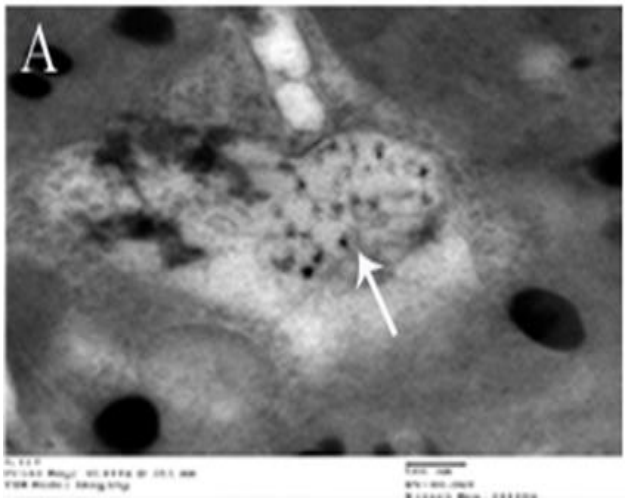
locations	Areas	Samples of stored products collected No.	infested Samples No.		Rice weevils		Darkling beetle		Drugstore beetle		Cigarette beetle	
			N	%	N	%	N	%	N	%	N	%
Jeddah	Elbawady	27	27	100	12	44.44	11	40.74	18	66.67	10	37.04
	Heraa	27	24	88.89	11	40.74	11	40.74	20	74.08	12	44.44
	Bab Makkah	27	25	92.59	16	59.26	15	55.56	24	88.89	15	55.56
	Elrawda	27	24	88.89	13	48.15	10	37.04	15	55.56	9	33.33
	Total Samples	108	100	92.59	52	48.15	47	43.52	77	71.29	46	42.59
Makkah	El-Azezia	27	25	92.59	18	66.67	13	48.15	19	70.37	12	44.44
	Elatebia	27	24	88.89	14	51.85	11	40.74	13	48.15	8	29.63
	Batha korish	27	22	81.48	12	44.44	9	33.33	14	51.85	9	33.33
	Elawaly	27	23	85.19	13	48.15	10	37.04	13	48.15	8	29.63
	Total Samples	108	94	90.38	57	52.78	43	39.82	59	54.63	37	34.26
Taif	Naghab	27	19	70.37	18	66.67	10	37.04	20	74.08	11	40.74
	Howaia	27	23	85.19	17	62.96	11	40.74	19	70.37	7	25.93
	Karwy	27	20	74.07	14	51.85	10	37.04	22	81.48	10	37.04
	Shahar	27	24	88.89	12	44.44	12	44.44	19	70.37	8	29.63
	Total Samples	108	86	79.63	61	56.48	43	39.82	80	74.10	36	33.33
Aljumum	Elnasem	27	15	55.56	11	40.74	9	33.33	13	48.15	10	37.04
	Hay- Elnaghel	27	12	44.44	10	37.04	7	25.93	11	40.74	7	25.93
	Abo Shoeab	27	14	51.85	10	37.04	8	29.63	14	51.85	9	33.33
	Elnoarea	27	14	51.85	11	40.74	8	29.63	12	44.44	7	25.93
	Total Samples	108	55	50.93	42	38.89	32	29.63	50	46.30	33	30.56
Rabigh	Mastora	27	22	81.48	19	70.37	12	44.44	20	74.08	12	44.44
	Alabwaa	27	24	88.89	17	62.96	11	40.74	18	66.67	11	40.74
	Hager	27	24	88.89	18	66.67	11	40.74	21	77.78	11	40.74

	Alkadema	27	23	85.19	19	70.37	12	44.44	18	66.67	10	37.04
	Total Samples	108	93	86.11	73	67.6	46	42.59	77	71.30	44	40.74
Bahra	Elmorshedia	27	19	70.37	11	40.74	10	37.04	18	66.67	12	44.44
	Titan	27	22	81.48	11	40.74	8	29.63	19	70.37	10	37.04
	Heda	27	20	74.07	10	37.04	9	33.33	18	66.67	11	40.74
	Alfag Elkaremy	27	20	74.07	11	40.74	10	37.04	18	66.67	10	37.04
	Total Samples	108	81	75	43	39.82	37	34.26	73	67.60	43	39.82
Khulais	kaded	27	26	96.29	23	85.19	15	55.56	23	85.19	9	33.33
	setarh	27	24	88.89	19	70.37	15	55.56	25	92.59	10	37.04
	Om Algrm	27	24	88.89	21	77.78	14	51.85	22	81.48	8	29.63
	Elghewar	27	25	92.59	20	74.08	11	40.74	22	81.48	9	33.33
	Total Samples	108	99	91.67	83	76.85	55	50.93	92	85.19	36	33.33
	Total	756	608	80.42	411	54.37	303	40.10	508	67.20	275	36.38
	Mean infection rate			80.42		54.37		40.10		67.20		36.38

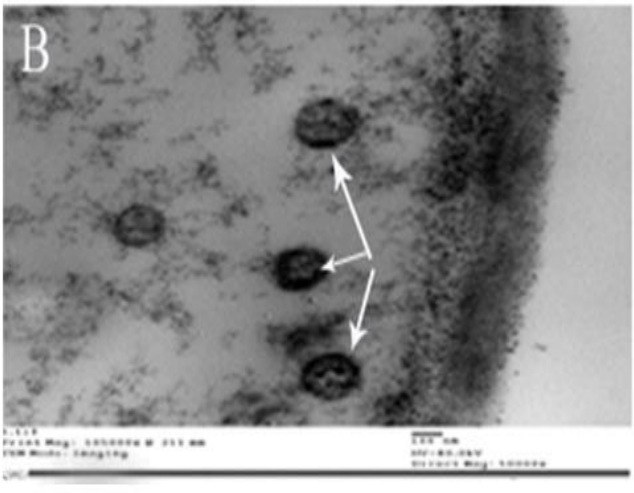
Effect of (Cypovirus1) infection on rice weevils, darkling beetle, drugstore beetle, and cigarette beetle larvae

Identification of pathogen

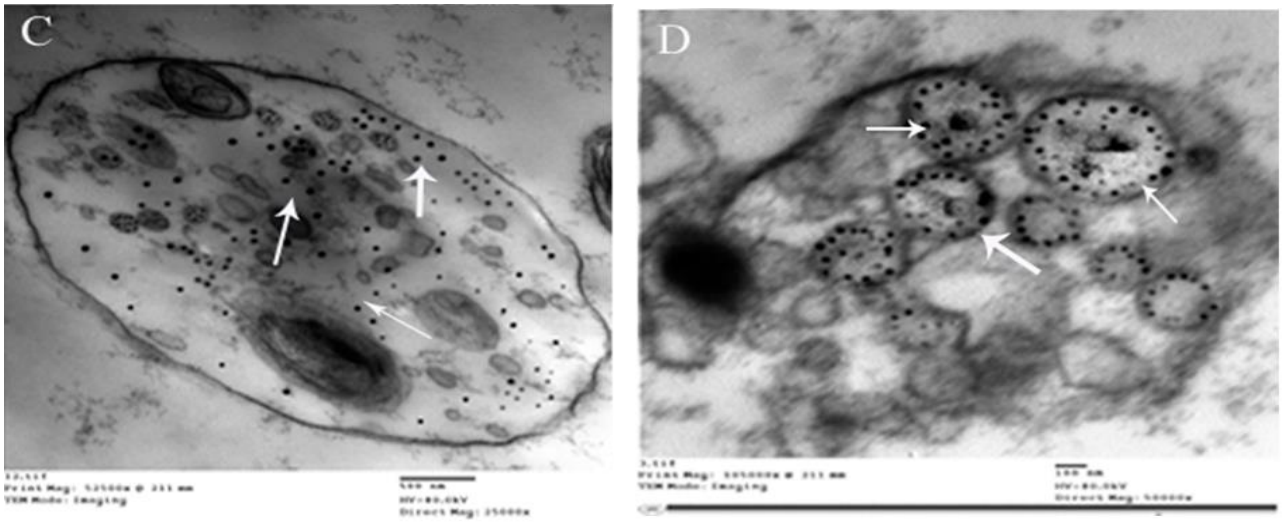
Samples containing suspected virus isolates (Cypovirus1) following examination of larval smears, were examined by symptoms appearance and Cypovirus1 is can be diagnosed from intact Drugstore beetle larvae host cells because the polyhedra are formed only in the cell cytoplasm. This was confirmed by transmission electron microscopy studies, which showed typical cytoplasmic polyhedral inclusion bodies **(Figure 2)** demonstrated that the occlusion bodies were of irregular shape and ranged from 2.2 to 4.9 µm in diameter [22]. whose dimension that the electron microscopy studies showed typical cytoplasmic polyhedral inclusion bodies that are icosahedral, and ranged from 2.4 to 5.3 µ m in diameter [23, 24]. The three-dimensional structures of full and empty Cypovirus1 by electron microscopy show identical outer shells but differ inside **(Figure 2)**.



a)



b)



c) d)

Figure 2. Electron micrographs of typical cytoplasmic polyhedral inclusion bodies from Stegobium paniceum A, B, C, and D. Transmission electron micrograph showing inclusion bodies demonstrated that the occlusion bodies were of irregular shape and ranged from 2.4 to 5.3 µm in diameter.

Cypovirus1 viral particles are icosahedral in shape and have 12 spikes or projections at each of the 12 vertices of the particles. Cypovirus1 has been isolated from Samples of living and dead stem and cob borer larvae and pupae. Unlike the multiple-shelled organization of other Reoviridae members, Cypovirus1 has a single-shelled capsid [13, 22] who mention that A cytoplasmic polyhedrosis virus (CPV) was isolated from the larvae of *Thaumetopoea pityocampa* and shown to cause an infection of midgut cells. On the other hand, this viral infection revealed several important diagnostic symptoms, including reduced feeding, discoloration of the posterior midgut, and extended development time of the larvae.

Electrophoretic Analysis of dsRNA

An initial Cypovirus1 genome analysis by 1% agarose gel using a 14-cm gel at 75 V for 4 h generated seven RNA bands, some of which stained more intensely and appeared to contain more than one genome segment each (the first intense band contained segments 1, 2, and 3 and the fourth band contained segments 6 and 7). Analysis using a longer agarose gel with a lower voltage resolved the first band into three bands and the fourth band into two single bands, confirming that the genome contains a total of 10 segments. Approximate sizes of segments were estimated with size markers as follows: Seg-1, 3,846 bp; Seg-2, 3,612 bp; Seg-3, 3,431 bp; Seg-4, 3,100 bp; Seg-5, 2,972 bp; Seg-6, 2,523 bp; Seg-7, 2,115 bp; Seg-8, 1,756 bp; Seg-9, 1,275 bp; Seg-10, 754 bp. **(Figure 3)** [22].

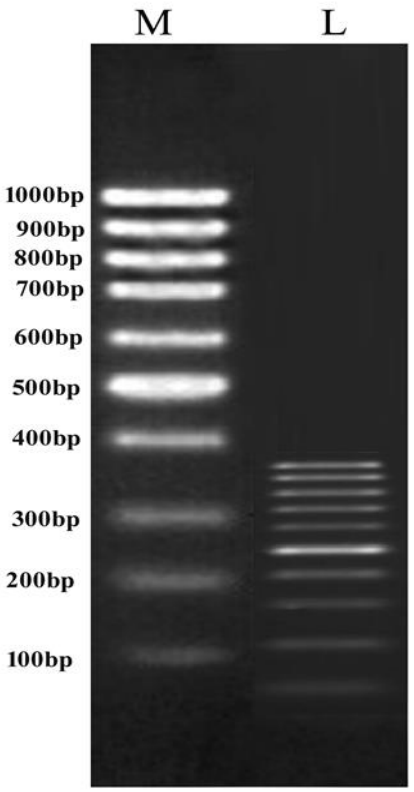


Figure 3. Electrophoretic separation of Cypovirus1:1 total genome in 1% agarose gel. Lane 1: DNA molecular weight marker (1 kb); lane 2: Cypovirus1 genome segments. The arrows indicated that viral segments are Seg-1, 3,846 bp; Seg-2, 3,612 bp; Seg-3, 3,431 bp; Seg-4, 3,100 bp; Seg- 5, 2,972 bp; Seg-6, 2,523 bp; Seg-7, 2,115 bp; Seg-8, 1,756 bp; Seg-9, 1,275 bp; Seg-10, 754 bp. in size, respectively.

Effect of (Cypovirus1) infection on stored products beetles

We are studying the effects of different concentrations of a new Bio - component of Cypovirus1 infection on the rice weevil adults, tobacco beetle, drugstore beetle, and darkling beetle larvae study their impact on the rates and severity of the infection. The highest percentage of mortality was recorded by concentrate 10/100 on tobacco beetle, drugstore beetle, darkling beetle larvae, and rice weevil adults (96.67%, 96.11%, 92.78%, and 90.00%) respectively, followed by concentrate 10/500 recorded 94.44% and concentrate 10/1000 recorded 70.56 %, on tobacco beetle larvae. On the other hand, a lower percentage of mortality was recorded by concentrate 1/25000 on the adult stage of rice weevil (33.33%), **(Table 2)**, these results were illustrated with [14-16].

Table 2. Effect of (CPV) infection on four-stored product beetles pests.

Treatments	Rice weevils (*Sitophilus orizae*) adults							
	Infected weevils / Total weevils treated (I/T)			Mean	Percentage of mortality (%)			Mean
	R1	R2	R3		R1	R2	R3	
10/100	54/60	53/60	55/60	54.00/60	90.00	88.33	91.67	90.00
10/500	51/60	51/60	50/60	50.67/60	85.00	85.00	83.33	84.44
10/1000	45/60	46/60	43/60	44.67/60	75.00	76.67	71.67	74.45
10/10000	32/60	30/60	32/60	31.33/60	53.33	50.00	53.33	52.22
10/25000	23/60	17/60	20/60	20.00/60	38.33	28.33	33.33	33.33
Inactivated integrate	9/60	10/60	10/60	9.67/60	15.00	16.67	16.67	16.11
Control	4/60	6/60	7/60	5.67/60	06.67	10.00	11.67	9.45
Tobacco beetle (*Lasioderma serricorne*) Larvae								
Treatments	**Infected weevils / Total weevils treated (I/T)**			**Mean**	**Percentage of mortality (%)**			**Mean**
	R1	**R2**	**R3**		**R1**	**R2**	**R3**	
10/100	59/60	57/60	58/60	58.00/60	98.33	95.00	96.67	96.67
10/500	56/60	57/60	57/60	56.67/60	93.33	95.00	95.00	94.44
10/1000	50/60	49/60	51/60	50.00/60	83.33	81.67	85.00	83.33
10/10000	43/60	40/60	44/60	42.33/60	71.67	66.67	73.33	70.56
10/25000	32/60	33/60	32/60	32.33	53.33	55.00	53.33	53.89
Inactivated integrate	12/60	13/60	11/60	12.00/60	20.00	21.67	18.33	20.00
Control	5/60	4/60	4/60	4.33/60	08.33	6.67	06.67	7.22
Drugstore beetle (*Stegobium paniceum*) Larvae								
Treatments	**Infected weevils / Total weevils treated (I/T)**			**Mean**	**Percentage of mortality (%)**			**Mean**
	R1	**R2**	**R3**		**R1**	**R2**	**R3**	
10/100	58/60	57/60	58/60	57.67	96.67	95.00	96.67	96.11
10/500	54/60	52/60	55/60	53.67	90.00	86.67	91.67	89.45
10/1000	49/60	48/60	51/60	49.33	81.67	80.00	85.00	82.22
10/10000	39/60	43/60	44/60	42.00	65.00	71.67	73.33	70.00
10/25000	33/60	20/60	27/60	26.67/60	55.00	33.33	45.00	44.44
Inactivated integrate	10/60	12/60	8/60	10	16.67	20.00	13.33	16.67
Control	8/60	7/60	8/60	7.67	13.33	11.67	13.33	12.78
Darkling beetle (*Tribolium castaneum*) Larvae								
Treatments	**Infected weevils / Total weevils treated (I/T)**			Maen	**Percentage of mortality (%)**			Maen
10/100	**R1**	**R2**	**R3**		**R1**	**R2**	**R3**	
10/500	55/60	56/60	56/60	55.67	91.67	93.33	93.33	92.78
10/1000	47/60	51/60	53/60	50.33	78.33	85.00	88.33	83.89
10/10000	37/60	33/60	32/60	34.00	61.67	55.00	53.33	56.67
10/25000	29/60	17/60	21/60	22.33	48.33	28.33	35.00	37.22
Inactivated integrate	15/60	14/60	12/60	13.67	25.00	23.33	20.00	22.78
Control	9/60	10/60	10/60	9.67	15.00	16.67	16.67	16.11

Effect of (Cypovirus1) on protein pattern of all larva stages

Electrophoresis and densitometry analysis of protein bands by SDS-PAGE and illustrated in **Figure 4**. The results tabulated in **Table 3** showed various distinguishable sole bands in both healthy and infected adult weevils *Sitophilus oryzae*, larvaes of *Lasioderma serricorne*, *Stegobium paniceum*, and *Tribolium castaneum* for which it could be used according to it is presented assign for the Cypovirus1 infection and effect of Cypovirus1 on protein pattern in infected weevils compared with healthy weevils. This results practically like be with [12].

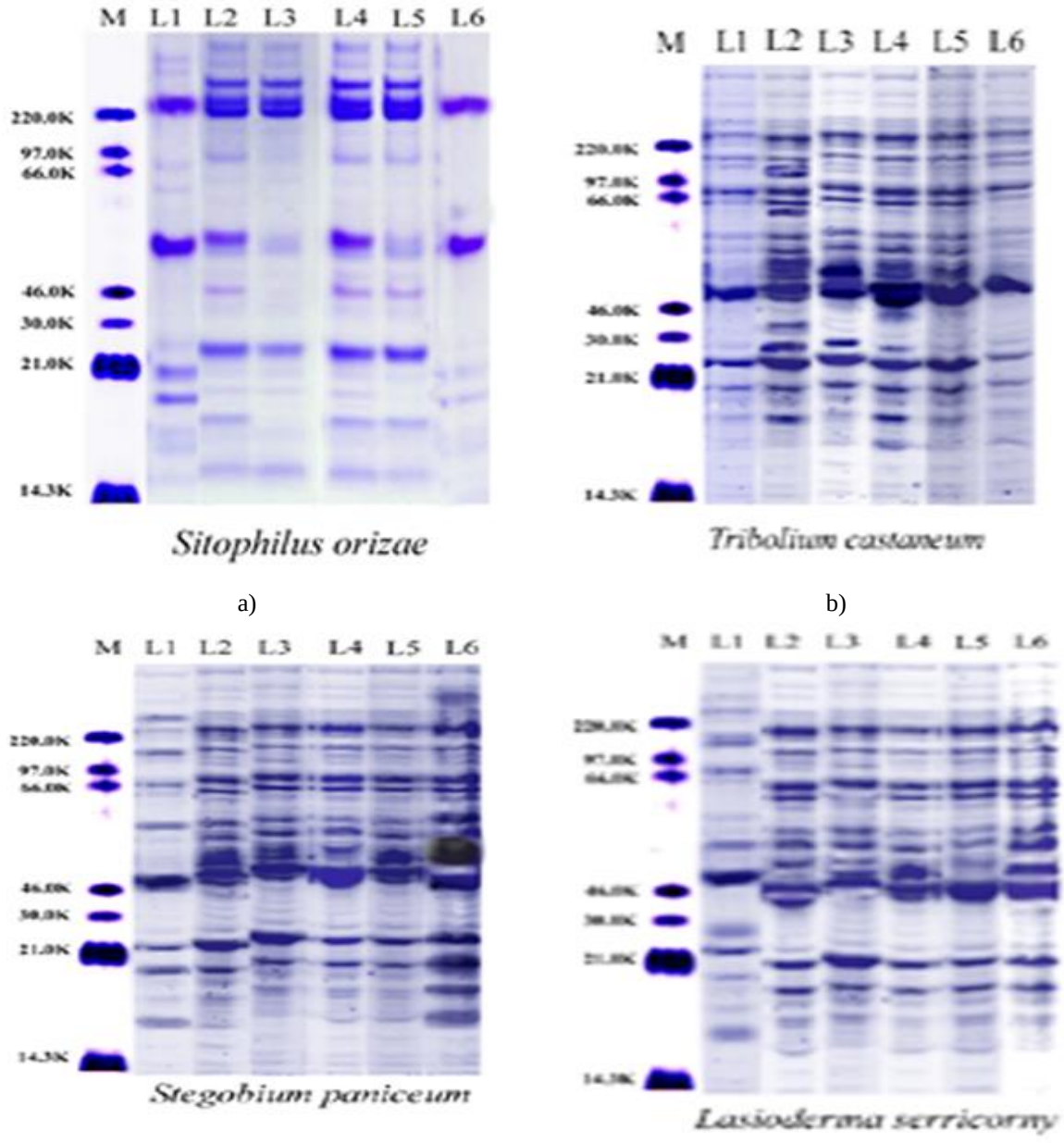


Figure 4. SDS–PAGE profile showing the changes in protein patterns of (A) *Sitophilus arise*, (B) *Tribolium castaneum*, (C) *Stegobium paniceum*, and (D) *Lasioderma serricorne* infected with different concentrations of (Cypovirus1). The protein profiling image of the SDS–PAGE electrophoresis M. protein ladder marker, L1. Control healthy of stored product beetle, L2. Weevils treated with 10gm/100gm CPV, L3. Weevils treated with 10gm/500gm CPV, L4. Weevils treated with 10gm/1000gm CPV, L5. Weevils treated with 10gm/10000gm CPV and L6. Weevils treated with 10gm/25000gm CPV.

Table 3. Hypothesized protein markers for the determined effect of different concentrations treatments from the Cypovirus1 on protein bands in infected *Sitophilus orizae*, *Lasiderma serricorny*, *Stegobium paniceam*, and *Tribolium castaneum*

Bands No.	RF	MW	Bands presented in healthy and absented in infected	Bands presented in infected and absented in healthy				
			Healthy	Different CPV concentrations				
			Sitophilus orizae					
			L1	L2	L3	L4	L5	L6
42	0.309	96	-	+	+	+	+	
50	0.416	65	-	-	+	+	+	+
55	0.503	48	-	+	+	+	+	+
57	0.537	42	+	-	-	-	-	-
61	0.622	31	-	+	+	+	+	+
65	0.663	27	+	-	-	-	-	-
66	0.673	26	-		+	+	+	+
67	0.691	24	+	-	-	-	-	-
			Lasiderma serricorny					
40	0.266	112	-	+	+	+	+	+
43	0.312	95	+	-	-	-	-	-
47	0.394	71	-	+	+	+	+	+
51	0.421	64	+	+	+	-	-	-
53	0.474	53	-	+	+	+	+	+
54	0.493	49	+	-	-	-	-	-
56	0.504	47	-	+	+	+	+	+
62	0.631	30	-	+	+	+	+	+
63	0.644	29	+	-	-	-	-	-
			Stegobium paniceam					
49	0.400	69	+	+	-	-	-	-
52	0.470	54	-	+	+	+	+	+
53	0.474	53	+	-	-	-	-	-
58	0.554	40	+	-	-	-	-	-
60	0.618	32	-	+	+	+	+	+
62	0.631	30	+					
64	0.647	28	-	+	+	+	+	+
68	0.721	22	-	+	+	+	+	+
74	0.989	8	-	+	+	+	+	+
			Tribolium castaneum					
46	0.32	92	+	-	-	-	-	-
48	0.395	70	-	+	+	+	+	+
59	0.559	39	-	+	+	+	+	+
63	0.644	29	-	+	+	+	+	+
67	0.691	24	-	+	+	+	+	+
70	0.799	16	+	-	-	-	-	-
71	0.923	11	-	+	+	+	+	+
72	0.925	10	+	-	-	-	-	-

Inference brought about in **Table 3** hinted to consideration that it would be acceptable if we assume there is a possible correlation between the virus infection and the band presence and / or absence and showed summary for protein bands markers suggested for detection of Cypovirus1 infection in adult weevils *Sitophilus oryzae*, larvaes of *Lasioderma serricorne*, *Stegobium paniceum*, and *Tribolium castaneum* were presented in healthy larvae and absented in infected , on the other hand, showed bands presented in infected larvae and absented in healthy such as the protein bands No. (42, 50, 55, 61 and 66), (40, 47, 53, 56 and 62), (52, 60,64, 68 and 74) and (48, 59, 63, 67 and 71) were presented in infected weevils *(Sitophilus oryzae*, larvaes of *Lasioderma serricorne*, *Tribolium castaneum* and *Stegobium paniceum*), respictivily, compared with healthy weevils which have protein bands No. (57, 65 and 67), (43, 51, 54 and 63), (49, 53, 58 and 62) and (46, 70 and

72) were absented in infected weevils *(Sitophilus oryzae*, larvaes of *Lasioderma serricorne*, *Tribolium castaneum* and *Stegobium paniceum*), respictivily, [12].

CONCLUSION

This study was carried out to incidence four different larvae's attack stored products were collected from a different location in Mecca regions, KSA and identify. Viral identity was confirmed by host range, indirect ELISA, electron microscopy examination, and RT-PCR test. On the other hand, we are studying the effects of different concentrations of new Bio-component of Cypovirus1 infection on four different larvae's study their impact on the rates and severity of the infection. SDS-polyacrylamide gel electrophoresis test showed sole bands become variously distinguishable between infected cells by Cypovirus1 comparing with healthy cells in these larvae under study.

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Conflict of interest: None

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REFERENCES

[1] Gilles AF, Schinko JB, Averof M. Efficient CRISPR-mediated gene targeting and transgene replacement in the beetle Tribolium castaneum. Development. 2015;142(16):2832-9. doi: 10.1242/dev.125054.

[2] Ngom D, Fauconnier ML, Malumba P, Dia CA, Thiaw C, Sembène M. Varietal susceptibility of maize to larger grain borer, Prostephanus truncatus (Horn)(Coleoptera; Bostrichidae), based on grain physicochemical parameters. PloS one. 2020;15(4):e0232164. doi: 10.1371/journal.pone.0232164.

[3] Grünwald S, Adam IV, Gurmai AM, Bauer L, Boll M, Wenzel U. The Red Flour Beetle Tribolium castaneum as a Model to Monitor Food Safety and Functionality. Adv Biochem Eng Biotechnol. 2013;135:111-22. doi: 10.1007/10_2013_212.

[4] Özberk F, Özberk İ, Yücel A, Atlı A, İzol D. Makarnalık buğdayda (Triticum durum Desf) kapra böceği (Trogoderma granarium Everts, 1898): Bazı tane özellikleri ve pazarlama fiyatlarına etkileri. Turk Entomol Derg. 2017;41(2):207-18.

[5] Querner P, Oberthaler E, Strolz M. Biological Pest Control of a Biscuit Beetle (Stegobium paniceum) Infestation in an Old Masters Paintings Storage Area. Stud Conserv. 2019;64(7):373-80. doi: 10.1080/00393630.2018.1537352.

[6] Nasir H, Noda H. Yeast-like symbiotes as a sterol source in anobiid beetles (Coleoptera, Anobiidae): possible metabolic pathways from fungal sterols to 7-dehydrocholesterol. Arch Insect Biochem Physiol: Published in Collab Entomol Soc Am. 2003;52(4):175-82. doi: 10.1002/arch.10079.

[7] Farber IM, Kudryashova MA, Galstyan LA, Shatalina SI. Current aspects of antibacterial drug administration when treating nosocomial Pneumonia. J Adv Pharm Educ Res. 2021;11(1):29-34.

[8] Soboleva MS, Loskutova EE, Kosova IV, Amelina IV. Problems and the Prospects of Pharmaceutical Consultation in the Drugstores. Arch Pharm Pract. 2020;11(2):154-9.

[9] Bond EJ, Bond EJ. Manual of Fumigation for Insect Control: FAO Plant Production and Protection Paper No. 54. FAO; 1984. 432 p.

[10] Gani M, Hassan T, Saini P, Gupta RK, Bali K. Molecular Phylogeny of Entomopathogens. In: Khan M., Ahmad W. (eds) Microbes for Sustainable Insect Pest Management. Sustainability in Plant and Crop Protection. 2019 (pp. 43-113) Springer, Cham. doi: 10.1007/978-3-030-23045-6_3.

[11] Zhan Z, Guan L, Wang J, Liu Z, Guo Y, Xiao Y, et al. Isolation and genomic characterization of a cypovirus from the oleander hawk moth, Daphnis nerii. J Invertebr Pathol. 2019;163:43-7. doi: 10.1016/j.jip.2019.03.002.

[12] Swevers L, Feng M, Ren F, Sun J. Antiviral defense against Cypovirus 1 (Reoviridae) infection in the silkworm, Bombyx mori. Arch Insect Biochem Physiol. 2020;103(3):e21616. doi: 10.1002/arch.21616.

[13] Altinli M, Schnettler E, Sicard M. Symbiotic Interactions Between Mosquitoes and Mosquito Viruses. Front Cell Infect Microbiol. 2021;11:694020. doi: 10.3389/fcimb.2021.694020.

[14] He L, Hu X, Zhu M, Liang Z, Chen F, Zhu L, et al. Identification and characterization of vp7 gene in Bombyx mori cytoplasmic polyhedrosis virus. Gene. 2017;627:343-50. doi: 10.1016/j.gene.2017.06.048.

[15] Zhang G, Yang J, Qin F, Xu C, Wang J, Lei C, et al. A reverse genetics system for cypovirus based on a bacmid expressing T7 RNA polymerase. Viruses. 2019;11(4):314. doi: 10.3390/v11040314.

[16] Kelland K. Cold virus hitches a ride to kill cancer: study. Reuters. 2012.
[17] Djaman K. Le role des maladies des insectes dans l'ecosysteme des foreurs de tige et mineurs d'épi du mais: inventaire et efficacité des entomopathogènes: Cas du Bénin. Ing. Agr. thesis, Ecole Superieure D'Agronomie, Lomé-Togo, 1997.
[18] Larsen RC, Duffus JE. A Simplified Procedure for the Purification of Curly Top Virus and the Isolation. Phytopathology. 1984;73:114-8.
[19] Dollet M, Accotto GP, Lisa V, Menissier J, Boccardo G. A geminivirus, serologically related to maize streak virus, from Digitaria sanguinalis from Vanuatu. J Gen Virol. 1986;67(5):933-7.
[20] Hames BD, Dickwood D. Gel electrophoresis of proteins. IRL-Press. 1981.
[21] Freedman DA, Pisani R, Purves R. Statistics, 4th edition. 2007. W.W. Norton & Company ISBN 978-0-393-92972-0.
[22] Jakubowska AK, Nalcacioglu R, Millán-Leiva A, Sanz-Carbonell A, Muratoglu H, Herrero S, et al. In search of pathogens: transcriptome-based identification of viral sequences from the pine processionary moth (Thaumetopoea pityocampa). Viruses. 2015;7(2):456-79. doi: 10.3390/v7020456.
[23] Marzban R, He Q, Liu X, Zhang Q. Effects of Bacillus thuringiensis toxin Cry1Ac and cytoplasmic polyhedrosis virus of Helicoverpa armigera (Hübner)(HaCPV) on cotton bollworm (Lepidoptera: Noctuidae). J Invertebr Pathol. 2009;101(1):71-6.
[24] Marzban R. Midgut pH profile and energy differences in lipid, protein and glycogen metabolism of Bacillus thuringiensis CRY1Ac toxin and cypovirus-infected Helicoverpa armigera (Hübner)(Lepidoptera: Noctuidae). J Entomol Res Soc. 2013;15(1):45-53.