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ORIGINAL ARTICLE

Effect of selected baculoviruses on oviposition preference by *Trichogramma chilonis* (Trichogrammatidae: Hymenoptera)

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KEYWORDS

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Abstract Microbial insecticides are effective, environmental friendly and are widely used worldwide to control insect pests. Nucleopolyhedroviruses and granuloviruses belonging to family *Baculoviridae* are widely used for control of caterpillar pests on wide varieties of crops and vegetables. The selected baculoviruses (BVs) were evaluated for oviposition preference by *Trichogramma chilonis* (Ishii) of virus treated and untreated (water: control) host eggs (*Sitotroga cerealella* Olivier), which revealed no significant difference among the used concentrations regarding oviposition preference. All the used concentrations of *Helicoverpa armigera* nucleopolyhedrovirus (HaNPV), *Spodoptera exigua* nucleopolyhedrovirus (SeMNPV) and *Cydia pomonella* granulovirus (CpGV) including 12.5×, 6.25×, 2.5×, 1.25× and 0.625× were harmless ($E > 30\%$) for parasitism by *T. chilonis* as comparison of virus treated and untreated control eggs showed similar parasitism i.e., $\leqslant 15\%$ reduction over control in parasitism. Thus it was concluded that all three types of baculoviruses were compatible with the parasitism by *T. chilonis* at all treated concentrations.

1. Introduction

Biological control is globally preferred over synthetic pesticides for its effective role to suppress the population of insect pests (Omkar and Kumar, 2016). Determination of adverse impacts of pesticide on beneficials is required to find chemicals not compatible with natural enemies, in order to effectively integrate both chemical and biological controls (Croft, 1990; Ruberson et al., 1998; Stark et al., 2007; Shoeb, 2010; Khan et al., 2014; 2015a,b). Integrated pest management and sensible use of pesticides are needed to keep the losses caused by pests under economic threshold levels (Karuppuchamy and Venugopal, 2016).

Insecticides resistance to the broad-spectrum pesticides led to limit the effectiveness of many such chemicals and this resulted in intensive efforts to find out alternate methods of control (Nathan et al., 2004; Sagheer et al., 2008). Selective insecticides may be valuable to effectively overcome increasing pesticide resistance (Nabil and El-Wakeil, 2013). Thus use of selective insecticides to manage pests contributes to the conservation of natural enemies associated with crops (Thomson et al., 2000).

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Biopesticides including botanical insecticides and microbial pesticides are safe, ecologically acceptable, and are highly effective against target pest but are relatively safe to natural enemies (Sagheer et al., 2008). The microbial insecticides can be equally as effective as synthetic chemicals to control insect pests (Khan et al., 2014).

Nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) are microbial insecticides belonging to the major group of viruses known as baculoviruses. They are widely used as natural enemies of insect pests (Moscardi, 1999; Khan et al., 2014), and have been used since the early 1890s (Huber, 1986; Khan et al., 2014). They are obligate pathogens and are commonly used to control Lepidoptera and Hymenoptera (Mazzone, 1985; Khan et al., 2014). They are host specific (Federici, 1997; Khan et al., 2014), and replicate in the host cells. They usually infect their larval hosts following ingestion (Andreadis, 1987; Khan et al., 2014).

Trichogramma species are the most widely used among the parasitoids for pest management worldwide (Jalali et al., 2016). They have been extensively used as natural enemies (Shoeb, 2010), and have achieved appreciable pest control success in many crop ecosystems, while their role in the biological control programs of pest management is well understood (Smith, 1996; Sorokina, 1999; Hussain et al., 2010). They were recognized as biological control agents in the 1900s, which led to their mass rearing, aiming to use them in pest control programs (Smith, 1996; Bastos et al., 2006). They control pests particularly among the Lepidoptera (Khan et al., 2015a). Around 18 different species of *Trichogramma* are being mass reared in 16 countries to control insect pest on 18 million of hectares (Hassan, 1994).

Trichogramma chilonis (Ishii) is widely distributed throughout the Indian subcontinent and has been effectively used to control caterpillar pests in the field (Manjunath et al., 1985; Khan et al., 2014). They control common pests in Pakistan including sugarcane borer (*Chilo sacchariphagus*) in sugar cane, diamondback moth (*Plutella xylostella*) in cabbage and other vegetables, and cotton bollworms (*Helicoverpa armigera*) in cotton and corn.

The baculoviruses used in this work are: HaNPV (HELICOVEX), SeMNPV (SPEXIT) and CpGV (MADEX), two of them are NPVs and the other is a GV. They were evaluated for their effect on the oviposition preference by *T. chilonis*.

2. Materials and methods

2.1. Rearing of *Sitotroga cerealella*

The young larvae of grain moth *S. cerealella* hatched and infested the wheat grain within a week host eggs were sprinkled on sterilized grains in a plastic/metal tray (30 × 18 cm) in the laboratory of Entomology Division, NIFA, Peshawar, (Pakistan). The infested wheat was then shifted to plastic rearing jars (15 × 20 cm), and their openings were subsequently covered with a piece of cotton cloth, and were maintained in the laboratory at average conditions of 24 ± 6 °C, 65 ± 10% relative humidity (RH) and 16:8 (L:D) until adults' emergence after 20–25 days.

Regular collections of emerged moths from the rearing jars every 24 h were carried out by an electric suction apparatus in the oviposition jar (10 × 15 cm) covered at bottom by mesh (mesh No. 30–40 pore size). The jar containing adult moths was placed over the corn flour in a metal/plastic tray, and was given a single turn to adhere the flour to the jar mesh at the bottom for egg laying. The jar was then carefully placed on metal/plastic tray until next day (24 h) allowing the moths to lay eggs in the flour. Next day, the host eggs were collected by sieving the flour and the eggs were used in the experimental work as well as for maintenance of *S. cerealella* culture in the laboratory.

2.2. Rearing of *Trichogramma chilonis*

Approximately 1000–1300 eggs of *S. cerealella* (less than 24 h old) were glued onto a hard paper card (5 × 8 cm). Several cards were prepared and dried for one h. and each card was subsequently exposed for parasitism in glass jar (5 × 12 cm) for 24 h containing approximately 30–40 adults (mixed-gender) of *T. chilonis*. The opening of the glass jars was tightly covered with muslin cloth to prevent escape of the adults. Droplets of honey were scattered on the inner surface of the glass jar walls as food for the parasitoid. The jar was placed in the lamp light in order to obtain good parasitism by the tiny wasp. Subsequently, the parasitized card was removed and was transferred to another glass jar of the same size, and the jar was incubated at the 23 ± 3 °C, 70 ± 10% RH and 14:10 (L:D) conditions until adult emergence. Stock culture of *T. chilonis* was produced for use in the experimental work.

2.3. Preparation of different concentrations of pesticides solution

Commercially available three types of BVs (Table 1) including HaNPV, SeNPV and CpGV were diluted with tap water to prepare their respective stock solutions. The stock solution was diluted (serial dilutions) and 5 different concentrations (12.5×, 6.25×, 2.5×, 1.25× and 0.625×) of insecticides were prepared for use in the experiments by the formula: $C_1V_1 = C_2V_2$, where C_1 and V_1 are the concentration and volume of commercial pesticides/stock solution, respectively, while C_2 and V_2 are the concentration and volume of the required pesticide solutions (diluted), respectively.

2.4. Testing for oviposition preference by Trichogramma chilonis

Approximately 60–65 fresh *S. cerealella* eggs were glued on the hard paper card (5 × 8 cm). The card was dried for 1–2 h and was subsequently cut into six card strips (0.9 × 8 cm each) each containing 10 host eggs. Card strips were treated by dipping for 1–2 s in the different solution of each type of BVs or control in the laboratory. Each card was dried at the aforementioned laboratory conditions, and subsequently one card containing virus treated host eggs and the other containing water treated (control) host eggs were exposed to a pair of *T. chilonis* (< 24 h old) in the glass vial (1 × 10 cm) to evaluate their effect on oviposition preference in choice design. The vial was exposed to light for 3 h for completion of parasitization. The trial consisted of six replications for each concentration and treatment. The exposed parasitizing female was removed after 3 h from each vial and all the vials were incubated at aforementioned conditions until pupae formation. The data were recorded by counting darkened eggs (pupae) 7 days after exposure to the parasitoids separately for each card, and data

Table 1 Label description of selected baculoviruses used in the experiments.

Virus and formulation	Active ingredient	Conc	Trade name	Manufacturer/supplier	Condition of storage	FRC
HaNPV (suspension concentrate)	*Helicoverpa armigera* nucleopolyhedrovirus	7.5×10^{12} NPV/liter	HELICOVEX	Andermatt Biocontrol (Switzerland)	−10–37 °C, RH (70 ± 10%) (protected from light)	50–200 ml
SeNPV (suspension concentrate)	*Spodoptera exigua* nucleopolyhedrovirus	3.75×10^{12} NPV/liter	SPEXIT	Andermatt Biocontrol (Switzerland)	Same as above	100–200 ml
CpGV (suspension concentrate)	*Cydia pomonella* granulovirus	3×10^{13} GV/liter	MADEX	Andermatt Biocontrol (Switzerland)	Same as above	100 ml

were compared for both virus treated and control to determine oviposition preference by the parasitoid.

2.5. Data analysis

The data were analyzed using GLM (Statistix 9) on average parasitization. Tukey HSD test ($p = 0.05$) were used for mean separation. Reduction in parasitism (%) over controls were evaluated by toxicity categories of International Organization for Biological Control (IOBC)/West Palaearctic Regional Section (WPRS) (Hassan et al., 1994; Sterk et al., 1999): 1 = harmless ($E < 30\%$); 2 = slight harmful ($30 \leqslant E \leqslant 79\%$); 3 = moderately harmful ($79 < E \leqslant 99\%$); 4 = harmful ($>99\%$), where "E" stand for effect of the pesticide on the biological control agent measured as the reduction in percentage of parasitism over control.

3. Results and discussion

All three types of BVs including HaNPV, SeMNPV and CpGV were evaluated for oviposition preference of host eggs (*S. cerealella*) by *T. chilonis*. Their active ingredient, supplier of such products and the conditions used for their storage are given in Table 1. The analysis of variance revealed (Table 2) no significant difference for parasitism among the used concentrations regarding both viruses treated and untreated (control: water) host eggs ($p > 0.05$).

All the used concentrations of selected BVs were found harmless for parasitism by *T. chilonis* based on the comparison of virus treated and control host eggs which showed $\leqslant 15\%$ reduction over control in parasitism (Fig. 1). Furthermore, both types of host eggs were similarly preferred for parasitism by tiny parasitoid, and all used concentrations of BVs showed statistically at par with control (Table 2). Therefore, results demonstrated that both virus treated and untreated host eggs observed similar parasitism.

Sufficient published studies are not available on effects of the pesticides on *Trichogramma* spp. However, few experiments were conducted by various scientists assessing toxicity of microbial insecticides to beneficials including *Trichogramma* (Khan et al., 2014). The compatibility of BVs with *T. chilonis* in the current study were supported by Moscardi (1999) and Khan et al. (2014), who described viral insecticides as not harmful to humans and are compatible with natural enemies of target pests. Similarly, Khan et al. (2014) described HaNPV as very safe microbial insecticide for emergence of as well as parasitism by *T. chilonis* and can effectively manage the target pests. For example, Ramteke and Gangurde (2011) described that both fresh HaNPV (2 × 109 POBs/ml @ 250 ml/ha and 1 × 109 POBs/ml @ 500 ml/ha), and stored HaNPV formulations (stored for 1 year (2 × 109 POBs/ml @ 250 ml/ha and 1 × 109 POBs/ml @ 500 ml/ha) led to effectively reduced larval populations of *H. armigera* and led to higher yields of pigeon pea. Similarly, treatment of bacterium *Pseudomonas fluorescens* did not exhibit adverse impacts on the parasitism and emergence success of the *T. chilonis* (Gandhi et al., 2005; Khan et al., 2014). Sagheer et al. (2008) reported that integration of bioinsecticides (neem and *Bacillus thuringiensis*-Bt) and *Trichogramma* spp. can enhance effectiveness of the parasitic wasps against rice leaf folder *Cnaphalocrocis medinalis*.

Table 2 Oviposition preference (mean ± SE) of previously virus treated and untreated (control) host eggs (*S. cerealella*) by single female *T. chilonis* under choice design, and means comparison (Tukey's HSD, $p = 0.05\%$ or 5%).

Type of BVs	Concentration (mean ± SE); virus treatments and control									
	12.5×		6.25×		2.5×		1.25×		0.625×	
	T	C	T	C	T	C	T	C	T	C
HaNPV	8.83 ± 0.75a	9.00 ± 0.63a	8.83 ± 0.48a	9.17 ± 0.48a	8.17 ± 0.91a	9.17 ± 0.40a	8.17 ± 0.60a	8.50 ± 0.43a	7.83 ± 1.60a	8.00 ± 0.45a
SeMNPV	8.67 ± 0.80a	9.00 ± 0.26a	7.50 ± 1.63a	7.50 ± 0.72a	8.00 ± 0.89a	8.00 ± 0.93a	8.17 ± 0.98a	8.17 ± 0.48a	7.50 ± 0.89a	8.83 ± 0.31a
CpGV	9.17 ± 0.40a	9.50 ± 0.34a	9.83 ± 0.17a	9.83 ± 0.17a	9.83 ± 0.17a	10.0 ± 0.00a	7.83 ± 0.95a	9.17 ± 0.65a	9.17 ± 0.48a	9.50 ± 0.22a

ANOVA results for HaNPV							
Concentration	df		f		p		Remarks
	T	C	T	C	T	C	
	4	4	0.22	1.10	0.9246	0.3775	Not significant

ANOVA results for SeMNPV							
Concentration	df		f		p		Remarks
	T	C	T	C	T	C	
	4	4	0.21	1.07	0.9321	0.3905	Not significant

ANOVA results for CpGV							
Concentration	df		f		p		Remarks
	T	C	T	C	T	C	
	4	4	2.49	0.85	0.0690	0.5081	Not significant

All means sharing same letter "a" within a column/among columns, are not significantly different (Tukey's HSD, $p > 0.05$). T stands for virus treatment and C stands for control.

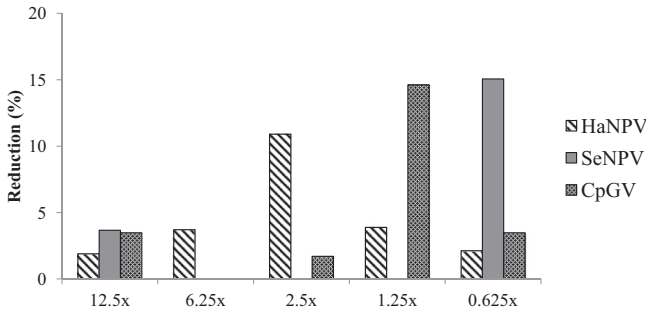

Figure 1 Percent reduction in parasitism over control by *T. chilonis* in oviposition preference test (choice design).

Plant extracts and microbial formulations may effectively replace conventional synthetic insecticides (Khan et al., 2014). Biopesticides replaced synthetic pesticides based on their generally low environmental pollution, low toxicity to humans, and other benefits (DeBach and Rosen, 1991; Qi et al., 2001; Gandhi et al., 2005), and are effective under field conditions when integrated with biological control (Huffaker, 1974; Beddington et al., 1978; Barclay, 1982; DeBach and Rosen, 1991; Van Driesche and Bellows, 1996; Qi et al., 2001; Gandhi et al., 2005).

Viruses belonging to family *Baculoviridae* have been used as pesticides for biological control of pests (Copping and Menn, 2000; Souza de et al., 2007). They have narrow specificity, and are harmless to people and wildlife and have been used in many countries around the world (Souza de et al., 2007). More than 600 species of baculoviruses attack pests belonging to order Lepidoptera, Hymenoptera and Diptera (Souza de et al., 2007). Successful pest controls rely on the use of chemicals and several viruses (Prasad and Srivastava, 2016). According to modern classification based on ICTV (International Committee on Taxonomy of Viruses): the family *Baculoviridae* have been divided into four genera: (1) Alphabaculovirus (lepidopteran-specific NPV), (2) Betabaculovirus (lepidopteran-specific Granuloviruses), (3) Gammabaculovirus (hymenopteran-specific NPV) and (4) Deltabaculovirus (dipteran-specific NPV) (Jehle et al., 2006).

Nucleopolyhedrovirus belonging to baculoviruses in the family *Baculoviridae*, consists of large rod-shaped nucleocapsids with circular double-stranded DNA (Bilimoria, 1986; Khan et al., 2014). The outer lipoprotein envelope surrounds the nucleocapsid (Khan et al., 2014). The virions are invisible by light microscope, however, large occlusion bodies (OB) produced in the host cell, range from 1 to 15 μm, are visible in a compound microscope, and occlude many virions protecting them to some degree during host-to-host transfer (Benz, 1987; Ignoffo et al., 1989; Khan et al., 2014). They are commonly associated with the Lepidoptera and Hymenoptera (Mazzone, 1985) including cotton bollworm, beat armyworm and codling moth. They are very effective to manage a variety of pest insects, although some insects survive and show only sublethal effects ranging from deformed pupae (Peng et al., 1997) to slower development, lower weight, reduced reproduction and shorter life span (Rothman and Myers, 1996). Attempts at controlling insect populations with nucleopolyhedroviruses (NPVs) date to at least the early 1890s (Huber, 1986). *Cydia pomonella* granulosis virus (CpGV) is a granulovirus with double-stranded DNA and forms small bodies called granules containing a single virion. CpGV is a biological control agent of Codling moth *C. pomonella*, and kills its host in the same instar as infection.

H. armigera nucleopolyhedrovirus or HaNPV is a microbial pesticide, marketed as "HELICOVEX" in the world including Pakistan, and effectively control caterpillar pests including, *H. armigera* (Hubner) in cotton, tomato, pea, tobacco, maize, sweet corn and lettuce. Pulses, sunflower, wheat, lucerne, potato and other crops are hosts of *H. armigera* larvae in Pakistan (Ahmed et al., 1992; Khan et al., 2014). The virus kills young instars (L1–L3) and infects older larvae. It is well suited for organic and integrated pest management strategies and resistance management programs (Andermatt and Andermatt, 2015).

Spodoptera exigua nucleopolyhedrovirus available as SPEXIT worldwide including Pakistan is a highly specific/selective insecticide which effectively controls beet armyworms *Spodoptera exigua* (Hübner) on various greenhouse and open field crops including corn, cotton, soybean, alfalfa, sweet pepper, tomato, melon, cucurbit, strawberry, sugar-beet, bean, cabbage, citrus, garlic, groundnut, lettuce, maize, onion, potato, pea, rice and tobacco in many parts of the world. The use of SPEXIT significantly reduces crop damage and pest population (Andermatt and Andermatt, 2015).

MADEX, a bioinsecticide containing *C. pomonella* Granulovirus (CpGV) as active ingredient, is a highly specific/selective insecticide against the codling moth (*C. pomonella* (Linnaeus) (Andermatt and Andermatt, 2015). They kill the larvae in their early instars before causing damage to the plants (Andermatt and Andermatt, 2015). At a lower dosage, the larvae will be killed at a later instar providing excellent population control. It can be used by organic growers, but is also an effective product for use in IPM and conventional control programs against codling moths in apple, pear, walnuts, quinces, apricots, peaches, almonds, kakis, medlars, oranges and others (Andermatt and Andermatt, 2015).

4. Conclusion

Selected baculoviruses were assessed for their effect on the oviposition preference of virus treated and untreated host eggs of *Sitotroga cerealella by Trichogramma chilonis*. All the three types of baculoviruses including HaNPV, SeNPV and CpGV tested at concentrations including 12.5x, 6.25x, 2.5x, 1.25x and 0.625x against *T. chilonis* did not demonstrated oviposition preference of untreated host eggs compared to virus treated eggs. Therefore, it was concluded that all the three types of BV are compatible with parasitism by parasitoids at the used concentrations (under the choice).

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