



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2019; 7(2): 1006-1014

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Received: 01-01-2019

Accepted: 03-02-2019

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## Behaviour of solitary endoparasitoids *Glyptapanteles agamemnonis* and *Meteorus pulchricornis* towards virus infected and parasitized *Spilarctia obliqua* larvae and the interaction thereof

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#### Abstract

The interactions between two solitary endoparasitoids, *Glyptapanteles agamemnonis* (Wilkinson 1932) (Hymenoptera: Braconidae) and *Meteorus pulchricornis* (Wesmael 1835) (Hymenoptera: Braconidae) and the parasitoid - virus were explored towards healthy vs. *Nucleopolyhedrovirus* infected and parasitized vs. unparasitized *Spilarctia obliqua* (Walker 1855) (Lepidoptera: Arctiidae) larvae to understand the outcome of within-host competition. The results revealed lack of discrimination in the behaviour of both the parasitoids towards virus infected *S. obliqua* larvae within 48 hrs (from ingestion of virus contaminated diet upto 48 hrs) of viral infection. However, discrimination became evident 48 hours post-infection in the form of antennal contacts. Interestingly, no significant difference was observed in the number of ovipositor insertions. The parasitoids also failed to discriminate between unparasitized larvae and larvae parasitized by either of the two parasitoids. It was found that almost all (>99 per cent) the host larvae which were first infected with virus and then exposed to parasitoids for parasitisation succumbed to virus. However, when the host larvae were treated with virus 4 to 6 days post-parasitism, more than fifty per cent of parasitoids managed to complete their development. The effect of virus on the parasitoids was therefore dependent on the interval between parasitism and viral infection and postponing the exposure of pre parasitized larvae to baculovirus infection increased the survival of both the parasitoid species. In case of simultaneous parasitisation of the host larvae by both the wasp species, *M. pulchricornis* out competed *G. agamemnonis*. In general, the parasitoids oviposition behaviour was not affected by the virus-infected and parasitized hosts. However, for the sake of successful parasitoid emergence, viral applications must be done few days after parasitoid release in augmentative bio control programmes.

**Keywords:** Arctiidae, baculovirus, *Braconidae*, interspecific competition, multiple parasitism, *Nucleopolyhedrovirus*, Parasitoids.

#### Introduction

Biological control with natural enemies has been increasingly followed due to environmental, economical, social and ecological problems with insecticides [1]. Since various natural enemies also coexist for natural control of insect pests, it is imperative to understand the interaction between baculoviruses and parasitoids to effectively integrate them in biological control programs [2, 3]. These biological control agents may act synergistically, additively or antagonistically. Synergetic interactions between pathogens and insect natural enemies can enhance control efficacy, whereas antagonistic interactions can reduce total control efficacy [4, 5]. Although baculoviruses do not infect insect natural enemies, an important aspect in the development of baculovirus as bio pesticides is the evaluation of their possible impact on predators and parasitoids [6]. When a host is simultaneously infected by virus and parasitized by an insect parasitoid, possible interactions include the death of the parasitoid due to virus-induced host mortality [7], or due to toxic factors produced by the virus in infected hosts [8]. Besides, virus production can also be impaired due to competition for host resources from the developing parasitoid [9].

The bihar hairy caterpillar, *Spilosoma* (= *Spilarctia*) *obliqua* is a serious pest attacking nearly 126 plants species such as oilseeds, pulses, vegetables, fodder, fiber crops, and fruit trees in Bangladesh, Myanmar, India, Pakistan, Phillipines and Sri Lanka [10, 11]. At present,

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parasitoids along with other natural enemies such as *Nucleopolyhedrovirus* (NPV) are being evaluated to manage early instars of *S. Obliqua* [12]. The braconid parasitoids viz, *Glyptapanteles agamemnonis* and *Meteorus pulchricornis* are most crucial parasitoids which regulate the population of *S. obliqua* on various hosts [13]. In addition, *S. obliqua* *Nucleopolyhedrovirus* (SoNPV) is also being applied for the management of *S. obliqua* populations in many cropping systems. Number of theoretical and laboratory studies showed parasitoids and pathogens of insects as important antecedents of their host's population dynamics and structure [14]. The interaction between the host and the natural enemies has been examined independently, but these parasites are unlikely to act alone and aggregate of interactions might be anticipated between them [15]. The impact of parasitism on viral insecticidal characteristics will be particularly relevant when virus production involves the field application of virus, followed by harvesting of virus-infected larvae and the subsequent recycling of this inoculum for pest control throughout the growing season. Although these natural enemies play an important role in pest population dynamics, however, several factors viz., insect stages attacked, duration of infection or parasitism, toxin production by the virus, parasitoid discrimination between healthy and infected hosts, parasitoid mediated manipulation of the host immune response and virus dispersal by parasitoids should taken into consideration to make their integration into pest management programs effective [16]. Such types of interactions are crucial for devising the management strategy against insect pests and have broad ecological implications. In accordance with this the present study was planned to understand the outcome of within-host competition between parasitoid species and between the parasitoids and the virus in order to use them most effectively.

### Materials and methods

*Spilosoma* (= *Spilarctia*) *obliqua* larvae were collected between August to October 2011 from Chatha, Jammu. Identification of insect was checked and confirmed throughout larval development while promising parasitoids that emerged from them were identified by T. C. Narendran as *Glyptapanteles agamemnonis* and *Meteorus sp.* [13] while the identity of this virus was confirmed through molecular characterization as multiple *Nucleopolyhedrovirus* [17].

### Insect rearing

#### Host insect: *Spilosoma (Spilarctia) obliqua*

The nucleus culture of *S. obliqua* was established by collecting the adults using light trap during August, 2011 at SKUAST- J, FOA- Chatha. These adults were kept in glass jars (50×30 cm diameter) lined with filter paper and covered with muslin cloth. The moths were fed on 10 per cent sucrose solution and allowed to mate for egg laying on glass surface or paper. Eggs obtained from these adults were kept in Petri plate. Newly hatched larvae were placed in plastic dishes (35 × 10 mm) until they reached sufficient size to be reared in glass Petrie dishes (100 × 10 mm). Larvae that hatched at the same day were reared in groups of up to 30 larvae. Larvae that showed synchronised development were isolated in a different container and maintained on castor leaves that were previously washed and air dried before being fed to larvae. Uneaten foods along with faeces were removed regularly in order to maintain hygiene in the rearing containers. The feed

was changed daily and rearing space was increased regularly by using more number of jars for avoiding overcrowding of the larvae for promoting uniform growth and development of the larvae until pupation. The pupae were sexed and the progeny of individual females were marked and reared in separate dishes as laboratory cohort at temperature of 26 ± 2°C and 70 ± 10 % RH and L: D (16:8) photoperiod for further studies.

### Parasitoids

*G. agamemnonis* and *M. pulchricornis* used in this study emerged from naturally parasitized field collected *S obliqua* larvae. For each parasitoid species, groups of one hundred cocoons were placed and maintained in 400 ml glass vials and when required adult females were removed for experiments. Adults were provided with honey solution (10%). On the third day after adult parasitoid emergence, one isolated female wasp was allowed to parasitize one isolated third instar larva of *S obliqua*. After the first sting, the host was removed from the parasitoid and individually kept, until emergence of the parasitoids, in a plastic container (4.5 x 3cm). Each larval host was maintained on castor (*Ricinus communis*) leaves as described earlier. After larval parasitoid emergence and cocoon formation, each group was maintained in a plastic container (4.5 x 3cm) with a hole covered by muslin cloth. Emerged adults were supplied with honey solution (10%) until their death. The parasitoids that were initially collected from field served as nucleus culture and were maintained in the laboratory on *S. obliqua* larvae. The parasitized larvae were reared on tender castor leaves in glass jars (50 × 30 cm diameter) covered with muslin cloth. The cocoons that emerged from the laboratory parasitized hosts were collected, harvested and placed in glass jars (20 × 15 cm) covered with muslin cloth. Upon emergence, adult parasitoids were transferred into separate test tubes (5 × 2 cm) with the help of aspirator for further experiments. They were fed on honey streaks.

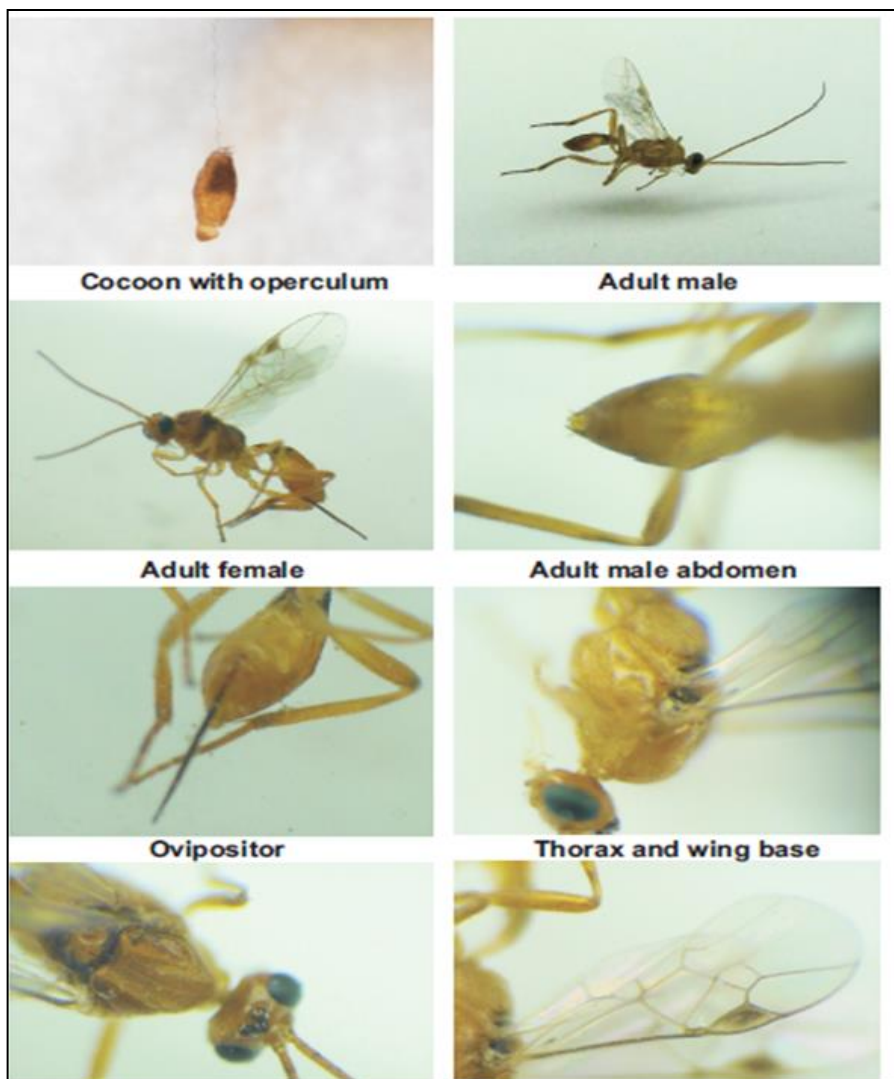
### Identification of Parasitoids

The identification of the parasitoids was done through visual sampling (morphological features) and molecular techniques as well. The identification of *G. agamemnonis* was well established [13]. However, since the identification of *M. pulchricornis* couldn't be established to species level. The samples were identified through molecular techniques from Department of Entomology, Swedish Museum of Natural History (Plate 1). The mitochondrial protein-coding gene cytochrome oxidase I (COI) was selected because this gene exhibits interspecific variability in other insect genera, but low intraspecific variability [18]. The DNA was extracted from the legs of parasitoids and the COI gene was sequenced. The COI primers used in the study include (LCO 5'GGT CAA CAA ATC ATA AAG ATA TTG G3'; HCO 5'TAA ACT TCA GGG TGA CCA AAA AAT CA3') (665 bp) [19]. The polymerase chain reaction (PCR) program for COI had an initial 5-min denaturation at 94°C, followed by 40 cycles at 94°C for 15 s, 46°C for 15 s and 72°C for 15 s and ending with a 10-min extension period at 72°C. PCR products were purified using EXOFAP (EXO1 and Fast AP). Gene regions were sequenced with the same primers as in the PCRs using the Big Dye TM Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems) and cycle sequencing reactions were run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) (all accordingly to the manufacturer's

instructions). Sequencing reactions were purified using the Dye Ex 96 kit (QIAGEN). For assembling and viewing the sequence data and contigs the Pregap4 and Gap4 modules of the Staden package [20] and Geneious v 7.0.4 were used. The sequences were further processed in Bio Edit [21]. COI was aligned by eye. VoSeq\_1.7.0 database was used to store voucher and sequence data. The voucher specimens JS10\_00462 and JS10\_00463 and their respective DNA were kept at the Swedish Museum of Natural History in Stockholm

Sweden.

The sequences of these two specimens were run together with sequences of several other *Meteorus* species. The data were run using MrBayes 3.1.2 [22] under the GTR + I + Gamma model using default settings. The analysis was run with 5 000 000 generations with sample frequencies every 1000 generations. After the analysis 25% of the generations were eliminated in burn-in. Two separate analyses were performed, which produced similar trees.



**Plate 1:** Morphological features of new species of *Meteorus* that lies closely to *Meteorus pulchrichornis* but it has differential feature like the shape of the clypeus looks flattened and the length of the ovipositor looks too long in comparison to *Meteorus pulchrichornis*.

### Virus preparation

The multiple *Nucleopolyhedrovirus* used in this study was originally isolated from naturally field-infected *S. obliqua* larvae collected from fields in Jammu, India (BLJSO-2007). The virus was propagated in third instar *S. obliqua* larvae maintained on castor leaves. Viral occlusion bodies (OBs) were extracted by homogenizing virus-killed larvae in 0.1 % sodium dodecyl sulphate (SDS), followed by filtration through muslin cloth and subsequent pelleting through continuous sucrose gradient centrifugation for 1 hour at 50,000g [23]. After several washes in TE (10 mM Tris-HCl, pH 8, 115 mM EDTA) the OBs were resuspended in 0.75 ml of distilled water and stored in aliquots at  $-20^{\circ}\text{C}$ . The virus was quantified using a haemocytometer and phase contrast microscope at  $\times 400$  magnification under oil immersion.

### Parasitoids ovipositional behaviour

The ovipositional behaviour of *G. agamemnonis* females were evaluated in two different experiments to determine whether ovipositing females were able to discriminate hosts which were already parasitized or infected with virus. As ovipositional experience can affect parasitoid host discrimination responses [24], six to eight days old *G. agamemnonis* mated females which had prior oviposition experience on healthy second instar *S. obliqua* larvae were used in the experiment. In both experiments, healthy larvae were marked with a small spot of white correcting paint to distinguish them from parasitized or diseased larvae. Prior experiments had demonstrated that the paint spot had no effect on the probability of parasitism of marked larvae [25]. The first experiment was conducted to determine if *G. agamemnonis* females showed any ovipositional preferences

to the hosts which were parasitized by *M. pulchricornis* 24 h earlier and also to unparasitized hosts. In this experiment, laboratory reared newly moulted third instar parasitized/unparasitized *S. obliqua* larvae were provided to the gravid female of each parasitoid for parasitisation. The host larvae were simultaneously exposed in groups of 7 (14 larvae in total) to a single mated *G. agamemnonis* female in a plastic Petri dish (115 mm in diameter, 45 mm in height) for 30 min. The parasitized larvae were fed on tender castor leaves in separate containers and maintained in laboratory at  $26 \pm 2^\circ\text{C}$  and  $70 \pm 10\%$  relative humidity and 10: 14 (L: D) h photoperiod. A second experiment was performed to determine whether parasitoid females were able to discriminate among hosts that had been virus-infected 12, 24, 36, 48, and 60 h earlier. A combination of 7 healthy and 7 infected larvae were exposed in groups to individual *G. agamemnonis* females in a plastic Petri dish (115 mm in diameter, 45 mm in height) for 30 min. The experiments were replicated twenty times using a different female at each occasion. Following exposure to parasitoid females, larvae were dissected to determine the presence of parasitoid eggs. The behaviour of parasitoid females towards both virus-infected and healthy larvae and parasitized and unparasitized larvae were observed continuously and the number of contacts and ovipositor insertions with each type of larvae was recorded. Probing of oviposition was recorded when the female inserted her ovipositor into a larvae. Similar procedure was followed with *M. pulchricornis*.

#### Parasitoids survival in parasitized and virus infected hosts

The parasitoids survival was compared in the host larvae parasitized singly either by *G. agamemnonis* or *M. pulchricornis* and in the host larvae parasitized simultaneously by both the parasitoid species. In each case, laboratory reared newly moulted third instar *S. obliqua* larvae were offered to the gravid females of parasitoids for parasitisation in clean glass tubes ( $5 \times 2$  cm) for 30 min. For multiple parasitism third instar host larvae were simultaneously exposed to *G. agamemnonis* and *M. pulchricornis*. Twenty such larvae that were parasitized successfully by parasitoids were reared until emergence of the parasitoids. A second experiment was conducted to determine the survival of *G. agamemnonis* and *M. pulchricornis* in virus-infected and parasitized hosts. The newly moulted third instar *S. obliqua* larvae were parasitized as described earlier and were inoculated a virus concentration of  $1.00 \times 10^7$  OBs/ml at 0, 2, 4 and 6 days post-parasitism. In another batch the larvae that were fed on virus-contaminated diet were subjected to parasitisation 12, 24, 36, 48, and 60 h post-infection. Since the larvae stop feeding within 24-48 hours of viral infection and the larval death started 3 days onwards, the further parasitisation of larvae after 60 h of viral infection was not possible. Larvae were checked daily for death due to parasitoid emergence, viral infection, or other causes. Larvae that died from causes other than parasitoid emergence were dissected to determine the presence of parasitoid immature stages.

#### Statistical analyses

All analyses were performed utilizing SPSS version 16. The behaviour of parasitoids towards virus-infected and healthy larvae and parasitized and unparasitized larvae were analysed by paired-samples t-test. Data on mortality (%) and emergence (%) of *M. pulchricornis* and *G. agamemnonis* from parasitized and virus infected hosts were subjected to ANOVA followed by Tukey's post-hoc test for comparison of means.

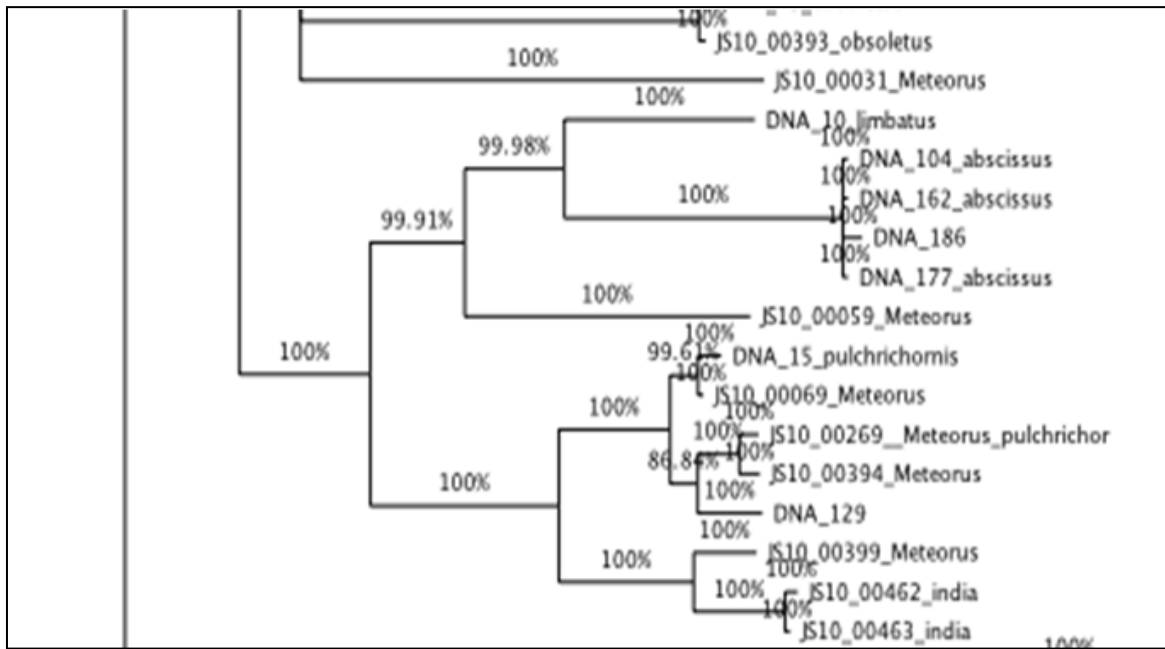
#### Results

On the basis of these sequences, the specimens were considered as a new species that lies closely to *M. pulchricornis* (Plate 2) but most close to a specimen from Vietnam. The detailed sequences are cited hereunder:  
Specimen: JS10\_00642

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TTTATTATATTTTATTTTTGGTTTTTGGATCTGGGATAC
TGGGATTATCTTTAAGTATACTTATTCGTATAGAGTT
GTCTGGAGTTGGTAGATTTTTAGGGGATGATCAAAT
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ATTTTTTTTATGGTTATGCCAATTATAATTGGTGGAT
TTGGAAATTGATTAATTCCTTTGATATTAGGGGCTCC
AGATATGGCTTTTCCTCGTATAAATAATATAAGGTTT
TGATTATTAGTTCCTTCTGTAATAATATTATTAATAA
GAAGAGTTACTAATATGGGAGTAGGAACTGGGTGAA
CAGTTTATCCTCCATTATCTTTAAATATTAGTCATGG
TGGGATATCAGTTGATTTAGCTATTTTTTCTTTACATT
TAGCAGGGGCTTCTTCAATTATAGGGGCTGTAAATTT
TATTACAACCTATTATTAATATACATTTGATAGGATTA
AAATTGGATAATGTGACATTATTTATTTGATCTGTTT
TATTAACGGCTATTTTATTATTATTATCTTTGCCAGTT
TTAGCAGGTGCAATTACTATATTATTAACCTGATCGAA
ATTTAAATACTTCATTTTTTGGATCCGGCAGGGGGAGG
AGATCCTATTTTATATCAACATTTGTTT
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Specimen: JS10\_00643

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TTATAATAGTGTGTTACAGCTCATGCTTTTATTATA
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TTGGAAATTGATTAATTCCTTTGATATTAGGGGCTCC
AGATATGGCTTTTCCTCGTATAAATAATATAAGGTTT
TGATTATTAGTTCCTTCTGTAATAATATTATTAATAA
GAAGAGTTACTAATATGGGAGTAGGAACTGGGTGAA
CAGTTTATCCTCCATTATCTTTAAATATTAGTCATGG
TGGGATATCAGTTGATTTAGCTATTTTTTCTTTACATT
TAGCAGGGGCTTCTTCAATTATAGGGGCTGTAAATTT
TATTACAACCTATTATTAATATACATTTGATAGGATTA
AAATTGGATAATGTGACATTATTTATTTGATCTGTTT
TATTAACGGCTATTTTATTATTATTATCTTTGCCAGTT
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AGATCCTATTTTATATCAACATTTGTTT
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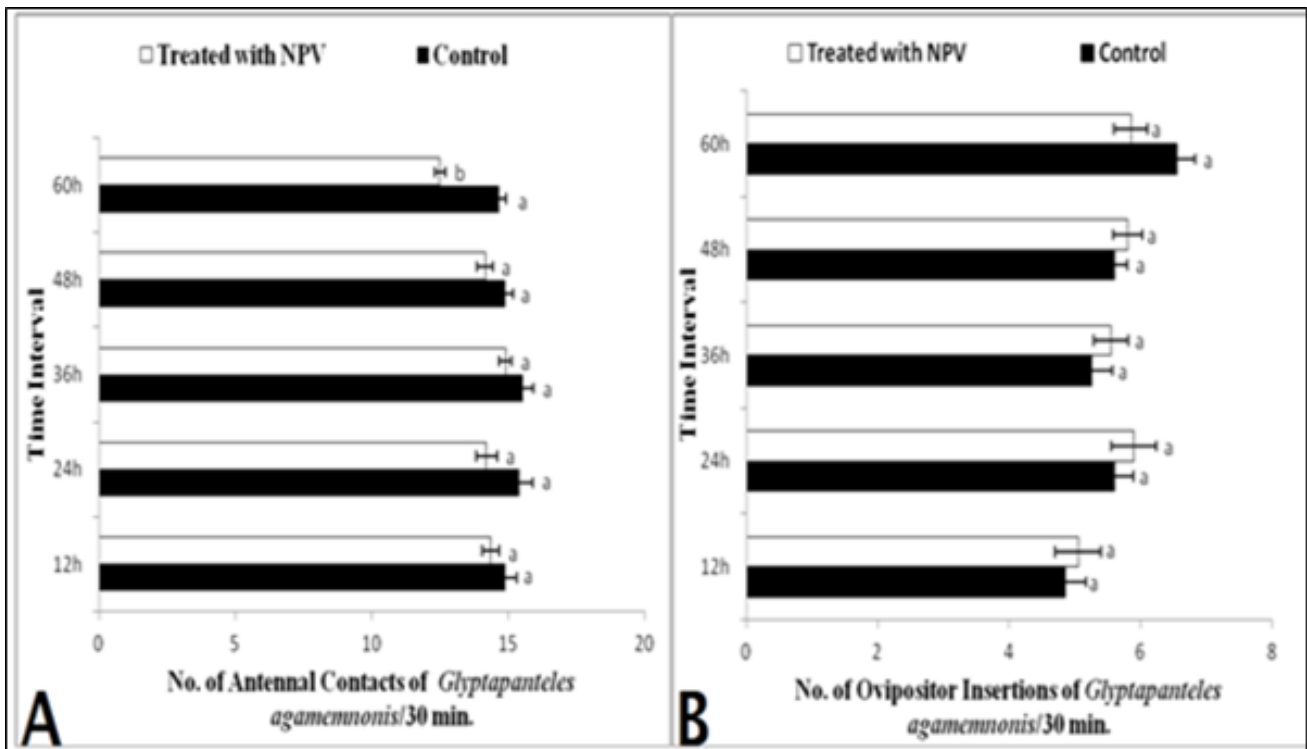
**Plate 2:** Molecular Identification of *Meteorus* based on sequences run Bayesian analysis with 213 other specimens performed at Department of Entomology, Swedish Museum of Natural History, Stockholm, Sweden. The species appear closer to *Meteorus pulchrichornis*.

**Ovipositional behaviour of parasitoid females towards Healthy and virus infected larvae**

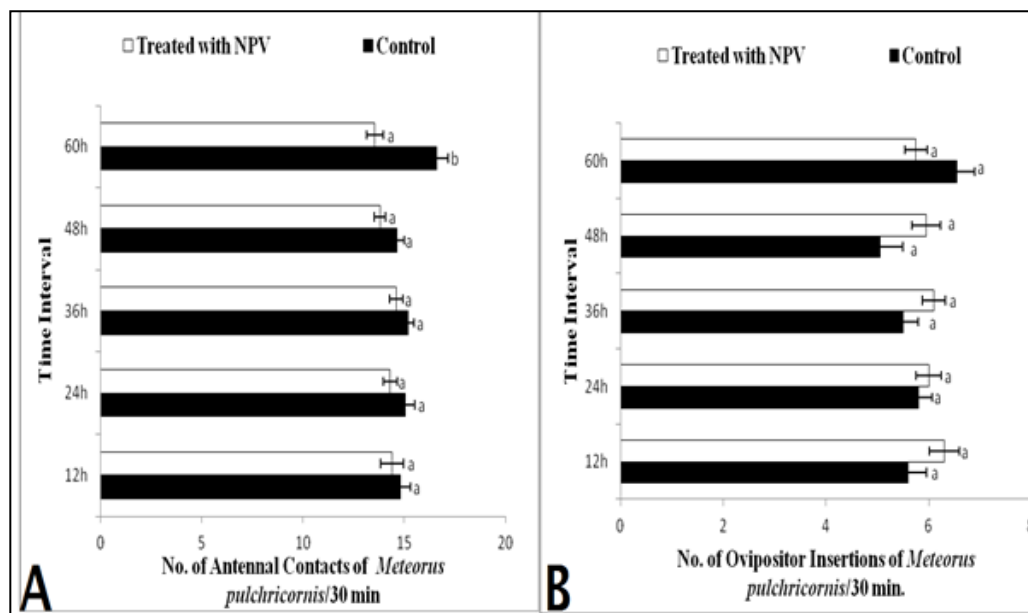
The number of antennal contacts made by female *G. agamemnonis* towards So NPV treated and untreated larvae were not significantly different during 12 ( $t = 1.16, df = 1, 38, P > 0.05$ ), 24 ( $t = 0.49, df = 1, 38, P > 0.05$ ), 36 ( $t = 0.60, df = 1, 38, P > 0.05$ ) and 48 ( $t = 0.50, df = 1, 38, P > 0.05$ ) hours post-infection. Similar results were obtained in case of *M. pulchricornis* towards So NPV treated and untreated larvae. However, at 60 hours post-infection, significantly more antennal contacts were made towards healthy larvae than So

NPV treated larvae of *S. obliqua* by both the parasitoid females i.e. *G. agamemnonis* ( $t = 42.27, df = 1, 38, P < 0.05$ ) and *M. pulchricornis* ( $t = 19.03, df = 1, 38, P < 0.05$ ).

Although the females of both the species attempted more number of ovipositor insertions towards SoNPV treated larvae but when compared to healthy larvae, the number of insertion did not vary significantly during 12 ( $t = 2.45, df = 1, 38, P > 0.05$ ), 24 ( $t = 2.32, df = 1, 38, P > 0.05$ ), 36 ( $t = 2.10, df = 1, 38, P > 0.05$ ), 48 ( $t = 3.76, df = 1, 38, P > 0.05$ ) and 60 ( $t = 3.27, df = 1, 38, P > 0.05$ ) hours post-infection (Fig. 1, 2).



**Fig 1:** Mean ( $\pm$  SE) number of Antennal contact (A) and Ovipositor insertions (B) exhibited by *Glyptapanteles agamemnonis* towards virus-infected and healthy larvae of *Spilarctia obliqua*. Within bars, means followed by the same letter do not differ significantly (Paired T-test;  $P < 0.05$ )



**Fig 2:** Mean ( $\pm$  SE) number of Antennal contact (A) and Ovipositor insertions (B) exhibited by *Meteorus pulchricornis* towards virus-infected and healthy larvae of *Spilarctia obliqua*. Within bars, means followed by the same letter do not differ significantly (Paired T-test;  $P < 0.05$ )

**Parasitized and unparasitized larvae**

It was found that the number of antennal contacts ( $t = 3.37$ ,  $df = 1, 38$ ,  $P > 0.05$ ) and ovipositor insertions ( $t = 2.49$ ,  $df = 1, 38$ ,  $P > 0.05$ ) were apparently more towards unparasitized larvae as compared to larvae already parasitized by *G.*

*agamemnonis*, but the observed differences were non-significant. Similar results were observed when *M. pulchricornis* females were offered unparasitized host larvae and larvae already parasitized by *G. agamemnonis* (Table 1).

**Table 1:** Mean ( $\pm$  SE) number of Antennal contact (A) and Ovipositor insertions (B) exhibited by respective female parasitoid towards larvae parasitized previously by other species and unparasitized larvae of *Spilarctia obliqua*.

| S No | Parasitoid                        | Larval Treatment                                   | No. of Antennal Contacts | No. of Ovipositor Insertions |
|------|-----------------------------------|--|--------------------------|------------------------------|
| 1    | <i>Meteorus pulchricornis</i>     | Parasitized with <i>Glyptapanteles Agamemnonis</i> | 13.90 $\pm$ 0.57a        | 4.40 $\pm$ 0.28a             |
|      |                                   | Unparasitized                                      | 15.75 $\pm$ 0.82a        | 5.10 $\pm$ 0.33a             |
| 2    | <i>Glyptapanteles agamemnonis</i> | Parasitized with <i>Meteorus pulchricornis</i>     | 14.85 $\pm$ 0.59a        | 4.35 $\pm$ 0.31a             |
|      |                                   | Unparasitized                                      | 15.90 $\pm$ 0.44a        | 5.05 $\pm$ 0.32a             |

Means with in a column followed by same letters do not differ significantly (Paired T test;  $P < 0.05$ )

**Parasitoids survival in parasitized or virus-infected hosts**

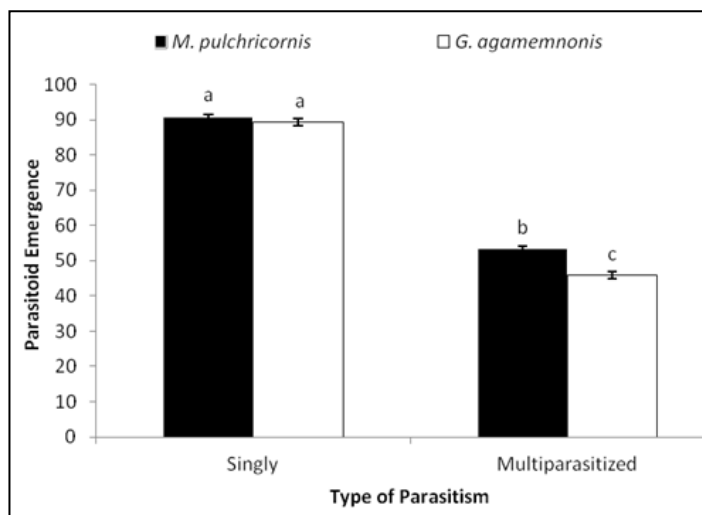
The parasitoids survival increased significantly with increase in intervals between parasitism and viral infection. In general, it was found that almost all the host larvae died from polyhedrosis disease when infected and then parasitized. The larvae that were first parasitized and then infected at 0 and 2 days also succumbed to viral infection. However, the virus mortality was significantly reduced in larvae that were first parasitized and then infected at 4 and 6 days post-parasitism compared to the mortality in larvae exposed to viral infection at earlier intervals ( $F = 204.90$ ,  $df = 4, 20$ ,  $P = 0.00$ ). Since the larvae stop feeding within 24-48 hours of viral infection and the larval death started 3 days onwards, the parasitisation of larvae after viral infection was not possible (Table 2). The

larvae that were subjected to NPV infection after 4 days of parasitization resulted in considerable emergence of both the parasitoids which ranged from 52.60 to 80.00 %. Similarly, the percentage of parasitism by the female parasitoids, *G. agamemnonis* and *M. pulchricornis* did not differ significantly when provided unparasitized host larvae and larvae parasitized previously by other species. The percentage of emergence of *G. agamemnonis* and *M. pulchricornis* from singly parasitized larvae varied from 84.2 to 92.4 % and 84.5 to 94.3 %, respectively. However, when the host larvae were parasitized simultaneously by both the wasp species, *M. pulchricornis* out competed *G. agamemnonis* as the percentage of emergence of *G. agamemnonis* (45.8%) was significantly lower than *M. pulchricornis* (53.4%) (Fig. 3).

**Table 2:** Comparative mortality induced by virus/parasitism (Mean  $\pm$  SE) by two different parasitoids exhibited at different time intervals between Parasitism and Viral Infection

| Larval treatment                   | Status               | Parasitoids                       |                                 |
|------------------------------------|----------------------|-----------------------------------|---------------------------------|
|                                    | Infected/parasitized | <i>Glyptapanteles agamemnonis</i> | <i>Meteorus pulchricornis</i>   |
| Infected then parasitized          | Infected Parasitized | 100 $\pm$ 0.00a 0.00 $\pm$ 0.0a   | 99.8 $\pm$ 0.2a 0.20 $\pm$ 0.2a |
| Parasitized then infected (0 days) | Infected Parasitized | 98.4 $\pm$ 0.50a 1.60 $\pm$ 0.50a | 98.4 $\pm$ 0.5a 1.60 $\pm$ 0.5a |
| Parasitized then infected (2 days) | Infected Parasitized | 93.8 $\pm$ 0.58a 6.20 $\pm$ 0.58a | 97.8 $\pm$ 0.8a 2.20 $\pm$ 0.8a |
| Parasitized then infected (4 days) | Infected Parasitized | 47.4 $\pm$ 1.2b 52.60 $\pm$ 1.2b  | 44.8 $\pm$ 2.2b 55.2 $\pm$ 2.2b |
| Parasitized then infected (6 days) | Infected Parasitized | 20.2 $\pm$ 1.0b 79.80 $\pm$ 1.0b  | 20.0 $\pm$ 1.7b 80.0 $\pm$ 1.7b |

Means with in a column followed by same letters do not differ significantly (ANOVA, Tukey's HSD;  $P < 0.05$ )



**Fig 3:** Mean emergence of *Meteorus pulchricornis* and *Glyptapanteles agagemnonis* in singly and multiparasitized hosts of *Spilarctia obliqua*. Means with in a series followed by same letters do not differ significantly (ANOVA, Tukey's HSD;  $P < 0.05$ )

### Discussion

Thorough investigation of interactions between the *Nucleopolyhedrovirus* and the parasitoid species is important when considering them for use in a pest management program. Parasitoid may alter behaviour towards larvae already parasitized by another species or those infected by insect viruses. Further, the death of the parasitoid can occur within virus-infected hosts due to virus-induced host mortality [26] or due to toxic factors produced during viral replication in the host insect [27] or because of a physiological incompatibility of the infected host for parasitoid development [28]. Based on our own observations and as reported by Karamaouna and Copland [29], that lack of host discrimination by parasitoid may result after antennation or ovipositor insertion. Such discrimination may also be influenced by wasp's previous experience with unparasitized host [30], therefore we used "gravid female parasitoids," that had not yet oviposited (inexperienced parasitoids) in both unparasitized and parasitized hosts. It was found that both the parasitoids exhibited lack of discrimination within 48 hours of infection. However, beyond this interval, the discrimination became evident in the form of antennal contacts whereas no significant difference was observed in the no. of ovipositor insertions. The experimental data obtained demonstrated that parasitoids discriminating behaviour was strongly influenced by post-infection period. The parasitoid's ability to discriminate between healthy and infected hosts increased as virus concentration increased and as the time between exposure of hosts to virus and subsequent exposure to parasitoids increased [31]. Further, both the parasitoid species *G. agagemnonis* and *M. pulchricornis* were not able to make a difference between parasitized and unparasitized host larvae in the form of number of antennal contacts and ovipositor insertions within 60 hours of exposure. These results are in line with the observations of Marktl *et al.* [32] who revealed that in interspecific competition between the braconid endoparasitoids *G. porthetriae* and *G. liparidis* in *Lymantria dispar* larvae, both wasp species do not discriminate between unparasitized host larvae and larvae parasitized previously by the same or the other species. Nevertheless, host discrimination, *i.e.* the ability to distinguish unparasitized hosts from parasitized ones, and to reject the latter for egg laying is evident in many parasitic wasp species. However, different species do not react to each other's marks and lay

eggs in hosts parasitized by the other species. Apparently, the marks used for recognition are specific and many wasps cannot distinguish hosts parasitized by themselves from those parasitized by others [33]. In the present situation, the unique defensive behaviour of the host larvae during parasitisation might have influenced the decision of a parasitoid for repeated insertion. It was evident that *S. obliqua* larvae displayed aggressive behaviour towards both the parasitoid females which might have encouraged parasitoid females to oviposit under pressure once they encounter the host without any discrimination. Nufio and Papaj [34] reported that the stimulus for discrimination becomes more pronounced with the lapse of time of parasitisation and as the parasitoid larva undergoes further development in the host, an alteration of the haemolymph of the host enables the parasitoids to undergo discrimination

Since *Nucleopolyhedrovirus* es and insect parasitoids are two important biological entities which exploit a shared host resource and when these two natural enemies occur simultaneously in a given host, the result can be the elimination of one enemy by the competitor in all co-infected hosts or it can be some intermediate outcome [35]. The present study revealed that the host larvae that were first infected and then exposed to parasitism died from polyhedrosis disease and the effect of So MNPV on *G. agagemnonis* and *M. pulchricornis* was dependent on the intervals between parasitism and viral infection. Also, delaying the exposure of parasitized larvae to So MNPV increased the percentage of successful parasitoid development. One of the possible explanations for this is the premature death of hosts as has been reported by Nguyen *et al.* [36] for the parasitoid *M. pulchricornis* and NPV-infected larvae of *Spodoptera litura*. The host dies from the viral infection before development of the parasitoid is completed. The premature death of parasitoids in virus-infected hosts for various combinations of larval parasitoid and viruses had been reported by several authors [37, 38, 39, 40]. When there is no interval or an insufficient interval between parasitism and virus infection, the parasitoids cannot complete development because the larvae die of viral infection quickly, and fewer parasitoids emerge. The present study revealed that all the third instar larvae of *S. obliqua* died due to viral infection within 5 to 12 days but if the virus inoculums were administered six days post parasitism, more than 75 per cent of parasitoid were able to

complete their development successfully. The parasitoid progeny develops slowly during the larval stage, whereas the virus replicates so quickly that it kills the host in the instar following infection<sup>[41]</sup>. Thus, scramble competition in dually infected and parasitized hosts led to death of the parasitoid. Therefore, we speculate that the parasitoids did not get enough time to complete development in the SoMNPV-inoculated hosts.

It was found that when subjected to multiple parasitism by *G. agamemnonis* and *M. pulchricornis*, emergence of *G. agamemnonis* (45.8%) was highly reduced in *S. obliqua*. Since egg-larval period of *G. agamemnonis* is 18 -21 days in comparison 9-12 days for *M. Pulchricornis*<sup>[42]</sup>, rapid development of *M. pulchricornis* probably enabled it to outcompete *G. agamemnonis* in multiparasitized hosts. Although viral infection can result in the premature death of parasitized hosts, parasitoids that do manage to complete their development and succeed emergence from virus infected insects can act as efficient vectors for virus dispersal<sup>[43, 44, 45]</sup>. This supports the assertion that, generally, baculoviruses and insect parasitoids are compatible in nature. However, for the sake of successful parasitoid emergence, viral applications must be done few days after parasitoid release in augmentative biocontrol programmes.

### Conclusion:

The study revealed that the Parasitoids exhibited lack of discrimination within 48 hours of infection. However, beyond this interval the discrimination becomes evident in the form of antennal contact while no significant difference was observed in ovipositor insertions. Apparent lack of discrimination between already parasitized and unparasitized hosts was seen in both the parasitoids.

Interestingly, virus mortality was accredited to the virus infection when larvae were treated first with virus and then parasitized. However, postponing the exposure of parasitized larvae (4 and 6 d post-parasitism) to baculovirus increased the percentage of successful parasitoid development. When host larvae were parasitized simultaneously by both the wasp species, it was seen that *Meteorus pulchricornis* outcompeted *Glyptapanteles agamemnonis* altogether.

### Acknowledgement

We are deeply grateful to Scott R. Shaw, Professor and Curator UW Insect Museum, Department of Ecosystem Science (3354) 1000 East University Avenue Laramie, Wyoming 82071 USA for facilitating identification of parasitoids. Financial assistance from the Department of Science and Technology, Government of India and Director Research SKUAST-J is sincerely acknowledged. Our special thanks are also due to National Bureau of Agriculturally Important Insects (NBAIL) for encouragement and co-operation.

### References

- Desneux N, Decourtye A, Delpuech JM. The Sublethal Effects of Pesticides on Beneficial Arthropods. Annual Review of Entomology. 2007; 52:81-106.
- Cossentine JC. The parasitoid factor in the virulence and spread of Lepidopteran baculoviruses. Virologica Sinica. 2009; 24:305-314.
- Chailleux A, Mohl EK, Teixeira Alves M, Messelink GJ, Desneux N. Natural enemy-mediated indirect interactions among prey species: potential for enhancing biocontrol

- services in agroecosystems. Pest Management Science. 2014; 70:1769-1779.
- Roy HE, Pell JK. Interactions between entomopathogenic fungi and other natural enemies: Implications for biological control. Biocontrol Science and Technology. 2000; 10:737-752.
- Lu Z, Li Z, Lu Z, Li J, Yang Y, Zhang Q, et al. Interaction between endoparasitoid *Microplitis mediator* (Hymenoptera: Braconidae) and *Nucleopolyhedrovirus* in larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae). Biological Control. 2017; 115:152-156.
- Brooks WM. Host-parasitoid-pathogen interactions. In: Beckage N E, Thompson S N, Federici B (ed): Parasites and pathogens of insects, 2<sup>nd</sup> edn., Academic Press, San Diego, USA, 1993, 231-272.
- Okuno S, Nakai M, Hiraoka T, Kunimi Y. Isolation of a protein lethal to the endoparasitoid *Cotesia kariyai* from entomopoxvirus-infected larvae of *Mythimna separata*. Insect Biochemistry and Molecular Biology. 2002; 32:559-566.
- Hotchkiss PG, Kaya HK. Pathological response of the parasitoid, *Glyptapanteles militaris*, to nuclear polyhedrosis virus-infected armyworm hosts. Journal of Invertebrate Pathology. 1983; 42:51-61.
- Stoianova E, Williams T, Cisneros J, Munoz D, Murillo R, Tasheva E et al. Interactions between an ectoparasitoid and a *Nucleopolyhedrovirus* when simultaneously attacking *Spodoptera exigua* (Lepidoptera: Noctuidae). Journal of Applied Entomology. 2012; 136:596-604.
- Goel SC, Kumar S, Bhardwaj DK. Salient features and bibliography of Bihar hairy caterpillar, *Spilosoma obliqua* Wlk. (Lepidoptera: Arctiidae). Pakistan Journal of Zoology. 2004; 24:1-31.
- Parui A, Roy N. Ecofriendly and sustainable management of *Spilosoma obliqua* Walker on sesame (*Sesamum indicum* L.) crop by new botanicals. Journal of Entomology and Zoology Studies. 2016; 4:349-354.
- Varatharajan R, Singh MI, Reeta L. Cross infectivity of baculovirus, *Spilarctia obliqua*, nuclearpolyhedrosis against mulberry pest, *Porthesia xanthorrhoea* Kollar. Indian Journal of Experimental Biology. 2006; 44:419-421.
- Gupta RK, Narendran TC. Field parasitism and biological characteristics of potent larval endoparasitoids of *Spilosoma obliqua* Walker in Jammu and Kashmir, India. Journal of Biological Control. 2007; 21:43-52.
- Briggs CJ, Godfray HCJ. The dynamics of insect-pathogen interactions in stage-structured populations. American Naturalist. 1995; 145:855-887.
- Hochberg ME, Hassell MP, May RM. The dynamics of host-parasitoid-pathogen interactions. American Naturalist. 1990; 135:74-94.
- Brooks WM. Host-parasitoid-pathogen interactions. In: Beckage NE, Thompson S N, Federici B (ed): Parasites and pathogens of insects, 2<sup>nd</sup> edn., Academic Press, San Diego, USA, 1993, 231-272.
- Kumar SCM, Jacob TK, Devasahayam S, D'Silva S, Jinsha J, Rajna JS. Occurrence and characterization of a tetrahedral *Nucleopolyhedrovirus* from *Spilarctia obliqua* (Walker). Journal of Invertebrate Pathology. 2015; 132:135-141.
- Vogler AP, Desalle R, Assmann T, Knisley CB, Schultz TD. Molecular population genetics of the endangered



- tiger beetle *Cicindela dorsalis* (Coleoptera: Cicindellidae). - Annals of Entomological Society of America. 1993; 86:142-152.
19. Folmer O, Black M, Hoeh W, *et al.* DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*. 1994; 3:294-299.
  20. Staden R, Kathryn FB, James K. Bonfield the Staden Package, Computer Methods in Molecular Biology. In: Stephen M and Steve AK Bioinformatics Methods and Protocols. The Humana Press Inc, New Jersey. 1998; (132):115-130.
  21. Hall TA. Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic acids symposium series. Oxford University Press, USA. 1999; 41:95-98.
  22. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*. 2001; 17:754-755.
  23. Gupta RK, Gani M, Jasrotia P, Srivastava K. Development of the predator *Eocanthecona furcellata* on different proportions of *Nucleopolyhedrovirus* infected *Spodoptera litura* larvae and potential for predator dissemination of virus in the field. *Bio Control*. 2013; 58:543-552.
  24. Vanlenteren JC. Host discrimination by parasitoids. In: Norlund DA, Jones RL, Lewis WJ. Semi chemicals, their role in Pest Control, John Wiley & Sons, New York, 1981, 153-179.
  25. Escribano A, Williams T, Goulson D, Cave RC, Caballero, P. Parasitoid Pathogen-Pest Interactions of *Chelonus insularis*, *Campoletis sonorensis*, and a *Nucleopolyhedrovirus* in *Spodoptera frugiperda* Larvae. *Biological Control*. 2000; 19:265-273.
  26. Okuno S, Nakai M, Hiraoka T, Kunimi Y. Isolation of a protein lethal to the endoparasitoid *Cotesia kariyai* from entomopoxvirus-infected larvae of *Mythimna separata*. *Insect Biochemistry and Molecular Biology*. 2002; 32:559-566.
  27. Hotchkiss PG, Kaya HK. Pathological response of the parasitoid, *Glyptapanteles militaris*, to nuclear polyhedrosis virus-infected armyworm hosts. *Journal of Invertebrate Pathology*. 1983; 42:51-61.
  28. Brooks WM. Host-parasitoid-pathogen interactions. In: Beckage NE, Thompson S N, Federici B (ed): Parasites and pathogens of insects, 2<sup>nd</sup> edn., Academic Press, San Diego, USA, 1993, 231-272.
  29. Karamaouna F, Copland MJW. Conspecific and heterospecific host discrimination in two parasitoid species of the mealybug *Pseudococcus viburni*, the solitary *Leptomastix epona* and the gregarious *Pseudaphycus flavidulus*. *Entomologia Hellenica*. 2009; 18:17-34.
  30. Bai B. Conspecific superparasitism in two parasitoid wasps, *Aphidius ervi* Haliday and *Aphelinus asychis* Walker: reproductive strategies influence host discrimination. *Canadian Entomologist*. 1991; 123:1229-1237.
  31. Jiang JX, Bao YB, Ji XY, Wan NF. Effect of *Nucleopolyhedrovirus* infection of *Spodoptera litura* larvae on host discrimination by *Microplitis pallidipes*. *Biocontrol Science and Technology*. 2014; 24:561-573.
  32. Marktl RC, Stauffer C, Schopf. Interspecific competition between the braconid endoparasitoids *Glyptapanteles porthetriae* and *Glyptapanteles liparidis* in *Lymantria dispar* larvae. *Entomologia Experimentalis et Applicata*. 2003; 105:97-109.
  33. Bakker K, Van Alphen JJM, Van Batenburg FHD, Van der Hoeven N, Nell HW, Van Strien-van Liempt WTFH, *et al.* The function of host discrimination and superparasitization in parasitoids. *Oecologia*. 1985; 67: 572-576.
  34. Nufio CR, Papaj DR. Host marking behavior in phytophagous insects and parasitoids. - *Entomologia Experimentalis et Applicata*. 2001; 99:273-293.
  35. Hochberg ME, Hassell MP, May RM. The dynamics of host-parasitoid-pathogen interactions. - *American Naturalist*. 1990; 135:74-94.
  36. Nguyen DH, Nakai M, Takatsuka J, Okuno S, Ishii T, Kunimi Y. Interaction between a *Nucleopolyhedrovirus* and the braconid parasitoid *Meteorus pulchricornis* (Hymenoptera: Braconidae) in the larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). *Applied Entomology and Zoology*. 2005; 40:325-334.
  37. Hochberg ME. Intra-host interactions between a braconid endoparasitoid, *Apanteles glomeratus* and a baculovirus for larvae of *Pieris brassicae*. *Journal of Animal Ecology*. 1991; 60:51-63.
  38. Escribano A, Williams T, Goulson D, Cave RC, Caballero, P. Parasitoid Pathogen-Pest Interactions of *Chelonus insularis*, *Campoletis sonorensis*, and a *Nucleopolyhedrovirus* in *Spodoptera frugiperda* Larvae. *Biological Control*. 2000; 19:265-273.
  39. Nguyen DH, Nakai M, Takatsuka J, Okuno S, Ishii T, Kunimi Y. Interaction between a *Nucleopolyhedrovirus* and the braconid parasitoid *Meteorus pulchricornis* (Hymenoptera: Braconidae) in the larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). *Applied Entomology and Zoology*. 2005; 40:325-334.
  40. Azam A, Kunimi Y, Inoue MN, Nakai M. Effect of granulovirus infection of *Spodoptera litura* (Lepidoptera: Noctuidae) larvae on development of the endoparasitoid *Chelonus inanitus* (Hymenoptera: Braconidae). *Applied Entomology and Zoology*. 2016; 51:479-488.
  41. Escribano A, Williams T, Goulson D, Cave RC, Caballero, P. Parasitoid Pathogen-Pest Interactions of *Chelonus insularis*, *Campoletis sonorensis*, and a *Nucleopolyhedrovirus* in *Spodoptera frugiperda* Larvae. *Biological Control*. 2000; 19:265-273.
  42. Gupta RK, Narendran TC. Field parasitism and biological characteristics of potent larval endoparasitoids of *Spilosoma obliqua* Walker in Jammu and Kashmir, India. *Journal of Biological Control*. 2007; 21:43-52.
  43. Caballero P, Vargas-Osuna E, Santiago-Alvarez C. Parasitization of granulosis-virus infected and noninfected *Agrotis segetum* larvae and the virus transmission by three hymenopteran parasitoids. *Entomologia Experimentalis et Applicata*. 1991; 58:55-60.
  44. Jiang J, Zeng A, Ji X, Wan N, Chen X. Combined effect of *Nucleopolyhedrovirus* and *Microplitis pallidipes* for the control of the beet armyworm, *Spodoptera exigua*. *Pest Management Science*. 2011; 67:705-713.
  45. Guo HF, Fang JC, Zhong WF, Liu BS. Interactions between *Meteorus pulchricornis* and *Spodoptera exigua* multiple *Nucleopolyhedrovirus*. *Journal of Insect Science*. 2013; 13:1-12.