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Effect of Nuclear Polyhedrosis Virus (NPV) Infection on *Culex pipiens* Mosquito Larvae: Relative Quantification of Vitellogenin Gene Expression and Protein Electrophoretic Analysis of Ovary Homogenates in Emerged Females

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ABSTRACT

Mosquitoes are important vectors of various diseases, and their control is essential to minimize their impact on human health. Traditional methods of mosquito control have relied on the use of insecticides, which have negative effects on the environment and human health. Biological control has emerged as an effective alternative, and nuclear polyhedrosis virus (NPV) has shown potential as a biocontrol agent against mosquito populations. This study aimed to investigate changes in vitellogenin (Vg) gene expression and protein profiles in female *Culex pipiens* mosquitoes treated, in the larval stage, with nucleopolyhedrovirus (NPV) before and after blood feeding. Real-time quantitative PCR was used to measure Vg gene expression. In the untreated females, the Vg gene expression increased in the 24 hrs post-blood meal (pbm) then decreased at 48 hrs pbm while in the treated females, the level of Vg gene expression declined in both 24 & 48 hrs pbm. SDS-PAGE analysis of ovary homogenates revealed differences in protein composition between normal and treated blood-fed females, with a range of molecular weights observed in the blood-feeding stages in 24 to 48 hrs pbm. The study concludes that NPV treatment significantly reduces Vg gene expression and protein profiles in *Cx. pipiens* mosquitoes. Our research emphasizes the possible application of NPV to control mosquito populations effectively, and further research on the mechanism underlying the pathogenicity of NPV and its effect on vitellogenesis in adult females could open new avenues for mosquito control.

INTRODUCTION

Mosquitoes represent a significant public health concern due to their role as primary vectors of pathogens affecting both humans and animals. These pathogens are responsible for diseases such as malaria, dengue, filariasis, chikungunya, Zika, Japanese encephalitis, West Nile fever, and yellow fever, which contribute to millions of deaths annually **(Rawal, 2019)**. Over half of the world's population is at risk from mosquito-borne diseases (MBD), which make up roughly 17% of the overall burden of all infectious diseases (**Khezzani *et al.*, 2023**). The *Culex pipiens* complex (Diptera: Culicidae) are vectors for the West Nile virus, St Louis encephalitis virus, and lymphatic

filariasis. *Culex pipiens* assumes a pivotal role in Egypt's ecosystem due to its significant economic impact as a disease-carrying vector **(Abdel-Hamid *et al.*, 2011).**

One of the most important physiological processes related to mosquito reproduction is vitellogenesis. This process is triggered after female mosquitoes consume a blood meal from a suitable host. They use blood not only for their nourishment but also as a source of protein for their egg maturation. During the process of vitellogenesis, the fat body tissues synthesize Vitellogenin (Vtg) protein, which serves as the primary yolk protein that is internalized by the oocyte **(Raikhel *et al.*, 2002; Attardo *et al.*, 2005; Wu *et al.*, 2021)**. Vitellogenin proteins and the expression of their coding genes have received significant attention in scientific research across multiple mosquito vector species due to their crucial role in facilitating egg maturation **(Isoe & Hagedorn, 2007; Costa-Da-Silva *et al.*, 2014; Shaalan *et al.*, 2017)**.

The extensive use of insecticides in mosquito control has led to the development of many problems. Of these negative effects, the development of insecticide resistance and the residual effect of insecticides on the environment, non-target species, and human health have shown remarkable significance **(Hamed *et al.*, 2022; Ahmed *et al.*, 2023)**. To diminish the dramatic effect of the indiscriminate use of chemical insecticides, the potential of biological control, especially microbial insecticides, has proven its effectiveness as one of the insect vector management components **(Hegazy *et al.*, 2021)**. The most outstanding advantage of using such alternatives over traditional insecticides is environmental safety **(Erayya *et al.*, 2013)**. Recently, environmental changes have led to adverse effects on vector distribution, alongside the threat of reintroducing and re-emerging disease vectors worldwide (**El-Sayed & Kamel, 2020**; **Brugueras *et al.*, 2020)**.

Baculoviridae is one of the pathogenic DNA virus families that their organisms belong to and that infect insects. They are arthropod-specific viruses that have been isolated from insect species of different orders, including Lepidoptera, Hymenoptera, Diptera, and Coleoptera. According to the International Committee on Taxonomy of Viruses (ICTV), Baculoviridae is divided into four genera, including *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus* and *Deltabaculovirus* (**Harrison *et al.*, 2018**). Of these four genera, the nucleopolyhedrovirus group (NPV) can infect mosquitoes, which are characterized by having more than one virion per occlusion body and are known as occluded viruses. The advancement of the use of such pathogenic viruses in mosquito control is limited due to their poor ability to transmit viruses inside the body of the mosquito larva. The efficacy of incorporating divalent cations such as magnesium ions in larva feeding to facilitate the transmission of NPV to mosquito larvae **(Becnel, 2006)**.

To minimize biocontrol's drawbacks and further extend its advantages, further research is required to understand the mechanism underpinning the pathogenicity of such viruses and their effect not only on the immature stages but also on reproduction and vitellogenesis in adult female mosquitoes. Therefore, we focus on this study to determine

the effect of NPV as an entomopathogenic virus on the expression level of the vitellogenin gene and protein profile of female *Culex pipiens* ovaries after engorging a blood meal.

MATERIALS AND METHODS

Culex culture

The *Culex pipiens* colony used in this study was obtained from the Research and Training Centre on Vectors of Disease (RTC) at the Faculty of Science, Ain Shams University, Cairo, Egypt. The mosquitoes were reared in a rearing room with controlled environmental conditions of 27 ± 1 °C, a relative humidity of 65% and a daily photoperiod of 14 hours of light and 10 hours of darkness. Egg batches from the RTC were provided for the rearing process. The larvae were reared in white enamel plates filled with dechlorinated tap water. Their diet consisted of a finely ground fish food called Tetramine®, which was suspended in distilled water. Pupae were collected and transferred to beakers, where they were reared in insect cages sized $35 \times 35 \times 35$ cm until adult mosquitoes emerged, according to **Gerberc (1970)**. Upon emergence, newly emerged adult mosquitoes were provided with a 10% sugar solution for feeding. Female mosquitoes, aged 4-5 days, were fed on pigeon blood. Fully engorged female mosquitoes laid their eggs in oviposition cups, and these eggs were then transferred to enamel containers filled with dechlorinated tap water **(Farag *et al.*, 2021).**

Nuclear polyhedrosis virus (NPV) bioassay

Nuclear polyhedrosis Virus (NPV) suspension was isolated from 3rd instar *Spodoptera littoralis* larvae obtained from the Plant Protection Research Institute, Dokki, Giza, Egypt. The infected larvae were ground, and the homogenate was suspended in 50 mM Tris/HCl buffer (pH 8). After filtration, the supernatant was obtained by centrifugation at 12,000 rpm for 20 minutes. Further purification was achieved by centrifuging the virus suspension through a sucrose gradient, followed by washing and additional centrifugation steps. The virus concentration was determined using a hemocytometer, and the suspension was stored at -20 °C **(Backwad & Pawar 1981)**.

The bioassays were conducted in the Entomology Department, Faculty of Science, Ain Shams University. The third instar larvae of *Cx. pipiens* were treated with a serial concentration of NPV extract. Stock solutions of NPV extract were prepared in Tris-HCl buffer, and 10 mM $MgCl_2$ was added to each treatment to enhance the infectivity of the virus in the larval mosquitoes **(Becnel, 2006)**. Serial dilutions of NPV extract ranging from 10^5 to 10 polyhedra/mL were prepared using distilled water, resulting in 100 mL of each concentration. Three replicates, each comprising 25 larvae, were exposed to different concentrations. Mortality rates were recorded 24 hours after treatment. A control group received distilled water without any viral particles.

Extraction and quantitative expression of the vitellogenin gene (Vg)

Qualitative molecular characterization of Vg in normal and treated Cx. pipiens

Third-instar larvae treated with the LC_{50} of Nuclear Polyhedrosis Virus (NPV) were allowed to complete their life cycle, and the emerged females represented the treated females. For biochemical and molecular characterization of the target vitellogenin gene in *Cx. pipiens*. A series of 200 larvae, 40 newly emerged unfed females, and 80 blood-fed females at 24, and 48 post blood feeding were selected under normal and treated conditions and kept at -80°C for RNA extraction and profiling of gene and protein expression.

Vg primer design

The primers used in this study were based on primers previously designed by **Shaalan *et al.* (2017)**. To identify suitable forward and reverse primers, an alignment was performed between two isoforms of the Vg gene, namely Vg-C1 (GenBank accession AY691324) and Vg-C2 (GenBank accession AY691325), in the closely related species *Cx. quinquefasciatus*. The alignment was conducted on the second large exon, which spans a length of 5054 bp. From this alignment, primers named VgF and VgR were chosen, targeting a specific region expected to yield a 127 bp amplification product.

Total RNA extraction and quantitative detection of the Vg gene using real-time PCR.

Total RNA was isolated from pools of 100 larvae in the third instar and of 10 adult females at different time points of newly emerged and 24 & 48 hrs pbm during normal and treated conditions. Extraction was performed using the Trizol reagent method (Invitrogen, Cat. No. BSC51M1 Carlsbad, CA, USA) according to the manufacturer's instructions. The quality and quantity of the isolated total RNA samples were examined by Nanodrop spectrophotometer (ND-2000c, Thermo Scientific) for determining their concentration (ng/µl) and the ratio between the wavelength readings at 260 and 280ºA to determine the degree of impurity. Samples were stored at -80ºC until use.

To study the expression profile of Vg gene expression in different mosquito stages, The quantitative real-time PCR (qPCR) analysis was conducted using an Mx3005P qPCR system from Agilent Technologies. The experiment was carried out in 96-well optical reaction plates, following the manufacturer's instructions with slight modifications **(Green & Sambrook, 2017).** The expression of the Vg gene was normalized by comparing it to the expression of β-actin, a commonly used reference gene. Primers specific to β-actin were utilized for this purpose.

Forward, VgF 5'- (CCATCAAGGGWCTGTACGTCGAG)-3'

Reverse, VgR 3'- (ARCTGGTTGTACTTGGACTTGAT)- 5'

β-actin primers were:

Forward, β-actin F 5'- (ATGTTTGAGACCTTCAACTCGC) -3'

Reverse, β-actin R 3'-(TAACCTTCRTAGATTGGGACG)-5' according to **Provost-Javier *et al.* (2010)**.

The reactions were performed in triplicate with a volume of 25 µl each, using the Quantitect SYBR Green PCR kit. This kit consisted of 1 ml of 2x QuantiTect SYBR Green PCR Master Mix and 2 ml of RNase-free water (Cat. No. 204141, Qiagen). Total RNA was extracted in one step by using RevertAid Reverse Transcriptase (Cat. No. EP0441, Thermo Fisher) at 50^0 C for 30 min. Each reaction was adjusted to 25 µl total volume containing 12.5 µl SYBR Green PCR Master Mix, 0.25 µl reverse transcriptase, and each primer set with a final concentration of 50 pmol at a volume of 0.5 µl. Additionally, 4.25 µl of RNase-free water and 7 µl of total RNA were included in the reaction mixture. For each time point, three independent replicates were performed. The PCR conditions consisted of an initial incubation cycle at 50°C for 2 minutes, followed by a single cycle of incubation at 94°C for 5 minutes. This was followed by 35 cycles, each consisting of denaturation at 94°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. The Stratagene MX3005P software (Agilent Technologies, Inc. 2009, version 4.10) was utilized to analyze the amplification curves and determine the cycle threshold (Ct) values. To assess the variation in gene expression among the RNA samples, the cycle threshold (Ct) value of each sample was compared to that of the control group using the ΔΔCt method, as described by **Yuan *et al.* (2006)**. Quantitative measurements were performed in triplicates and normalized to β-actin gene as the reference gene for each sample.

Total protein concentration and protein electrophoretic analysis

Estimation of total protein

To analyze the vitellogenin protein in *Cx. pipiens*, ovaries were dissected from mosquito females after 24 hrs and 48 hrs post blood feeding in both normal and treated samples. The dissected ovaries were collected in cold phosphate buffered saline (PBS) buffer on ice (0.2 g KCl, 8 g NaCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 in 1 liter deionized water, pH 7.4) (Sigma) to process them to measure the total protein concentration using the Nanodrop apparatus (2000, Thermo Scientific) at wavelength 280 °A using the apparatus default settings. The apparatus was first blanked by the buffer used in sample dilution (PBS), and then each sample was determined by taking only two microliters from each.

Protein electrophoretic analysis

The total protein profiles of dissected female ovaries of *Cx. pipiens* were determined using denaturing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using the VS10WCBS apparatus from (Cleaver Scientific). Electrophoretic conditions and procedures were carried out according to the method of **Laemmli (1970)**.

The samples were loaded onto 10% polyacrylamide gels that were stained with 0.1% Coomassie Brilliant Blue R-250. Afterward, the gels were destained in a solution consisting of 50% methanol and 10% acetic acid until the protein bands became visible.

The gel was then scanned using a gel documentation system, and the resulting images were analyzed using Gel-Pro Analyzer software, specifically version 6.0 (MediaCybernetics Inc., Bethesda, MD company).

Statistical Analysis

The median lethal concentration (LC_{50}) was calculated at 95% confidence limit according to Finney (1971) using the statistical software LDP-line. The differences in vg gene expression and total protein concentration between different stages of control and treated groups were analyzed by one-way analysis of variance (One-way ANOVA). The means were compared by Tukey's multiple comparisons tests using SPSS software version 26. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

1. Bioassay test

The results of the bioassay demonstrated a concentration-dependent mortality effect of the nuclear polyhedrosis virus (NPV) extract on *Culex pipiens* larvae. Mortality rates increased with increasing concentrations of the NPV extract. The control group, which received distilled water, exhibited negligible mortality. The LC_{50} value of the NPV extract was determined to be $1.31x10^3$ polyhedra/mL (Figure 1).

2. Relative quantification of vitellogenin gene expression using real-time quantitative PCR

To assess changes in gene expression patterns of vitellogenin (Vg) in *Cx. pipiens* mosquitoes during their immature and adult stages, quantitative real-time PCR was performed. The aim was to measure fold changes in gene expression compared to control samples. To ensure accurate and reliable results, it is crucial to employ a stable reference gene for normalization purposes.

In adult females at different feeding statuses (sugar fed, 24 and 48 hrs pbm), total RNA was isolated and subjected to relative quantification to determine alterations in gene expression between normal and infected groups. Adult and immature stages were selected to observe changes in gene expression at both the early (larva stage) and late (blood-fed female) stages of infection.

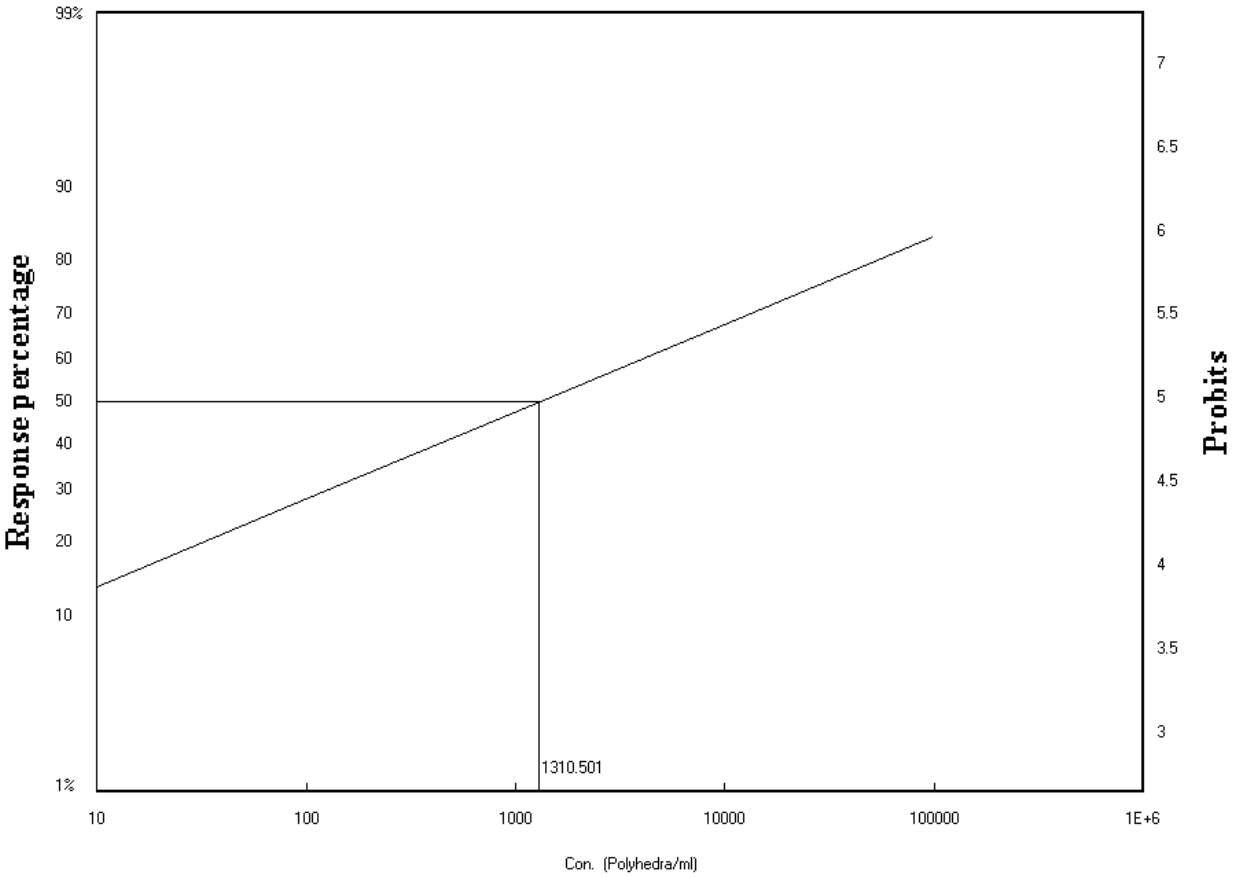

Figure 1. The regression line of the relation between the pathogenic effect of Nuclear polyhedrosis Virus (NPV) and the percentage mortalities in *Culex pipiens* larvae.

The vitellogenin gene expression pattern in NPV-treated mosquitoes was also significantly affected by viral infection.

2.1 Transcript expression analysis in developmental stages

According to **Shaalan *et al.* (2017)**, the Vg primers were designed based on the vitellogenin gene sequences of the closely related species *Culex quinquefasciatus*. These sequences, specifically Vg-C1 and Vg-C2, were previously identified by **Isoe and Hagedorn (2007)** and can be found in GenBank under the accession numbers AY691324 and AY691325, respectively.

The normal larval stage was established as the baseline of Vg for other stages. Expression analysis of the vitellogenin gene was detected in larvae and adult females at different feeding statuses (sugar-fed females and blood-fed females at 24 & 48 hrs pbm). The level of expression was normalized with the β-actin mRNA, an internal control.

Data in Table 1 and Figure 2 show that the level of vitellogenin gene expression reaches its maximum at 24 hrs pbm with ~79.34 fold and then declines to ~ 26.18-fold during 48 hrs post blood meal, respectively.

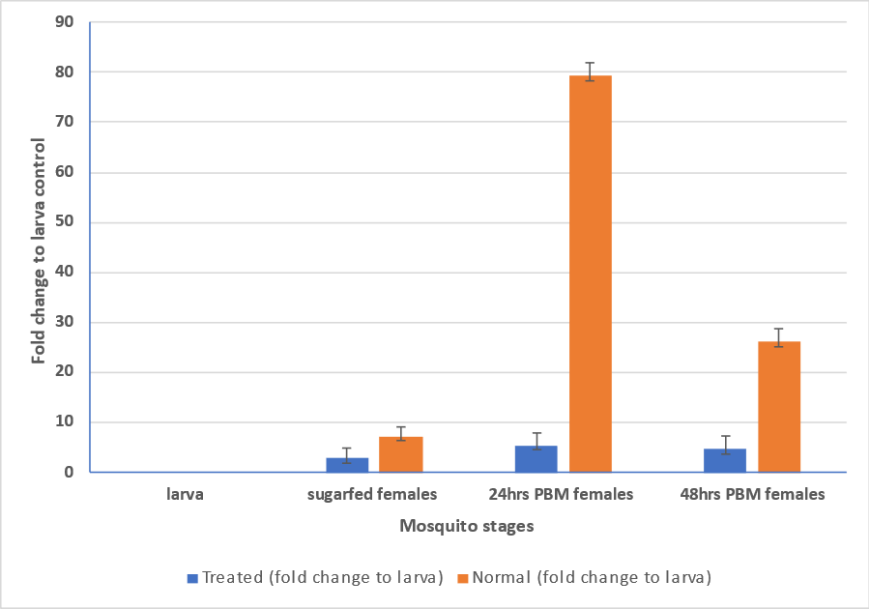

Figure 2. Histogram represents the Vg gene expression in both normal and treated *Cx. pipiens* in different mosquito stages relative to fold changes in larvae as baseline control.

Table 1. Mean cycle threshold (Ct) of both Vg and β-actin expression in larvae, sugar fed females, and blood feeding females at 24 & 48 hrs pbm in accordance with the fold change to the normal 24 hrs pbm blood fed females (N: normal females, T: Treated females).

Mosquito stages	β- actin Ct (mean value)	Vg Ct (mean value)	Fold change to control (larva)
Larva stage (Control)	19.12	23.12	-
Sugar fed females (N)	23.51	24.66	7.21
Sugar fed females (T)	20.42	22.90	2.87
24 hours pbm females (N)	22.69	20.38	79.34
24 hours pbm females (T)	19.71	21.27	5.43
48 hours pbm females (N)	23.72	23.01	26.18
48 hours pbm females (T)	19.65	21.43	4.66

3. Total protein and protein electrophoretic analysis of the ovary homogenates of mosquito *Cx. pipiens*

Data in Figure 3 show that the total protein concentration in the ovary homogenates of normal females at 24 & 48 hrs pbm was 39.1 and 52.2 mg/ml respectively. In comparison with normal, these protein concentration levels were significantly reduced to 2.03 and 2.66 mg/ml in the treated females of both intervals at 24 & 48 hrs pbm, respectively.

Protein profiles of ovary homogenates of female mosquito *Cx. pipiens* were separated electrophoretically for the normal and treated blood-fed females at two different time intervals (24 hrs & 48 hrs pbm). Comparisons of electrophoretic profiles of *Cx. pipiens* revealed differences in the protein composition of the ovary among the normal and treated phases. In normal samples, the number of protein bands determined ranged from 9 to 11, with molecular weights ranging from approximately 336 to 29 KDa. While in treated samples, the number of protein bands greatly declined to only 2 or 3 bands with moderate molecular weight ranging from approximately 88 to 77 KDa (Figure 4) (table 2).

SDS-PAGE analysis in Figure 4 and Table 2 showed that the protein profiles of the ovary homogenate of blood-fed females of *Cx. pipiens* at 24 hrs pbm electroporated into eleven major protein bands according to their molecular weight ranged from 336.52 to 29.29 KDa. The highest protein fraction percentage was detected in band number 6, with a molecular weight of 89.84 KDa and 30.9% at this stage. At normal 24 hrs pbm, protein was fractionated into three unique bands of high molecular weight ranging from 336 to 158 KDa, which indicated a large subunit of vitellogenin protein that clearly disappeared in the treated 24 hrs pbm.

Proteins of *Cx. pipiens* ovaries at 48 hrs pbm were separated electrophoretically into nine bands. Densitometry scanning of the electropherogram of SDS revealed that band No. 3 showed the highest concentration (30.28%) in normal 48 hrs pbm with a molecular weight of 93.01 KDa. In comparison with normal, the NPV treated females after 48 hrs pbm showed an extensive decline in total protein level. In the treated 48 hrs pbm sample, two protein fractions were detected. Protein bands with molecular weights of 89.04 and 77.76 KDa were the only ones evident. The 2 bands were detected in both normal and treated female ovary homogenates, with the disappearance of the rest of the 7 bands.

Overall results revealed that the protein bands of the ovaries of *Cx. pipiens* were less in both treated 24 & 48 hrs pbm female stages than the same normal one, referring to the effect of NPV on the protein content.

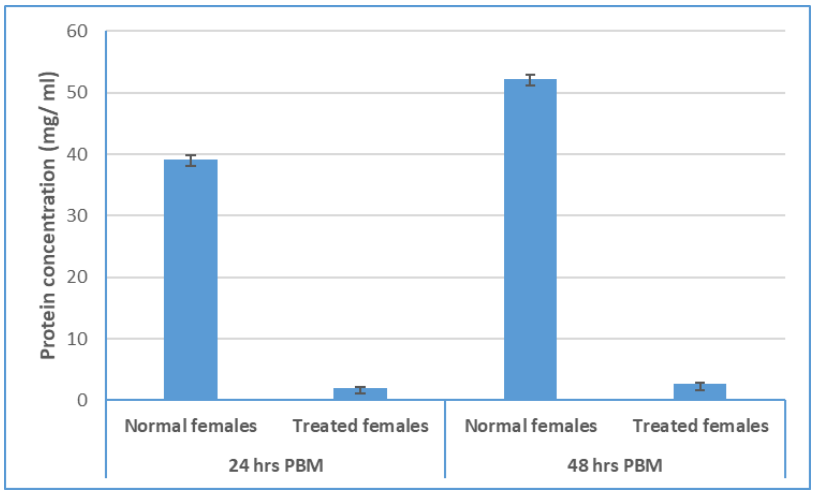

Figure 3. Protein concentration of normal and treated *Cx. pipiens* female ovaries after 24 hrs & 48 hrs post-blood meal (pbm) using nanodrop.

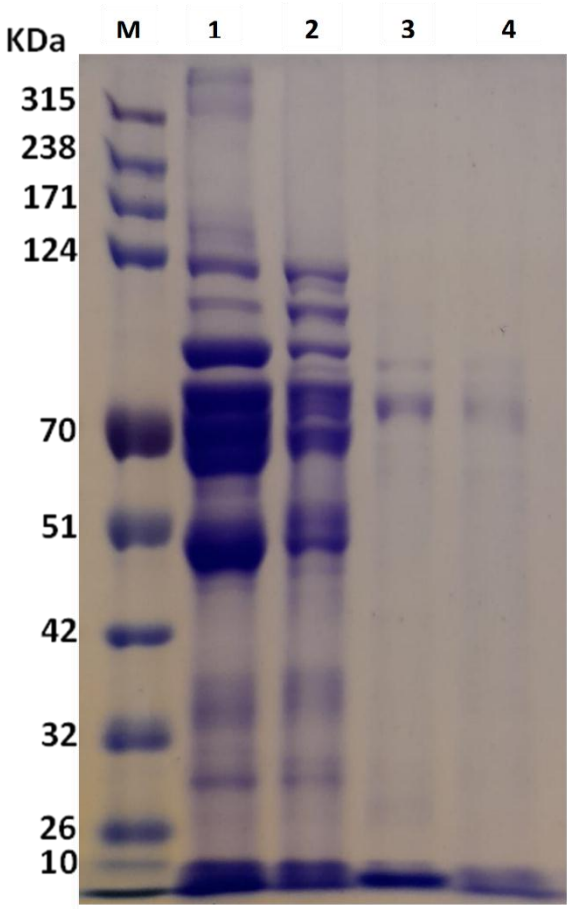

Figure 4. SDS-PAGE of ovaries homogenates of female mosquito *Cx. pipiens* after 24 & 48 hrs of taking a blood meal. Lanes M: Protein marker (with a wide range of molecular weight, ranged from 10 to 315 KDa; Lanes, 1: 24 hrs pbm females (Normal), 2: 48 hrs pbm females (Normal), 3: 24 hrs pbm females (Treated), 4: 48 hrs pbm females (Treated).

Table 2. Comparative analysis of molecular weight, percentage amount (% raw vol.) of protein bands of the ovary homogenate of female *Cx. pipiens* at different feeding statues

Lanes	Number	Molecular weight	% raw vol.
Marker	1	315	13.14
	2	238	9.38
	3	171	9.62
	4	124	12.39
	5	70	4.28
	6	51	5.98
	7	42	17.92
	8	32	9.74
	9	26	11.57
	10	10	5.98
24 hrs pbm (Normal)	1	336.52	0.18
	2	297.83	0.00
	3	158.07	0.06
	4	120.71	26.43
	5	107.09	15.77
	6	89.84	30.96
	7	78.20	8.29
	8	56.16	0.03
	9	49.48	6.34
	10	34.47	0.67
	11	29.30	11.27
48 hrs pbm (Normal)	1	118.18	17.78
	2	104.72	23.39
	3	93.01	30.29
	4	80.78	3.95
	5	70.95	9.18
	6	55.08	0.04
	7	50.45	10.31
	8	35.90	0.12
	9	29.34	4.95
24 hrs pbm (Treated)	1	88.64	35.60
	2	77.07	64.07
	3	27.44	0.33
48 hrs pbm (Treated)	1	89.04	30.91
	2	77.76	69.09

DISCUSSION

Mosquitoes are vectors of numerous pathogens that cause severe diseases in humans and animals **(Benelli, 2015)**. The traditional methods of controlling mosquito populations mainly rely on the use of insecticides, which have had several negative impacts on the environment, non-target species, and human health **(Walia *et al.*, 2017)**. Hence, there is a need for alternative mosquito control strategies, and the use of biological control is one of the most effective strategies to reduce the use of insecticides **(Yakob & Walker, 2016; Aly *et al*., 2023)**.

Vitellogenesis is a vital physiological process in mosquito reproduction. It involves the synthesis of vitellogenin (Vg) protein by the fat body tissues, serving as the primary yolk protein internalized by oocytes. Due to their significant role in egg maturation, extensive research has been conducted on vitellogenin proteins and their corresponding gene expression in various mosquito vector species **(Wu *et al.*, 2021)**.

This study focused on two main areas: the real-time quantitative PCR analysis of vitellogenin gene expression and the protein electrophoretic analysis of the ovary homogenates of *Cx. pipiens* mosquitoes untreated and treated with NPV.

The real-time quantitative PCR analysis of vitellogenin gene expression was performed on *Cx. pipiens* mosquitoes at different stages of development to observe changes in gene expression. Depending on previous studies, the level of vitellogenin in the larval stage is expressed at a low trace level **(Shaalan *et al.*, 2017)**. Therefore, in our study, we established the larval stage as the baseline of Vg for other stages. The results showed that the level of vitellogenin gene expression starts to increase in females post blood feeding, reaching its maximum at 24 hours post blood meal with ~79 fold, and then declining to ~26 fold for 48 hours post blood meal. This finding is consistent with a previous study that showed an increase in Vg gene expression after blood feeding in mosquitoes **(Hansen *et al.*, 2004)**. Moreover, the study evaluated the effect of viral infection on the expression of the vitellogenin gene. The results indicated that the vitellogenin gene expression pattern in NPV-treated mosquitoes was significantly affected by viral infection. This finding suggests that viral infection could alter the normal gene expression signature in mosquitoes, which could have implications for their fitness and survival. **Shaalan *et al.* (2017)** found that Vg genes were expressed in *Cx. pipiens* females 24 hours post-blood meal and that Vg protein was present in the ovarian tissues of blood-fed females but not in sugar-fed females. The results suggest that Vg plays a crucial role in egg development in *Cx. pipiens* complex mosquitoes and may be an important target for control interventions against disease-transmitting mosquito vectors. After a female mosquito feed on blood, the fat body undergoes structural modifications that trigger a cascade of transcriptional events, leading to the production of significant quantities of vitellogenin (Vg) in the ovaries. In our study, we observed the highest levels of Vg in female *Cx. pipiens* mosquitoes at 24 hours post-blood meal (pbm),

followed by a gradual decline at 48 hours pbm. This pattern aligns with the transcriptional profile of Vg observed in *Aedes aegypti* mosquitoes, demonstrating consistency across different mosquito species **(Kokoza *et al.*, 2001)**. Our results align with other studies that have documented low levels of residual Vg transcripts in female mosquitoes prior to blood feeding, as well as in immature mosquitoes of *Cx. tarsalis* and *A. aegypti* species **(Isoe & Hagedorn, 2007; Provost-Javier *et al.*, 2010)**.

Protein electrophoretic analysis of the ovary homogenates of *Cx. pipiens* mosquitoes was also performed. The results showed that the protein composition of the ovary differed among the normal and treated phases. In normal samples, the analysis revealed a range of 9 to 11 protein bands, with molecular weights varying from ~29.3 to 336.52 KDa. The study investigated the protein content and electrophoretic profiles of ovary homogenates from female *Culex pipiens* mosquitoes under normal and treated blood-fed conditions at two post-blood meal (pbm) time points (24 and 48 hours). The results revealed differences in protein composition between the normal and treated phases. Treated samples had a reduced number of bands (2-3) with moderate molecular weights (77.76 to 88.64 KDa). SDS-PAGE analysis showed distinct protein profiles, with normal 24 hrs pbm samples showing 11 major bands, while treated samples showed the disappearance of high molecular weight bands. Normal 48 hrs pbm samples exhibited 9 bands, with band 3 having the highest concentration. Treated 48 hrs pbm samples showed a decline in total protein levels, and only two bands were detected. The disappearance of several bands in the treated samples suggests a specific effect of NPV treatment on the protein composition of the ovaries. These results contribute to a better understanding of the molecular changes occurring in the ovaries during different feeding statuses and treatments, which can have implications for reproductive biology and mosquito population control strategies.

The mechanism by which nuclear polyhedrosis virus (NPV) affects the expression of the vitellogenin gene in mosquitoes is not yet fully understood. However, there is some evidence to suggest that the virus may interfere with the hormonal regulation of vitellogenin synthesis. NPV infection may disrupt the hormonal signaling pathways that regulate vitellogenin gene expression in mosquitoes. Specifically, NPV may interfere with the synthesis or signaling of juvenile hormone (JH), a hormone that plays a key role in regulating vitellogenin production in mosquitoes **(Raikhel *et al.*, 2005)**. Some studies have shown that NPV can reduce JH synthesis in infected insects, which could in turn lead to a decrease in vitellogenin production. **Zhang *et al.* (2015)** investigated the effects of NPV infection on the expression of juvenile hormone (JH)-related genes in *Helicoverpa armigera* and demonstrated a significant decrease in the expression levels of genes associated with juvenile hormone (JH) biosynthesis and signaling following infection with NPV, suggesting that the reduction in JH signaling may be a mechanism used by NPV to inhibit host growth and development and promote its own replication. **Lu *et al.* (2016)** found that the synthesis of vitellogenin was directly regulated by JH, as

treatment with a JH analogue led to an increase in vitellogenin expression in the fat body of female *Nilaparvata lugens*.

NPV replicates within insect cell nuclei and manipulates host gene expression to promote viral structural protein production. They use viral transcription factors to activate transcription of viral and host genes. NPV also affects host gene expression post-transcriptionally via microRNAs, which degrade mRNAs involved in antiviral defenses. Moreover, NPV modified host protein synthesis machinery to promote the production of viral proteins, including encoding viral translation factors to selectively promote viral mRNA translation. This creates an environment favorable for virus replication and structural protein production **(Rohrmann, 2019)**.

In mosquitoes, the fat body plays a critical role in nutrient storage and metabolism, serving as a site for the synthesis and secretion of yolk protein precursors (YPPs) during the process of vitellogenesis. YPPs are taken up by developing oocytes and serve as the major source of nutrients for the developing embryo. The fat body synthesizes and secretes YPPs in response to hormonal signals triggered by a blood meal, and their regulation is influenced by hormonal signals, nutritional status, and other physiological factors **(Roy *et al.*, 2018)**. Once NPV enters the insect's body, it targets and infects the fat body cells. The virus replicates inside the infected cells, eventually causing the cells to burst open and release new viral particles. As the infection progresses, the fat body tissue becomes increasingly damaged, and the insect's metabolism and immune response are disrupted **(Rohrmann *et al.*, 2019).**

It should be noted that the success of the biocontrol approach depends on understanding the mechanism underlying the pathogenicity of NPV and its effect not only on the immature stages but also on reproduction and vitellogenesis in adult females. In this study, the effect of NPV on vitellogenesis was demonstrated, which could open new avenues for further research on the utilization of NPV as a biocontrol agent and demonstrate its effectiveness in controlling mosquito populations.

CONCLUSION

This study highlights the potential use of NPV as a biocontrol agent against mosquito populations. The findings of this study indicate that NPV infection significantly affects the expression of the vitellogenin gene and alters the protein profile of the ovaries in female mosquitoes. These findings provide valuable insights into the mechanisms underlying the regulation of vitellogenin gene expression and protein synthesis in mosquitoes, which can contribute to the development of new strategies for mosquito-borne disease control.

ETHICAL APPROVAL

This research paper was approved by the research ethics committee from Faculty of Science, Ain Shams University (ASU-SCI/ENTO/2023/5/1).

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