J. ISSAAS Vol. 25, No. 2: 23-30 (2019)

SUSCEPTIBILITY OF ONION ARMYWORM, *Spodoptera exigua* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE), LARVAE TO *Spodoptera exigua* MULTIPLE NUCLEOPOLYHEDROVIRUS (SeMNPV)

Melissa P. Montecalvo* and Marcela M. Navasero
National Crop Protection Center, College of Agriculture and Food Science,
University of the Philippines Los Baños, 4031 College, Laguna, Philippines
*Corresponding author: mpmontecalvo@up.edu.ph

(Received: July 19, 2019; Accepted: October 24, 2019)

ABSTRACT

Bioassays were carried out to determine the effect of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) to the different larval instars of *Spodoptera exigua* from May to September 2018 at the National Crop Protection Center, University of the Philippines Los Baños. Fresh castor leaves were treated with crude SeMNPV concentrations of 10^3-10^8 polyhedral inclusion body (PIB)/ml and were fed to five larval instars. Larval mortality increased with increasing crude SeMNPV concentrations. The 1st instar larvae were the most susceptible with >80% mortality, while 10-93% mortality was recorded in older larvae. The virus caused disintegration of larval body and distortion in pupae. Based on probit analysis, lethal dose $(LD)_{50}$ ranged from 7.05×10^5 to 1.71×10^9 PIB/ml. Higher SeMNPV doses are required to cause lethal infection in older larvae population. The LD_{50} and LD_{95} from 1st to 5th larval instar increased by 94 and 33,333 times, respectively. The virus significantly affected the growth and development of the larvae by prolonging the larval period and decreasing pupation rate (0-60%) and adult emergence (0-40%). Less than 50% of the treated 4th and 5th larval instars pupated and emerged into adults. This research suggests that SeMNPV is infective to various larval instars of *S. exigua* resulting in high mortality and reduced growth and development.

Key words: SeMNPV, onion armyworm, polyhedral inclusion bodies, biological control, *Spodoptera exigua*

INTRODUCTION

Onion armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), had an outbreak in onion growing provinces in the Philippines particularly in Nueva Ecija, Pangasinan, and Tarlac in 2016 (Navasero et al. 2017). About 4,089 onion farmers in 14 municipalities with a total land area of 5,330 ha were affected by the pest outbreak. Armyworm infestation resulted in defoliation, blighting, and drying of onion leaves. Other crops such as *Capsicum annuum*, *Cucurbita maxima*, *Momordica charantia*, *Zea mays*, and *Vigna unguiculata* were also damaged by this insect pest (Candano et al. 2019). Likewise, this polyphagous insect was observed in several weed hosts such as *Achyrantes aspera*, *Amaranthus viridis*, *Centrocema pubescens*, *Commelina benghalensis*, *Eleusine indica*, *Synedrella nodiflora*, *Rottboellia cochinchinensis*, and *Tagetes sp*. The severe infestation of armyworm resulted in increased cost in farm production and crop loss.

Long distance migration from other countries northeast of Philippines as triggered by El Niño may have caused the pest outbreak (Navasero et al. 2017). Likewise, misapplication of pesticide and use of smuggled and banned insecticides were believed to contribute to the outbreak of this pest. Onion growers reported that the pest easily withstand chemical spray, which may suggest pesticide resistance development due to malpractices in pesticide application. The control of insect pests such as armyworm is mainly based on the use of chemical insecticides. However, an integrated pest management program (IPM) should be implemented to eliminate reliance to chemical insecticides and possible development of pesticide resistance. Thus, the use of biological control agents must be an option to manage this serious pest. The damage of armyworm is limited by the natural parasitoids and predators (Capinera 2017). The fungal species (*Erynia sp*. and *Nomuraea rileyi*) and nuclear polyhedrosis virus (NPV) also cause larval mortality (Ruberson et al. 1994).

NPVs cause epizootic death to lepidopteran pests by infecting the midgut cells that leads to chronic infection (Kumar et al. 2008). They are host specific and virulent making them promising biological control agents. An in-depth study on the use of NPVs for beet armyworm had been explored by Smits (1987). Among the 5 multiple NPVs evaluated, *S. exigua* multiple NPV (SeMNPV) had the highest biological activity against beet armyworm. The application of SeMNPV at 1 x 10^8 polyhedra/ml resulted in 95-100% larval mortality in chrysanthemum, gerbera, kalanchoe, and tomato crops. SeMNPV was also identified to infect *S. exigua* larvae in the Philippines (Cayabyab, personal communication, 2019). Filtered and crude SeMNPV extracts caused 62.5% and 72% mortality *in vitro*.

With the promise on the use of NPVs in controlling the pest, preliminary studies were conducted to assess the potential of SeMNPV as biological control agent against onion armyworm *in vitro*. Primarily, this research work investigated the effects of SeMNPV on larval mortality and development of *S. exigua*.

MATERIALS AND METHODS

Preparation of crude *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV). SeMNPV infected larvae were macerated and filtered with cheesecloth to prepare the stock suspension. Dilutions were made by adding distilled water to the stock suspension to enable counting of polyhedral inclusion bodies (PIB) using a haemacytometer under 400x magnification. Six (6) concentrations of SeMNPV with 10^3 to 10^8 PIB/ml were prepared by diluting the stock suspension with specific volume of distilled water.

Bioassay procedure. Different concentrations of crude SeMNPV were used in the assays and each was replicated three times. A total of 10 larvae of *S. exigua* were used per instar in each replicate. Ten 1st and 2nd larval instars were initially cultured in sterile disposable Petri plates and were single-cultured when they grew as 3rd instar. For 3rd, 4th, and 5th larval instars, 1 larva was cultured per plate.

Leaf discs of fresh castor leaves were prepared using a cork borer. These leaves were surface sterilized in 0.5% sodium hypochlorite solution for 10 minutes and washed thrice in sterile distilled water (Magholi et al. 2014). After airdrying, leaf discs were dipped in crude concentrations of SeMNPV for 1min then airdried prior to introducing them to the larva. Leaf discs dipped in distilled water were used for control. In each Petri plate, the tested larvae were placed in between castor leaves and allowed to feed for 24h. After which, fresh untreated and surface-sterilized castor leaves were given to the tested larvae daily. The experimental set-up was incubated and overlaid in blotter to maintain humidity under ambient temperature.

Mortality was observed daily until the larvae either died, pupated, or emerged. Percentage mortality was corrected using the equation: $M [\%] = [(t - c)/ (100 - c)] \times 100$, where: M – corrected mortality; c – percentage mortality in controls; t – percentage mortality in treatments (Abbott, 1925). Lethal dose (LD) estimates were calculated using PriProbit ver 1.63. The effect on growth and development of SeMNPV on larval instars of onion armyworm were also observed. Percentage pupation, larval period, and adult emergence were calculated. One-way ANOVA was performed and means were compared using Tukey's Honestly Significant Difference Test (HSD).

RESULTS AND DISCUSSION

Effect of crude SeMNPV on larval mortality of *Spodoptera exigua.* Crude SeMNPV concentrations effectively caused mortality to onion armyworm, *S. exigua*. Mean values of mortality are shown in Figure 1. Increasing concentrations of the virus affected more larvae. Highest mortality was recorded in 1st instar larvae (>80%), which appeared to be the most susceptible stage. Larval mortality ranged from 10-93%, 17-93%, 17-47%, and 57-63% for 2nd, 3rd, 4th, and 5th instars, respectively. Briese (1986) noted that susceptibility of lepidopteran insects to NPVs decreases with age and correlated to the increase in larval weight as development proceeds.

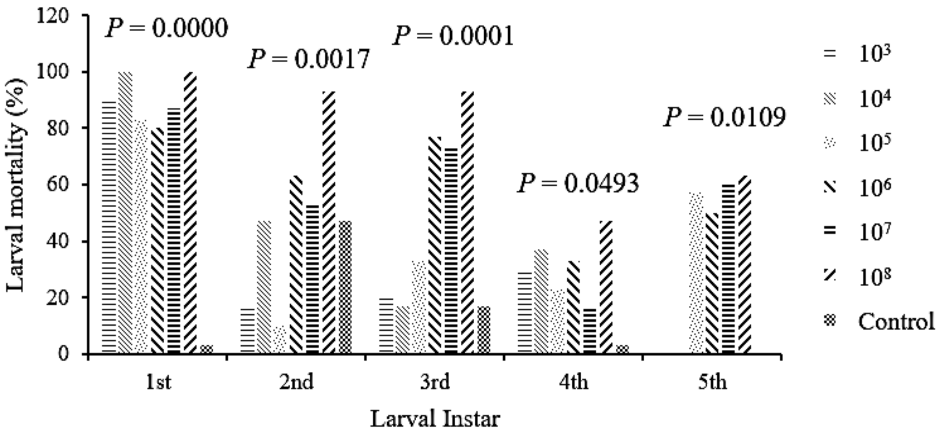

Fig. 1. Mean mortality of different instars of *Spodoptera exigua* larvae due to crude SeMNPV at 7 days after treatment. Means are significant at $P < 0.05$.

Dead larvae showed discoloration of a portion of the larval body (Fig. 2). SeMNPV also caused infection to half of the larval body as well as complete disintegration of the exoskeleton. These observations can be attributed to the infection of the midgut epithelial cells due to ingestion of NPVs along with the food (Smits 1987). The proteinaceous occlusion bodies dissolved under the alkaline conditions (pH >9) in the larval gut allowing the virions to spread and infect the cells and tissues resulting in cell lysis. The infected larvae died several days later after most tissues have been infected and completely disintegrated. In our bioassays, we also observed that infected pupae were distorted and failed to emerge into adults.

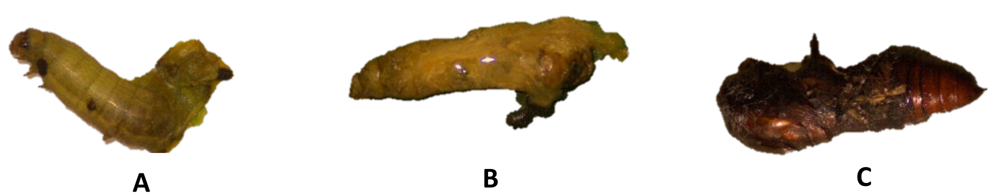

Fig. 2. Pathological appearance of *Spodoptera exigua* due to crude SeMNPV showing infection of half of larval body (a), disintegration of the body (b), and distorted pupa (c).

Probit analysis of mortality resulted in calculation of lethal dose (LD) estimates (Table 1). First instar larvae were susceptible to SeMNPV doses at 1.61 x 10^7 to 2.13 x 10^7 polyhedral inclusion body

(PIB)/ml, which could infect 10-95% of its larval population. However, LD_{10} and LD_{50} values of 1st (1.61 x 10^7 and 1.82 x 10^7 PIB/ml) larval instar are higher than the 2nd (2.53 x 10^5 and 6.70 x 10^6 PIB/ml) and 3rd (8.46 x 10^3 and 7.05 x 10^5 PIB/ml) larval instars. The 1st larval instar is usually one day only (Navasero et al. 2019) and feeds in small amount, hence, the need to be exposed to higher concentration of the virus. Their low feeding ability as compared with older larval instars also imply the need for longer exposure to SeMNPV treated leaves.

Table 1. Calculated lethal dose estimates of crude SeMNPV treated larval instars of *Spodoptera exigua*.

Larval Instar	**Lethal Dose (LD) Estimates**			**Slope**	**Intercept**
	10	**50**	**95**		
1st	1.61 x 10^7	1.82 x 10^7	2.13 x 10^7	24.264	-176.155
2nd	2.53 x 10^5	6.70 x 10^6	4.49 x 10^8	0.901	-6.148
3rd	8.46 x 10^3	7.05 x 10^5	2.06 x 10^8	0.667	-3.902
4th	7.66 x 10^7	1.31 x 10^8	2.62 x 10^8	5.481	-44.492
5th	1.56 x 10^7	1.71 x 10^9	7.10 x 10^{11}	0.628	-5.800

The calculated LD estimates also suggest that increasing crude SeMNPV doses are required to kill higher number of larvae in the later stages (4th to 5th instars). Calculated LD_{50} ranged from 7.05 x 10^5 to 1.71 x 10^9 PIB/ml. LD_{50} increased by 94 times from 1st (1.82 x 10^7 PIB/ml) to 5th (1.71 x 10^9 PIB/ml) larval instar. LD_{95} also rose to 33,333 fold from 1st (2.13 x 10^7 PIB/ml) to 5th (7.10 x 10^{11} PIB/ml) larval instar. High LD values calculated in our assay particularly for 5th instar imply that lethal infection at later larval age will occur if older larvae consume more virus-treated foliage and higher virus dosages. This was also observed in *S. exigua* (Smits and Vlak 1988), for *Pieris rapae* granulosis virus (Payne et al. 1981), and for *Mamestra brassicae* MNPV (Evans 1981).

Previous findings support our result on increasing LD values during larval development (Smits 1987; Trang and Chaudhari 2002). Growing larva increases its foliage consumption and changes its feeding behavior resulting in higher intake of polyhedra (Smits 1986). LD_{50} between 1st and 2nd instar of *M. brassicae* larvae rose to about 7 to more than 900 polyhedra with the corresponding MbMNPV (Evans 1981). LD_{50} values for older instars were 5 to 250 times of the 2nd instars. On the other hand, the LC_{50} for *S. litura* increased by 1,500,000 fold from 1 x 10^3 PIB/ml for 2-day old larvae to 1.5 x 10^9 PIB/ml for 8-day old larvae (Trang and Chaudhari 2002). This observation suggests that insects like beet armyworm become less susceptible to SeMNPV with age (Smits and Vlak 1988). The LD_{50} values for *S. exigua* due to SeMNPV were 4, 3, 39, 102, and 11,637 occlusion bodies for the neonate, second-, third-, fourth-, and fifth-instar larvae (Smits and Vlak 1988). On the other hand, reported LD_{50} values for *S. exigua* were 2.5, 11.2, 5.5, 32.4, and 181.8 polyhedral occlusion bodies for neonate, second-, third-, fourth-, and fifth-instar larvae (Takatsuka and Kunimi 2002). The lower calculated LD_{50} of 3rd instar in our experiment conforms with the observation of Takatsuka and Kunimi (2002) wherein the median lethal dose of SeMNPV increased with host instar of *S. exigua* except for the third instar.

The calculated LD_{95} value (7.10 x 10^{11} PIB/ml) for 5th instar in our assay can be explored in field trials to meet the potential field control efficacy. Weekly application of technical grade lyophilized preparation of SeMNPV at rates of 2.5 x 10^{11} and 12.5 x 10^{11} occlusion bodies/ha significantly reduced plant damage due to beet armyworm (Kolodny-Hirsch et al. 1993).

Effect of crude SeMNPV on growth and development of *Spodoptera exigua*. The growth and development of the larval instars were significantly affected by SeMNPV (Fig. 3 to 5). Generally, the virus prolonged the larval duration of *S. exigua*. Larval duration was extended for up to 5 days in virus-infected 3rd and 4th larval instars. Both treated and control 1st instar larvae did not pupate (data not presented). Pupation rate and adult emergence of 2nd to 5th larval instars significantly decreased when treated with crude SeMNPV particularly at higher concentrations. Pupation rate was reduced in SeMNPV treated larvae (0-60.00%) as compared to control (43.33-90.00%). No pupation was observed when treated with higher crude extract at 10^6-10^8 PIB/ml in 2nd instar and 10^7-10^8 PIB/ml in 3rd instar larvae. Likewise, exposure to the virus resulted to less pupae emerging into adults. Adult emergence was remarkably lowered in SeMNPV (0-40.00%) than in control (43.33-66.67%). Less than 50% of the treated larvae pupated and emerged into adults in 4th and 5th larval instars.

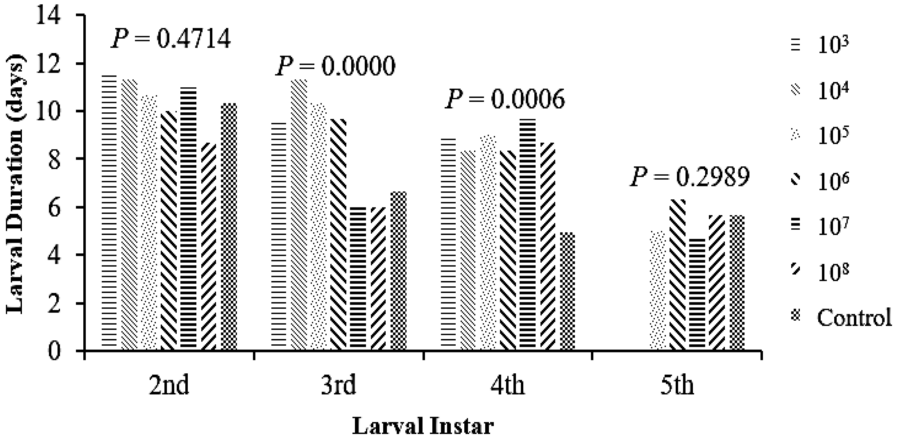

Fig. 3. Mean duration of the larval instars of *Spodoptera exigua* exposed to crude SeMNPV. Means are significant at $P< 0.05$.

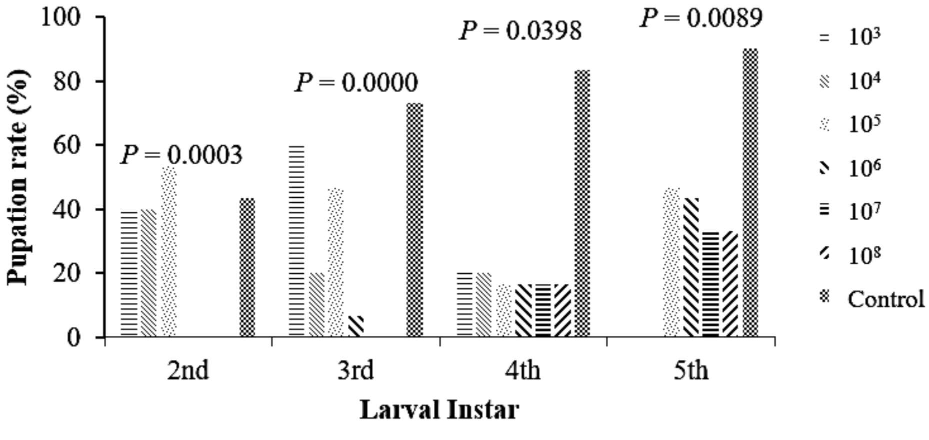

Fig. 4. Mean percent pupation rate of *Spodoptera exigua* exposed to crude SeMNPV. Means are significant at $P< 0.05$.

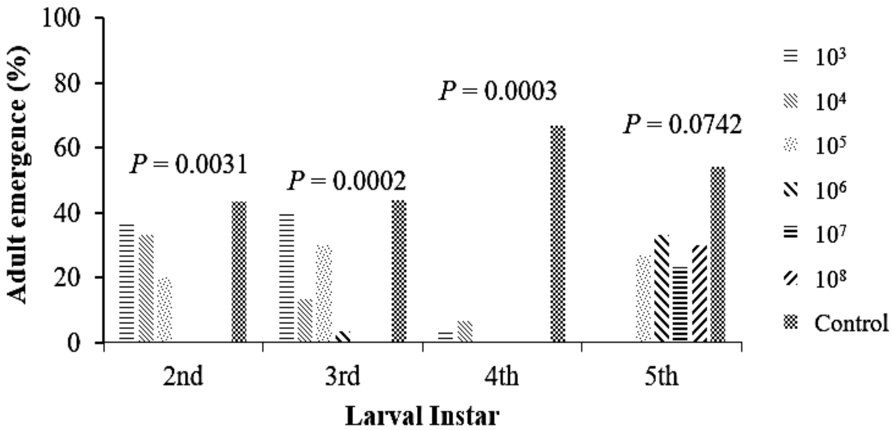

Fig. 5. Mean percent adult emergence of *Spodoptera exigua* exposed to crude SeMNPV in their larval stages. Means are significant at $P < 0.05$.

Our findings on the effect of NPVs in prolonging larval period and reducing pupation rate and adult emergence agree with earlier findings (Magholi et al. 2014). NPV infections result in debilitating effects such as prolonged development, lower pupal weight, and reduced reproductive capacity (egg production and hatching success of eggs) (Rothman and Myers 1996). The impaired development and reproductive success result in an additional 22% reduction in population growth. The prolonged developmental time has great impact on the reduction of the rate of population buildup and allow more time of exposure to the natural enemies, insecticides, and environmental stresses. Likewise, fewer generations per season can be expected due to longer generation time for the insects, hence, less potential damage to crops.

The lethal effect of SeMNPV as shown in our bioassays confirms its potential for *S. exigua* management. SeMNPV was reisolated from beet armyworm populations in California (Gelernter and Federici 1986), Florida (Kolodny-Hirsch et al. 1993), and Japan (Takatsuka and Kunimi 2002). Likewise, the efficacy of SeMNPV has been demonstrated for the control of beet armyworm in several vegetable crops (Kolodny-Hirsch et al. 1993). NPV was also effective against *S. litura* in onion field trials in Bongabon, Nueva Ecija, Philippines in 1993 (Cultural Practice 2019). This microbial along with *Bacillus thuringiensis* (Bt) and NPV + Bt remarkably reduced the larval counts and improved yield than insecticide (Karate) application.

CONCLUSION AND RECOMMENDATION

Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) was infective against different larval instars of *Spodoptera exigua* infesting onion. Larval feeding with crude SeMNPV-treated castor leaves resulted in high larval mortality and reduced growth and development. Our significant observations on the potential of SeMNPV as entomopathogen for *S. exigua* implies that this biocontrol agent can be considered as a management option for onion armyworm. Hence, further experiments must be conducted to formulate the SeMNPV and evaluate its control efficacy in the field.

ACKNOWLEDGEMENT

The authors appreciate the funding support provided by the Department of Agriculture – Bureau of Agricultural Research (DA-BAR), Philippines. They would also like to thank Mr. Randolph N. Candano, Ms. Jovel P. Ybañez, Ms. Lerma dP. Oguan, Mr. Arcangel P. Cueto, and Ms. Sylvia M. Calderon for their assistance during the conduct of the research.

REFERENCES CITED

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265–267.

Briese, D.T. 1986. Insect resistance to baculoviruses. In: Granados, R.R. and B.A. Federici (Eds.). The Biology of Baculoviruses, Vol. 2. CRC Press, Boca Raton, Florida, 237– 263 p.

Candano, R.N., W.N. de Panis, M.V. Navasero, and M.M. Navasero. 2019. Survey of host plants of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) in Luzon, Philippines. Submitted for publication.

Capinera, J.L. 2017. Beet armyworm in featured creatures. Retrieved January 21, 2019 from the World Wide Web: http://entnemdept.ufl.edu/creatures/veg/leaf/beet_ armyworm.htm.

Cultural Practice. 2019. Potential of nuclear polyhedrosis virus (NPV) and *Bacillus thuringiensis* (Bt) for *Spodoptera* control in yellow granex onions (Microbial control of *Spodoptera* in onions). Retrieved June 7, 2019 from the World Wide Web: http://crsps.net/resource/potential-of-nuclear-polyhedrosis-virus-npv-and-bacillus-thuringiensis-bt-for-spodoptera-control-in-yellow-granex-onions-microbial-control-of-spodoptera-in-onions.

Evans, H.F. 1981. Quantitative assessment of the relationships between dosage and response of nuclear polyhedrosis virus of *Mamestra brassicae*. J. Invertebr. Pathol. 37:101-109.

Gelernter, W.D. and B.A. Federici. 1986. Isolation, identification and determination of virulence of a nuclear polyhedrosis virus from beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) on head lettuce. Environ. Entomol. 15:240-245.

Kolodny-Hirsch, D.M., D.L. Warkentin, B. Alvarado-Rodriguez, and R. Kirkland. 1993. *Spodoptera exigua* nuclear polyhedrosis virus as a candidate viral insecticide for the beet armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol., 86:314-321.

Kumar, N.S., K. Murugan, and W. Zhang. 2008. Additive interaction of *Helicoverpa armigera* nucleopolyhedrovirus and azadirachtin. BioControl 53: 869–880.

Magholi, Z., H. Abbasipour, and R. Marzban. 2014. Effects of *Helicoverpa armigera* nucleopolyhedrosis virus (HaNPV) on the larvae of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Plant Protect. Sci. 50(4): 184-189.

Navasero, M.V., M.M. Navasero, B.F. Cayabyab, R.N. Candano, M.D. Ebuenga, G.A.S. Burgonio, N.M. Bautista, E.M. Aquino, and G.G. Gaspar. 2017. Investigation on the 2016 outbreak of the onion armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), in onion growing areas in Luzon. Abstracts. The Annual Scientific Conference of the Philippine Association of Entomologists, Inc. The 49th Annual Convention of the Pest Management Council of the Philippines, Inc. May 10-12, 2017. Malay, Aklan, Philippines: Philippine Ent. 31(2): 141-142.

Navasero, M.M., M.V. Navasero, R.N. Candano, and W.N. de Panis. 2019. Comparative life history, fecundity and survival of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) on *Allium cepa* L. and other host plants on the Philippines. Philipp. Ent. 73-84.

Payne, C.C., G.M. Tatchell, and C.F. Williams. 1981. The comparative susceptibilities of *Pieris brassicae* and *P. rapae* to a granulosis virus from *P. brassicae*. J. Invertebr. Pathol. 38: 273-280.

Rothman, L.D. and J.H. Myers. 1996. Debilitating effects of viral diseases on host lepidoptera. J. Invertebr. Pathol. 67: 1–10.

Ruberson, J.R., G.A. Herzog, W.R. Lambert, and W.J. Lewis. 1994. Management of the beet armyworm (Lepidoptera: Noctuidae) in cotton: Role of natural enemies. Florida Entomologist 77: 440-453.

Smits, P.H. 1986. Calculations on the intake of polyhedra by beet armyworm larvae feeding on virus sprayed chrysanthemums. In: Samson, R.A., J.M. Vlak, and D. Peters (eds.). Fundamental and applied aspects of invertebrate pathology. Proc. 4^{th}. Int. Coll. Invertebr. Pathol., Veldhoven, the Netherlands, 161-619 p.

Smits, P.H. 1987. Nuclear polyhedrosis virus as biological control agent of *Spodoptera exigua*. [PhD Dissertation]. Wageningen Agricultural University. Netherlands. 121 p. Retrieved June 1, 2018 from the World Wide Web: http://edepot.wur.nl/201983.

Smits, P.H. and J.M. Vlak. 1988. Biological activity of *Spodoptera exigua* nuclear polyhedrosis virus against *Spodoptera exigua* larvae. J. Invertebr. Pathol. 51(2): 107-114.

Takatsuka, J. and Y. Kunimi. 2002. Lethal effects of *Spodoptera exigua* nucleopolyhedrovirus isolated in Shiga Prefecture, Japan, on larvae of the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae). Applied Entomology and Zoology 37(1): 93-101.

Trang, T.T.K. and S. Chaudhari. 2002. Effect of NPV and NPV in combination with insecticides on *Spodoptera litura*. Science and Technology Journal of Agriculture and Rural Development 6: 491-493.