Int. J. Indust. Entomol. Vol. 21, No. 1, 2010, pp. 139~143

International Journal of
Industrial Entomology

Formulation of *Mamestra brassicae* Nucleopolyhedrovirus-K1 as Viral Insecticide

Jae-Bang Choi, Tae-Young Shin, Sung-Min Bae and Soo-Dong Woo*

Department of Agricultural Biology, College of Agriculture Life & Environment Sciences, Chungbuk National University, Cheongju 361-763, Korea

(Received 20 July 2010; Accepted 27 August 2010)

The objective of our study was the formulation of a local strain of *Mamestra brassicae* nucleopolyhedrovirus-K1 (MabrNPV-K1) for the development of viral insecticide to control *M. brassicae*. To formulate MabrNPV-K1, feeding toxicities of various supplements and ultraviolet (UV)-protection were investigated. Optical brightener Tinopal UNPA-GX (Tinopal) as UV protectant and Bentonite had some toxicity themselves to increase the mortality. The protection of polyhedra from UV light radiation was observed only by Tinopal. The MabrNPV-K1 was formulated as a wettable powder form. The mortality of the formulation was higher and rapid than that of the un-formulated. This suggested the possibility of MabrNPV-K1 formulation as an effective biological control agent for *M. brassicae*.

Key words: *Mamestra brassicae*, Formulation, MabrNPV-K1, Tinopal

Introduction

Baculoviruses are members of a single family, the baculoviridae, which has been proposed recently for the revision family into four genera based on molecular phylogeny and host insects (Jehle *et al*., 2006). According to this revision, *Nucleopolyhedrovirus* (NPV), one of two genera of lepidopteran specific Baculoviruses, has been given a new genus name, *Alphabaculovirus*. Baculoviruses are virulent pathogens of insects and have been well characterized due to their potential as biological control agents (Moscardi, 1999; Erlandson and Theilmann, 2009). An important factor contributing to the success of baculoviruses as biological control agents against insect pests is the ability of the virus to persist in the environment (Hughes *et al*., 1997). This is achieved largely by protection of virus particles in proteinaceous occlusion bodies (OBs) or polyhedra (Harrap *et al*., 1977; Hunter *et al*., 1984). Polyhedra are composed mainly of a single virus-encoded polypeptide, polyhedrin, which forms a quasi-crystalline lattice into which the virus particles become embedded (Rohrmann, 1986). Survival may be achieved by persistence of polyhedra in soil or decaying leaf matter, particularly during periods when the insect host is not available (Hughes *et al*., 1997). Following application to plant surfaces, polyhedra are rapidly inactivated by solar ultraviolet (UV) radiation, particularly in the UV-B range of 280~320 nm (Killick, 1990; Morris, 1971). Fluorescent (optical) brighteners are known for their characteristics of protecting baculoviruses against deactivation by UV light and enhancing the activity of these agents as microbial insecticides on hosts and semipermissive hosts (Lauro *et al*., 2001). Chief of all, there are some reports which Stilbene optical brighteners can significantly improve OB persistence by absorbing UV radiation and re-emitting the energy as visible blue light (Dougherty *et al*., 1996; Shapiro, 1992; Shapiro and Farrar, 2003). Also, Stilbene derived optical brighteners could improve the insecticidal properties of baculoviruses (Lasa *et al*., 2007).

The cabbage armyworm, *Mamestra brassicae* is a serious insect pest of numerous vegetables and ornamental plants in Europe and Asia including Korea (Kwon *et al*., 2005). *M. brassicae* is a polyphagous insect species that survives on many species of plants (Ulland *et al*., 2008). The larval stage is highly polyphagous and is known to feed on more than 70 species of host plants from 22 fam-

*To whom the correspondence addressed
Department of Agricultural Biology, College of Agriculture Life & Environment Sciences, Chungbuk National University, Cheongju 361-763, Korea.
Tel: +82-43-261-2553; Fax: +82-43-271-4414;
E-mail: sdwoo@cbnu.ac.kr

ilies, including *Brassica* species, lettuce, beet, onion, potato, pea, tomato, apple, chrysanthemum, beech and oak (Popava, 1993; Rojas, 1999). Feeding by the caterpillars causes severe damage on the plants in monocultures and the species is an economically important pest in agriculture (Ulland *et al*., 2008). Although it can be controlled by some chemical insecticide application, their control is not easy because rapid development of resistance and the use of pesticide sprays on vegetables such as cabbage is considered undesirable by consumers (Kwon *et al*., 2005; Chougule *et al*., 2008). Several NPVs isolated from *M. brassicae* have been considered useful biological-control agents for *M. brassicae* and one of them has been commercialized (Aruga *et al*., 1960; Akutsu, 1972; Okada, 1977; Vlak and Groner, 1980; Brown *et al*., 1981; Evans and Allaway, 1983).

Recently, we reported the isolation of novel MabrNPV-K1 which has higher pathogenicity than commercialized MabrNPV (Lee *et al*., 2008). For the practical use of MabrNPV-K1 as a viral insecticide, it is necessary to determine the supplements including fluorescent (optical) brighteners toward MabrNPV-K1 in formulation. Here we report the efficacy of the supplements and formulated MabrNPV-K1 against *M. brassicae* larvae.

Material and Methods

Insect and virus

M. brassicae larvae from National Institute of Highland Agriculture (Pyeongchang, Korea) were maintained on a Chinese cabbage diet. All experiments were conducted at 25°C, 60% relative humidity under a 16 h light/8 h dark photoperiod. The MabrNPV-K1 was propagated in third instars larvae of *M. brassicae* by oral infection of polyhedra. Purification of polyhedra from dead larvae was performed as follows. The infected larva was homogenized and then filtered through sterile cheesecloth. After centrifugation of the mixture, the polyhedra pellet was resuspended in washing buffer (50 mM Tris-HCl, pH8.0, 10 mM EDTA, 5% β-mercaptoethanol, 4% SDS), and was re-centrifuged. Purified polyhedra pellet was resuspended into distilled water. The concentration of polyhedral inclusion bodies (PIBs) in the stock suspension was determined by using a Thoma haemocytometer under a phase-contras microscope. PIBs were maintained at 4°C until use.

Optical brightener and formulation

The fluorescent brightener Tinopal (Fluorescent brightener 28, Tinopal UNPA-GX; Sigma) was used as an optical brightener at a final concentration of 1% (w/v). The purified polyhedra were formulated as a wettable powder (WP) using previously reported supplements (Table 1). The WP formulation was prepared by direct mixing of polyhedra suspension with the addition of supplements. After the mixing of all components, the material was dried at room temperature and ground with an air mill.

Table 1. Composition of formulation using *Mamestra brassicae* Nucleopolyhedrovirus-K1

Ingredient	Formulation	Ingredient/last volume (%)[a]
Tinopal UNPA-GX	1 g	1
Sucrose	5 g	5
Polyvinylalcohol	0.5 g	0.5
Triton X-100	0.1 ml	0.1
Bentonite	0.1 g	0.1
Virus (1.0×10^{10})	10 ml	1.0×10^{10} PIBs/ml

[a]Add the ingredients of the formulation to 100 ml of water for laboratory use

Bioassay

The feeding toxicities of various supplements including fluorescent brightener and formulate were determined. Bioassay was carried out with 3rd instar larvae of *M. brassicae*. Before bioassay, *M. brassicae* larvae were maintained at insect breeding dish (SPL Co., Korea) without diet for 24 h. After 24 h, *M. brassicae* larvae were inoculated by using a droplet-feeding method with or without a virus (1×10^6 PIBs/larva) to 20 larvae per dose. Mortality was tabulated daily and data were analyzed on the basis of mortality on day 10 post-infection. The median lethal Time (LT_{50}) values were determined by Probit analysis (Finney, 1971).

Ultraviolet radiation

To determine of UV protection provided by fluorescent brighteners and sucrose, MabrNPV-K1 was diluted to a final concentration of 1×10^6 PIBs/larva, in either 1% Tinopal or 5% sucrose suspension. Virus suspension, with and without Tinopal and sucrose, was pipette onto the diet in insect breeding dish and was exposed to UV light radiation for 90 min. UV light radiation was provided by a 30-W germicide mercury lamp (Model NIS G30 T8; Sankyo Denky Co., Japan) and was positioned 40 cm above the surface of the diet. Insect breeding dish with the same treatment, but not exposed to UV, were used as controls. Mortality was assessed up to 10 days after UV exposure, when surviving insects had reached the prepupal stage. The level of protection conferred by the formulations to the pathogen exposed to UV light was also evaluated with same method in laboratory condition.

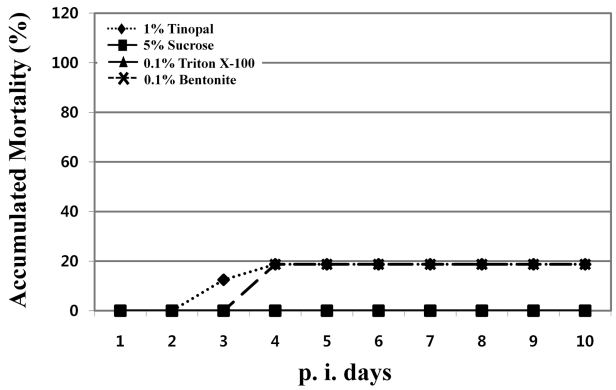

Fig. 1. Effect of supplements on *Mamestra brassicae* nucleopolyhedrovirus-K1 infection of 3rd instar of *M. brassicae.*

Table 2. Values of lethal time (LT_{50}) of *Mamestra brassicae* nucleopolyhedrovirus-K1 by different additives against 3rd instar of *M. brassicae*

Treatment (PIBs/larva)	LT_{50} (95%CL)	LT_{95} (95%CL)
MabrNPV-K1 1.0×10^6	6.17 (6.0-6.3)	7.87 (7.5-8.2)
+1% Tinopal UNPA-GX	5.07 (4.9-5.2)	7.85 (7.4-8.4)
+5% Sucrose	5.96 (5.8-6.1)	7.88 (7.5-8.3)
+0.1% Bentonite	5.36 (5.1-5.5)	9.15 (8.6-9.8)
+0.1% Trion X-100	5.69 (5.5-5.8)	8.58 (8.1-9.1)

Result and Discussion

Effects of supplements on virus mortality

The toxicity of supplements was evaluated against to *M. brassicae* 3rd instar larvae. No mortality was observed in 3rd instar larvae treated with 5% sucrose or 0.1% Triton X-100. However, mortality was observed in 3rd instar larvae treated with 1% Tinopal and 0.1% Bentonite showed 18%, respectively (Fig. 1). This suggest that the already reported toxicity of Tinopal and Bentonite to insect (Morales *et al*., 2001) is effective to *M. brassicae* larvae. The addition of each supplement to MabrNPV-K1 some decreased the mean time to death of *M. brassicae* larvae. The median lethal time (LT_{50}) values of MabrNPV-K1 with supplements were determined as 5.96 to 5.07 (Table 2). The addition of Tinopal was most effective like its toxicity. These results indicated that the supplements can enhance the mortality of virus. These correspond with the previous similar experiments for other NPVs (Im *et al*., 1990).

Effects of protection from UV radiation

UV light radiation reduced MabrNPV-K1 caused mortality from 100% to 12.5%, but the addition of fluorescent brightener Tinopal protected it up to 85% (Fig. 2). 5% sucrose did not provide good protection to MabrNPV-K1 and provided some UV radiation protection. Analogous data were shown by Shapiro (1992) using brighteners as radiation protectant for the gypsy moth NPV. Substances that might be used as UV protection for insect pathogens may be separated into two groups (Shapiro *et al.* 1983). In the first group, including zinc oxide, titanium oxides, silicates, and talcum, radiation is reflected by these materials. In the second group, these chemicals act as UV protectant by selectively absorbing UV-B rays while transmitting UV-A rays and visible light or by additionally absorbing UV rays. Fluorescent brighteners readily absorb UV light. In this case, light is absorbed in the UV region of the spectrum, converted to longer wavelengths, and emitted at 440 nm in the blue portion of the visible spectrum (Villaume, 1958). The Stilbene group, significantly enhanced larval mortality, reduced the mean time to death, and provided significant protection against UV radiation (Morales *et al*., 2001). These results indicate that

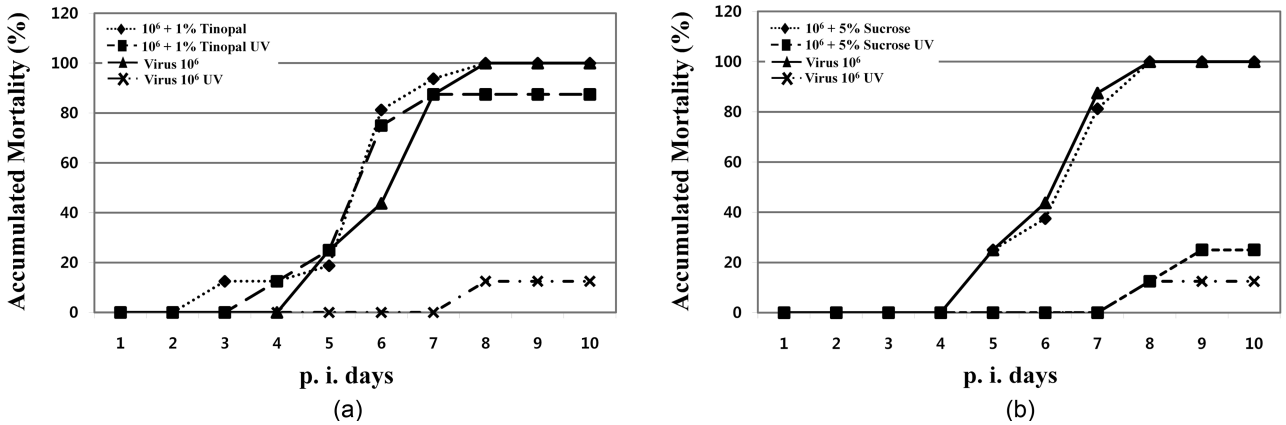

Fig. 2. Activity of *Mamestra brassicae* nucleopolyhedrovirus -K1, with UV protectants Tinopal (a) and Sucrose (b).

Table 3. Values of lethal time (LT_{50}) of *Mamestra brassicae* nucleopolyhedrovirus-K1 by formulation and unformulated virus

Treatment (PIBs/larva)	LT_{50} (95%CL)	LT_{95} (95%CL)
Formulation 1.0×10^6	3.93 (3.8-4.0)	5.14 (4.9-5.4)
Formulation 1.0×10^5	4.15 (4.0-4.3)	5.59 (5.3-5.9)
MabrNPV-K1 1.0×10^6	6.17 (6.0-6.3)	7.87 (7.5-8.2)
MabrNPV-K1 1.0×10^5	6.82 (6.7-6.9)	7.67 (7.5-7.9)
Formulation 1.0×10^6 UV 90 min	4.29 (4.2-4.4)	5.75 (5.5-6.1)
MabrNPV-K1 1.0×10^6 UV 90 min	12.59 (11.2-15.5)	27.20 (20.4-46.3)

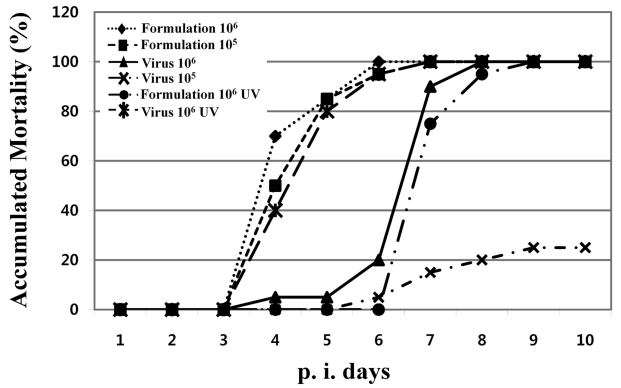

Fig. 3. Pathogenicity and UV protectant of *Mamestra brassicae* nucleopolyhedrovirus -K1 formulation.

fluorescent brightener Tinopal is useful in formulations of the MabrNPV-K1, either to increase activity and speed up killing.

Efficacy of formulation in laboratory condition

Formulations provided mortalities from the dose leaf assay that were greater than the corresponding unformulated virus treatment (Table 3 and Fig. 3). The difference of virus dose did not influence significantly the mortality of *M. brassicae* when it was formulated or not. However, it was clear that formulation caused higher and more rapid mortality than unformulated MabrNPV-K1. The LT_{50} values of MabrNPV-K1 were determined as 4.15 to 3.93 days at formulation and 6.82 to 6.17 days at unformulated virus (Table 3). This difference was clearer after exposure to UV light radiation. The mortality of unformulated virus was reduced from about 100 to 25%, but it was not at formulated virus (Fig. 3). The LT_{50} value of formulated virus was also less changed from 3.93 to 4.29 days (Table 3). On the other hand, UV light radiation increased significantly the LT_{50} value of unformulated virus about two times higher. This leaded the about three times higher LT_{50} value of unformulated virus than formulation. These results are similar to those of Jones (1988) who reported that pure *Spodoptera littoralis* NPV (*Sl*NPV), formulated with commercially available UV protectant, had no better persistence than unformulated and unpurified *Sl*NPV suspensions. Solar UV radiation is a major cause of activity loss in field-applied baculoviruses. Jones (1988) reported that solar UV radiation accounted for up to 88% of the loss of *Sl*NPV activity in the first four days after spraying on cotton in Egypt. UV mediated inactivation of NPV is likely to be a very important factor in the poor persistence of NPV and other microbial (Cherry *et al.*, 2000).

These our results suggest that the formulation of MabrNPV-K1 with various supplements including Tinopal may have high efficacy and stability in filed condition. The efficacy of formulation in field condition should be evaluated, and it will be utilized effectively to control *M. brassicae* larvae.

Acknowledgements

This work was supported by the research grant of the Chungbuk National University in 2009

References

Akutsu K (1972) The use of viruses for the control of vegetable pests. Plant Prot 26, 19-23.

Aruga H, Yoshitake N, Watanabe H, Hukuhara T (1960) Studies on nuclear polyhedrosis and their induction in some Lepidoptera. Japan J Appl Entomol Zool 4, 51-56.

Brown DA, Evans HF, Allen CJ, Kelly DC (1981) Biological and biochemical investigations on five European isolates of *Mamestra brassicae* nuclear polyhedrosis virus. Arch Virol 69, 209-217.

Cherry AJ, Rabindra RJ, Parnell MA, Geetha N, Kennedy JS, Grazywacz D (2000) Field evaluation of *Helicoverpa armigera* nucleopolyhedrovirus formulation for control of the chickpea pod borer, *H. armigera* (Hubn.) on chickpea (*Cicer arietinum* var. Shoba) in Southern India. Crop Prot 19, 51-60.

Choi JB, Lee JK, Bae SM, Shin TY, Koo HN, Kim JI, Kwon M, Woo SD (2009) Pathogenicity and production of *Mamestra brassicae* nucleopolyhedrovirus (MabrNPV)-K1. Int J Indust Entomol 19(2), 237-241.

Chougule NP, Doyle E, Fitches E, Gatehouse JA, (2008) Biochemical characterization of midgut digestive proteases from *Mamestra brassicae* (cabbage moth; Lepidoptera: Noctuidae) and effect of soybean Kunitz inhibitor (SKTI) in feeding assays. Insect Physiol 54(3), 563-572.

Dougherty EM, Guthrie KP, Shapiro M (1996) Optical bright-

eners provide baculovirus activity enhancement and UV radiation protection. Biol Control 7, 71-74.

Erlandson MA, Theilmann DJ (2009) Molecular Approaches to Virus Characterization and Detection. In: Insect Pathogens: Molecular Approaches and Techniques. Patricia Stock SP, Vanderberg J Boemare N, Glazer I, (eds), pp. 3-31, CABI.

Evans HF, Allaway GP (1983) Dynamic of baculovirus growth and dispersal in *Mamestra brassicae* L. (Lepidoptera: Noctuidae) larval populations introduced into small cabbage plots. Appl Environ Microbiol 45, 493-501.

Finney DJ (1971) Probit analysis. 3rd (ed), pp. 33, Cambridge University Press, Cambridge.

Harrap KA, Payne CC, Robertson JS (1977) The properties of three baculoviruses from closely related hosts. Virology 79(1), 14-31.

Hughes DS, Possee RD, King LA (1997) Evidence for the presence of a low-level, persistent baculovirus infection of *Mamestra brassicae* insects. J Gen Virol 78(7), 1801-1805.

Hunter FR, Crook NE, Entwistle PF (1984) Viruses as pathogens for the control of insects. In: Microbial Methods for Environmental Biotechnology. Grainger JM, Lynch JM (eds), pp. 323-347.

Im DJ, Jin BR, Choi KM, Kang SK (1990) Microbial control of the tobacco cutworm, *Spodoptera litura* (Fab.), using S. litura nuclear polyhedrosis virus. J Appl Entomol 29, 244-251.

Jehle JA, Blissard GW, Bonning BC, Cory JS, Herniou EA, Rohrmann GF, Theilmann DA, Thiem SM, Vlak JM (2006) On the classification and nomenclature of baculoviruses: a proposal for revision. Arch Virol 151, 1257-1266.

Jones KA (1988) Studies on the persistence of spodoptera nuclearpolyhedrosis virus on cotton in Egypt. Ph.D. University of Reading.

Killick HJ, Warden SJ (1991) Ultraviolet penetration of pine trees and insect virus survival. Entomophaga 36, 87-94.

Kwon M, Kwon HJ, Lee SH (2005) Temperature-dependent development and seasonal occurrence of Cabbage armyworm (*Mamestra brassicae* L.) at highland chinese cabbage fields. J Appl Entomol 44, 225-230.

Lasa R, Ruiz-Portero C, Alcazar MD, Belda JE, Caballero P, Williams T (2007) Efficacy of optical brightener formulations of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) as a biological insecticide in greenhouses in southern Spain. Biol Control 40, 89-96.

Lee JK, Shin TY, Bae SM, Choi JB, Oh JM, Koo HN, Kim JI, Kwon M, Woo SD (2008) Characterization of *mamestra brassicae* nucleopolyhedrovirus (MabrNPV)-K1 isolated in Korea. Int J Indust Entomol 17, 125-129.

Morales L, Moscardi F, Sosa-Gomez DR, Paro FE, Soldorio IL (2001) Fluorescent brighteners improve *anticarsia gemmatalis* (Lepidoptera: Noctuidae) nucleopolyhedrovirus (AgMNPV) activity on AgMNPV susceptible and resistant strains of the insect. Biol Control 20, 247-253.

Morris ON (1971) Effect of sunlight, ultraviolet and gamma radiations, and temperature on infectivity of a nuclear polyhedrosis virus. J Invertebr Pathol 18, 292-294.

Moscardi F (1999) Assessment of the application of baculoviruses for control of lepidoptera. Annu Rev Entomol 44, 257-289.

Okada M (1977) Studies on the utilization and mass production of *Spodoptera litura* nuclear polyhedrosis virus for control of the tobacco cutworm, *Spodoptera litura* Fabricius. Agric Exp Stn Sere 12, 1-66.

Popova TA (1993) A study of antibiotic effects of cabbage cultivars on the cabbage moth *Mamestra brassicae*. Entomol Rev 72, 125-132.

Rohrmann GF (1986) Polyhedrin structure. J Gen Virol 67, 1499-1533.

Rojas JC (1999) Influence of host plant damage on the host-finding behavior of *Mamestra brassicae* (Lepidoptera: Noctuidae). Environ Entomol 28, 588-593.

Shapiro M, Agin PP, Bell RA (1983) Ultraviolet protectants of the gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus. Environ Entomol 12, 982-985.

Shapiro M, (1992) Use of optical brighteners as radiation protectants for Gypsy-moth (Lepidoptera, Lymantriidae) nuclear polyhedrosis virus. J Econ Entomol 85, 1682-1686.

Shapiro M, Farrar RR (2003) Fluorescent brighteners affect feeding rates of the *corn earworm* (Lepidoptera: Noctuidae) and act as enhancers and sunlight protectants for its nucleopolyhedrovirus. J Entomol Sci 38, 286-299.

Ulland S, Ian E, Stranden M, Borg-Karlson AK, Mustaparta H (2008) Plant volatiles activating specific olfactory receptor neurons of the cabbage moth *Mamestra brassicae* L. (Lepidoptera, Noctuidae). Chem Senses 33, 509-522.

Vlak JM, Groner A, (1980) Identification of two nuclear polyhedrosis viruses from the cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae). J Invertebr Pathol 35, 269-278.

Villaume FG (1958) Optical brighteners in soaps and detergents. J Am Oil Chem Soc 35, 558-566.