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Pathogenicity and Production of *Mamestra brassicae* Nucleopolyhedrovirus (MabrNPV)-K1

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The objective of our study was the evaluation of pathogenicity of a local strain of *Mamestra brassicae* nucleopolyhedrovirus-K1 (MabrNPV-K1) derived from a diseased larva of *M. brassicae* found in Korea. The effect of temperature and larval instar on the pathogenicity and production of MabrNPV-K1 was determined under laboratory conditions. The median lethal concentration (LC_{50}) values of MabrNPV-K1 for 3rd instar larvae were 3.7×10^4 PIBs/larva at 20°C, 9.9×10^2 PIBs/larva at 25°C and 3.8×10^2 PIBs/larva at 30°C, respectively. The LC_{50} for the 4th instar larvae was similar to that for the 3rd instar larvae. However, the pathogenicity to the 3rd instar larvae was higher than that to the 4th instar larvae. The median lethal time (LT_{50}) values of MabrNPV-K1 were 11.4 to 5.0 days at 30°C and 18.3 to 5.5 days at 25°C for the 3rd instar larvae. The LT_{50} value was lowered as temperature went up to 30°C and dependent on viral concentration. In production efficiency of MabrNPV-K1 using *M. brassicae* larvae, the mortality of the 3rd instar larvae was 100% when inoculated with 1.0×10^5 PIBs/larva and the yield of MabrNPV-K1 was maximal. Regarding the mortality, yield of polyhedra, inoculation doses and required time, the 1.0×10^4 PIBs/larva at 30°C was determined as optimal conditions producing polyhedra efficiently.

Key words: *Mamestra brassicae*, Nucleopolyhedrovirus, MabrNPV-K1, Pathogenicity

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Introduction

Baculoviruses are insect pathogenic viruses, mainly lepidopteran larvae including mosquitoes and sawflies (Bonning, 2005). 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*. Nucleopolyhedroviruses form two groups, group I and II, by phylogenetic analysis of several genes (Zanotto *et al*., 1993; Herniou *et al*., 2001). Many of characteristics including the infection process of NPV have been well characterized mainly using the group I NPVs, such as *Autographa californica* NPV, *Orgyia pseudotsugata* NPV and *Bombyx mori* NPV (Williams and Faulkner, 1997; Bonning, 2005). The group II NPVs on the other hand, such as *Lymantria dispar* NPV (Riegel and Slavicek, 1997), *Helicoverpa zea* NPV (Granados *et al*., 1981), *Mamestra brassicae* NPV (MabrNPV; Kondo *et al*., 1995), *Spodoptera exigua* NPV (Hara *et al*., 1994) and *Spodoptera frugiperda* NPV (Knudson and Tinsley, 1974), have different multiplication strategies comparing group I NPVs because of the difference of entry mechanism of budded virus (BV) in haemocoel, and are effective NPVs for most important pests.

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). In spite of many efforts, their control is not easy because rapid development of resistance to chemical insecticides (Kwon *et al*., 2005). Several NPVs isolated from *M. brassicae*

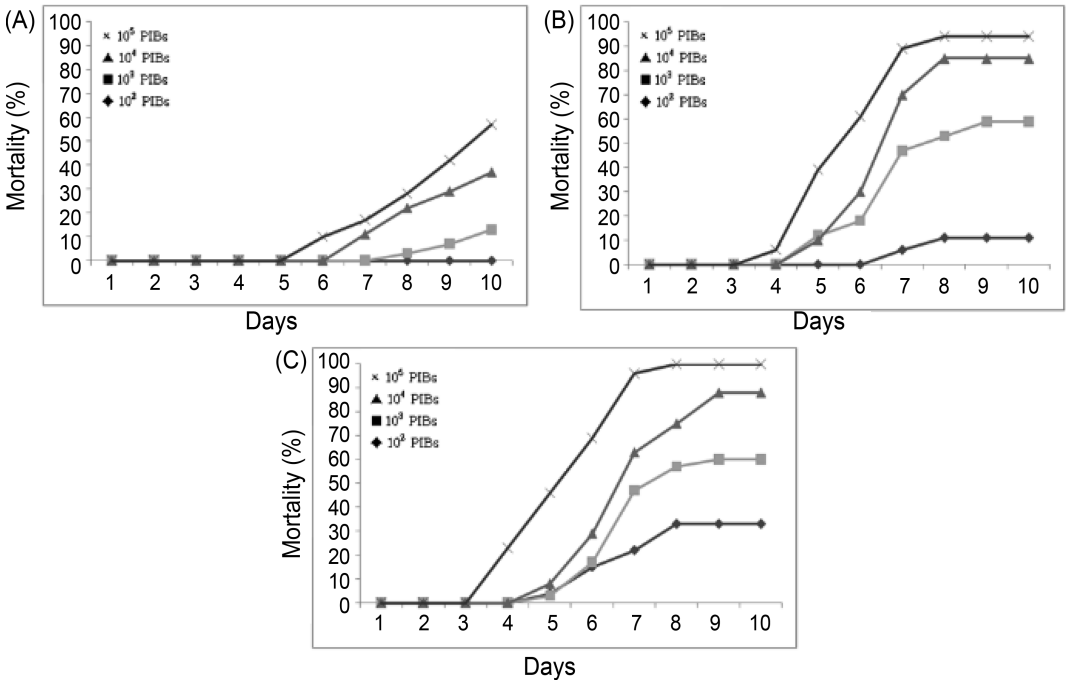

Fig. 1. Mortality of *M. brassicae* 3rd instar larvae at 20°C (A), 25°C (B) and 30°C (C) for the inoculation of MabrNPV-K1.

(Aruga *et al*., 1960; Okada, 1977; Vlak and Groner, 1980; Brown *et al*., 1981) have been considered useful biological-control agents for *M. brassicae* (Akutsu, 1972; Brown *et al*., 1981; Evans and Allaway, 1983). Recently, we reported the isolation of novel MabrNPV-K1 which has higher pathogenicity than commercial MabrNPV (Lee *et al*., 2008). For the practical use of MabrNPV-K1 as a viral insecticide, it is necessary to evaluate its pathogenicity under the various conditions and mass production of polyhedra. The objective of our study was the evaluation of pathogenicity according to the temperature and larval instar, and optimal condition of polyhedra.

Materials and Methods

Insect and viruses

M. brassicae larvae from National Institute of Highland Agriculture (Pyeongchang, Korea) were maintained on a Chinese cabbage diet at 25°C, 60% relative humidity and an 16L:8D 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 and maintained at 4°C until to use.

Bioassay

Bioassay was carried out with 3rd and 4th instar of *M. brassicae* larvae by using a droplet-feeding method with four virus doses (1×10^2, 1×10^3, 1×10^4 and 1×10^5 PIBs/larva) at various temperature conditions (20°C, 25°C and 30°C). Mortality was tabulated daily and data were analyzed on the basis of mortality on day 10 post-infection. The median lethal concentration (LC_{50}) values were determined by Probit analysis (Finney, 1971). The bioassay was performed in three repetitions.

Polyhedra production

To produce polyhedra of MabrNPV-K1 using *M. brassicae* larvae, the efficiency of in vivo production of polyhedra was analyzed for the inoculums concentration and mortality. In order to mass production of MabrNPV-K1, the inoculated larvae of different densities were reared on the same cage at 30°C.

Results and Discussion

Pathogenicity of MabrNPV-K1

The pathogenicity of MabrNPV-K1 was evaluated against to *M. brassicae* larvae according to the larval instar and

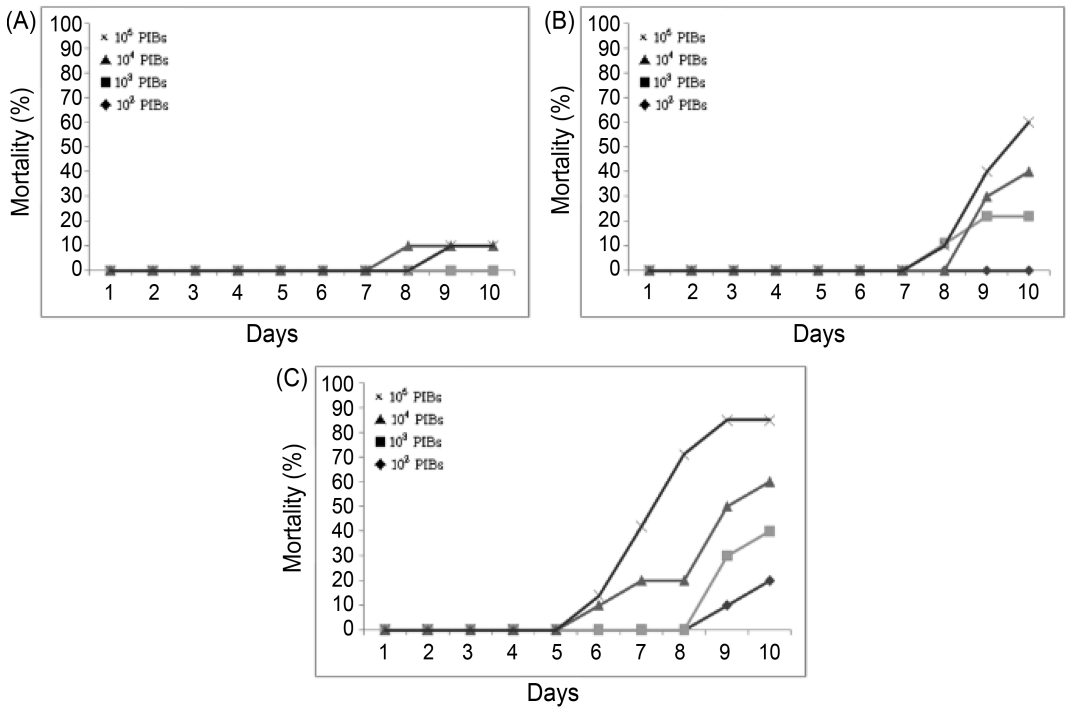

Fig. 2. Mortality of *M. brassicae* 4th instar larvae at 20°C (A), 25°C (B) and 30°C (C) for the inoculation of MabrNPV-K1.

Table 1. Values of lethal concentration (LC_{50}) of MabrNPV-K1 by different temperatures against 3rd instar of *M. brassicae*

Temperatures (°C)	LC_{50} (PIBs/larva)	Regression	95% fiducial limits		χ^2
			lower	upper	
20	3.7×10^4	4.5+0.7×	2.3×10^4	6.8×10^4	4.57
25	9.9×10^2	5.1+0.9×	6.8×10^2	1.4×10^3	9.83
30	3.8×10^2	5.3+0.9×	2.4×10^2	5.6×10^2	3.0

Table 2. Values of lethal concentration (LC_{50}) of MabrNPV-K1 by different temperatures against 4th instar of *M. brassicae*

Temperatures (°C)	LC_{50} (PIBs/larva)	Regression	95% fiducial limits		χ^2
			lower	upper	
20	ND[a]	-	-	-	-
25	2.9×10^4	4.5+0.7×	1.8×10^4	5.5×10^4	9.1
30	2.7×10^3	5.0+0.6×	1.6×10^3	4.5×10^3	0.9

[a]ND- Not Determined

Table 3. Values of lethal time (LT_{50}) of MabrNPV-K1 by different temperatures against 3rd instar of *M. brassicae*

Temperatures (°C)	Concentrations (PIBs/larva)	LT_{50} (95%CL)	LT_{95} (95%CL)
20	1.0×10^2	ND[a]	-
	1.0×10^3	ND	-
	1.0×10^4	10.7(10.0~11.8)	17.9(15.2~23.7)
	1.0×10^5	9.5(9.1~10.2)	14.9(13.1~18.2)
25	1.0×10^2	18.3(13.6~52.6)	46.5(24.7~466.1)
	1.0×10^3	7.9(7.5~8.4)	14.8(12.8~18.8)
	1.0×10^4	6.5(6.4~6.8)	9.6(9.1~10.3)
	1.0×10^5	5.5(5.3~5.7)	8.0(7.5~8.5)
30	1.0×10^2	11.4(10.4~13.5)	23.0(17.8~38.2)
	1.0×10^3	7.8(7.5~8.1)	12.6(11.5~14.6)
	1.0×10^4	6.8(6.6~7.0)	9.8(9.3~10.6)
	1.0×10^5	5.0(4.8~5.2)	7.4(7.0~7.9)

[a]ND- Not Determined

temperature conditions. The mortality generally increased with increase of temperatures and also dependent on viral concentration (Fig. 1 and 2). The mortality of 3rd instar larvae showed 94% and 100% at 25°C and 30°C, respectively, on the concentration of 10^5 PIBs (Fig. 1). However, at 20°C, it showed only 57% at 10^5 doses. In the 4th instar larvae, the 10^5 doses at 30°C only showed meaningful mortality as 85% (Fig. 2). The LC_{50} values of MabrNPV-K1 for 3rd instar larvae were 3.7×10^4 PIBs/larva at 20°C, 9.9×10^2 PIBs/larva at 25°C and 3.8×10^2 PIBs/larva at 30°C, respectively (Table 1). The LC_{50} for the 4th instar larvae showed similar pattern with that for the 3rd instar larvae, but they were about 10 times higher (Table 2). The

Table 4. Values of lethal time (LT_{50}) of MabrNPV-K1 by different temperatures against 4th instar of *M. brassicae*

Temperatures (°C)	Concentrations (PIBs/larva)	LT_{50} (95%CL)	LT_{95} (95%CL)
20	1.0×10^2	ND[a]	-
	1.0×10^3	ND	-
	1.0×10^4	ND	-
	1.0×10^5	ND	-
25	1.0×10^2	ND	-
	1.0×10^3	ND	-
	1.0×10^4	10.1 (9.8~10.6)	12.6 (11.8~14.4)
	1.0×10^5	9.5 (9.3~9.8)	11.8 (11.3~12.8)
30	1.0×10^2	11.2 (10.6~12.9)	14.3 (12.6~19.7)
	1.0×10^3	10.1 (9.8~10.6)	12.6 (11.8~14.4)
	1.0×10^4	9.4 (9.0~10.0)	15.8 (13.9~19.4)
	1.0×10^5	7.4 (7.1~7.6)	10.7 (10.1~11.5)

[a]ND- Not Determined

median lethal time (LT_{50}) values of MabrNPV-K1 were determined as 11.4 to 5.0 days at 30°C and 18.3 to 5.5 days at 25°C for the 3rd instar larvae (Table 3). In the 4th instar larvae, the LT_{50} values were 11.2 to 7.4 days at 30°C, and 10^4 and 10^5 doses showed only 10.1 and 9.5 days, respectively, at 25°C (Table 4). The LT_{50} value was lowered as temperature went up to 30°C and dependent on viral concentration. These results correspond with those of the previous similar experiments for other NPVs (Im *et al.*, 1990; Kim *et al.*, 2004). The higher pathogenicity at 30°C suggests that *M. brassicae* could be controlled effectively in the field conditions because the *M. brassicae* appears in summer season.

Production of MabrNPV-K1

Through the evaluation of pathogenicity, we could determine the 3rd instar for the optimal larval instar to produce MabrNPV-K1 polyhedra because of higher and faster mortality than 4th instar larvae. Therefore we determined the optimal temperature and concentration of inoculation against 3rd instar *M. brassicae* larvae. The results showed that the mortality was only 100% when inoculated with 1.0×10^5 PIBs/larva at 30°C and the yield of MabrNPV-K1 was maximal with 5.6×10^7 PIBs/larva (Table 5). The yield of polyhedra was directly proportional to the temperature and inoculation doses. Regarding the mortality, yield of polyhedra, inoculation doses and required time, the 1.0×10^4 PIBs/larva at 30°C was determined as optimal conditions producing polyhedra efficiently. Under these conditions, the yield of polyhedra rises up to 1,000 times over than that of initial concentration, and the mortality is more than 91%. These results also correspond with the previous results for the production of *S. exigua* NPV polyhedra in *S. exigua* larvae (Kim *et al.*, 2005).

As *M. brassicae* is increasingly serious pest in Korea owing to the spreading of organic farming, the development of effective biological control method is needed. We are going to investigate the methods for the mass production and formulation of MabrNPV-K1.

Table 5. Yields of polyhedra of MabrNPV-K1 after inoculation at different temperatures and viral concentrations against 3rd instar *M. brassicae*

Temperatures (°C)	Concentration (PIBs/Larva)	No. of total polyhedra (±SD)	No. of polyhedra per larva (±SD)	No. of death larvae	Mortality (%)
20	10^2	0	0	0	0
	10^3	$3.4\pm2.2\times10^6$	$3.4\pm2.2\times10^6$	1	10.5
	10^4	$2.3\pm0.2\times10^7$	$9.5\pm1.5\times10^6$	3	31
	10^5	$6.8\pm1.5\times10^7$	$1.1\pm0.1\times10^7$	6	68.5
25	10^2	$5.5\pm0.6\times10^5$	$2.8\pm2.8\times10^5$	1	10
	10^3	$5.3\pm2.0\times10^6$	$1.1\pm1.5\times10^6$	5	60
	10^4	$1.2\pm4.0\times10^8$	$1.5\pm3.0\times10^7$	9	85
	10^5	$5.6\pm3.8\times10^8$	$6.5\pm4.5\times10^7$	9	95
30	10^2	$3.3\pm0.5\times10^7$	$9.3\pm0.1\times10^6$	4	40.5
	10^3	$3.2\pm0.6\times10^8$	$4.9\pm3.7\times10^7$	6	55
	10^4	$4.7\pm1.0\times10^8$	$5.4\pm4.1\times10^7$	7	91.5
	10^5	$9.3\pm0.7\times10^8$	$5.6\pm4.3\times10^7$	9	100

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