THESIS TITLE : MODIFICATION OF ARTIFICIAL DIET, REARING CONTAINER AND INOCULATION METHOD FOR MASS PRODUCTION OF NUCLEAR POLYHEDROSIS VIRUS OF COTTON BOLLWORM (*Helicoverpa armigera* Hubner)

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ABSTRACT

Study on the factors affecting the efficiency of the mass production and commercial production of nuclear polyhedrosis virus (HaNPV) was divided into three parts.

Part I. The study focused on two factors affecting the rearing of cotton bollworm as insect stock under laboratory conditions (26±1°C, 70-85% RH, 8 hr. light/day). Rearing containers and formulations of artificial diet were compared.

Among three rearing containers (2 oz. plastic, 35 mm. film cup : 24 cups/set and plastic box : 18 x 25 x 5.6 cm., 24 cells/box), plastic box was found to be the most suitable for cotton bollworm stock rearing, since the amount of expected hatching larvae was consistently high in all three generations. They were 16,455.60 in the

1^{st} generation, 21,118.49 in the 2^{nd} generation and 21,928.30/larva in the 3^{rd} generation. The cost for plastic box was relatively high (8.12 baht/cell) but the extra cost was justified by the high rate of expected hatching larvae. The 35 mm. film cup was the least suitable for cotton bollworm stock rearing because it resulted in of the lowest amount of expected hatching larvae.

Three formulations of artificial diet were compared for insect stock rearing : based on a slight development of that modified from Sothorn *et al.*, 1970 (I : mixed vitamin stock; II : mixed baby vitamin Bodivitin® and III : mixed baby vitamin Kiddi®). It was found that formula I was the most suitable for cotton bollworm stock rearing since the amount of expected hatching larvae was high and continuous for at least three generations, was 2-3 times of those from formulations II and III in the second generation and about 19-179-times in the third generation, and the larval growth was very even. Although the most expensive was formula I (1.26 baht/larva) and the least expensive was formula II (0.47 baht/larva).

Part II. The study in investigated the most appropriate inoculation concentration of HaNPV for larva reared in the laboratory and collected from the field; inoculation method; rearing container and artificial diet for HaNPV mass production. The 3^{rd} instar larva (7-day old) was reared with artificial diet (formula I) under laboratory conditions ($26 \pm 1^{\circ}C$, 70-85% RH, 8 hr. light/day). The inoculation conditions were assessed with HaNPV isolate RT., using the diet plug method and the 3^{rd} instar larva (7-day old) reared on artificial diet formula IV (mixed vitamin stock) in 2 oz. plastic cup at $30 \pm 1^{\circ}C$ for 16 hr. dark and $27 \pm 1^{\circ}C$ for 8 hr. daylight, 70-85% RH.

Concerning the appropriate HaNPV concentration for larva in the laboratory, four virus concentrations were tested. The 2×10^{8} PIBs/ml. gave the highest total amount of PIBs (1.63×10^{11}), whereas 2×10^{7}, 2×10^{6} and 2×10^{5} PIBs/ml. gave 6.38×10^{10}, 3.97×10^{10} and 0 PIBs, respectively (significant at P=0.05).

For the most suitable HaNPV concentration for larva collected from the field, the same conditions as for larva in the laboratory were used, but with 2nd instar larva (6-day old). The $2x10^6$ PIBs/ml. gave the highest total amount of PIBs ($5.05x10^{10}$), whereas $2x10^5$, $2x10^7$ and $2x10^8$ PIBs/ml. gave $3.68x10^{10}$, $3.34x10^{10}$ and $2.83x10^{10}$ PIBs, respectively (significant at P=0.05).

Three inoculation methods, diet plug, surfaced layer and incorporated method, were tested with virus concentration of $2x10^8$ PIBs/ml., 30, 30 and 60 μl/larva for each method, respectively. The diet plug method gave the highest total amount of PIBs ($7.90x10^{10}$), whereas the surfaced layer and incorporated methods gave $4.08x10^{10}$ and $3.16x10^{10}$ PIBs, respectively (significant at P=0.05). In addition, the lowest cost was obtained with the diet plug method (703.29 baht/$2x10^{12}$ PIBs/l.).

Four rearing containers : 2 oz. plastic cup, 35 mm. film cup (24 cups/set), 1 oz. plastic cup and plastic box (14 x 19 x 4 cm., 24 cells/box) inoculated with the concentration of $2x10^8$ PIBs/ml. were compared for their practical use and suitability for mass production based on the total amount of HaNPV polyhedra produced. The total PIBs obtained from each container were significantly different at $2.89x10^{11}$ (2 oz. plastic cup), $2.75x10^{11}$ (35 mm. film cup), $2.25x10^{11}$ (1 oz. plastic cup) and $1.74x10^{11}$ (plastic box). The 35 mm. film cup was found to be the most suitable because it was the cheapest at 308.16 baht/$2x10^{12}$ PIBs/l.

Concerning formulations of artificial diet for HaNPV mass production, the 2nd instar larva (6-day old) were reared separately from the time of hatching in three artificial diet formulae (IV, V : mixed baby vitamin Bodivitin® and VI : mixed baby vitamin Kiddi®) using the same as previously diets but without formalin solution inoculated with $2x10^6$ PIBs/ml. in 35 mm. film cup. Formula V was considered most suitable because of its lowest cost (137.91 baht/$2x10^{12}$ PIBs/l.) and relatively high result. The total production was significantly different (P=0.05) among those obtained from formula IV ($1.94x10^{11}$ PIBs), formula V ($1.63x10^{11}$ PIBs) and VI ($1.23x10^{11}$ PIBs).

Part III. The combination of the most suitable factors for mass production techniques identified in parts I and II was studied. Insect stock rearing was undertaken in a plastic box (18 x 25 x 5.6 cm.) and using artificial diet formula I. For HaNPV production the diet plug method with 2×10^{6} PIBs/ml. was used together with artificial diet formula V for 2nd instar larva (6-day old) in a 35 mm. film cup. Testing was carried out under the same laboratory conditions as in part II. The total PIBs obtained from each generation were significantly different. They were 1.40×10^{11} PIBs (1st generation), 1.68×10^{11} PIBs (2nd generation) and 8.29×10^{10} PIBs (3rd generation). The costs for 2×10^{12} PIBs/l. were 160.57, 133.81 and 271.17 baht in the 1st, 2nd and 3rd generation, respectively.

This study reveals that when the most appropriate factors were combined, the total HaNPV PIBs production from each generation (8.29×10^{10} - 1.68×10^{11} PIBs) was in the range of the total production from each factor separately (7.90×10^{10} - 2.29×10^{11} PIBs). The combination of the most appropriate factors also results in the cheapest production process.