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Effect of vegetable oils on the yield of nucleopolyhedrovirus of *Helicoverpa armigera* (HearNPV)

A. MEHRVAR[1], R. J. RABINDRA[2], K. VEENAKUMARI[2] and G. B. NARABENCHI[3]

[1]Department of Plant Protection, Faculty of Agriculture, University of Maragheh, Maragheh 5518183111, Iran.

[2]National Bureau of Agriculturally Important Insects, Post Bag No. 2491, H. A. Farm Post, Bellary Road, Hebbal, Bangalore 560024, Karnataka, India.

[3]Biocontrol Research Laboratories, Pest Control (India) Pvt. Ltd., Bangalore 560064, Karnataka, India.

E-mail: alimehrvar@yahoo.co.uk

ABSTRACT: Effect of dietary incorporation of three vegetable oils, *viz.*, sunflower, soybean and coconut, on the yield productivity of seven geographic isolates of nucleopolyhedrovirus of *Helicoverpa armigera* at 1965.78 OB / mm^2 was evaluated to select the most efficient vegetable oil and the highly promising virus isolate(s). Among the seven isolates, Coimbatore isolate recorded the highest yield of 6.895 x 10^9 OB / larva, followed by Negamum, Ooty, Parbhani, Mumbai, Hyderabad and Rahuri, when the larvae were fed with virus inoculated standard diet without vegetable oils. Among the different oils tested, sunflower oil enhanced the larval mortality (92.03–94.07% for different isolates tested). However, the yield of virus per larva was significantly increased when both sunflower oil and soybean oil were used as additives along with standard diet. The productivity ratio was also highest when both sunflower oil and soybean oil were used as additives in all the isolates tested. So it can be concluded that sunflower oil can be used as a dietary adjuvant in HearNPV production systems.

KEY WORDS: *Helicoverpa armigera*, mass production, nucleopolyhedrovirus, vegetable oils

INTRODUCTION

Indiscriminate use of chemical pesticides has led to several problems such as development of insecticide resistance in insect pests, their resurgence and accumulation of toxic residues in the ecosystem rendering the agriculture system unsustainable (Pawar, 2002). The pressing need for sustainable crop production has established the importance of microbial pesticides (Rabindra, 2002), of which baculoviruses hold great promise in IPM programmes. However, the availability of this viral biopesticide is far from the demand in developing countries. This is due to the non-availability of methods to scale up the virus production process to the desired levels (Moscardi, 1999)

Production of nuclear polyhedrosis virus of *Helicoverpa* spp., which began in 1961 has progressed through various research and developmental phases and attained technical realization as the first commercial viral pesticide (Ignoffo, 1973). While propagation of the virus in cell culture continues to receive increasing attention (Shuler *et al.*, 1995), *in vivo* production has been found to be the only economical method for large scale propagation of the virus presently (Hunter *et al.,* 1998). In general, any factor that influences the larval growth rate after virus inoculation will affect the virus yield (Shapiro, 1982). Subsequent development and industrialization for mass rearing process, improvements in viral recovery procedures and formulation of the virus made it possible for commercialization of HearNPV (Shieh, 1978). The optimization of the production factors in *in vivo* propagation methods is crucial to minimize the cost. Several factors like type of host insect, its biology and behavior, age, stage and sex of the larvae used for virus production, the rearing environment which includes factors like temperature, humidity, photoperiod and nutritional quality of the insect diet greatly influence the production and also the quality of the virus produced (Shapiro *et al.*, 1981; Shapiro, 1982, 1986). Hence, the present investigations were undertaken to evaluate the effect of dietary incorporation of vegetable oils on the yield productivity of different geographic isolates of nucleopolyhedrovirus of *Helicoverpa armigera* (Hübner).

MATERIALS AND METHODS

The semi-synthetic diet developed by Shorey and Hale

(1965) was adopted with suitable modifications (Srinivasan *et al.,* 1994) for mass culturing of the larvae of *H. armigera.* Seven geographical isolates of HearNPV from the microbial repository of the Project Directorate of Biological Control (PDBC), Bangalore, India, were used in this study (Table 1). These isolates were passaged through early fifth instar larvae of the host insect at 25 ± 2˚C.

Origin	Abbreviation
Parbhani. Maharashtra	PRB
Mumbai, Maharashtra	MUM
Rahuri, Maharashtra	RHI
Ooty, Tamil Nadu	OTY
Coimbatore, Tamil Nadu	CMB
Negamum, Tamil Nadu	NGM
Hyderabad, Andhra Pradesh	HYD

Three batches of semi-synthetic diet incorporating 6000 ppm of three different vegetable oils, *viz.*, sunflower, soybean and coconut, were prepared. The neonate larvae of *H. armigera* were separately reared on these modified semi-synthetic diets. The larvae after reaching third instar were transferred individually to multicavity trays (25 × 25cm) containing modified semi-synthetic diet (with respective oil). When the larvae reached fifth instar, the bioassays were conducted. Larvae weighing 66–71mg were used in the studies. Semi-synthetic diets containing oils and lacking formaldehyde were prepared and filled in 5ml glass vials (dia. 2cm) up to ⅓rd height of the vial. Suspensions of different viral isolates containing 5×10^5 OB were prepared and 10µl of virus was applied onto the diet surface using a micropipette providing a dose of 1965.78 OB mm^{-2}. The suspension was spread uniformly over the diet surface. The larvae were weighed individually in an electronic balance and transferred to the treated diet. The treatments were replicated three times and a control was maintained for each oil and isolate. In addition to this, the larvae reared on semi-synthetic diet (without oil) served as another control. Each treatment had 40 larvae. After inoculation, the larvae were incubated at 25 ± 2°C. Four days after treatment the mortality was recorded at 24 hours interval. The cadavers were collected individually, transferred to sterile vials and weighed again to arrive at cadaver weight and were frozen immediately. The cadavers were homogenized individually and the volume was made up to 25ml with distilled water. The polyhedra in these solutions were enumerated using an improved Neubauer haemocytometer. After enumeration the following parameters were calculated.

$$\text{Yield larva}^{-1}\text{ (OB)} = \frac{\text{OB.ml}^{-1} \times \text{Suspension volume}}{\text{Total no. of cadavers}}$$

$$\text{Yield gram}^{-1}\text{ body weight (OB)} = \frac{\text{Mean yield.larva}^{-1}}{\text{Mean weight of cadavers}} \times 1000$$

$$\text{Productivity ratio (OB)} = \frac{\text{Yield.larva}^{-1}}{\text{OB inoculated.larva}^{-1}}$$

RESULTS AND DISCUSSION

Yield of different HearNPV isolates (Table 2) was assessed to identify the most promising isolate with and without vegetable oils as additives. The viral isolate CMB recorded the highest yield of 6.895×10^9 OB/larva when inoculated on semi-synthetic diet without any oil, followed by NGM, OTY, PRB, MUM, HYD and RHI. NGM was statistically on par with CMB isolate (P = 0.05) with respect to yield and productivity ratio (Table 2). Two isolates, *viz*., HYD and RHI recorded the least yield. Among the oils, sunflower oil recorded the highest mortality of 92.03–94.07 per cent for all the tested isolates at the dose of 1965.78 OB.mm^{-2} against early fifth instar larvae (Table 3). Soybean and coconut oils were on par with standard semi-synthetic diet showing no enhancement in larval mortality. There was a significant increase in the larval weights when the larvae were fed with sunflower oil incorporated semi-synthetic diet. Sunflower oil recorded the maximum body weight ranging from 335.71 to 339.45 mg and weight gain of 267.64–271.57 mg in the tested isolates and was statistically superior to other oils. This data further confirms that larval weights and yield of virus are directly proportional to each other. With all the viral isolates, the yield per larva was significantly higher in diets incorporated with both sunflower and soybean oils. However, coconut oil was not able to enhance the yield of virus and was on par with that of standard diet. The productivity ratio obtained from sunflower oil was the highest and on par with that of soybean oil in the case of all the isolates tested (Table 3). Among all the isolates tested, CMB isolate recorded the highest yield (both with and without incorporation of vegetable oils) followed by NGM, OTY, PRB, MUM, HYD, and RHI. Altering the ingredients of artificial diets to enhance the OB productivity of NPVs was one of the approaches already followed by several authors (Shapiro *et al.*, 1981; Rabindra and Jayaraj, 1988; Kelly *et al.*, 1989;

Table 2: Effect of dietary incorporation of oils on the larval mortality and yield of HearNPV isolates

HearNPV isolates	Standard diet		Standard diet + Sunflower Oil		Standard diet + Soybean oil		Standard diet + coconut oil	
	Corrected larval Mortality (%)	Productivity Ratio (x 104 OB)	Corrected larval Mortality (%)	Productivity Ratio (x 104 OB)	Corrected larval Mortality (%)	Productivity Ratio (x 104 OB)	Corrected larval Mortality (%)	Productivity Ratio (x 104 OB)
CMB	82.90^{b}	1.379^{a}	93.31^{ab}	1.640^{a}	85.98^{abc}	1.622^{a}	85.27^{abc}	1.437^{a}
NGM	84.62^{a}	1.270^{ab}	94.07^{a}	1.562^{ab}	87.63^{a}	1.546^{ab}	86.73^{a}	1.353^{ab}
OTY	81.21^{c}	1.222^{bc}	93.64^{ab}	1.501^{bc}	86.49^{abc}	1.490^{bc}	86.13^{abc}	1.317^{bc}
PRB	84.21^{a}	1.118^{cd}	93.34^{ab}	1.424^{cd}	86.38^{abc}	1.383^{cd}	85.44^{abc}	1.225^{cd}
MUM	84.68^{a}	1.083^{d}	92.65^{ab}	1.347^{d}	87.25^{ab}	1.328^{d}	86.39^{ab}	1.191^{d}
HYD	83.35^{ab}	1.021^{de}	92.30^{b}	1.303^{de}	85.46^{c}	1.264^{de}	84.79^{bc}	1.138^{de}
RHI	83.33^{ab}	0.955^{e}	92.03^{b}	1.231^{e}	85.67^{bc}	1.197^{e}	84.46^{c}	1.065^{e}

Means followed by the same letter are not significantly different (P = 0.05) by DMRT.

Table 3. Effect of dietary incorporation of vegetable oils on production of HearNPV isolates

HearNPV isolates	Oil (@ 6000ppm)	Corrected larval mortality (%)	Mean initial larval weight (mg)	Mean cadaver weight (mg)	Weight gain (mg)	Yield larva^{-1} ($\times 10^9$ OB)	Yield gram 1 body weight ($\times 10^{10}$OB)	Productivity ratio ($\times 10^4$ OB)
CMB	Standard Diet	82.90^b	66.39	316.86^b	250.4^b	6.895^b	2.109^b	1.379^b
	Sunflower	93.31^a	67.49	338.83^a	271.34^a	8.230^a	2.429^{ab}	1.646^a
	Soybean	85.98^b	69.04	326.87^b	257.83^b	8.112^a	2.560^a	1.622^a
	Coconut	85.27^b	69.37	318.01^b	248.64^b	7.187^{ab}	2.260a^b	1.437^b
NGM	Standard Diet	84.62^b	67.67	317.32^b	249.65^b	6.350^b	1.954^b	1.270^b
	Sunflower	94.07^a	68.37	338.26^a	269.89^a	7.812^a	2.309^{ab}	1.562^a
	Soybean	87.63^b	66.37	325.01^b	258.64^b	7.730^a	2.436^a	1.546^a
	Coconut	86.73^b	66.58	313.37^b	246.79^b	6.763^{ab}	2.158^{ab}	1.353^b
OTY	Standard Diet	81.21^b	69.58	317.36^b	247.78^b	6.109b	1.865^b	1.222^b
	Sunflower	93.64^a	68.52	339.45^a	270.93a	7.507^a	2.212^{ab}	1.501^a
	Soybean	86.49^b	71.20	327.59^b	256.39^b	7.452^a	2.348^a	1.490^a
	Coconut	86.13^b	67.74	317.07^b	249.33^b	6.584^{ab}	2.077^{ab}	1.317^b

* For each isolate, means followed by the same letter are not significantly different (P = 0.05) by DMRT.

Table 3. (contd.)

HearNPV isolates	Oil (@ 6000ppm)	Corrected larval mortality (%)	Mean initial larval weight(mg)	Mean cadaver weight (mg)	Weight gain (mg)	Yield larva^{-1} ($\times 10^9$ OB)	Yield gram^{-1} body weight ($\times 10^{10}$OB)	Productivity ratio ($\times 10^4$ OB)
PRB	Standard Diet	84.21^{b}	68.23	318.59^{b}	250.36^{b}	5.591^{b}	1.716^{b}	1.118^{b}
	Sunflower	93.34^{a}	69.64	341.21^{a}	271.57^{a}	7.119^{a}	2.086^{ab}	1.424^{a}
	Soybean	86.38^{b}	67.33	325.74^{b}	258.41^{b}	6.915^{a}	2.171^{a}	1.383^{a}
	Coconut	85.44^{b}	68.59	315.49^{b}	246.90^{b}	6.127^{ab}	1.942^{ab}	1.225^{b}
MUM	Standard Diet	84.68^{b}	67.92	316.31^{b}	248.39^{b}	5.413^{b}	1.679^{b}	1.083^{b}
	Sunflower	92.65^{a}	67.23	335.71^{a}	268.48^{a}	6.734^{a}	2.006^{ab}	1.347^{a}
	Soybean	87.25^{b}	66.57	322.30^{b}	2555.73^{b}	6.641^{a}	2.100^{a}	1.328^{a}
	Coconut	86.39^{b}	70.03	320.32^{b}	250.29^{b}	5.953^{ab}	1.859^{ab}	1.191^{b}
HYD	Standard Diet	83.35^{b}	67.33	315.61^{b}	248.28^{b}	5.103^{b}	1.561^{b}	1.021^{b}
	Sunflower	92.30^{a}	68.59	336.23^{a}	267.64^{a}	6.513^{a}	1.937^{ab}	1.303^{a}
	Soybean	85.46^{b}	69.39	326.95^{b}	257.56^{b}	6.318^{a}	2.002^{a}	1.264^{a}
	Coconut	84.79^{b}	67.69	313.15^{b}	245.46^{b}	5.692^{ab}	1.818^{ab}	1.138^{b}
RHI	Standard Diet	83.33^{b}	68.48	316.39^{b}	247.91^{b}	4.773^{b}	1.469^{b}	0.955^{b}
	Sunflower	92.03^{a}	67.78	337.11^{a}	269.33^{a}	6.156^{a}	1.826^{ab}	1.231^{a}
	Soybean	85.67^{b}	68.05	324.86^{b}	256.81^{b}	5.983^{a}	1.891^{a}	1.197^{a}
	Coconut	84.46^{b}	69.61	315.75^{b}	246.14^{b}	5.325^{ab}	1.686^{ab}	1.065^{b}

* For each isolate, means followed by the same letter are not significantly different (p=0.05) by DMRT.

Srinivasan *et al.*, 1994). Manipulating the concentration of vitamins in the diet of *Lymantria dispar* larvae resulted in 30 % increase in yield of *Ld*NPV (Shapiro *et al.*, 1981). Likewise, Kelly *et al.* (1989) developed a high wheat germ diet for the production of *Euproctis chrysorrhoea* NPV. Srinivasan *et al.* (1994) manipulated the diets of *H. armigera* for mass multiplication of HearNPV and found that a diet based on French bean flour was better than that with chickpea flour. Rabindra and Jayaraj (1988) reported that oils of groundnut and cotton-seed were more effective than palm oil in increasing the mortality of *H. armigera* larvae due to virus infection. This study on vegetable oils as additives in the semi-synthetic diet of HearNPV showed that sunflower oil enhanced the larval mortality and yield of virus per larva. As some oils may inhibit larval growth, use of oil as an additive to the semi-synthetic diet for virus production has to be a selective matter. Accordingly, it can be concluded that use of CMB isolate (for inoculation of larvae) and addition of sunflower oil to the standard diet in HearNPV mass production systems can maximize the virus yields.

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