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Production and Formulation of Baculoviruses from Some lepidopterous Insects in Egypt

By

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ABSTRACT

In laboratory study two formulation of Baculovirsues *Spodoptera littoralis* nuclear polyhedrosis viruses (NPVs) and *Pthorimaea operculella* granulosis virus (Gvs)were produced in Egypt. Formulation of Baculoviruses, like chemical insecticides, is essential for consistent and effective control. Formulation additives such as wetting and thickening, and antievaporation agents were added to improve physical performance by aiding dispersion and suspension of the virus in the spray liquid, to improve the deposition of the virus spray and to aid the spread of spray droplets on the target. Many additives many also be included in a formulation to maximize persistence (e. g. Stickers, U V protectants) and to encourage ingestion of the viruses or to improve its potency. Finally with liquid concentrates of virus antibacterial and anti-fungal agents were added to prevent growth of contaminant microorganisms. Moreover the goals required for a formulation, is to ensure their length of persistence.

INTRODUCTION

In a previous work, a large number of viruses have been reported to occur as insect pathogens in nature (Kurstak *et al* ., 1978) . Such viruses, together with other naturally occuring biological control agents, including parasites, predators and other insect pests, play an important role as pest suppressants in natural ecosystems (Steinhaus, 1963, Burges and Hussay, 1981). Of some 200 viral diseases identified as insect pathogens, 29 viruses were isolated from lepidoptera and have been identified as Baculoviruses. These viruses of arthropoda are evaluated most often, and it has been demonstrated that, they have control potential over most insects (Yearian and Young, 1982). Control of insects on cotton with Baculoviruses has been attempted or recommended in more than 20 countries and has been directed against some of the major insect pests, Which have mainly been from lepidoptera. Formulation of Baculoviruses, like chemical insecticides, are essential for consistent and effective control. Although novel methods of application of Baculoviruses have been suggested such as the release of contaminated adults or the spreading of solid baits containing virus (Mckinley, 1985). Baculoviruses are DNA viruses, which replicate in the nucleus of insect cells. Therefore, the current study was carried out to explore the feasibility of using the Baculoviruses for controlling insect populations, Baculoviruses are surprisingly host specific within the class insecta. There seems to be a high level of host specific within the class insecta. There seems to be a high level of host specificity, which is especially marked in the granulosis viruses (GVS) in general, and the majority of nuclear polyhedrosis viruses.

MATERIALS AND METHODS

Production of *S. littoralis* (SNPV) :

Infecting and harvesting of larvae :

Surface contamination of the artificial diet was carried out using standard suspension of purified polyhedra. The homologous inoculum contained $1x10^6$ PIBs / ml; 2 ml were sprayed on the prepared diet tray. Larvae (7days old) were placed on treated diet trays (500 larvae l tray). Harvesting larvae is the most labor-intensive operation in the virus production process. In involved the daily collection of larvae from all the trays after infection. Live larvae (about to die due to virus infection) were collected using soft forceps, placed in 8 pots and weighed. The pots were stored at 10°C for 7 days to allow the maximum development of the disease and then placed in a deep freeze until the larvae were required for freeze drying as next step.

Freeze drying of diseased larvae :

After defrosting the larval material, each 400-g were blended in 250 ml of distilled water. This amount of mixture was poured on trays and placed in a deep freezer for 24 hrs. The freeze-drying machine was prepared on the next day by allowing it to cool to – 40°C (for about 45 min.); then the trays were introduced in the machine. The vacuum pump was then switched on until a constant pressure is reached (for about 1 h). One hour later, the heater unit should be switched on. Freeze drying continued for 24 h. the freeze-dried material was removed and packed in polyethylene bags labeled and weighted.

Preparation of wettable Powder of (SNPV) :

A wetting complex was first prepared by mixing 250g of Neosyl powder into a plastic bucket with 250-g acetone until smooth slurry was obtained. Then was added and stirred until mixed. The mixture was poured into shallow trays and left over night to let the acetone evaporate. There after a drying powder was obtained. Lumps were gently broken up and this complex (nearly 500g) was packed and sealed in a polyethelene bag until required. An amount of 250g freeze dried larvae was added to mixture of 150g of China clay (spes white) and 100 g of the prepared wetting complex and mixed very well. A coarse powder should be obtained with a uniform grey color. Using an ultra-centrifugal mill with vacuum cleaner, the produced powder was processed into the desired fine wettable powder formulation of (SNPV).

Production of *Phthorimaea operculelle* (G V's)

The *Phthoriumaea operculella* granulosis virus can be multiplied using mass – reared potato moth larvae hosts. Mass rearing provides sufficiently large larvae and adult population, which can then be infected. Harvesting the infected larvae then Freeze drying of diseased larvae and preparation of wettable powder of *Phthorimaea operculelle* granulosis viruses (G V's) as described before in *Spodoptera littoralis*.

RESULTS AND DISCUSSION

Insect pathogenic Baculoviruses affair a means of pest control that is safe, specific and environmentally benign (Jones, 1988a). This group of viruses compressing of the nuclear polyhedrosis viruses (N P V's) and granulosis viruses (G V's) are highly suitable for use as one component of an I P M strategy, and have been shown to be effective and economically valuable for controlling a number of pests (Winstanley and Rovesti, 1993, Jones et al, 1993). Cotton leafworm Larvae, 7 - days old were obtained from the laboratory reared colony and were left to develop on artificial media (800 ml for one tray). The contaminated with 1 x 10^6 PIB / larva of nuclear polyhedrosis virus. Number of larvae were harvested from 50 trays in five groups of 10 trays was 15097 larvae (Table 1). The concentrations of virus used was 1 x 10^6 /2ml. The total weight of harvested larvae was 7028.8g, with an average of 0.46 for one larva. Mean while, the total numbers of dead larvae were 3467 larvae. The mean percentage of mortality for each group of trays was 18.6%. The same procedure was repeated for another 60 trays, but, in this case all of the trays were assessed individually. The results indicated that, total no. of harvested larvae live were 23627, where as the total weight of larvae live was 12080g. The mean weight of larvae was 0.51 ± 0.0451g. with range of 0.49 - 0.59. The results showed that there was little variation in the mean weight of the harvested larvae, all trays producing a mean weight between 400 and 600mg. There was, however, a large variation in the number of live Larva harvested per tray for example in the 2nd experiment which ranged from 227 to 509 larvae per tray, and number of dead larvae (9-34% mortality).

Obviously the higher number of that harvested, the more virus will be produced the consistency in the mean weight of the harvested larvae shows that this is an optimum for the diet used , therefore , increased virus production must rely on improvement in the technique which will give large number of survival larvae up to harvesting and reduces the possibility of larval escaping from the trays before harvest . The potential production (total weight of larvae) which can be produced per tray can be estimated , if we assure 100% recovery of larvae at harvesting as 560X 0,49g : 274,4g/tray. A more realistic from to aim for is a 90% survival role which give 247g / tray . Later experiments described in this study attempted to achieve optimum results .

Table (1);details of larval harvesting

No of trays	Total no. of larvae live	Total wt. of larvae live (g)	Mean of one larva live (g)	Total no. of larvae dead	Mean mortality %
10	4043	1681.0	0.41	582	12.6
10	1789	906.2	0.50	928	34.0
10	2508	1184.6	0.47	739	22.0
10	4144	1650	0.40	460	9.0
10	2613	1607	0.55	758	22.0
50	15097	7028.8	0.46	3467	18.6

Table (2);percentage of freeze- dried *spodoptera littorals* larvae .

No of trays	Wt. of freeze infected larvae (g)	Wt. of freeze dried larvae (g)	%
10	25573	2629	9.7
10	21471	2300.5	9.3
10	14777.5	1465.3	10
8	20022	2048	9.8
38	81843.5	8442.8	9.7

Freeze- drying : data in Table (2) indicated that the total weight of 38 samples of larvae after harvested were 8442.8g . the mean percentage of drying in these samples were 9.7% the highest percentage was 10% where as the lowest percentage was 9.3 To prepare 50% NPV wettable powder formulation of virus additives such as about 60% of spes white and 40% Neosy Etocas to dry larvae as the weight . Twenty samples were repeated , for example , taking 1013g from dried larvae added to 607.8g of spes white , 405.2g of Neosyl / Etocas . all these amounts become sufficient to prepare 2026g from the 50% wettable powder (Table 3). This result was proved by Ignoffo (1965& 1979) who manually collected cabbage looper larvae cotton bollworm larvae sparated them into living or dead , and stored them later . Ignoffo and coush (1981) collected larvae with a suction tube , slurred through water and the suspension then was filtered through a screen to remove large particles and insect debris , gypsy moth cadavers were collected by vacuum and blended in water . The virus suspension was then filtered and refrigerated (smith *et al* 1976) . Virus killed pine sawfly larvae manually collected and refrigerated (4° C) until processed (Lewis , 1971) . Gypsy moth Larvae were collected manually 24 hours prior to death and were placed in clean plastic containers under refrigeration . The insects were placed in the containers to prevent wilting of the larvae on the diet , with subsequent loss of recoverable virus (Lewis , 1971) . virus – killed douglas – fir tussock moth larvae were collected , lyophilized and placed in sealed glass containers under vacuum until processed (Chauthani and Claussen,1968) Ignoffo and Shapiro (1978) compared the harvest of infected bollworms with the harvest of diet plus infected bollworms . Whether it was processed or removed , similar trends were noted : 1) Virus from dead larvae was more virulent than virus from living infected larvae , and 2) No significant differences in the moisture levels or in the lipid levels were found between the preparations . In one instances (i.e virus from living infected larvae) there was some indication that virulence was greater in the preparation containing the diet .

NB . polyhedrosis of previous prepared samples were counted by aid of Haemocytometer Alice . freeze – dried insect powder assayed at 2.98 X 10^9 PIB / g .

Table (3) : Fifty Percentages NPV wettable powder formulation

No	Wettable powder of larvae (g)	Spes white (g)	Neosyl / Etocas (g)	Total wt 50% W.P. (g)
1	1013	607.8	405.2	2026
2	245	147.0	98.0	490
3	88	52.8	35.2	176
4	253	151.8	101.2	506
5	242	145.2	96.8	584
6	271.5	162.9	108.6	543
7	288	172.8	115.2	576
8	267	160.2	106.8	534
9	253	151.8	101.2	506
10	259.5	155.7	103.8	519
11	214	128.4	85.6	428
12	541.5	324.9	216.6	1083
13	240	144.0	96.0	480
14	128.5	77.1	51.4	257
15	252.5	161.5	101.0	505
16	258.0	154.8	103.2	516
17	402.5	241.5	161.0	805
18	528.5	317.1	211.4	1057
19	231.5	192.9	38.6	463
20	517.0	310.2	206.8	1034

2-Production of *Phthorimaea operculella* granulosis virus :-

The *P. operculella* granulosis virus can be a multiplied mass-reared potato moth larvae as hosts. Mass rearing provides sufficiently large larvae and adult populations , which can then be infected , the viral agent in a liquid suspension is applied to the feeding substrate obtained from tubers or foliage . In the laboratory , larvae or adults were multiplied on tubers .

Alternatively , adults were multiplied on foliage . you can use either method depending on the development stage of the moth at infestation and on the type of feeding substrate . Multiplication can also be carried out in the field by applying a liquid suspension of the granulosis virus to a highly infested potato crop After 2-3 weeks , you can collect the infected larvae and use them to prepare the bioinsecticide either in powder , or as a suspension for the field . the freeze – dried virus – insect mixture now had to be formulated for field use. Additives were required to improve suspension and wettability and to maximize persistence in the field .

Table (4) Production of *Phthorimaea operculella* granulosis virus.

Treatment	Mean ± SE	Range
10 larvae / litter	66.5 ± 15.4	0 – 211
5 larvae + dry larvae / litter	64.18 ± 15.66	0 – 210
10 freeze-dried larvae	104.18 ± 15.05	31 - 300

The obtained data indicated that the easiest and most effective formulation from the point of view of virus persistence was simply to use unpurified viruswhich persisted for at least two weeks in the regions of the plant where the insects are located. Additives were included in the formulation to improve the flowability, wettability and suspensibility of the powder. Speswhite China clay (F- ECC PLC) was added primarily as a diluent. The clay also acts as an UV protectant by coating the virus.

Table (4) summarized the freeze-dried larvae prepared for production – higher average (Anova shows that this is not significant) and lower variation, but future research should look at better production, e.g. artificial diet. This result also shows that application of freeze – dried wettable powder is more effective than suspension. Effective control has been demonstrated in several developing countries, Where the economics of production make viruses an attractive alternative to chemicals. Local production of viruses for cotton pest control in developing countries is likely to be the most appropriate strategy for the future . Future developments include the possible use of genetically engineered Baculoviruses (Ali, 1991). Already *B. Thuringiensis* and other toxin genes have been engineered into Baculoviruses in an attempt to increase speed of skill or to stop the feeding of infected insects. Several other genes are also being considered.

However, such products may prove to be difficult to register, a problem not encountered with naturally occurring viruses. For this reason, where possible, the use of genetically engineered viruses should be avoided.

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الملخص العربى

إنتاج المستحضر الفيروسى من بعض آفات حرشفية الأجنحة فى مصر

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استهدفت الدراسة مراحل إنتاج الفيرس النيوكيار بولى هيدروسيسز فيرس لمكافحة دودة ورق القطن وكذلك فيرس الجرانيولوسيسز فيرس لمكافحة دودة درنات البطاطس محلياً كوسيلة عملية وفعالة وبديلة لاستخدام المبيدات .
ربيت يرقات دودة ورق القطن العمر الثالث على بيئة صناعية رشت بواسطة فيرس النيوكليار بولى يدروسيسز بتركيز 1×10^6 ml بمعدل 2 ml للصنية الواحدة ثم وضعت اليرقات بمعدل ٥٠٠ يرقة للصنية الواحدة لمدة أسبوع فى حضانة على درجة حرارة (٢٦ درجة منوية ورطوبة نسبة ٧٠%) لمدة سبعة أيام ثم جمعت اليرقات الحية ووضعت فى الحضانة على درجة حرارة ١٠ درجة منوية لمدة أسبوع ثم طحنت اليرقات وتم تجفيفها بواسطة جهاز freeze dried ثم أضيف لها المواد الحاملة والمواد الحافظة من أشعة الشمس وتم تحضير مسحوق قابل للبلل بمعدل ٥٠% .
كما شملت الدراسة أيضاً إنتاج الجرانيولوسيسز فيرس من دودة درنات البطاطس بتربية الآفة تربية موسعة على درنات البطاطس وجمع البيض بأعداد كبيرة ثم يوضع على البطاطس التى غمرت فى مسحوق فيرس بمعدل ١٠ يرقات مصابة للتر ماء لمدة دقيقة وجففت البطاطس بتركها فى الهواء لمدة عشرين دقيقة وتوضع فى حجرة على درجة حرارة ٢٦م ورطوبة نسبية ٦٠ – ٦٥% لمدة تتراوح من ١٨ – ٢١ يوم ثم تجمع اليرقات المصابة الحية ثم تطحن وتجفف ويضاف إليها المواد الحاملة والحافظة من أشعة الشمس وتجهز لتكون جاهزة للتطبيق داخل النوالات المصرية أثناء تخزين البطاطس فى العروة الصيفية.