Community-based mass production of *Maruca vitrata* multi-nucleopolyhedrovirus and its comparative efficacy against *Maruca vitrata*, cowpea insect pest

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Abstract

Cowpea (*Vigna unguiculata* (L.) Walp is an important crop with low yield due to insect pests.

The present study evaluated the feasibility of *Maruca vitrata* multi-nucleopolyhedrovirus (*Mavi*MNPV) (potential biological agent specific to cowpea pod borer *M. vitrata)* mass production in field conditions (Dassa and Glazoué locations) with farmers' participation. This is of great interest in context of side effects of chemical pesticides namely insect pests resistance and environmental pollution. *Mavi*MNPV mass production virus was assessed using starved (starving for 24hours 12hours 06hours and 00hour) and fed three-days-old *Maruca vitrata* larvae. Alive larvae were fed using cowpea seed pre-germinated for 72, 48 and 24hours. Virus-dead *M. vitrata* larvae were collected daily and the viral concentration estimated after purification using centrifuge (ALC-PK121). The effect of the mixture *Mavi*MNPV + neem oil on the virulence of virus was studied by combining ten ml of *Mavi*MNPV to ten ml of neem oil at room temperature. The efficacy of *Mavi*MNPV was studied in field conditions, in comparison with neem oil, Decis and untreated control. Treatments consisted of single application or combination of these biological products, were arranged in a complete random block design with four replicates. Results revealed that starving larvae before virus infection; negatively affected the production. Non-starving larvae fed with 72hours pre-germinated cowpea seeds were the most effective for *Mavi*MNPV mass production as well as in laboratory or in field conditions. Furthermore, storing *Mavi*MNPV in neem oil didn't alter the virulence of virus within seven days. Cowpea yield was similar in plots treated but was significantly higher than untreated control plot. *Mavi*MNPV mass production in field conditions was possible and should be encouraged in context of integrated pest management.

Keywords: *Mavi*MNPV; Cowpea; *Maruca vitrata*; Bénin

Résumé

Production en masse du *Mavi*MNPV au niveau de la communauté locale et son efficacité comparée contre *Maruca vitrata*, ravageurs du niébé

Le niébé (*Vigna unguiculata* (L.) Walp est une importante culture avec de faible rendement causé par les insectes ravageurs. La présente étude a évalué la faisabilité de la production locale de *Maruca vitrata* multi-nucleopolyhedrovirus (potentiel agent biologique spécifique à *M. vitrata* ravageur du niébé) à (Dassa et Glazoué) avec la participation des producteurs. Ceci est d'un grand intérêt face aux effets secondaires des pesticides chimiques sur l'environnement et la résistance des insectes ravageurs à ces pesticides. La production a été évaluée en utilisant les larves L3 de *M. vitrata* affamées pendant 24h, 12h, 06h et 00h. Les larves vivantes sont nourries avec les graines de niébé prégermées en 72h, 48h et 24h. Les larves mortes par infection virale sont collectées quotidiennement et la concentration virale est déterminée après purification à la centrifugeuse (ALC-PK121). L'effet du mélange *Mavi*MNPV + huile de neem sur le virus a été étudié en mélangeant dix ml de *Mavi*MNPV à dix ml de l'huile de neem et conserver en température ambiante. L'efficacité du virus a été également comparée à l'huile de neem, Decis et un témoin en testant ces produits sur *Maruca vitrata* séparément ou combinés. Un bloc aléatoire complet avec quatre traitements a été réalisé. Les résultats ont montrés que les larves affamées affectent négativement la production. Au laboratoire comme en conditions ambiantes les larves non affamées nourries avec les graines de niébé prégermées en 72h sont les plus efficaces pour la production. Aussi, la conservation du virus dans l'huile de neem pendant 7 jours n'a pas d'effets négatifs sur la virulence du virus. Le rendement grain du niébé était similaire sur les parcelles traitées, mais était significativement supérieur au témoin. La production locale de *Mavi*MNPV était possible et devrait être encouragée dans le contexte de la gestion intégrée des ravageurs.

Mots clés : *Mavi*MNPV ; Niébé ; *Maruca vitrata* ; Bénin

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Introduction

Cowpea (*Vigna unguiculata* (L.)) Walp. (Fabales: Fabaceae) is a grain legume with high nutritional value in Africa where cereal are the most stuff food (Bal, 1992; Ouedraogo, 2000; Atachi et al., 2002). As a good source of proteins (22-26%), it the most widely cultivated (Pasquet and Baudoin, 1997) and contributes to balanced diet. Furthermore, it provides nutritious dry grains and fodder for livestock (Tarawali et al., 1997) and maintains soil fertility through nitrogen fixing (Asiwe et al., 2009). However this role is limited by several factors inducing reduced yield. In Benin, cowpea yield did not exceed 600 Kg/ha, lower than its potential (OBOPAF, 2004; IITA, 2004). Pressure from insect pests remains one of

the limiting factors to cowpea production. The cowpea pod borer *Maruca vitrata* Fabricius (Lepidoptera: *Crambidae*) is one of the most destructive insects causing up to 80% of yield losses in cowpea (Nampala et al., 2002). Control of this pest has been achieved mainly by the application of synthetic chemicals. Even the use of chemicals has booted cowpea production; it induced several side effects including human hazards, environmental pollution and pest resistance. Alternative methods to control cowpea insect pests were developed to overcome these effects. Of these, biological control is the main environmental sound alternative method. It implies the use of natural enemies such as parasitoids, predators and micro-organisms. In 2005, a classical biological control programme against *M. vitrata* has started via the importation of several parasitoids species and the virus *Mavi*MNPV from Taiwan to Benin by the IITA. The strategy built by IITA Benin involved the release of suitable biological control agents (preventive) followed by application of the virus *Mavi*MNPV in the case of high infestation of cowpea field (curative) (Tamò et al.,2012). The use of biopesticides in crop protection has become an attractive control option in the context of insect pest resistance to synthetic chemicals. Thus, *Maruca vitrata* multi-nucleopolyhedrovirus (*Mavi*MNPV) has been identified as a specific entomopathogen to *M. vitrata* with high control potential (Miller et al., 1983; Dent, 1991). Furthermore, *MaviMNPV* doesn't affect the environment, human, other vertebrates and natural enemies of pests. This positive attribute is a great interest for *Mavi*MNPV use as biological pesticides in management of *M. viitrata*. The proper way for applying entomolopathogenic virus is to build a formulation ready for use by farmers. In this perspective previous studies were carried out in Benin to build an easier and cheaper method to mass produce *Mavi*MNPV based on the use of different cowpea varieties as feeding substrate (Gouissi, 2013). The current study aims to assess the possibility of local mass production of *Mavi*MNPV, specifically by testing the effect of contaminated host larval diet and host fasting with farmers' participation. Moreover, the effectiveness of the combination of *Mavi*MNPV and neem oil in controlling *M. vitrata* has been evaluated in field conditions to validate previous laboratory findings (Sokame et al;, 2015).

Material and methods

Study site

The mass production of *Mavi*MNPV in field condition was done in two locations of central Benin namely Mangoumi and Odo-Otchèrè in Glazoué and Dassa districts respectively. Glazoué is 234Km far from Cotonou between 7°90 and 8°30 north latitude, 2°05 and 2°22 east longitude, while Dassa is located at 193Km from Cotonou between 7°30 and 7°57 north latitude, 1°46 and 1°29 east longitude. These localities were selected for the experiments because of the importance of cowpea production in the areas and the pressure of *M. vitrata* (Arodokoun et al., 1997).

Mass culture of MaviMNPV

The mass culture of *Mavi*MNPV took place at the laboratory of IITA-Benin (located in Abomey Calavi, 12km north-western of Cotonou) under temperature of 25±0.5°C with relative humidity of 76±0.1%. The third instar larvae of *M. Vitrata* were obtained from a stock culture at the laboratory of IITA and used for the mass culture of *Mavi*MNPV. They were inoculated with a *Mavi*MNPV suspension (obtained from Taiwan and continuously cultured at IITA-Benin) containing $6.7x10^9$ occlusion bodies (OB)/ml (Lee et al., 2007). Natural rearing diet (pre-germinated cowpea seed (local variety Tawa) of 72 hours old) (Gouissi, 3013) was infused with *Mavi*MNPV formulation and fed to the larvae. They were placed in plastic containers (20cm diameter with an opening of 127cm circumference and 15cm depth) covered with fine white mesh and kept at 25±0.5°Cand 76±0.1% relative humidity. The dead larvae caused by viral infection were collected daily after 48 hours of incubation, and transferred in small cylindrical plastic transparent containers (4cm diameter × 6cm depth) and stored in fridge at -4°C. Virus-dead larvae were thoroughly macerated in distilled water and homogenized using tween solution 0.1%. They were thoroughly grinded using a mortar (Moulinex) and the paste was washed with tween solution 0.1%. The viral suspension was filtered through a sterilized sieve of 90µm mesh, and the filtrate was centrifuged at 6680tr/min for 30 min (Harrap et al.,1997; Hunter et al.,1984). The supernatant was discarded and the sediment, containing the OB, was diluted with distilled water and centrifuged again at 6680tr/min for 30min. The concentration of the viral suspension was determined by counting in a Neubauer haemocytometer. Serial dilutions were then made with distilled water to produce the concentrations.

Assessment of optimal starving duration with different larval numbers

For optimal starving duration third stage larvae were put singly in group of 100, 200, 300, 400, 500 and 600 in plastic containers (20cm diameter with an opening of 127cm circumference and 15cm depth), each covered with fine white mesh and submitted to starving with different times (24h, 12h, 6h and 00h (not starving)). They were kept at 25°C±0.5°C and 76±0.1% relative humidity (laboratory conditions). The numbers of larvae alive by group after 24 hours of starving (64, 123, 180, 221, 253, 284), 12hours (83, 150, 243, 303, 380, 410) and after 06hours (94, 181, 262, 327, 409, 462) were fed using respectively 35, 70, 100, 135, 170 and 200g of substrates (pre-germinated cowpea seeds of 72 hours old) prior mixed with the virus suspension containing $6.7x10^9$OB/ml. The ones non-starved (100, 200, 300, 400, 500, 600) were also fed using respectively 35, 70, 100, 135, 170 and 200g cowpea sprouts. Volumes of virus suspension applied were 3, 2.5, 2, 1.5, 1 and 0.5ml for 200, 170, 135, 100, 70 and 35g of feeding substrate respectively. The virus suspensions volumes of 3, 2.5, 2, 1.5, 1 and 0.5 ml were diluted in 7, 6, 5, 3.5, 2, and 1 ml of distilled water respectively. Experiments were repeated three times. Plastic containers were covered with fine white mesh and incubated at 25°C±0.5°C and 76±0.1% relative humidity for 48hrs. After incubation, the dead larvae caused by viral infection were collected daily and kept in fridge at - 4°C. The time when dead larvae because of virus infection was maximum, was noted. The final virus concentration was estimated after extraction and purification for each larval quantity and starving duration.

Germination test of cowpea seeds on water hyacinth fibers and toilet paper

The germination of cowpea seeds was performed at ambient temperature. Fine and wide water hyacinth fibers and toilet paper were used as matrix in plastic containers for the germination test. Two hundred (200)g of cowpea seeds were

sorted and washed using tap water and transferred in bleach solution at 10% for five minutes. Cowpea seeds were washed again and transferred in tap water for six hours. Then, cowpea seeds were collected and spread onto the water hyacinth fibers or toilet paper in the plastic containers. Seeds were watered twice daily for two days. The cowpea seeds spread onto toilet paper were watered daily till the first leaves appeared (Gouissi, 2013). Experiments were repeated three times for each modality.

Effect of the cowpea sprouts age on the virus production

Cowpea sprouts of 24, 48 and 72hours age taken from 200g germinated seeds were mixed with 3ml of viral suspension containing 6.7×10^9OB/ml diluted in 7ml sterilized distilled water. Six hundred (600) *M. vitrata* third stage larvae (L3) were fed using each feeding substrate age (24, 48 and 72 hours hold sprouts) placed in plastic containers. Containers were covered with fine mesh and incubated at 25°C±0.5°C and 76±0.1% relative humidity. The dead virus larvae were collected daily after 48 hours of incubation and stored in fridge at -4°C. Experiments were repeated three times. The final virus concentration was estimated by grinding dead larvae in a mortar (Moulinex), extracting using 90µm fine sieves and purifying with the centrifuge ALC-PK121.

Production of the virus in field conditions

The virus production in field conditions was performed with famers' participation using special wire-made containers of 16m^2. Six hundred (600) *M. Vitrata* non-fasting third instar larvae (L3) obtained from local mass rearing unit consisted of farmers with technical support from IITA staff were feed using substrate consisted of 200g of 72 hours old cowpea sprouts (local variety Tawa provided by farmers) (Gouissi, 2013). The substrate was prior mixed to 3ml of *Mavi*MNPV containing 6.7_x10^9OB/ml diluted in 7ml tap water. All experiment was carried out under ambient temperature. In the first village Odo-Otchèrè (Dassa), the mean daily temperature ranged between 26°C and 28°C with relative humidity of 73-84%. But in Mangoumi (Glazoué), the mean temperature varied from 24°C to 28°C and the relative humidity from 77% to 85,5%. Six hundred (600) dead larvae because of virus infection were collected daily into small cylindrical plastic boxes (04cm of diameter × 06 cm depth) and transferred to IITA laboratory to estimate the final virus concentration. A pilot farmers unit was trained and was associated to the current study. This will contribute to the local availability of cheap *Mavi*MNPV-made biopesticide ready for use.

Assessment of the virulence of MaviMNPV in combination with neem oil on M. vitrata larvae

Ten (10) ml of *Mavi*MNPV solution containing 35×10^{10}OB/ml were mixed with 10ml of neem oil (100% neem oil manufactured by the enterprise BioPhyto Collines SARL) in boxes covered and incubated for 0, 3, 5 and 7 days at room temperature. Control treatment consisted of 10ml of the virus suspension only. Treatments were repeated 4 times. At the term of each incubation period in the case of mixture (*Mavi*MNPV and neem oil) the final virus concentration was estimated through centrifugation procedure for baculovirus (Hunter et al.,1984, Harrap et al.,1997). To assess the virulence capacity of *Mavi*MNPV extracted from mixture and the one of control, three (3) ml of each solution according to incubation date were diluted in 7ml distilled sterilized water and mixed with 200g of cowpea seedlings for 72 hours. Six hundred (600) non-starving *M. Vitrata* larvae of the third stage were fed using these contaminated cowpea seedlings. Mortality was checked, 48 hours after inoculation for each modality.

Occlusion bodies counting

The occlusion bodies were counted using improved Neubaver hematimeter, adapted for highly purified baculovirus suspension (Wigley, 1980; Hunter-Fujita et al*.*,1998). The hematimeter is kind of thicker glass slide with an area 25 tiles split each in 16 sub-tiles of 0.00025mm^2. After the occlusion bodies counting, the virus concentration was determined by method described by Grzywacz (1987):

Effectiveness of MaviMNPV for cowpea protection

These experiments were performed in field at IITA-Bénin station, to compare the efficacy of *Mavi*MNPV suspension, its combination with neem oil and Decis, a synthetic chemical. The experiment was arranged in a complete randomized block design consisting of six treatments, each replicates four times. The experimental units consisted of 7m x 4m plot separated by alleys of 2m width. Two cowpea seeds were sown per hill at 25cm x 75cm spacing. Weeding was carried out twice with a hoe during the vegetative stage of the crop. The treatments constituted of **T_0**: Control (untreated plots), **T_1:** neem oil (N)**, T_2:** half proportion Neem oil + half proportion *Mavi*MNPV suspension (**$N_{1/2}$ +$V_{1/2)}$**, **T_3:** half proportion Neem oil + recommended volume of *Mavi*MNPV (**$N_{1/2}$ + V_E**), **T_4:** recommended Neem oil volume+ half proportion volume *Mavi*MNPV (**N_E+$V_{1/2}$**) and **T_5:** Decis (D). The application of the different products was conducted using a manual backpack sprayer, starting at flowers buds onset (at the 33rddays after sowing (DAS)), and was repeated at weekly interval until 54th (DAS). *Mavi*MNPV containing 6.7_x10^9 OB/ml was applied at 106 ml/ha, neem oil and Decis were applied at the dose of 1L/ha each in 115 Liters water.

Data collection started at flowers buds onset till pod maturity (54thDAS) by taking twenty flowers buds, twenty flowers and pods. These organs were taken from ten randomly selected plants per plot and treatment. Two organs were collected per plant. These plants were identified to avoid double counting on the same plant. Only larvae were considered for *M. vitrata* counting. Flowers buds and flowers collected were put into cylindrical plastic containers transparent (2cm diameters × 6cm depth) containing ethanol diluted to 70%, and the pods were put into envelopes. The whole transferred to the laboratory for binocular enumeration of *M. vitrata* larvae the same day after dissection of various organs.

Cowpea yield was estimated per treatment by harvesting cowpea in defined quadrants of 1m^2. No organ harvesting wasn't done on these spaces. Grains harvested were then dried, shelled and weighed on the scale. Yield losses caused by pest's damages were assessed by attack percentage (Cruz *et al*., 1988). They were evaluated according to the counting and weighing method (MCP) described by Boxall, (2002).

Data analysis

Excel software was used for data entry and organization, and figures realization. The effect of starving duration of the larvae, virus volume/concentration, germination seed rate, the effect of virus + neem oil mixture on *Mavi*MNPV virulence, were analyzed by performing ANOVA using GLM

procedure of SAS version 9.2 followed by the SNK test at 5% for the separation of the means. To normalize the data and stabilized the variance, the starving duration and the number of starving larvae were transformed using $\arcsin\sqrt{p}$ Were P is the proportion, prior to ANOVA. Virus concentrations were Log_{10}-transformrd before ANOVA.

Concentration of viral suspensions were calculated using formula (Grzywacz (1987))

$$C = \frac{FxXm}{KV}$$

Were **C** = Virus concentration; $\mathbf{X_m}$=Average of occlusion bodies; **F=1/D** = dilution factor with D= dilution level; **K**=number of tiles on the hematimeter (16x25=400); V=Volume of a sub-tile; V=0.1mm x 0.00025mm^2 =2.5x10^{-7}mm^3. The effect of different treatments on *M. vitrata* population density and yield, were analyzed by performing ANOVA using GLM procedure of SAS version 9.2 followed by the SNK test at 5% for the separation of the means. Cowpea grains losses were evaluated as percent damage with the count and weigh methods (Boxall, 2002):

$$P\% = \frac{NaPs - NsPa}{(Ns + Na)Ps} \times 100$$

Were P%= loss weight; Na= attacked seeds number; Ns= non-attacked seeds number; Pa=attacked seeds weight; Ps= non-attacked seeds weight.

Results

Optimum M. vitrata larvae starving duration in relation with larval number

Larval mortality rate varied with starving duration for a given larval number (Figure1). Significant differences were observed between the starving duration of 6h, 12h and 24h when 100 ($F_{(2,\ 4)}$=10.06 ; P=0.0275), 200 ($F_{(2,\ 4)}$=89.32 ; P=0.0005), 400($F_{(2,\ 4)}$=8.39 ; P=0.0371), 500 ($F_{(2,\ 4)}$=17.41 ; P=0.0106) and 600 ($F_{(2,\ 4)}$=56.62 ; P =0.0012) larvae were reared together, exception for 300 larvae ($F_{(2,\ 4)}$=1.68 ; P=0.2948). Larval cannibalism was therefore significantly higher when larvae were starved for 24hours, regardless of larval number. Comparison of the different larval number for a given starving duration revealed that cannibalism was higher when 600 larvae were together with the starving duration of 24hours (Figure2). However, no difference was observed between 300, 400, 500 and 600 larvae when the starving time was 24hours or 06hours. Consequently, the remaining number of larvae infected with *Mavi*MNPV, the number of virus-dead larvae recorded, the virus solution obtained, the final concentration of the viral solution and the concentration in OB/ml/larva were significantly higher for non-starving larvae compared to the staving duration of 06hours, 12hours and 24hours (Table1). During the experimental observation period, the maximum dead larvae was recorded 5 days after the virus inoculation, regardless of starving or non-starving larvae (Figure 3).

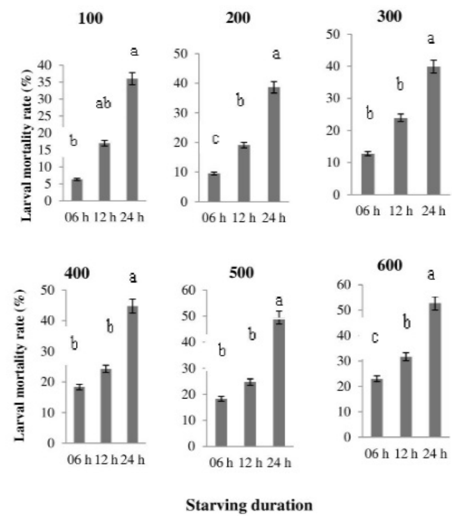

Figure1: Cannibalism rate of *M. vitrata* larvae L3 for different starving durations

Bars followed by the same letter are not significantly different after ANOVA followed by SNK at 5%

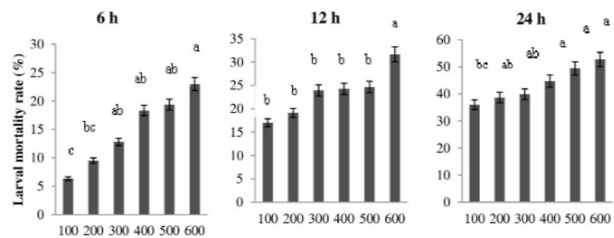

Figure 2: Cannibalism effect when different *M. vitrata* larval number were reared together

Bars followed by the same letter are not significantly different after ANOVA followed by SNK at 5%

Cowpea grains germination test

Visual observation of cowpea seeds germination on toilet paper and water hyacinth fiber indicated the apparition of leaves and cotyledons three days after sowing. The germination rate did not show any different when toilet paper was used as matrix in laboratory and field conditions (P=0.45). Likewise, no differences were observed between water hyacinth fiber and toilet paper when used as layer for the germination of cowpea grains (P=0.34) (Table2).

Table1: Number of larvae remained infested by virus (mean ± SE), number of dead larvae (mean ± SE), volume of viral solution (mean ± SE) and total virus concentration (mean ± SE) for different starving duration of *M. vitrata*

Starving duration	Number of larvae subjected to starving	Number of larvae remained infected by virus	Number of dead larvae	Volume of viral solution (ml)	Virus concentration (OB/ml)	Virus concentration per larva (OB/ml)
24 h	6300	3383±0.33^d	1428±0.34 ^{d}	123.76±0.8^d	2.55 x10^8±0.45^c	1.78 x10^5± 0.23^c
12 h	6300	4163±0.51^c	2545±0.5^c	220.6±0.41^c	1.43 x10^9 ± 0.36^b	1.78 x 10^5± 0.23^c
06 h	6300	5201±0.70^b	3430±0.53^b	297.27±0.23^b	6.40 x10^9±0.23^b	1.86 x 10^6± 0.24^b
Control (non-starving larvae)	6300	6300±0.00^a	5530±0.71^a	480.27±0.25^a	7.56 x10^{11}± 0.52^a	1.36 x10^8± 0.21^a
Probability		**<0.0001**	**<0.0001**	**<0.0001**	**0,0321**	**0,042**

*Means followed by the same letter in each column are not significantly different after ANOVA followed by SNK at 5%

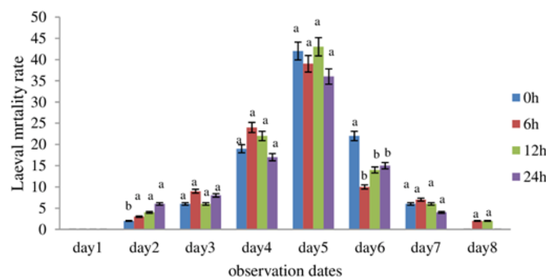

Figure3: Mortality peak of *M. vitrata* larvae after virus infection

Bars followed by the same letter are not significantly different after ANOVA followed by SNK at 5%

Effect of larval feeding substrate on the virus MaviMNPV production

Of the three larval feeding substrates tested only substrate constituted of 72 hours pre-germinated cowpea seeds was the most effective. The number of dead larvae, the volume of viral solution and the final concentration of the viral solution were significantly higher when 72 hours pre-germinated cowpea seeds were used for *Mavi*MNPV production compared to 24 hours and 48 hours germinated cowpea seeds (Table 3).

Table2: Germination rate (mean ± SE) of cowpea grains on different layer substrates

Locations	Lyers	
	Toilet paper (%)	Fibers of water hyacinth (%)
Mangoumi	85±1.3^{a}	84±1.5^{a}
Odo-Ochtèrè	83±1.23^{a}	82±1.34^{a}
IITA laboratory	86±0.54^{a}	85±0.63^{a}
Probability	0.45	0.34

*Means followed by the same letter are not significantly different after ANOVA followed by SNK at 5%

Table3: Number of virus dead larvae collected (mean ± SE), volume of viral solution (mean ± SE) and concentration (mean ± SE) for effect of *M. vitrata* larval feeding substrate on the virus production

	Feeding substrates			Probability
	24h pre-germinated cowpea seeds	48h pre-germinated cowpea seeds	72h pre-germinated cowpea seeds	-
Number of infected larvae	600	600	600	-
Number of virus dead larvae collected	421±0.72^{c}	450±0.63^{b}	560±0.78^{a}	**0.001**
Volume of viral solution (ml)	36±0.44^{c}	40.6±0.65^{b}	54.75±0.73^{a}	**0.025**
Concentration (OB/ml)	2.10x10^{5}±0.40^{b}	7.10x10^{5}±0.40^{c}	6.21x10^{7}±0.51^{a}	**0.032**

*Means followed by the same letter are not significantly different with ANOVA followed by SNK at 5%

Production of Mavi*MNPV in laboratory and field conditions using M. vitrata larvae L3*

The weight of virus-infected larvae and virus volume were statistically similar in laboratory and field conditions. On the other hand, the total virus concentration (OB/ml) and the concentration per larva (OB/ml) were significantly higher in laboratory compared to field conditions (Table 4).

Table4: Weight of 600 dead virus larvae (mean ± SE), viral solution volume (mean ± SE) and concentration (mean ± SE) of the production at Mangoumi, Odo-Otchèrè and IITA laboratory

Locations	Dead virus larvae	Weight of 600 dead virus larvae (g)	Viral solution volume (mL)	Concentration in viral solution (OB/ml)	Concentration /larva (OB/ml)
Mangoumi	600	34 ±0.57^{a}	51.66 ±1.2^{a}	6.95x10^{9} ±0.08^{b}	1.33 x10^{7} ±0.087^{b}
Odo-Otchèrè	600	32.33 ±1.20^{a}	53 ±0.57^{a}	8.28 x10^{9}±0.089^{b}	1.34 x10^{7} ±0.087^{b}
IITA laboratory	600	33.33 ±0.67^{a}	53 ±0.57^{a}	3.46 x10^{12} ±0.026^{a}	1.376 x10^{11} ±0.307^{a}
Probability		0.466	0.483	<0.0001	<0.0001

*Means followed by the same letter are not significantly different with ANOVA followed by SNK at 5%

Effect of neem oil on the virulence of the virus MaviMNPV stored in neem oil

Neem oil increased the conservation time for the virus *MaviMNPV* when the virus suspension was mixed with neem oil. Seven days after mixing *MaviMNPV* with neem oil, the number of occlusion bodies was higher compared to control where the virus was stored alone (Table5). The highest final virus concentration was recorded in the combination virus-neem oil compared to the control with the lower mortality rate.

Effect of the different products on Maruca vitrata population density (mean ±SE)

Maruca vitrata population density evolution indicated the same trend for all plots treated using *Mavi*MNPV suspension combined with neem oil (Figure 4).Indeed, The curves show peak at 47th DAS for most of the treatments (exception for Decis, peak of 15.00±3.67 at 51st days after sowing) with the lower larval numbers in plots treated with *Mavi*MNPV suspension combined with neem oil (10.00±0.40 for $N_E+V_{1/2}$, 7.50±3.92 for $N_{1/2}+V_E$ and 9±3.90 for $N_{1/2}+V_{1/2}$) compared to control (16.5±3.59) and neem oil (15.00±2.82). A significant difference was noted between the treatments (P=0.0525, F=2.86). After the fifth treatment (47th days after sowing), the number of *M. vitrata* larvae recorded on mixture treated plots decreased 51st day after sowing (2.00±1.08 for $N_E+V_{1/2}$, 1.75±.62 for $N_{1/2}+V_E$ and 1.25±1.10 for $N_{1/2}+V_{1/2}$) while the number was high in untreated control plots (12.25 ± 1.65), treated neem oil plots (15.00 ± 2.38) and treated Decis plots (15.00 ± 3.67). A significant difference was noted between the treatments (P=0.0002, F=9.92). Cowpea yield (Kg per ha) (mean ±SE) was significantly higher in treated plots compared to the control with product application (Figure5). Likewise grain losses (% ±SE) were lower in treated plots compared to the control (Figure 6)

Table5: Mean number of occlusion bodies t_0 (mean ± SE), mean number of occlusion bodies t_f (mean ± SE), concentrations (mean ± SE) and mortality rate (mean ± SE) for effect of neem oil on the virulence of *Mavi*MNPV at different storage length

Incubation length	Treatments	Mean number of occlusion bodies at t_0	Mean number of occlusion bodies at t_f	Initial concentration C_0 (OB/ml)	Final concentration Cf (OB/ml)	Mortality rate (%)
07 days	Neem oil + virus	135±0^{a}	132±173^{ab}	1.35 x10^{10} ± 0^{a}	1.32 x10^{10} ± 0.005^{a}	94±0.3^{a}
07 days	Virus only	135±0^{a}	3217±1.3^{d}	1.35 x10^{10} ± 0^{a}	3.22 x10^{9} ±0.017^{d}	23±1.43^{c}
05 days	Neem oil + virus	135±0^{a}	128±1.15^{b}	1.35 x10^{10} ± 0^{a}	1.28 x10^{10} ± 0.003^{a}	97±0.23^{a}
05 days	Virus only	135±0^{a}	34.5±0.28^{d}	1.35 x10^{10} ±0^{a}	3.45 x10^{9} ± 0.003^{c}	34±1.11^{c}
03 days	Neem oil + virus	135±0^{a}	131.33±0.67^{ab}	1.35 x10^{10} ± 0^{a}	1.31 x10^{10} ± 0.003^{a}	96±0.56^{a}
03 days	Virus only	135±0^{a}	38.67±0.88^{c}	1.35 x10^{10} ± 0^{a}	3.86 x10^{9} ± 0.008^{b}	66±0.22^{b}
0 day	Neem oil + virus	135±0^{a}	132.67±1.45^{a}	1.35 x10^{10} ± 0^{a}	1.33 x10^{10} ± 0.005^{a}	97±0.23^{a}
0 day	Virus only	135±0^{a}	134±1.15^{a}	1.35 x10^{10} ± 0^{a}	1.34 x10^{10} ± 0.003^{a}	95±0.57^{a}
Probability	-	-	**<0.0001**	-	**<0.0001**	**0.003**

*Means followed by the same letter are not significantly different with ANOVA followed by SNK at 5%

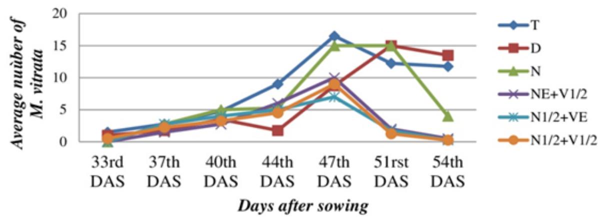

Figure 4: Population density of *M. vitrata* in plot treated with different products

Legends: $N_E+V_{1/2}$: Neem oil whole proportion + *Mavi*MNPV virus half proportion; $N_{1/2}+V_E$: Neem oil half proportion + *Mavi*MNPV virus whole proportion; $N_{1/2}+V_{1/2}$: Neem oil half proportion + *Mavi*MNPV, DAS (day after sowing)

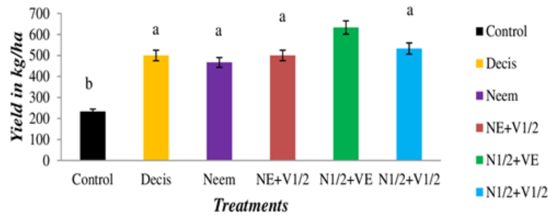

Figure 5: Cowpea yield

Bars followed by the same letter are not significantly different after ANOVA followed by SNK at 5%

Legends: $N_E+V_{1/2}$: Neem oil whole proportion + *Mavi*MNPV half proportion; $N_{1/2}+V_E$: Neem oil half proportion + *Mavi*MNPV whole proportion; $N_{1/2}+V_{1/2}$: Neem oil half proportion + *Mavi*MNPV

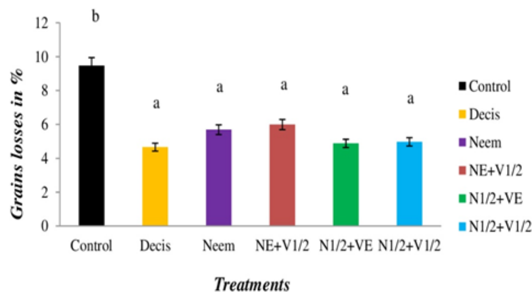

Figure 6: Grain losses.

Bars followed by the same letter are not significantly different after ANOVA followed by SNK at 5%

Legends: $N_E+V_{1/2}$: Neem oil whole proportion + *Mavi*MNPV half proportion; $N_{1/2}+V_E$: neem oil half proportion + *Mavi*MNPV whole proportion; $N_{1/2}+V_{1/2}$: Neem oil half proportion +*Mavi*MNPV

Discussion

The current study revealed that the volume of virus suspension and concentration varied with larval number and starving duration during a mass production of the virus *Mavi*MNPV. Indeed, cannibalism became higher when larval starving duration increased from 6h to 24h and when larval number reached 600 larvae per rearing box. Such findings were reported by Tanada (1964) in *M. vitrata.* Our results showed that *Mavi*MNPV virus could be transmitted through ingestion of contaminated feeding substrate. This transmission mode was identified as the main mode for *Mavi*MNPV (Kelly, 1981). Comparison of the virus production yield showed that the virus final concentration was higher in laboratory compared to field conditions (at Mangoumi and Odo-Otchere). These differences could be explained by differences in environmental factors. A lack of moisture coupled with higher temperature was known to affect entomopathogen survival (Utsa, 2013). Moreover, some entomopathogens were sunlight-sensitive (Dent,1991). However additional studies will be required for assessing the effect of temperature on *Mavi*MNPV production. At both laboratory and experimental sites, the final virus concentration obtained was higher than the lethal concentration (LC_{50}) of 2.39×10^3OB/ml (Lee et al,.2007) at 27±1°C temperature and 70±10% relative humidity. Likewise, this concentration was above the dose recommended per ha (X.10^8 OB/ml where X<10) for the control of *M. vitrata* in field conditions. Indeed, the influence of environmental factors differ with pathogen species in the cabbage hopper *Trichoplusiani* Hübner (Lepidoptera: Noctuidae) when infested with two baculovirus *Ac*MNPV (*Autographa cslifornica* multiple Nucleopolyhedrovirus) and *Tn*SNPV (*Trichoplusiani* single nucleopolyhedrovirus) (Shikano and Cory, 2015). The present study also revealed that *Mavi*MNPV virus could be mass produced using natural larval diet such as pre-germinated cowpea grains (sprouts) or semi-artificial larval diet (Vanderzant et al., 1962). The use of semi-artificial or natural diet reduced the virus production costs. Higher larval mortality caused by the virus was obtained regardless of larval number and starving duration, 5 days after inoculation. These observations were consistent with those reported by Kolani (2010). Storage of the virus in neem oil kept the concentration of the virus after 7 days suggesting the absence of negative effect of neem oil on the virus survival. This could solve the problem of viral solution conservation that is always done in fresh temperature. Viruses were known to be intact outside their host (Jacques, 1973; Fuxa, 1987). Moreover, the synergistic effect was observed between neem oil and *Mavi*MNPV for *M. Vitrata* control in field (Sokame et al., 2015). The curves depicting the temporal distribution of *M. vitrata* larvae showed similar trend for all treatments with a peak at 47th day after sowing (DAS) for most of them. However, a reduced population density of the pest was observed for treated plots compared to the control, contributing to an increase of cowpea yield. Grain yield was significantly higher in treated plots compared to the control. These results were consistent with those reported by Toffa-Mehinto et al.,(2014) and Sokame et al.,(2015) who demonstrated the effectiveness of the combination of *Mavi*MNPV virus with neem oil for the control of *M. vitrata.* Furthermore, storing *Mavi*MNPV in neem oil did not alter the virus virulence within 7 days. But prolonged storage needs to be investigated in future research works. The different products applied significantly improve cowpea yield compared to the control, even though no significant differences occurred between them. However, when considering the side effects of synthetic chemical, biological products may be preferred for a sustainable cowpea production. Moreover, using biopesticide as a component of Integrated Pest Management is of great importance in the context of insect resistance to chemicals (Chandler et al., 2011; Lacey et al., 2015). One of the major issue to make available a biopesticide from *Mavi*MNPV is the mass production cost. By testing the optimization of community-based mass production of *Mavi*MNPV, the current study found out that this virus could be cultured by producers using local materials. This mass production at community level would facilitate the technology adoption for an effective application of *Mavi*MNPV in cowpea production.

Conclusion

The interest of this study is to contribute to the biodiversity protection by reducing chemical pesticides use. The works achieved in this study showed that *M. vitrata* multi-nucleopolyhedrovirus could be mass cultured in field conditions with non-starving three-days-old *M. vitrata* larvae fed using 72hours pre-germinated cowpea seeds. This would be made available *Mavi*MNPV for an effective protection of cowpea against *M. vitrata* and could also be a great business opportunity for local young entrepreneurs. Viral particle (Occlusion bodies) obtained after extraction were effective in managing cowpea insect pests namely *M. vitrata.* Cowpea yield obtained after treatment was similar to the other products but was significantly higher compared to untreated control. The use of *Mavi*MNPV combined with neem oil give the same degree of control of *Maruca vitrata* as obtained with a chemical insecticide.

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