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ORIGINAL RESEARCH ARTICLE

Effects of *Maruca vitrata* multi-nucleopolyhedrovirus and neem oil, *Azadirachta indica* Juss on the eggs of the cowpea pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae)

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Abstract

Cowpea, *Vigna unguiculata* (L.) Walp., is the most cultivated and consumed legume in West Africa and is typically attacked by several insect pests, including *Maruca vitrata*, leading to reduced yields. This study assessed under laboratory conditions the efficacy of neem oil and *M. vitrata* multi-nucleopolyhedrovirus (*Mavi*MNPV) against *M. vitrata* eggs as alternatives to second generation pesticides. Hatching and mortality rates after biopesticide application of neem oil, *Mavi*MNPV, and the two in combination reduced the egg viability by 89%, 84% and 91%, respectively. Moreover, the combination of *Mavi*MNPV and neem oil induced 100% mortality among the hatched larvae, compared to 60% and 100% alone, respectively. Implications for using these biopesticides are discussed within an integrated pest management (IPM) context.

Keywords Baculoviruses · Biopesticides · Cowpea pod borer · Eggs · *Maruca vitrata* multi-nucleopolyhedrovirus · Neem oil

Introduction

Cowpea, *Vigna unguiculata* L. (Walp.) is the most important edible grain legume in West Africa. The typical cowpea yield in the field is often lowered by several insect pests, including the legume pod borer *Maruca vitrata,* whose populations attain damaging levels in southern Burkina Faso where the rainfall is between 900 and 1200 mm (Ba et al. 2009). Larvae of *M. vitrata* feed predominantly on reproductive organs such as the flowers and developing pods (Traoré et al. 2013). Chemical insecticides are effective in controlling *M. vitrata* (Amatobi 1995; Atachi and Sourokou 1989) but they are not widely applied because of the associated high cost, the lack of good quality products, and the challenges to safe-use practices especially for smallholder low-literate farmers (Pimentel et al. 1992; Tan et al. 1996) Therefore, it becomes imperative to provide cowpea farmers with alternative control methods, as part of an integrated pest management (IPM) system (Tamò et al. 2003).

Biopesticides constitute one alternative solution to the use of chemical insecticides and have the advantage of being biodegradable (Martinez 2002) with low, or no, toxicity to humans and animals (Valle Pinheiro and Dias Quintela 2010). Recent efforts have focused on the efficacy of a combination of biopesticides against *M. vitrata* larvae (Sokame et al. 2015). Among the biopesticides successfully assessed were neem oil (*Azadirachta indica* Juss) and the *M. vitrata* multi-nucleopolyhedrovirus (*Mavi*MNPV). Neem-based preparations have been reported to be effective for controlling *M. vitrata* (Jackai et al. 1992; Jackai and Oyediran 1991; Tanzubil 2000), but the effectiveness of neem has been variable from year to year (Bottenberg and Singh 1996) mainly because of the lack of a standardized product. A commercialized standardized neem oil is now available in Benin and has been successfully tested against *M. vitrata* in Benin and Burkina Faso (Drabo 2014; Ouédraogo 2013; Sokame et al. 2015).

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*Mavi*MNPV is a baculovirus. It was first isolated in Taiwan from infected larvae of *M. vitrata* on yard-long beans and found to be highly effective against *M. vitrata* larvae (Lee et al. 2007). The virus was introduced into the laboratories of the International Institute of Tropical Agriculture (IITA) in Benin, where its efficacy was confirmed, achieving over 95% *M. vitrata* larval mortality (Tamò et al. 2012). Moreover, field trials in Benin, Burkina Faso, and Niger indicated that the viral biopesticide could be as effective as conventional insecticides in controlling *M. vitrata* (Tamò et al. 2012).

Nucleopolyhedroviruses such as *Spodoptera litura* NPV and *Mavi*NPV combined with neem have been successfully assessed in the field against the larvae of *S. litura* (Nathan and Kalaivani 2006) and *M. vitrata* (Drabo 2014; Kadri et al. 2013), respectively. The combination of *Mavi*MNPV with neem and *Jatropha curcas* was effective in controlling *M. vitrata* larvae (Sokame et al. 2015). However, to our knowledge, no studies have been reported on the use of these biopesticides against *M. vitrata* eggs. Were such effectiveness demonstrated, they then might provide a basis for early, on-farm spraying to rapidly control pest populations. The current study was undertaken, therefore, to assess the effect of a combination of neem oil and the *Mavi*MNPV biopesticide against the eggs of *M. vitrata* under laboratory conditions.

Materials and methods

M. vitrata culturing

Eggs of *M. vitrata* used in the bioassays were obtained from a mass rearing facility at the INERA entomology laboratory at Kamboinsé in Ouagadougou, Burkina Faso (12°28'N, 32°1'W). The insect colony was established from wild *M. vitrata* moths collected from light traps in August 2014. Adults were kept in cups for oviposition in a room maintained at 24 °C with 58%–75% relative humidity. The eggs laid on the internal surface of the cups were collected and incubated until the emergence of the first instar larvae, which were subsequently reared on a modified European corn borer diet (Bio-Serv No. F9478B-M, without corn cob grits, Bio-Serv Co., Flemington, NJ, USA), supplemented with flour of the cowpea variety "Komcalle." Subsequent generations were regularly obtained after 24 days under the above mentioned conditions. For our experiment we used freshly laid eggs aged 0–12 h.

Biopesticides

A commercially available, emulsifiable neem preparation (Biophyto-Collines, Cotonou, Bénin) was diluted with tap water to obtain five concentrations for the bioassays: 0.5%; 0.62%; 0.74%; 0.87%, and 1%. Concentrations were made based on the recommendations of the International Institute of Tropical Agriculture (Benin) for the use of neem oil (1 l/ha) and those of the manufacturer (2 l/ha). The final volume of each solution was 250 ml.

The virus, *Mavi*MNPV, was provided by the IITA-Benin biocontrol laboratory where it is routinely cultured on *M. vitrata* larvae from the original Taiwan strain. For this experiment we used the standard IITA recommended concentration of 1.6×10^{11} Occluded Bodies/ml (OB/ml).

Bioassays procedures

Experiments were carried out using each of the above five neem solutions and *Mavi*MNPV solution alone, a combination of each of the five neem solutions with *Mavi*MNPV, and a tap water control. The numbers of eggs for each test solution were 6115 (neem oil), 2593 (*Mavi*MNPV), and 5120 (*Mavi*MNPV plus neem oil), and 2790 for the control.

To verify the specificity of *Mavi*MNPV to *M. vitrata* eggs, a complementary study was carried out with the eggs of two other lepidopterans, *Spodoptera frugiperda* and *Cirina butyrospermi*. One concentration of 1.6×10^{11} OB/ml virus *Mavi*MNPV was used on of 956 and 979 eggs, respectively, plus a tap water control.

For each trial, eggs were dipped in the biopesticide test solution (or tap water control) for 3 s and allowed to air dry at room temperature. Subsequently, in each of the treatment conditions, one third of the eggs were washed (dipped in tap water for 3 s) after 1 h, one third were washed (dipped in tap water for 3 s) after 12 h, and the final third were not washed. Washing was meant to simulate possible likely effects of rainfall in cowpea fields. The eggs were then incubated in a room maintained at 24 °C with 58%–75% relative humidity until hatching. The eggs were monitored daily, and the number of hatched eggs were recorded. Emerging first instars larvae were also counted, and motionless larvae were considered dead.

Data analyses

Microsoft Excel was used for data capture, storage, exploration, and management. All analyses were carried out using SAS software version 9 (SAS Institute 2003). The Shapiro-Wilk test was used to test for normality of data collected, with subsequent Analyses of Variance (ANOVA) to test for significant differences among the treatments. When ANOVAs were significant, means were separated by the Student–Newman–Keuls test at the 5% level.

Table 1 Effects of neem oil on the viability of *M. vitrata* eggs

Neem oil Conc. (%)	Mean Egg Viability After Washing (%±SE)			
	Unwashed	1 h	12 h	
Control: 0.0	77.28±10.3Aa	85.31±7.8Aa	88.09±4.2Aa	(F=0.60 P<0.56)
0.5	22.06±7.5Ab	29.61±2.8Ab	21.57±4.3Ab	(F=0.91 P<0.42)
0.62	13.07±5.5Ab	27.36±5.7Ab	13.69±3.2Abc	(F=2.71 P<0.11)
0.75	9.37±3.2Ab	14.20±3.1Ac	13.33±2.4Abc	(F=0.91 P<0.42)
0.87	7.25±2.5Ab	11.38±2.7Ac	7.34±2.9Acd	(F=0.92 P<0.42)
1.00	5.72±2.1Ab	9.94±2Ac	3.59±0.9Ad	(F=2.44 P<0.12)
	F=7.93; P < 0.0002	F=18.10; P < 0.0001	F=12.96; P<0.0001	

Means within a row followed by the same uppercase letter(s) and means within a column followed by the same lowercase letter(s) are not significantly different by SAS-SNK test (P < 0.05)

Results

Table 1 summarizes the significantly reduced *M. vitrata* egg viability from different test concentrations (Table 1). The rate of egg hatching decreased and was significantly different for increasing neem oil concentrations ($p < 0.05$) compared with the control. When eggs were washed with water either 1 or 12 h after being soaked in the neem solution, the biopesticide remained effective and significantly reduced the egg viability, with higher neem concentrations leading to higher egg mortality rate (Table 1). Egg washing time did not significantly ($p > 0.05$) affect the efficacy of the neem oil concentrations (Table 1).

Likewise, almost all of the different concentrations of neem oil resulted in 100% first instar larvae mortality, whether washed for 1 h, 12 h, or not (Table 2). All of the larvae from the control survived.

Table 3 summarizes the efficacy of MaviMNPV on egg hatch rate and shows a significant difference ($p < 0.05$) between washed, not washed, and control groups, with lower hatch rates in treatment groups compared to the control. A similar trend holds for hatched instar larvae mortality. In contrast, there was no significant difference ($p > 0.05$) in egg mortality between the control and MaviMNPV for the other tested lepidopterans (Table 4).

Overall, the combined MaviMNPV and neem oil solution significantly reduced egg viability and, in its highest concentrations, led to the lowest level of egg viability (Table 5). When eggs were washed with water either 1 or 12 h after being soaked in the combined MaviMNPV-neem solution, the biopesticide remained effective (Table 5). Similarly, when the eggs (washed or unwashed) hatched, all neonate larvae from the biopesticide treatment died while all of those from the control survived (Table 6).

Discussion

The present study confirms that when *M. vitrata* eggs were treated with neem oil, their viability is significantly reduced, similar to findings for several other insect species including *Atherigona soccata* Rondani (Zongo et al. 1993), *Pieris brassicae* (Hasan and Ansari 2011), *Diatraea saccharalis* (de Oliveira et al. 2013), *Clavigralla gibbosa* (Shukla and

Table 2 Effects of neem oil on *M. vitrata* hatched larvae mortality

Neem oil Conc. (%)	Mean Hatched Larvae Mortality After Washing (%)		
	Unwashed	1 h	12 h
Control: 0.0	0	0	0
0.5	100	97.60	100
0.62	100	97.63	100
0.75	100	100	100
0.87	100	100	100
1.00	100	100	100

ANOVA was not performed as all neem concentrations led to 100% mortality and the control did not have any mortality

Table 3 Effects of MaviMNPV and washing on *M. vitrata* egg viability and hatched larvae mortality

Treatments	Mean (%±SE)	
	Egg Viability	Hatched Larvae Mortality
Control: Water	86.74±2.5a	–
Unwashed Eggs	15.95±8.5b	59.09±17.1a
Eggs Washed after 1 h	21.74±9.3b	72.23±14.4a
Eggs Washed after 12 h	17.29±9.8b	69.86±15.2a
	F=15.72; P < 0.0001	F=0.20; P < 0.82

Means within a column followed by the same letter (s) are not significantly different by a SNK test (P < 0.05)

Kumar 2002), and stored product insects (Das 1987; Makanjuola 1989; Nukenine et al. 2011).

The ovicidal activity of neem oil could be due to its main chemical component, azadirachtin, as previously reported on *Corcyra cephalonica* (Pathak and Pandey 2011). On *M. vitrata* eggs, we recorded a > 90% mortality, which is similar to findings on *A. soccata* (Zongo et al. 1993) and much higher than reported on the sugarcane borer *D. saccharalis* (de Oliveira et al. 2013), a related Crambidae species. With crude extracts of 5%, 10% and 15% neem, a mortality range of 65–82% was reported on eggs of *M. vitrata* (Ekesi 2000). Other findings have indicated a limited effect of neem oil on the eggs of different insect pest species (Ahmad et al. 2015; Bruce et al. 2004; Marques et al. 2014; Seljåsen and Meadow 2006). Differences in the insecticidal effects of neem have been reported for several insect species because the contents of phytochemicals extracted from neem vary considerably due to biotic and abiotic factors and variations in product formulation (Gahukar 2014; Mouffok et al. 2008).

Our findings showed that neem oil remained highly effective even when the eggs were washed, indicating that the neem oil quickly penetrated the egg after treatment. In the case of head and body lice, an incubation time of only five minutes is sufficient to prohibit any larvae from hatching (Mehlhorn et al. 2011). In our case, the eggs were washed at 1 h and 12 h after being dipped in the neem solution, which likely gave enough time for the neem to enter the chorion. Specifically, neem enters eggs through aeropyles, tiny holes in the chorion, associated with the respiration of embryos (Pathak and Pandey 2011). It further induces chorion defects (Correia et al. 2013) and, as a consequence, the eggs dry up (Kaethner 1992). Interestingly, significant mortality occurred due to the residual activity on first instar larvae emergence from neem-treated eggs. Similar findings have been reported on *Bemisia tabaci* (Marques et al. 2014). Neem acts on the growth and molting of insect pests or as an anti-nutritional/anti-feedant (Blaney et al. 1990; Mouffok et al. 2008; Seljåsen and Meadow 2006). Neonate larvae may be killed by direct contact with the egg chorion when hatching or by consumption of neem present on the chorion.

When *M. vitrata* eggs were treated with *Mavi*MNPV, the viability of the washed and the unwashed eggs also decreased significantly. However, *Mavi*MNPV did not affect the egg hatchability of *C. butyrospermi* and *S. frugiperda* (see Table 4), suggesting its specificity to *M. vitrata* eggs. While *Mavi*MNPV, like other baculoviruses, is essentially pathogenic to larvae, this is the first time a larval entomopathogenic virus has been reported to have direct effects on egg viability. While the mechanism behind this observation remains to be investigated, we can hypothesize that the ovicidal action of *Mavi*MNPV may be physical, if viral bodies adhering to the egg obstruct the egg membrane and thus impede respiratory processes in the embryo. This might be peculiar to *M. vitrata* eggs, whose chorion has a slender appearance (Sharma et al. 1999) and might consequently be more fragile than the eggs of the two other lepidopterans tested in our study. Similar observations have been reported by Sato et al. (1980), who observed a strong binding of viral capsules to egg shells when

Table 4 Effect of MaviMNPV on the egg viability three lepidopterans

Virus Solution ($\times 10^{11}$ OB/ml)	Mean Eggs Hatched (%±SE)		
	Maruca vitrata	*Cirina butyrospermi*	*Spodoptera frugiperda*
0	90.94±2.63a	95.68±0.61a	88.12±2.13a
1.6	16.88±0.69b	90.9±2,82a	90.33±1.10a
	F=1515.03; *P*<0.0001	F=0.46; *P*=0.54	F=0.69; *P*=0.41

Means within a column followed by the same letter(s) are not significantly different by a SNK test (P < 0.05)

Table 5 Effect of the combination of neem oil and MaviMNPV on *M. vitrata* egg viability

Neem Oil Conc. (%)+Virus ($\times10^{11}$ PIB*/ml	Mean Egg Viability After Washing (%±SE)			
	Unwashed	1 h	12 h	
Control: 0.0	83.82±4.1Aa	73.67±5.4Aa	83.55±3.2Aa	F=1.79; P<0.21
0.5+1.6	21.02±1.3Bb	32.44±2.4Ab	19.87±1.6Bb	F=12.93; P<0.0010
0.62+1.6	12.62±0.6Bc	17.91±0.4Acb	13.13±0.7Bc	F=15.02; P<0.0005
0.75+1.6	5.03±0.4 Bd	17.74±3.7Acb	6.82±1.4 Bd	F=14.67; P<0.0006
0.87+1.6	4.11±0.4 Bd	15.29±5.9Ac	5.32±0.5 Bd	F=7.27; P<0.0085
1.00+1.6	2.10±0.9Be	10.53±3.5Ac	2.43±0.3Be	F=6.59 P<0.011
	F=59.85; P < 0.0001	F=14.05; P < 0.0001	F=139.18 P<0.0001	

*PIB, polyhedron inclusion bodies. Means within a row followed by the same uppercase letter(s) and means within a column followed by the same lowercase letter(s) are not significantly different by a SNK test (P < 0.05)

the eggs of the tea tortrix, *Homona magnanima* Diaknoff, were dipped in a granulovirus solution.

Interestingly, in our study, *Mavi*MNPV was also able to kill 59%–72% of the larvae that hatched from eggs dipped in the viral solution. An NPV virus was similarly found to inflict 93.6% mortality to larvae of *Helicoverpa armigera* hatching from eggs dipped in a viral solution immediately before hatching (Tuan et al. 1989). However, the maximum 72% larvae mortality recorded in our study was lower than that reported by Tamò et al. (2003) when directly spraying *Mavi*MNPV onto *M. vitrata* larvae. The contact time between the virus and larvae could also explain mortality rate differences. Between four to six days were needed to achieve 100% larval mortality (Laleye 2007) whereas, in our case, mortality was recorded only four days after incubation. The hatching larvae of *M. vitrata* may acquire *Mavi*MNPV by feeding on the egg chorion during emergence and ingesting the virus as reported on an unknown insect species (Ibarra and del Rincón Castro 2001), *Spodoptera exigua* (Yu and Brown 1997), *Heliothis virescens* (Jackson et al. 1992), and *H. armigera* (Tuan et al. 1989).

The combined application of *Mavi*MNPV with neem oil led to a significantly higher egg mortality than those components separately. As indicated above, while egg mortality is caused by neem's ovicidal action, neem may also obstruct egg membranes and limit respiration (Schmutterer 1990). The membrane obstruction by both the neem and *Mavi*MNPV in addition to neem's chemical actions may explain the higher ovicidal activities of the combined products. Likewise, the combination of neem and *Mavi*MNPV led to a higher larval mortality rate. This confirms previously noted synergetic effects of neem and *Mavi*MNPV on *M. vitrata* larvae (Sokame et al. 2015). Similar trends were also observed for *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) using a combination of azadirachtin and *Splt*NPV (Nathan and Kalaivani 2006).

Overall, our findings suggest that a compound solution of neem and *Mavi*MNPV could be sprayed early in cowpea when *M. vitrata* moths first appear in the field to coincide with egg laying and to provide a more efficient control. This is similar to controlling *Chilo suppressallis* in rice using early NPV applications (Zhai and Chai 2013).

Table 6 Effect of the combination of neem oil and MaviMNPV on *M. vitrata* hatched larvae

Neem Oil Conc. (%)+Virus ($\times10^{11}$ PIB*/ml	Mean Hatched Larvae Mortality After Washing (%)		
	Unwashed	1 h	12 h
Control: 0.0	0	0	0
0.5+1.6	100	91.91	100
0.62+1.6	100	96.42	100
0.75+1.6	100	97.89	100
0.87+1.6	100	98.57	100
1.00+1.6	100	100	100

*PIB, polyhedron inclusion bodies. ANOVA was not performed as similar numbers were recorded for all five treatments and zero mortality on the control

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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