Global journal of multidisciplinary and applied sciences

Available online at www.gjmas.com

©2013 GJMAS Journal-2013-1-1/19-25

ISSN xxxx-xxxx ©2013 GJMAS

Moringa, Rice bran as a New Additives for a Baculovirus against Ultraviolet Effect

A A. El- Helaly*

Entomology Department, Faculty of Agriculture, Cairo University, Egypt

***Corresponding author*:** A. El-Helaly

ABSTRACT: The addition of moringa, rice bran filtrates (1%) to the nucleopolyhedrovirus (*SpliMNPV*) of the cotton leaf worm *Spodoptera littoralis* (Bosid.) provided almost complete protection to the PIB's following exposure to artificial UV irradiation (30 min) in laboratory test. This work focuses on testing inexpensive additives that may sustain effectiveness of virus biocontrol agent; green tea filtrates and cacao were used as comparative additives. Polyhedra inclusion bodies were mixed with these plant extracts at (1%, 5% and 10%) concentrations exposed to artificial UV in two steps as a thin film in Petri dishes. The different treatments of NPV suspension were bioassayed using neonate healthy larvae. The concentration of 1% of Moringa additive preserved the activity of polyhedral inclusion bodies after UV-exposure resulting in 93.24% mortality of larvae and it was 91.66%, 90.54% and 66.43% for rice bran, cacao and green tea respectively while it was the lowest (15.06%) with virus alone treatments (positive control) 5 hr post application, similar trend was recorded in the second step using the 5, 10 % concentrations 5 hr post application. The mixtures of baculovirus PIB's and additives were measured with spectrophotometer under 400nm length before and 10 hr post application. The suspension absorbance at 400nm showed narrow differences with moringa followed by cacao, rice bran and green tea respectively. These findings indicate that these plant extracts could be promising UV protective additives for *SpliMNPV* and they should be further investigated in the field large scale to obtain the best formulation for the control of agriculture important insect pest.

***Keywords*:** antioxidants, Baculovirus activity, *Spodoptera littoralis* NPV, virus protection.

INTRODUCTION

Lepidopterous insects can damage agricultural crops, consume and/or damage harvested food, or transmit diseases to humans and animals. (Atkinson, 2010). In Egypt, several lepidopterous insects are important pests attacking agricultural crops. The Egyptian cotton worm is a highly polyphagous defoliator of many cultivated plants. It is one of the most destructive agricultural lepidopteron pests within its subtropical and tropical range. It is attacking plants belonging to 44 different families including grasses, legumes, crucifers and deciduous fruit trees all containing species of highly economic importance. In North Africa it is damaging vegetables, in Egypt cotton and in Southern Europe, the plant and flower production in glasshouses, and vegetables (CABI/EPPO1997, DEFRA 1999 and Ellis 2004, Alford, 2007, Anon, 2009 and Espinoss and Hodges, 2009). Insect viruses, as biocontrol agents, may play an important role in pest management, thus reducing dependency on chemical insecticides. Baculoviruses have some unique properties such as their virulence, specificity within the phylum Arthropoda, non-infectivity for beneficial insects and higher animals and their biodegradable nature, several of these insect viruses, have been commercialized for insect control (Moscardi 1999). The DNA in NPV is susceptible to degradation by ultra-violet (UV) light, and certain plant chemicals and high pH conditions can damage the protein body protecting the virus. For this reason, Virus Max applications generally provide relatively short residual control, particularly where the virus on the target crop is exposed to: high levels of UV (e.g. sorghum heads), inactivating plant chemicals (e.g. pulses) or high leaf pH (primarily cotton). Reynolds, S., 2001, Scott, P.J., 1991, Rabin *et al* 1989. One of the limitations of the use of baculoviruses as biological control agents is their loss of activity under field conditions due to inactivation by ultraviolet (UV) light, with most of the activity being lost within 24 h. Most of the studies to address this deficiency have centered on the use of microencapsulation and additives such as UV

protective additives (Ignoffo and Batzer 1971) and fluorescent brighteners (Shapiro and Vaughn 1995) to reduce or prevent inactivation by UV light. Chemical compounds have been devoted to protect baculoviruses, Several dyes (Shapiro, 1989; Ramakrichnan and Chaudhary, 1991; Reddy and Divakar, 2001; and Arivudainambi et al, 2000); optical brighteners (Baskaran, 2001; Kamaldeep & Battu, 2008). Lignin (Jacques, 1977, Tamez-Guerra *et al.,* 2000; El Salamouny *et al.,* 2002; Elnagar *et al*, 2003). Natural antioxidants folic acid (Deotale *et al*., 2007),riboflavin, Pyridoxine, eucalyptus and mango leaf extract (Deotale *et al*., 2003). Green tea (Shapiro *et al.,* 2008), Black tea (El Salamouny *et al.,* 2009a) and Cacao & coffee (El Salamouny *et al*., 2009 b). 20 other antioxidants (El Helaly *et al.*,2009) and Cacao, green coffee, green and red cabbage (El Helaly *et al.* 2013) The present study, evaluated two new promising natural products (Moringa and rice bran) for their suitability as UV protectants to *SpliMNPV* because their huge contents of deferent groups of antioxidants.

MATERIALS AND METHODS

2.1. Insect colony:

A laboratory colony of the cotton leaf worm, *Spodoptera littoralis* (Boisd.), was established as the test insect species on a semi synthetic diet of Shorey and Hale (1965).

2.2. Virus inoculum:

A Local isolate of *Spodoptera littoralis* multiple embedded nucleopolyhedrovirus (*Spli*MNPV) was originally isolated in Egypt by Abul Nasr (1956).

2.3. UV protective additives in the virus inoculum:

Four natural materials originated from plants, moringa, rice bran, cacao and green tea were obtained either fresh (moringa, rice bran and green tea), and where they were washed with distilled water, and dried under cooling or was already in a dry form (cacao). All products were ground to obtain powder, and the required concentrations were prepared using distilled water, and kept for 24 hours, then centrifuged. Suspensions were filtered through two layers of clean muslin cloth with a thin layer of cotton wool in-between them. The mixture of virus and tested additive was prepared at a final concentration of 1, 5 and 10 % additive in the tested mixture (Shapiro, 2008).

2.4. SIMULATED UV radiation in the sunlight

Sunlight UV (SUV) was simulated using a set of four UV lamps (Ultra-Vitalux, OSRAM, Germany), that were in vertical level at a distance of 160 cm from the exposed virus samples, and 60 cm between the centers of each two lamps. The biological effect is approximating 6-7 times greater than the natural sun-light if the distance between artificial sunlight - lamp and dry deposit is 50 cm. (Huber and Ludcke, 1996). A wetting agent (Teepol 2.5%) was added to the viral suspension, of which 50 µl was spread inside a Petri dish (10 cm in diameter) using a fine pipette (Jencons of Hemel Hempstead). After air drying, the dishes with the virus film were exposed to the tested irradiation source.

3. 5. Recovery of virus after treatment

After exposure of virus treatment to UV irradiation (2000 fold LC_{90} PIB's), the polyhedra deposits in the Petri-dish were re-suspended in 10 ml distilled water with 2.5% Teepol and standardized for use in bioassay tests.

3. 6. Bioassay

Diet incorporation bioassay technique was modified as two ml of collected PIB's suspension were applied on the surface of 50 ml semi-artificial diet, control; treated only with distilled water, was used for comparison. Neonate test larvae, of each treatment, were allowed to feed on the treated diet surface till pupation. Mortality caused by non irradiated virus treatments (in distilled water) was compared with those caused by irradiated ones (virus in distilled water or virus + additive) at different times after exposure to irradiation and up to 5hr post application (Fritsch and Huber, 1985). Standardization was based on the number of polyhedral inclusion bodies (PIB's)/ml of aqueous suspension. Treated insects were laboratory maintained at 25± 2 ºC and 65± 5 R. H.

2.7.Spectrophotometer test

The absorption of 1% concentration of the 4 tested plant-derived materials was measured using Spectrophotometer (Lambda EZ201). The UV absorbance under 300 nm of *SpliMNPV* at LC_{90} + Additive at 1%, before and after treatment with 10 hours of simulated UV irradiation.

2.8. Statistical analysis

Concentration-mortality regressions were calculated to determine the effectiveness of tested material as UV protective additives for the *SpliMNPV*. Slope and LC_{50} values were calculated according to the method described by Finney (1971).

RESULTS AND DISCUSSION

In the case of using SpliMNPV alone treatment, the recorded rates of mortality among S. littoralis neonate larvae after exposure periods of 0.5, 1, 2, 3, 4 and 5 hours to simulated sun light conditions were 60.00, 50.00, 20.54, 16.89, 15.75 and 15.06 %, respectively, compared to 97.88 % in case of un-irradiated virus (the calculated LIT_{50} was only hours) (Table 1, Fig 1).

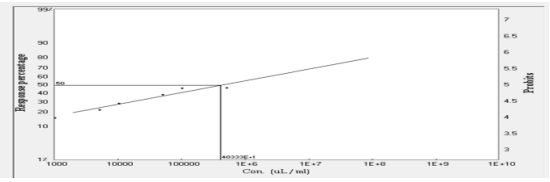
The screening of four new additive materials was carried out in three successive experimental steps. In the first step 4 plant-derived materials were tested with 1% concentrations. Then all materials were further tested together in step two with 5% concentrations finally all materials were further tested together in step three with 10% concentrations. The results of the present study indicated that, the estimated half life value of SpliMNPV deposits was 24.92 min tested in Petri dishes under simulated UV irradiation (SUV) and 1.0 day on cotton foliage under natural UV in sunlight.

Table 1. Average of mortality rate in virus activity among S. littoralis neonate larvae treated with SpliMNPV either alone or in combination with moringa, rice bran, cacao and green tea 1% concentration, both exposed to different UV irradiation periods.

Irradiation period(hours)	Mortality % among larvae tested with				
	SpliMNPV alone M%	SpliMNPV + moringa M%	rice bran M%	cacao M%	green tea M%
Zero time	97.88 (139/142)**	98.00 (147/150)	100.00 (150/150)	97.33 (146/150)	97.98 (146/149)
0.5	60.00 (90/150)	100.00 (149/149)	100.00 (146/146)	99.31 (146/147)	98.64 (146/148)
1	50.00 (74/148)	97.65 (144/148)	96.62 (143/148)	94.63 (141/149)	87.33 (131/150)
2	20.54 (30/146)	95.33 (143/150)	93.33 (140/150)	92.56 (137/148)	80.00 (120/150)
3	16.89 (25/148)	95.89 (140/146)	93.33 (140/150)	94.63 (141/149)	67.33 (101/150)
4	15.75 (23/146)	93.91 (139/148)	90.47 (133/147)	92.66 (141/149)	67.11 (100/149)
5	15.06 (22/146)	93.24 (138/148)	91.66 (132/144)	90.54 (134/148)	66.43 (97/146)
Control*	0.00 (0/148)	0.00 (0/148)	0.00 (0/150)	00 (0/146)	0.00 (0/149)

**Between brackets are the no. of virus-dead larvae / total no. tested. * Refers to either distilled water or additives alone at 1% M% = Mortality percentage.

Previous studies (El Salamouny et al., 2000, Tamez- Guerra et al., 2000 & Khattab, 2003) demonstrated that, baculoviruses were rapidly inactivated after exposure to SUV or natural sunlight, under natural field conditions. Also, under UV in sunlight, purified virus suspension was less effective than the crude extract as the latter contains coloring material (Elnagar and Abul-Nasr, 1980)



NPV conc. (PIB's*/ml)	No. of infected larvae / No. tested **	Mortality (%)
LC90	9.83 x 10^5 PIB's	
Slope	1.026	

Figure 1. Regression line of concentration-mortality response of S. littoralis neonate larvae towards S. littoralis NPV (SpliMNPV) using surface contaminated diet bioassay technique.

The first record of the use of plant extracts to increase the persistence of insect viruses was by (Shapiro et al. 2007a, b). Both of the green tea and black tea were reported to be UV protective additive to the beet armyworm nucleopolyhedrovirus (Shapiro, 2008 and El Salamouny et al., 2009). This effect could be due to the antioxidants present in tea. Also, mango leaf extract provided a protection effect to a baculovirus from the ultraviolet light (Deotale et al., 2007), and cacao, green tea, green cabbage and red cabbage (El-Helaly et al., 2011, 2013).

Table 2. Average of mortality rate in virus activity among S. littoralis neonate larvae treated with SpliMNPV either alone or in combination with moringa, rice bran, cacao and green tea 5% concentration, both exposed to different UV irradiation periods.

Irradiation period(hours)	Mortality % among larvae tested with				
	SpliMNPV alone M%	SpliMNPV + moringa M%	rice bran M%	cacao M%	green tea coffee M%
Zero time	98.66 (148/150)	97.33 (146/150)	100.00 (150/150)	100.00 (150/150)	100.00 (150/150)
1	53.33 (80/150)	100.00 (150/150)	99.33 (149/150)	100.00 (150/150)	87.33 (131/150)
2	33.33 (50/150)	99.33 (149/150)	95.91 (141/147)	100.00 (150/150)	88.00 (132/150)
3	19.72 (29/147)	96.00 (144/150)	92.66 (138/150)	96.66 (145/150)	81.33 (129/150)
4	20.00 (30/150)	95.33 (143/150)	92.00 (138/150)	93.33 (140/150)	80.00 (120/150)
5	13.69 (20/146)	97.97 (145/148)	92.66 (139/150)	90.00 (135/150)	73.33 (110/150)
Control*	0.00 (0/150)	0.00 (0/149)	0.00 (0/149)	0.00 (0/149)	0.00 (0/149)

**Between brackets are the no. of virus-dead larvae / total no. tested. * Refers to either distilled water or additives alone at 1% M% = Mortality percentage

However, the obtained results in present work showed that moringa and rice bran were the best UV protective to SpliMNPV when compared with cacao and green tea and all of previous candidates gave marginal difference in comparison with virus alone treatment. In conclusion, moringa showed the highest protection rate at all levels (1%, 5% and 10% concentrations of treated additives) Tables 1, 2 and 3 followed by rice bran and cacao under artificial UV while green tea toke the last level of virus protection, this can be also as it is discussed in literature were mentioned above due to the high antioxidants contents in moringa, according to Hong et al, 1996; Mahajan and Sharma 2004 and Nautiyal and Venkataraman 2005. There are over 46 antioxidants and 36 anti-inflammatory compounds all naturally occurring in the Moringa plant. Vitamin A, Vitamin C, Vitamin E, Vitamin K, Vitamin B (Choline), Vitamin B1 (Thiamin),Vitamin B2 (Riboflavin), Vitamin B3 (Niacin), Vitamin B6, Alanine, Alpha-Carotene, Arginine, Beta-Carotene, Beta-sitosterol, Caffeoylquinic Acid, Campesterol, Carotenoids, Chlorophyll, Chromium, Delta-5-Avenasterol, Delta-7-Avenasterol, Glutathione, Histidine, Indole Acetic Acid, Indoleacetonitrile, Kaempferal, Leucine, Lutein, Methionine, Myristic-Acid, Palmitic-Acid, Prolamine, Proline, Quercetin, Rutin, Selenium, Threonine, Tryptophan, Xanthins, Xanthophyll, Zeatin, Zeaxanthin, Zinc, besides by digging Moringa leaves into the soil before planting, damping off disease (Pythium debaryanum) can be prevented among seedlings. Cacao toke place as a good protectant under both laboratory and field conditions, (El-helaly et al 2009 and 2013). This work is the first record to use moringa in Baculovirus protection. Moringa plant in comparison with cacao higher in its antioxidant contents and Nautiyal and Venkataraman 2005, which prove the theory of the capability of plants containing antioxidant to protect the virus from the UV radiation but with lack in mechanism, and this part we did not study in this investigation. Our second candidate was rice bran. Rice bran shows strong antioxidant activities in various food systems (Nanua et al., 2000; Kim and Godber 2001) besides the high antioxidant capacity (Gerhardt and Gallo1998; Bramley et al., 2000; Cicero and Gaddi 2001; Jariwalla 2001). The high antioxidant capacity of light brown rice bran is mainly attributed to its lipophilic antioxidants, which include γ –oryzanol, tocopherols, and tocotrienols (Quereshi, 1997; Cicero and Gaddi 2001; Oki, 2002). These lipid-soluble antioxidants consist of a phenolic compound with hydroxyl groups,which are responsible for antioxidant activity and a hydrocarbon side chain or phytosterol, which provides these compounds with hydrophobic characteristics. Oki, (2002) indicate that the antioxidant capacity measured by oxygen radical absorbance capacity (ORAC) value in lipophilic fraction of the rice bran is significantly greater than that in its hydrophilic fraction. However, water soluble antioxidants was used in this investigation not the lipid and rice bran showed the second highest protection rate after Moringa additive all the way experiment at all levels (1%, 5% and 10% concentrations of treated additives) Tables 1, 2 and 3.

Table 3. Average of mortality rate in virus activity among S. littoralis neonate larvae treated with SpliMNPV either alone or in combination with moringa, rice bran, cacao and green tea 10% concentration, both exposed to different UV irradiation periods.

Irradiation period(hours)	Mortality % among larvae tested with				
	SpliMNPV alone M%	SpliMNPV + Moringa M%	Rice bran M%	Cacao M%	Green tea coffee M%
Zero time	98.66 (148/150)	98.00 (147/150)	96.66 (148/150)	100.00 (150/150)	100.00 (150/150)
1	53.33 (80/150)	100.00 (150/150)	89.79 (132/147)	100.00 (147/147)	96.66 (145/150)
2	33.33 (50/150)	100.00 (150/150)	93.28 (139/149)	100.00 (150/150)	93.33 (140/150)
3	19.72 (29/147)	99.33 (149/150)	94.00 (141/150)	99.33 (149/150)	93.19 (137/147)
4	20.00 (30/150)	98.62 (147/149)	99.33 (149/150)	93.33 (140/150)	88.43 (130/147)
5	13.69 (20/146)	97.95 (144/147)	94.59 (140/148)	93.33 (140/150)	80.00 (120/150)
Control*	0.00 (0/150)	0.00 (0/150)	0.00 (0/150)	0.00 (0/149)	0.00 (0/150)

**Between brackets are the no. of virus-dead larvae / total no. tested. * Refers to either distilled water or additives alone at 1% M% = Mortality percentage

Spectrophotometer measure of UV absorption level for the tested additives at tested concentration.

The mode of action of UV-protective additives is measured by its efficiency in absorbance of the ultraviolet light; UV-B region, 280-320 nm, UV-A region, 320-400 nm or both of them (Shapiro, 1989). The success of additive substances was thought to be due to its good absorption in the ultraviolet UV-B as well as UV-A (Shapiro, 1985).

Table 4. The UV absorbance under 300 nm of SpliMNPV + additives (moringa, cacao, rice bran and green tea) before and after 10 hours of simulated UV lamps.

	The UV absorbance under 300 nm of SpliMNPV LC90 + Additive	The UV absorbance under 300 nm of SpliMNPV LC_{90} + Additive after treatment with 10 hours of simulated UV lamps
moringa	3.6	3.4
cacao	2.9	2.6
rice bran	2.7	2.2
green tea	3.4	2.8

The test was conducted for the mixtures of baculovirus PIB's and additives at 1% concentration. The absorbance was detected at 300 nm, as the range 280-320 is the most destructive wave length recorded in literature (UV-B), therefore, the comparison was based on the median between 280 nm and the highest 320 nm wave length before and 10 hr post application. The suspension absorbance at 300nm showed narrow differences with moringa followed by cacao, rice bran and green tea respectively

Finally, moringa was the best candidate with a marginal difference from the second best followed by (Rice bran, cacao and green tea). Future studies should be done in order to evaluate the higher concentrations of moringa and rice bran from virus protection/ economically point of view besides studying their capability of protection under field sunny conditions and the role of protection. This investigation recommends Moringa (at 10% concentration).

REFERENCES

Abul-Nasr S. 1956. Polyhedrosis-virus disease on cotton leaf worm, Prodenia litura F. Bull. Entomol. Soc. Egypt, Econ, Ser.,40:321-332.,

Alford DV. 2007. Pests of fruit crops. Academic Press, Boston.

Anon. 2009. CAPS surveys for 2009. Ohio Department of Agriculture.(http://www.agri.ohio.gov/divs/plant/caps/docs/CAPS_Surveys_2009.pdf), application of nuclearpolyhedrosis virus with adjuvants and UV

Arivudainambi S, Selvanarayanan V and Vikash A. 2000. Enhancing the efficacy and persistency of Spodoptera litura (Fab.) nuclear polyhedrosis virus using UV irradiation protectants. Indian Journal of Experimental Biology. 38 (11): 1175-1176.

Arivudanainambi S, Selanarayanon V and Vikash A. 2000. Enhancing the efficacy and persistency of Spodoptera litura (Fab.) nuclear polyhedrosis virus using UV irradiation protectants. Indian Journal of Experimental Biology. 38 (11):1175-1176.

Baskaran RKM. 2001. In vivo screening of optical brighteners as UV protectants and their efficacy in enhancing the virulence of Maduri isolate of NPV of Amascata albistriga (Walker).Lepidoptera: Arctiidae. Journal of Biological Control.2 (1):169-174.

Baskaran RKM. 2007. In vivo screening of optical brighteners as UV protectants and their efficacy in enhancing the virulence of Madurai isolate of NPV of Amsacta albistriga (Walker) (Lepidoptera: Arctiidae). Journal of Biological Control. 21(1): 169-174.

Bramley PM, Elmadfa I, Kafatos A, Kelly FJ, Manios Y, Roxborough HE, Schuch W, Sheehy PJA and Wagner KH. 2000. Review: vitamin E. J Sci Food Agric 80:913–38.

CABI/EPPO. 1997. Quarantine pests for Europe, 2nd Ed. CAB International. Wallingford, UK

Cicero AFG and Gaddi A. 2001. Rice bran oil and γ -oryzanol in the treatment of hyperlipoproteinemias and other conditions. Phytother Res 15:277–89.

Control 3, 37–39.

DEFRA. 1999. Spodoptera species, PB 4641(a),1999. Department for Environment, Food and Rural Affairs.Central Science Laboratory. www.defra.gov.uk/planth/pestnote/spod.htm

Deotale RO, Dawane PN, Biswane KD and Borker SL. 2007. Effectiveness of UV protectants on the activity of NPV against Helicoverpa armigera (Hubner) on chickpea. Journal of Entomology Research. 31(1): 33-35.

Deotale RO, Dawane PN, Underwide DB, Deshmukh IB and Bisane KD. 2003. Performance of UV protectants for the effectivness of HaNPV against Helicoverpa armigera on chickpea. Journal of Soils and Crop. 13(1): 154-157.

El Salamouny S, Herz A and Huber J. 2002. Suitability of three lignin products as UVprotectantsto baculovirus.TTBulletinEntomological Society Egypt, Economic Series28, 103-111.

El Salamouny S, Shapiro M, Ling KS and Shepard BM. 2009a. Black tea and lignin as Ultraviolet protectants for the beet armyworm nucleopolyhedrovirus.

El Salamouny S, Ranwala D, Shapiro M, Shepard M and Farrar R. 2009b. Tea, coffee, and cocoa as ultraviolet radiation protectants for the beet armyworm nucleopolyhedrovirus. Journal of Economic Entomology. 102(5) :33-38

El Salamouny S, El-Sheikh MAK, Elnagar S and Huber J. 2000. Prolongation of the UV persistence of nucleopolyhedroviruses by the lignin derived product. 33rd Annual Meeting Society for Invertebrate Pathology. Guanajuats Mexico University of Guanajuata, 13-18 Aug., pp.39

El Salamouny S, Shapiro M, Ling KS and Shepard BM. 2009. Black tea and lignin as ultraviolet protectants for the beet army worm (Lepidoptera:Noctuidae) nucleopolyhedrovirus. Journal of Entomological Science. 44:50-58.

El-Helaly, El-Salamouny AS, Khattab M, El-Sheikh M and Elnagar S. 2009. Preliminary evaluation of natural antioxidants as UV- protectants of Spodoptera littoralis nucleopolyhedrovirus, (Baculoviridae). 4th conference on recent technologies in agriculture" challange of agriculture modernization" Cairo university, Faculty of Agriculture.1:7-13.

El-Helaly, Khattab M, El-Salamouny AS, El-Sheikh M and Elnagar S. 2013. P romising additives to protect the activity of Baculovirus biocontrol agent under field – sunlight conditions in Egypt.Journal of Life Sciences 7: 495-500.

Ellis SE. 2004. New Pest Response Guidelines: Noctuidae: Spodoptera worm Handbook, second ed. Natural Resources Institute,USDA APHIS PPQ PDMP.(http://www.aphis.usda.gov/ppq/manuals/)

Ellis SE. 2004. New Pest Response Guidelines: Spodoptera. USDA/APHIS/PPQ/PDMP. (http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/nprg_*spodoptera*.pdf).

Elnagar S, El-Sheikh MAK, El- Salamouny S, Amin A and Khattab M. 2003. Screening of four lignin products as UVprotectantsto baculovirus.TTBulletinEntomological Society Egypt, EconomicSeriesTT. 29:165-178.El

Elnagar S and Abul-Nasr S. 1980. Effect of direct sunlight on the virulence of NPV (nuclear polyhedrosis virus) of the cotton leaf worm Spodoptera littoralis (Bosid.). Z. Angew. Entomology. 90:75-80.

Espinosa A and Hodges C. 2009. *Spodoptera littoralis*. (http://wiki.bugwood.org/Spodoptera_littoralis)

Finney DJ. 1971. Probit analysis. 3rd ed., Cambridge Univ., Cambridge U.K.

Gerhardt AL and Gallo NB. 1998. Full-fat rice bran and oat bran similarly reduce hypercholesterolemia in humans. J Nutr 128:865–9.

Hong TD, Linington S and Ellis RH. 1996. Seed storage behaviour: a compendium. Handbooks for Genebanks: No. 4.

Huber J and Ludcke C. 1996. UV-inactivation of baculoviruses: the bisegmented survival curve. *Bulletin OILB/SROP*. Abstract. 19(9): 253-256.

Ignoffo CM, Batzer OF. 1971. Microencapsulation and ultraviolet protectants to increase sunlight stability of an insect virus. Journal of Economic Entomology. 1971;64:850–853.

importance of the African Armyworm. Desert Locust Control

Jacques RP. 1977. Stability of entomopathogeneic viruses. Miscell publication of the Entomological Society of America.10 (3): 99-116.

Jariwalla RJ. 2001. Rice-bran products: Phytonutrients with potential applications inpreventive and clinicalmedicine. Drugs Exp Clin Res 27:17–26.

Jones KA, Moawad G, McKinley DJ and Grzywacz D. 1993. The effect of natural sunlight on Spodoptera littoralis nuclear polyhedrosis virus. Biocontrol Science and Technology. 3(2): 189-197.

Kamaldeep S and Battu GS. 2008. Residual persistence of the HearNPV against Helicoverpa armigera (Hubner) on tomato as affected by two selected optical brighteners. Indian Journal of Entomology. 10(1):26-30.

Kim JS and Godber JS. 2001. Oxidative stability and vitamin E levels increased in restructured beef roast with added rice bran oil. J Food Qual 24:17–26.

Mahajan S and Sharma YK. 2004. Production of rayon grade pulp from Moringa oleifera. Indian Forester. 110(3): 303-306.

Moscardi F. 1999. Assessment of the application of baculoviruses for control of lepidoptera. Annual Review of Entomology. 1999;14:257–289.

Muro EM and Paul JI. 1985. Laboratory evaluation of new ultraviolet absorbers for protection of Douglas-fir tussock moth (Lepidoptera: Lymantriidae) baculovirus. Journal of Economic Entomology. 78. 951-957.

Nanua JN, McGregor JU and Godber JS. 2000. Influence of high oryzanol rice bran oil on the oxidative stability of wholemilk powder. J Dairy Sci 83:2426–31.

NAPIS. 2008. Reported status of Egyptian cotton worm—*Spodoptera littoralis*. Pest tracker, National Agricultural Pest Information System. (http://pest.ceris.purdue.edu/searchmap.php?selectName=ITBCFPA)

Nautiyal BP and Venkataraman KG. 2005. Moringa (Drumstick) - An ideal tree for social forestry; growing conditions and uses - part I: Myoforest. 23(1):53-58.

Oki T, Masuda M, Kobayashi M, Nishiba Y, Furuta S and Suda I. 2002. Polymeric procyanidins as radical-scavenging components in red-hulled rice. J Agric Food Chem 50:7524–9.Passoa, S. (2008). Field key to the larvae of *Spodoptera* of America north of Mexico.

Quereshi AA, Bradlow BA, Salser WA, Brace LD. 1997. Novel to cotrienols of rice bran modulate cardiovascular disease risk parameters of hypocholesterolemic humans. Toxicol Ind Health 9:93–122

Rabindra RJ, Muthuswami M, Jayaraj S. 1989. Controlled droplet Organisation East Africa Technical Report 100, 46pp.

Ramakrichnan N and Chaudhary S. 1991. Effectiveness of some ultraviolet light protectants on the activity of nuclear polyhedrosis virus of Spodoptera litura (Fabricius). Indian Journal of Entomology. 53(4): 548-551.

Ramakrishnan N and Chaudhary S. 1991. Effectiveness of some ultra violet light protectants on the activity of nuclear polyhedrosis virus of Spodoptera litura (Fabricius). Indian Journal of Entomology. 53 (4): 548-551.

Reddy AR and Divakar BJ. 2001. Effect of Rubin blue on the efficiency of nuclear polyhedrosis for management of *Helicoverpa armigera* (Hub) in ground nut crop. National perspectives in biotechnology Proceedings of a national symposium wrangler, India.(26): 41-43.

Reynolds S. 2001. From darkness into light. New Agriculturalist 23, 5

Rose DJW, Dewhurst CF, Page WW. 2000. The African Army-

Scott PJ. 1991. A general review of the evidence on the economic

Shapiro M, Shepard BM and Lopez R. 2007. Effect of medicinal herbs upon the biological activity of the gypsy moth nucleoplyhedrosis virus. Biocontrol Science and Technology. 18:605-617.

Shapiro M, Vaughn J. 1995. Enhancement in activity of homologous and heterologous baculoviruses infectious to cotton bollworm (Lepidoptera: Noctuidae) by an optical brightener. Journal of Economic Entomology. 88(2):265–269.

Shapiro M. 1989. Congo red as an ultraviolet protectant for the gypsy moth (Lipidoptera: Lymantriidae) nuclear polyhedrosis virus. Journal of economic Entomology.82(2):548-550.

Shapiro M, El Salamouny S and Shepard BM. 2008. Green tea extracts as ultraviolet protectants for the beet armyworm, Spodoptera exigua nucleopolyhedrovirus. Journal of Biocontrol Science and Technology. 18(6): 591-603.

Shapiro M, Shepard BM and Lopez R. 2007. Effect of species upon activity of gypsy moth (Lepidoptera;Lymantriidae). Journal of Entomological Science. 42:82-91.

Shapiro M, El Salamouny S and Shepard BM. 2008. Green tea extracts as ultraviolet protectants for the beet armyworm, Spodoptera exigua nucleopolyhedrovirus. Biocontrol Science and Technology., 18 (6): 591-603.

Shorey H and Hale RL. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. Journal of Economic Entomology., 58:522-524.

Tamez-Guerra P, McGuire M, Behle RW, Hamm JJ, Sumner HS and Shasha BS. 2000. Sunlight persistence and rain fastness of spray-dried formulations of baculovirus isolate from Anagrapha falcifera (Lepidoptera Noctuidae). Journal of Economic Entomology. 93(2): 210-218.

Villamizar L, Espinel C and Cotes AM. 2009. Effect of ultraviolet radiation on the insecticidal activity of Spodoptera frugiperda (Lepidoptera: Noctuidae) nucleopolyhedrovirus. Journal Revista colombiana de Entomologic. 35:2, 116-121