Applied Science Reports
www.pscipub.com/ASR
E-ISSN: 2310-9440 / P-ISSN: 2311-0139

App. Sci. Report.
4 (1), 2013: 187-191

Additives For A Baculovirus Against Ultraviolet Effect

A. El-Helaly

Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.
Corresponding author: A. El-Helaly

Key words

Antioxidants

Baculovirus activity

Spodoptera littoralis NPV

virus protection

A B S T R A C T

The addition of moringa, rice bran filtrates (1%) to the nucleopolyhedrovirus (*Spli*MNPV) 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 5hr post application.The mixtures of baculovirus PIB's and additives were measured with spectrophotometer under 400 nm length before and 10 hr post application. The suspension absorbance at 400nm showed narrow differences with moringa followed by cacco, rice bran and green tea respectively. These findings indicate that theses plant extracts could be promising UV protective additives for *Spli*NPV and they should be further investigated in the field large scale to obtain the best formulation for the control of agriculture important insect pest.

Introduction

In Egypt, several lepidopterous insects are important pests attacking agricultural crops. Insect viruses, as biocontrol agents, may play an important role in pest management, thus reducing dependency on chemical insecticides. Baculoviruses (NPV's and GV's) are considered promising microbial agents for the biological control of lepidopterous pests. These naturally occurring viruses are proved safe due to their specificity. Studies abroad and in Egypt determined the effectiveness of baculoviruses against agriculture pests. Currently, baculoviruses are produced commercially, however, their use as biological control agents is hampered by their susceptibility to inactivation by ultraviolet (UV) in sun-light which is a major limitation for field application. Loss of infectivity is the most striking result of the action of UV irradiation on virions. The loss of infectivity of nuclear polyhedrosis virus and granulosis virus (Baculovirus sub groups A and B) by exposure to the UV portion of sunlight is well documented. The medium-wave or erythemal UV portion of the sun's radiation (UV-B, 28-320 nm) is the most important factor contributing to the photo inactivation of baculoviruses. Inactivation occurs also in the near UV region of the solar spectrum (UV-A 320-360 nm), but comparatively greater energy doses are needed as the wave length increases. A number of natural UV absorbers provided UV protection to the virus product; such as Dyes, Fluorescent brightener or lignin derivates. Antioxidant or oxidative enzyme such as Dilodin, Inol, Vitamins, Folic acid, Riboflavin, and Pyridoxine are all promising compounds for the protection of entomopathogenic viruses from UV-rays. Recently, natural material containing antioxidant such as green tea, Black tea, eucalyptus and mango leaf extracts have became promising protective additives to baculoviruses, and therefore, present investigation focuses on finding inexpensive, local and natural products containing antioxidants to be used as standard protective additives to baculoviruses products used in field application, in order to sustain effectiveness of virus bio-control agents. The study is designed to recruit best additive materials.

Material And Methods

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).

Virus inoculum

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

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 *et al.*, 2008)**.**

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.

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 resuspended in 10 ml distilled water with 2.5% Teepol and standerdized for use in bioassay tests.

Bioassay

Diet incorporation bioassay technique was modified as two mls of collected PIB's suspension were applied on the surface of 50 ml semi-artificial diet. An un-treated control treated, with only 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.

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 *Spli* NPV at LC_{90} + Additive at 1%, before and after treatment with 10 hours of simulated UV lamps.

Statistical analysis

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

Results And Discussion

In the case of using *Spli*NPV 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).

UV-Protection of *Spli*NPV **using plant-derived additives containing natural antioxidants.**

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 *Spli*NPV 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.

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 colouring material (Elnagar and Abul-Nasr, 1980).

Table 1. Average of mortality rate in virus activity among *S. littoralis* neonate larvae treated with *Spli* NPV either alone or in combination with Moringa, Rice bran, Cacco and Green tea 1% concentration, both exposed to different UV irradiation periods.

Irradiation period(hours)	Mortality % among larvae tested with				
		*Spli*NPV +			
	*Spli*NPV alone	Moringa	Rice bran	Cacco	Green coffee coffee
	M%	M%	M%	M%	M%
Zero time	98.66	97.33	100.00	100.00	100.00
	(148.150)	(146.150)	(150.150)	(150.150)	(150.150)
1	53.33	100.00	99.33	100.00	87.33
	(80.150)	(150.150)	(149.150)	(150.150)	(131.150)
2	33.33	99.33	95.91	100.00	88.00
	(50.150)	(149.150)	(141.147)	(150.150)	(132.150)
3	19.72	96.00	92.66	96.66	81.33
	(29.147)	(144.150)	(138.150)	(145.150)	(129.150)
4	20.00	95.33	92.00	93.33	80.00
	(30.150)	(143.150)	(138.150)	(140.150)	(120.150)
5	13.69	97.97	92.66	90.00	73.33
	(20.146)	(145.148)	(139.150)	(135.150)	(110.150)
Control*	0.00	0.00	0.00	0.00	0.00
	(0.150)	(0.149)	(0.149)	(0.149)	(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.**

Table 2. Average of mortality rate in virus activity among *S. littoralis* neonate larvae treated with *Spli* NPV either alone or in combination with Moringa, Rice bran, Cacco and Green tea 5% concentration, both exposed to different UV irradiation periods.

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

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 *et al*., 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). However, the obtained results in present work showed that moringa and rice bran were the best UV protective to *SpLi*MNPV. In conclusion, moringa showed the highest protection rate followed by rice bran and cacco under artificial UV while green coffee toke the last level of virus protedtion, tis can be 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. Cacco toke place as a good protectant under both laboratory and field conditions, (El-helaly *et al* 2009 and 2011). This work is the first record to use moringa in Baculovirus protection. Moringa plant in comparison with cacco 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 which we did not study in this investigation. Our second candidate was rice bran. Rice bran shows strong antioxidant activities in various food systems (Nanua and others 2000; Kim and Godber 2001) besides the high antioxidant capacity (Gerhardt and Gallo1998; Bramley and others 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 and others 1997; Cicero and Gaddi 2001; Oki and others 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 and others (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.

Table 3. Average of mortality rate in virus activity among *S. littoralis* neonate larvae treated with *Spli* NPV either alone or in combination with Moringa, Rice bran, Cacco and Green tea 10% concentration, both exposed to different UV irradiation periods.

Irradiation period(hours)	Mortality % among larvae tested with				
	*Spli*NPV alone	*Spli*NPV +			
		Moringa	Rice bran	Cacco	Green coffee coffee
	M%	M%	M%	M%	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 Spli NPV + additives (moringa, cacco, rice bran and green coffee) before and after 10 hours of simulated UV lamps.

The test was conducted for the mixtures of baculovirus PIB's and additives at 1% concentration. The absorbance was

	The UV absorbance under 300 nm of *Spli* NPV LC_{90} + Additive	The UV absorbance under 300 nm of *Spli* NPV LC_{90} + Additive after treatment with 10 hours of simulated UV lamps
Moringa	3.6	3.4
Cacco	2.9	2.6
Rice bran	2.7	2.2
Green coffee	3.4	2.8

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 cacco, rice bran and green coffee respectively

Finally, moringa was the best candidate with a marginal difference from the second best followed by (Rice bran, cacao and green coffee). 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*.

Arivudainambi S, Selvanarayanan V, 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. 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, Wagner KH. 2000. Review: vitamin E. J Sci Food Agric 80:913–38.

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

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

Elnagar S, 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.*

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

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

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

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

Kim JS, 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, Sharma YK, 2004. Production of rayon grade pulp from *Moringa oleifera.* Indian Forester. *110(3): 303-306.*

Muro EM, 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, Godber JS. 2000. Influence of high oryzanol rice bran oil on the oxidative stability of wholemilk powder. J Dairy Sci 83:2426–31.

Nautiyal BP, 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, Kobayashi M , Nishiba Y, Furuta S, Suda I. 2002. Polymeric procyanidins as radical-scavenging components in red-hulled rice. J Agric Food Chem 50:7524–9.

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

Ramakrishnan N, 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.*

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

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

Shapiro M, El Salamouny S, 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, Hale RL. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. *Journal of Economic Entomology., 58:522-524.*

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