

Black Tea and Lignin as Ultraviolet Protectants for the Beet Armyworm Nucleopolyhedrovirus¹

S. El Salamouny,² M. Shapiro, K. S. Ling,³ and B. M. Shepard⁴

Coastal Research and Education Center, Clemson University, Charleston, South Carolina 29414 USA

J. Entomol. Sci. 44(1): 50-58 (January 2009)

Abstract A major constraint to the use of baculoviruses for biocontrol of insects is their sensitivity to UV degradation. In this study, we evaluated black tea (Lipton®, London, UK) and lignin (Reax 85A™, MeadWestvaco, Charleston, SC) as potential UV protectants for beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) multiple-embedded nucleopolyhedrovirus (SeMNPV). The original activity remaining (OAR%) from SeMNPV upon exposure to various lengths of time (up to 5 h) to a source of UVA and UVB was evaluated in bioassays using beet armyworm third-stage larvae under laboratory conditions. Beet armyworm mortality was measured after larvae fed on artificial diet treated with SeMNPV. Mortality of beet armyworm due to SeMNPV, with no UV protectants added, was reduced to 23, 11.3 and 2.1% upon UV exposure for 15, 30 or 60 min, respectively. To investigate the mechanism of reduction in the efficacy of SeMNPV when exposed to UV was due to the degradation of full-length viral genomic DNA, a modified DNA isolation technique was developed to measure levels of the full length viral genomic DNA of SeMNPV through electrophoresis on an agarose gel. The efficacy of SeMNPV on beet armyworm was lost after 2 h of UV exposure, and the full-length genomic DNA also was degraded to levels that were not visible on agarose gel. However, both black tea and lignin provided nearly 100% UV protection for SeMNPV as measured in bioassays even after 5 h of UV irradiation. SeMNPV efficacy against beet armyworm in samples containing black tea or lignin resulted in no significant visual reduction of the full length viral genomic DNA. To investigate the mechanism of UV protection for SeMNPV from black tea and lignin, absorption spectra of both protectants were measured with a spectrophotometer. High rate of absorption in the UV range, especially at the range of UVB (280-320nm), was detected for both materials. The absorption rate was higher with lignin than with black tea. Whereas lignin was a good absorber for both UVB and UVA radiation, black tea was primarily an absorber of UVB. Therefore, both black tea and lignin are potential natural UV protectants in the formulation of baculovirus-based biopesticides.

Key Words nucleopolyhedrovirus, *Spodoptera exigua*, ultraviolet light, black tea, lignin, protectants

Baculoviruses are considered promising microbial agents for biological control of insect pests due to their specificity and safety to nontarget organisms (Miller 1997). Field and laboratory studies, however, have shown that natural sunlight is one of the most significant environmental factors affecting the stability of baculoviruses (David

¹Received 04 January 2008; accepted for publication 30 June 2008.

²Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt.

³U. S. Department of Agriculture, Agricultural Research Service, U. S. Vegetable Laboratory, Charleston, SC 29414.

⁴Address inquires (email: mshprd@clemson.edu).

1969, Krieg et al. 1980, Elnagar and Abul Nasr 1980, Shapiro et al. 1983, Ignoffo et al. 1989, Ignoffo 1992, Ignoffo and Garcia 1992, Jones et al. 1993, McGuire et al. 2000) with the UV radiation (280-400 nm; UVB/UVA) being most detrimental (Bullock et al. 1970, Ignoffo et al. 1977, Griego et al. 1985, Ignoffo et al. 1997). A previous study (Ignoffo et al. 1989) showed that a significant increase in the breakdown of viral DNA and virions occluded in viral inclusion bodies (OBs) resulted from UV exposure. It was suggested that the inactivation of baculoviruses by UV from sunlight could result from UV-generated reactive oxygen species such as peroxides, single oxygen, or hydroxyl radicals (Ignoffo et al. 1989, Ignoffo and Garcia 1994). However, these reactive oxygen species could only be partially neutralized by oxidative enzymes (catalase, peroxidase and antioxidants), thus preventing deterioration of nucleopolyhedroviruses activity.

Thus, in order for baculoviruses to be effective in controlling insect pests under field conditions, they must be protected from the adverse effects of UV exposure. Although UV protectants have been developed from synthetic chemicals (such as sunscreens) to selectively absorb, block or reflect UV irradiation (Martignoni and Iwai 1985, Shapiro and Robertson 1990, Dougherty et al. 1996, Burges and Jones 1998), adverse effects of fluorescent brightener (sunscreens) on photosynthesis and pollination by sunscreen-covered plants diminish their benefits (Goulson et al. 2000, 2003). A number of natural UV absorbers, used as adjuvants in microbial products, also provided UV protection (Shapiro et al. 1983, Burges and Jones 1998). Only a few studies have focused on using natural plant products to improve virus activity (Shapiro et al. 1994). Recently, 29 medicinal herbs and 35 spices were evaluated for their effects upon activity of the gypsy moth nucleopolyhedrovirus (*LdMNPV*). Six of those species (paprika, cayenne, curry, dill, nutmeg and tarragon) and 7 herbs (dandelion, feverfew, sarsaparilla, skullcap, St. John's wort, tansy, and valerian) enhanced virus activity (Shapiro et al. 2007a, b).

However, tea, one of the oldest herbal plants in the world, has not been evaluated for enhancing or protecting biological activity of baculoviruses. It is known that black tea is a source of antioxidants (Langley-Evans 2000, Leung et al. 1991). During the past 15 yrs, tea has been shown to inhibit the effect of UVB on tumor formation in mice (Wang et al. 1994). Caffin et al. (2004) and Ding et al. (2005) reported the absorption effect of the black tea to UV light exposure. Shapiro et al. (2008) demonstrated that green tea acted as an effective UV protectant for *ScMNPV* under laboratory and field conditions. Other natural products, such as lignins, also were reported to be effective UV protectants (Martignoni and Iwai 1985, Shasha et al. 1998, Tamez-Guerra et al. 2000, McGuire et al. 2001, El Salamouny et al. 2002, El Salamouny and Huber 2004). The objectives of our study were to determine the effects of black tea (Lipton®, London, UK) and a new form of lignin (Reax85A) (MeadWestvaco, Charleston, SC) as UV protectants for the virus of beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (*SeMNPV*), and to elucidate the mechanism underlying these effects through DNA analysis and absorption spectra.

Materials and Methods

Source of larvae. Beet armyworm egg masses were supplied by Ron Meyer (USDA-ARS, Tifton, GA). Larvae were reared on a wheat germ-based multiple species semiarificial diet (Southland Products Inc., Lake Village, AR). Third-stage larvae were used in all laboratory bioassays.

Viral preparations. *Spodoptera exigua* multiple-embedded nucleopolyhedrovirus (SeMNPV), registered as SPOD-X® (Certis USA, Columbia, MD), was used for these studies. The virus was maintained and propagated in beet armyworm. Third-stage larvae were infected by feeding on surface contaminated diet [100 μ L of 2×10^5 occlusion bodies (OBs)/ml = 2×10^4 OBs/ 30ml cup (Jetplastica, Hatfield, PA)]. Virus OBs were purified using a sucrose gradient of 40-65% (w/w) (El Salamouny et al. 2003). A concentration of 2×10^5 OBs/ml was previously determined in laboratory bioassays to be the LC₉₅ of the virus in beet armyworm. However, a concentration of 1×10^6 OBs/ml was required for DNA extraction.

Preparation of solutions. Two grams of black tea or lignin product Reax 85A were blended in 100 ml of distilled water and filtered through 16 layers of muslin in a glass funnel to produce a 2% (w/w) stock aqueous solution. The filtrate was kept at 4°C until used. Although lignins have been shown to be effective UV protectants (Shasha et al. 1998, El Salamouny 1998, El Salamouny and Huber 2004), the present study evaluated a new lignin (Reax 85A) and compared it to black tea as a UV protectant for SeMNPV.

UV exposure. To determine the effect of UV on SeMNPV with or without the addition of black tea or Reax 85A, SeMNPV (2×10^5 OBs/ml) was irradiated in a Pelco™ cabinet box (Ted Pella Inc., Redding, CA) with both 15 W UVA and UVB tubes custom-mounted in parallel. Four ml of each suspension was placed in glass Petri dishes (5.5 cm diam, VWR International, GA). The irradiation source was 20 cm above the dishes, and the suspensions were irradiated for predetermined lengths of time of 0, 15, 30, 60, 120, 180, 240, and 300 min. Following the irradiation treatments, the volume in each dish was adjusted to 4 ml with de-ionized water.

Bioassay. A diet surface bioassay was used to evaluate the viral activity against beet armyworm from each treatment. An aliquot of 0.1 ml of the irradiated virus suspension was pipetted onto the surface of diet in 30-ml cups (surface area, 7 cm²) (Jetplastica, Hatfield, PA) containing 7 ml artificial diet. Ten third-stage beet armyworm larvae were used for each treatment and were maintained individually in the plastic cups until death or pupation at 26°C, 50% RH and photoperiod of 12:12 h (L:D). The test was repeated at least 3 times with 10 larvae per virus dilution per treatment per replicate. Ten untreated cups with larvae per replicate, and 10 larvae with black tea or Reax 85A without SeMNPV were used as controls.

Evaluating the viral genomic DNA after UV exposure. The DNA from virus was recovered from each individual treated sample post UV exposure using a method modified after Ignoffo et al. (1989). For each sample, OBs were harvested from 20 ml of the irradiated virus suspension (1×10^8 OBs / ml) through centrifugation (4,200 rpm, 25 min). The virus pellet was resuspended in 250 μ L of 1 molar (M) Tris buffer (pH 7.6), followed by 25 μ L of 1M Na₂CO₃ to solubilize OBs and incubated at 37°C for 60 min to release virus particles. Viral DNA was extracted from virus particles by addition of 30 μ L of 10% SDS. Purification of virus DNA was conducted using DNeasy plant kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The viral DNA was eluted from the column with a same amount (50 μ L) of sterile distilled water for each sample. It was assumed that the commercial DNA purification kit has the same efficiency in DNA purification among samples with equal numbers of initial virus OBs. Therefore, the difference in genomic DNA concentration was likely due to the UV breakdown process. To evaluate effects of black tea or lignin Reax 85A for protection of full virus genomic DNA from breakdown by sunlight UV exposure, 20 μ L aliquot of DNA preparation from each treatment was applied onto 1% agarose gel. After

electrophoresis, agarose gel was stained with 0.01% SyBr Safe (Invitrogen, Carlsbad, CA). The gel image was taken with a digital imaging system (ChemiDoc XRS, Bio-Rad, Hercules, CA).

Absorption spectra of solutions. To evaluate the efficiency of UV absorption by black tea and lignin Reax85A, UV absorption spectra ranging from 190-600 nm were measured on 1% solution of black tea or lignin using a Spectrophotometer (Molecular Devices Corporation, SPECTRA, max plus³⁸⁴, Sunnyvale, CA) following the method described by El Salamouny and Huber (2004).

Results

Effect of UVA/UVB on SeMNVP. Treating third-stage beet armyworm via surface inoculated diet with 2×10^4 OBs/cup of unirradiated SeMNVP caused 99% mortality within 10 d. A decrease in virus-caused mortality of beet armyworm occurred within the 1st hour of exposure of SeMNVP to UVA and UVB. Insect mortality was reduced to 23, 11.3 and 2.1% after 15, 30, and 60 min UV exposure, respectively (Fig. 1). Decreased virus activity was accompanied by the gradual degradation of full length SeMNVP DNA as UV exposure time increased; whereas full-length virus DNA on the gel was visible and intact in nonirradiated virus control. In both the 15 and 30 min exposure treatments, the concentration of full-length viral DNA was lower than that of the untreated control, but the full genomic DNA from these samples remained visible (Fig. 1). Full-length genomic DNA was, however, not visible from samples that received more than 2h of UV exposure (Fig. 1). The amount of the lower molecular weight DNA products, likely the degraded virus genomic DNA due to the prolong UV exposure, increased in samples with longer exposure as observed on the stained

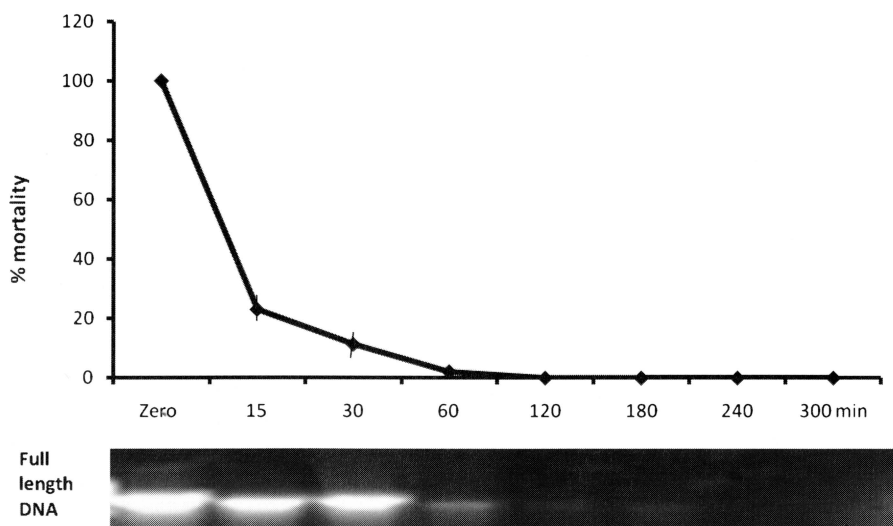


Fig. 1. Mortality of third-instar beet armyworm and the presence of full-length viral genomic DNA after UV exposure of *Spodoptera exigua* multiple-embedded nucleopolyhedrovirus (SeMNVP) without UV protectants

agarose gels (data not shown). In these latter samples, in vivo virus activity against beet armyworm larvae was also lost in bioassay (Fig. 1).

Black tea as UV protectant additive for SeMNPV. Addition of black tea extract (1%) to SeMNPV solution resulted in full protection of virus activity as determined by beet armyworm bioassay. Even with 5 h of UV exposure, virus-induced mortality remained >99% (Fig. 2). The high viral activity in the black tea-treated samples was also indicated with the presence of full-length virus genomic DNA after various length of exposure to UV. With black tea-treated samples, no significant reduction of the virus genomic DNA was observed even with 5 h of UVA/UVB irradiation (Fig. 2). Some general fluctuation in the DNA concentration from different samples (0 and 15 min) was likely due to handling variation. These results indicated that black tea was an effective UV protectant for SeMNPV.

Lignin Reax 85A as UV protectant for SeMNPV. Infection of *S. exigua* by SeMNPV occurred in lignin-treated samples exposed to UV for up to 5 h (Fig. 3). Virus-caused mortality remained at > 99% in samples even after 5 h of UV exposure. Analysis of full-length virus genomic DNA in lignin-treated samples upon different period of UV exposure again revealed no major reduction in DNA concentration (Fig. 3).

UV absorption spectra of the potential UV protectant additives. To investigate the mechanism of UV protection obtained, absorption spectra of black tea and lignin were measured using a spectrophotometer. A high rate of absorption, especially in the UV spectrum (280-320nm), was detected for both additives. UV absorption, however, was higher for lignin than for black tea. Moreover, lignin was a good absorber for both UVB and UVA radiation; whereas black tea was primarily an absorber of UVB.

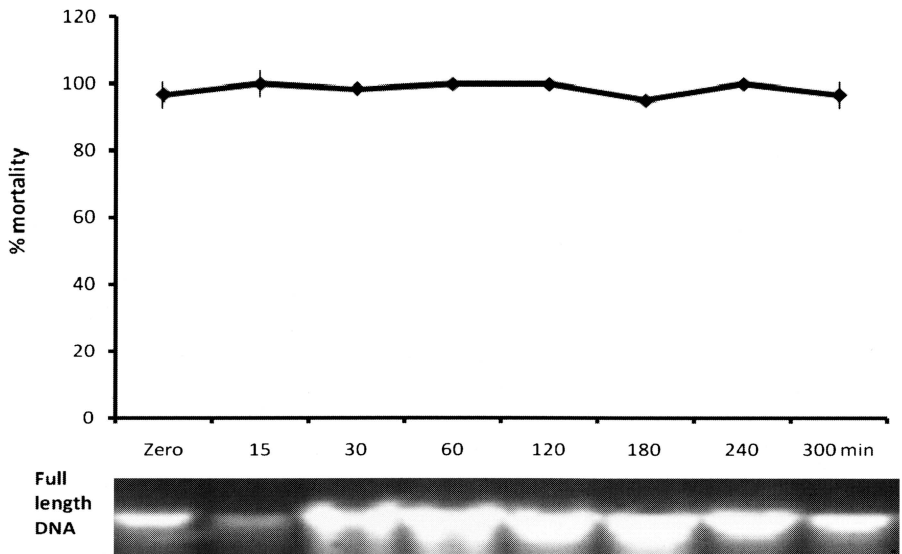


Fig. 2. Mortality of third-instar beet armyworm and the presence of full-length viral genomic DNA after UV exposure of *Spodoptera exigua* multiple-embedded nucleopolyhedrovirus (SeMNPV) with 1% black tea extract

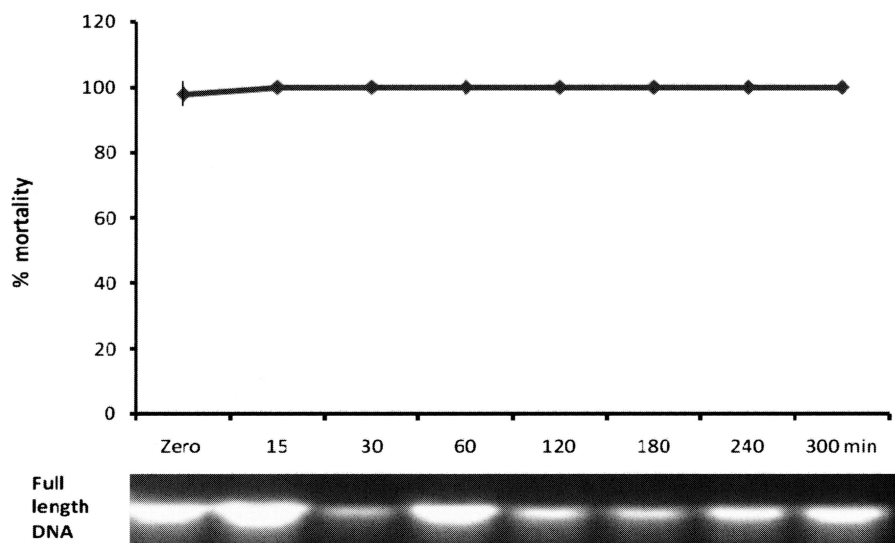


Fig. 3. Mortality of third-instar beet armyworm and the presence of full-length viral genomic DNA after UV exposure of *Spodoptera exigua* multiple-embedded nucleopolyhedrovirus (SeMNPV) with 1% lignin (Reax 85A) extract

Although the absorption for lignin remained high through the range of UV absorbance spectra, the absorption for black tea decreased rapidly in the UVA range (320-400 nm) (Fig. 4). These results suggest the UV protection was associated with UV absorption.

Discussion

Because UV radiation is the main constraint to using baculoviruses in plant protection, there is an urgent need to discover more effective UV protectants. This research focused on the use of plants or plant-derived sun screens for potential UV protectants. Our results demonstrated for the first time that black tea was an effective UV protectant for the SeMNPV.

To investigate the mechanism of UV exposure on the SeMNPV against beet armyworm, a new method was developed for DNA isolation from SeMNPV which can be used for studies to evaluate UV inactivation and UV protection of baculoviruses. The modified DNA purification method allowed a comparative evaluation of the full virus genomic DNA concentration among samples from different treatments. This improvement of DNA purification also avoided the use of harmful chemicals such as phenol, chloroform, or ethidium bromide as used previously (Ignoffo et al. 1989, Sambrook et al. 1989).

The decrease of virus activity after UV exposure was in general agreement with the results obtained in the studies of Krieg et al. (1980), Elnagar and Abul Nasr (1980), Shapiro et al. (1983), Ignoffo et al. (1989), Ignoffo (1992), Jones et al. (1993) and McGuire et al. (2000).

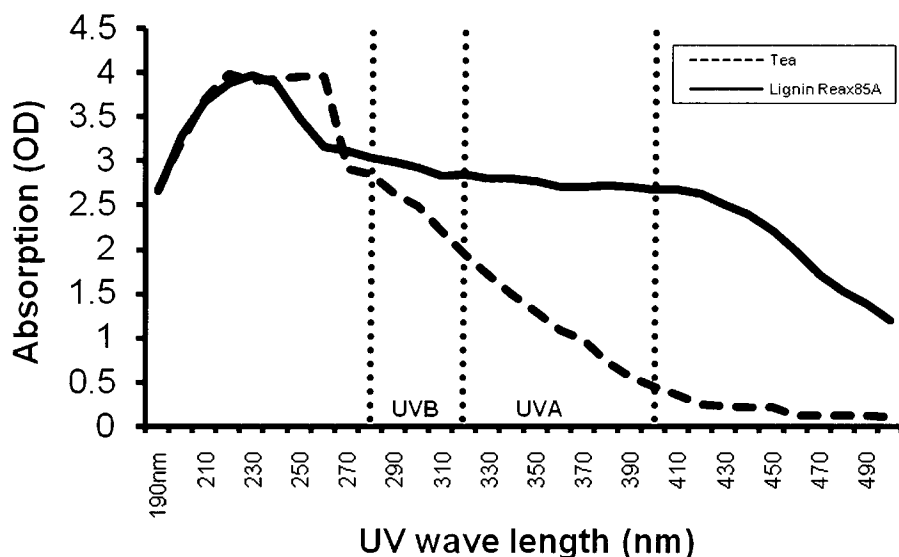


Fig. 4. Absorption spectra profiles of 1% black tea and lignin Reax85A when exposed to UV wavelengths (UVB=280-320nm, UVA=320-400nm)

This study demonstrated that UVA/UVB absorbance was important in protecting *SeMNPV* from breakdown by UV, but we recognize that antioxidants also may play an important role. Ignoffo and Garcia (1994) demonstrated that antioxidants and antioxidative enzymes act as UV protectants. Because black tea contains a high level of antioxidants (Leung et al. 2001, Langley-Evans 2000), future studies should investigate their effects, as well as those of green tea, as a UV protectant (Shapiro et al. 2008). Previously, lignin products were shown to protect baculoviruses (Martignoni and Iwai 1985, El Salamouny 1998, Shasha et al. 1998, Tamez-Guerra et al. 2000, McGuire et al. 2001, El Salamouny and Huber 2004); the present study confirms the efficiency of lignin Reax 85A as an effective UV protectant.

In summary, both black tea and lignin Reax 85A were effective in protecting *SeMNPV* from breakdown by UVA and UVB. They are desirable because both are safe natural products and are readily available. This study, along with our previous ones (El Salamouny 1998, El Salamouny et al. 2002, Shapiro et al. 2007a, 2007b, 2008), may lead to new research on plant-derived materials as UV protectants for baculoviruses. Future studies will focus on testing several teas, spices, medicinal herbs, lignins, oils and plant products as sunscreens in both the laboratory and in the field. This approach may offer potential for the discovery of viral enhancers as well as radiation protectants as adjuvants for insect pathogens in the control of agriculturally-important insect pest populations.

Acknowledgments

Funding for this work was provided by USDA-CSREES Special Grant "Pest Management Alternatives" South Carolina, US-Egypt Joint Fund, and the U.S.

Department of State, Fulbright Program. The authors thank Paul Goforth, Andrea Gilliard, Eric Marom, Mark Schaffer, Chad Smith and Nan Lu for their technical assistance and Ron Meyers (USDA, ARS, Tifton, GA) for supplying the beet armyworm eggs. Contribution number 5393 of the Clemson University Agricultural Experimental Station.

References Cited

- Bullock, H. R., J. R. Hollingsworth and A. W. Harstack. 1970.** Virulence of *Heliothis* nuclear polyhedrosis virus exposed to monochromatic ultraviolet irradiation. *J. Invertebr. Pathol.* 16: 419-422.
- Burges, D. H. and K. Jones. 1998.** Formulation of bacteria, viruses, and protozoa to control insects. *In* Burges, H. [ed.], *Formulation of Microbial Biopesticides*. Kluwer Academic Publisher. 412 p.
- Caffin, N., B. D'Arcy, L. Yao and G. Rintoul. 2004.** Developing an index of quality for Australian tea. Australian Government, Rural Industries Research and Development Corporation. The University of Queensland, RIRDC Publication No. 04/033.
- Dougherty, E. M., K. P. Guthrie and M. Shapiro. 1996.** Optical brighteners provide baculovirus activity enhancement and UV radiation protection. *Biol. Control* 7: 71-74.
- David, W. 1969.** The effect of ultraviolet radiation on known wavelengths on a granulosis virus of *Pieris brassicae*. *J. Invertebr. Pathol.* 14: 334-342.
- Ding, Z. T., X. F. Wang, H. N. Yu, H. Y. Zahng and S. R. Shen. 2005.** Color stability of oxidation products of tea polyphenols and their effects on the growth of PC-3 cells. *J. Tea Sci.* 25: 213-218.
- Elinagar, S. and S. Abul Nasr. 1980.** Effect of direct sunlight on the virulence of NPV (nuclear polyhedrosis virus) of the cotton leafworm, *Spodoptera littoralis* (Boisd.). *Z. Angew. Entomol.* 90: 75-80.
- El Salamouny, S. 1998.** Increasing efficacy of nucleopolyhedroviruses and a comparative study of an Egyptian isolate. Ph.D. Diss., Faculty of Agric. Cairo Univ. Egypt. 238 p.
- El Salamouny, S., A. Herz and J. Huber. 2002.** Suitability of three lignin products as UV protectants to baculovirus. *Bull. Ent. Soc. Egypt. Econ. Ser.* 28: 103-111.
- El Salamouny, S. and J. Huber. 2004.** Effect of lignin compounds as UV protectants for *Spodoptera littoralis* nucleopolyhedrovirus. *J. Agric. Sci. Mansoura Univ.* 29: 2893-2902.
- El Salamouny, S., M. Lange, M. Jutzi, J. Huber and J. Jehle. 2003.** Comparative study on the susceptibility of cutworms (Lepidoptera: Noctuidae) to *Agrotis segetum* nucleopolyhedrovirus and *Agrotis ipsilon* nucleopolyhedrovirus. *J. Invertebr. Pathol.* 84: 75-82.
- Goulson, D., A. M. Martinez, W. O. Hughes and T. Williams. 2000.** Effect of fluorescent brightener used in biopesticide formulations on the behavior of pollinators. *Biol. Control* 19: 232-236.
- Goulson, D., L. Derwent, D. Penagos and T. Williams. 2003.** Effect of optical brighteners included in biopesticide formulations on the growth of crops. *Agric. Ecosyst. Environ.* 95: 235-240.
- Griego, V. M., M. E. Martignoni and A. E. Claycomb. 1985.** Inactivation of nuclear polyhedrosis virus (Baculovirus: Subgroup A) by monochromatic UV radiation. *Appl. Environ. Microbiol.* 49: 709-710.
- Ignoffo, C. M. 1992.** Environmental factors affecting persistence of entomopathogens. *Fla. Entomol.* 75: 516-525.
- Ignoffo, C. M. and C. Garcia. 1992.** Combinations of environmental factors and simulated sunlight affecting activity of inclusion bodies of the *Heliothis* (Lepidoptera: Noctuidae) Nucleopolyhedrosis virus. *Environ. Entomol.* 21: 210-213.
- Ignoffo, C. M. and C. Garcia. 1994.** Antioxidant and oxidative enzyme effect on the inactivation of inclusion bodies of the *Heliothis* baculovirus by simulated sunlight-UV. *Environ. Entomol.* 23: 1025-1029.

- Ignoffo, C. M., C. Garcia and S. G. Saathoff. 1997.** Sunlight stability and rain fastness of formulation of baculovirus *Heliothis*. *Environ. Entomol.* 26: 1470-1474.
- Ignoffo, C. M., D. L. Hostetter, G. Sikorowski, G. Sutter and W. M. Brooks. 1977.** Inactivation of representative species of entomopathogenic viruses, a bacterium, fungus, and protozoan by an ultra-violet light source. *Environ. Entomol.* 6: 411-415.
- Ignoffo, C. M., W. C. Rice and A. H. McIntosh. 1989.** Inactivation of nonoccluded and occluded baculovirus-DNA exposed to simulated sunlight. *Environ. Entomol.* 18: 177-183.
- Jones, K. A., G. Moawad, D. J. Mickinley and D. Grzywacz. 1993.** The effect of natural sunlight on *Spodoptera littoralis* nuclear polyhedrosis virus. *Biocontrol Sci. Technol.* 3: 189-197.
- Krieg, A., A. Gröner, J. Huber and M. Matter. 1980.** Über die Wirkung von mittle- und langwelligen Ultravioletten Strahlen (UV-B und UV-A) auf Insekten Pathogene Bakterien und Viren und deren Beeinflussung durch UV- Schutzstoffe. *Nachr. bl. Dtsch. Pflanzenschutzd.* 32: 100-105.
- Langley-Evans, S. C. 2000.** Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. *Int. J. Food Sci. Nutr.* 51: 181-188.
- Leung, L. K., Y. Su, R. Chen, Z. Zhang, Y. Huang and Z. Y. Chen. 2001.** Theoflavins in black tea and catechins in green tea are equally effective antioxidants. *J. Nutr.* 131: 2248-2251.
- Martignoni, M. E. and P. J. Iwai. 1985.** Laboratory evaluation of new ultraviolet absorbers for protection of Douglas-fir Tussock moth (Lepidoptera: Lymantriidae) baculovirus. *J. Econ. Entomol.* 78: 982-987.
- McGuire, M. R., R. W. Behle, H. N. Goebel and T. C. Fry. 2000.** Calibration of a sunlight simulator for determining solar stability of *Bacillus thuringiensis* and *Anagrapha falcifera* nuclear polyhedrovirus. *Environ. Entomol.* 29: 1070-1074.
- McGuire, M. R., P. Tamez-Guerra, R. W. Behle and D. A. Streett. 2001.** Comparative field stability of selected entomopathogenic virus formulations. *J. Econ. Entomol.* 94: 1037-1044.
- Miller, L. K. 1997.** The baculoviruses. Plenum Press, New York, NY.
- Sambrook, J., E. F. Fritsch and T. Maniatis. 1989.** Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, New York, NY.
- Shapiro, M., P. P. Agin and R. A. Bell. 1983.** Ultraviolet protectants of the gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus. *Environ. Entomol.* 12: 982-985.
- Shapiro, M. and J. L. Robertson. 1990.** Laboratory evaluation of dyes as ultraviolet screens for the gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. *J. Econ. Entomol.* 83: 168-172.
- Shapiro, M., J. L. Robertson and R. E. Webb. 1994.** Effect of neem seed extract upon the gypsy moth (Lepidoptera: Lymantriidae) and its nuclear polyhedrosis virus. *J. Econ. Entomol.* 87: 356-360.
- Shapiro, M., B. M. Shepard and R. Lopez. 2007a.** Effect of spices upon activity of the gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrovirus. *J. Entomol. Sci.* 42: 84-91.
- Shapiro, M., B. M. Shepard and R. Lopez. 2007b.** Effect of medicinal herbs upon the biological activity of the gypsy moth nucleopolyhedrovirus. *J. Entomol. Sci.* 42: 426-429.
- Shapiro, M., S. El-Salimouny and B. Merle Shepard. 2008.** Green tea extracts as ultraviolet protectants for the beet armyworm, *Spodoptera exigua*, polyhedrosis virus. *Biocontr. Sci. Technol.* 18: 605-617.
- Shasha, B. S., M. McGuire and R. W. Behle. 1998.** Lignin based pest control formulations. US Patent No 5,750,467.
- Tamez-Guerra, P., M. McGuire, R. W. Behle, J. J. Hamm, H. R. Sumner and B. S. Shasha. 2000.** Sunlight persistence and rainfastness of spray-dried formulation of baculovirus isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 93: 210-218.
- Wang, Z. Y., M. T. Huang, Y. R. Lou, J. G. Xie, K. R. Reuhl, H. L. Newmark, C. T. Ho, C. S. Yang and A. H. Conney. 1994.** Inhibitory effect of black tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz (a) anthracene-initiated SKH-1 mice. *Cancer Res.* 54: 3428-3435.