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EFFECT OF LIGNIN COMPOUNDS AS UV PROTECTANTS FOR *Spodoptera littoralis* NUCLEO POLY HEDRO VIRUS

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ABSTRACT

Two new lignin products, desulfonated lignin and Lignin alkali were tested as UV protectant additives to *Spodoptera littoralis* nucleopolyhedrovirus (*Spli*MNPV) in comparison with magnesium lignosulfonate, Fluorescent brightener 28, Berberine and Nu-Film. Desulfonated lignin and lignin alkali exhibited a high rate of protection to *Spli*MNPV (3.1 fold) compared with the other tested UV lignin protectant additives. In contrast, Nu-Film did not show any rate of protection. The study recommends desulfonated lignin and lignin alkali as a natural UV protectant to be used for the improvement of virus formulations. The mechanism of the protection by lignin could be due to the dark color, which prevent penetration of the UV light that inactivate the virus. The study demonstrates the potential of desulfonated lignin and lignin alkali as UV protectant additives to baculoviruses and the mechanism of protection.

INTRODUCTION

Baculoviruses became important biocontrol agents, which can be used in plant protection. They are safe, environmentally friendly, effective and specific bioagents, which can be used instead of many agrochemicals (Burges *et al.,* 1980). However, inactivation of baculovirus under the natural sunlight in the field is considered the main constrain for the use of baculoviruses in practice. The effect of environmental factors on survival of microbial control agents has been well docum

ented by Ignoffo (1992) and Ignoffo & Garcia, (1992). Previous studies have reported that sunlight is the most destructive factor (Ignoffo *et al.,* 1997, Elnagar, 1983 and Jones *et al.,* 1993). *Spodoptera littoralis* MNPV sprayed on cotton plants in the Egyptian field lost much of its virulence on the next day of application, merely due to the sunlight effect. The crude extract persisted longer in the field (Elnagar and Abul Nasr (1980). Jones *et al.* (1993) reported that wave-lengths between 300 and 320*nm* were shown to be responsible for almost all of the inactivation attributed to sunlight, although there was some deleterious effect of wave lengths between 320 and 400*nm* and above 665*nm.* Different sunscreen additives were used by Burges and Jones (1998) in order to prolong the activity of baculoviruses.

Fluorescent brighteners act as UV protectant (Shapiro, 1992; Dougherty *et al.*, 1996& Martignoni and Iwai, 1985), but also as an excellent synergistic factor (El-Salamouny *et al.*, 1997 & Farrar and Ridgway, 1997, Okuno et al., 2003 & Shapiro and Dougherty 1994). Several natural materials were tested such as Carbon products as a blocking screen (Jaques, 1971). Coax is an excellent UV protectant (Shapiro *et al.*, 1983). Tinopal DCS (Fluorescent brightener) and Raymix powder (Lignosulfonate) protected NPV against UV radiation equally or better than did shade (Martignoni and Iwai, 1985). Berbrine increased the photo stabilization of *Spli*MNPV (Cohen *et al.*, 2001).

Recently, lignin derivatives have proven to be efficient natural UV protectants (Shasha *et al.*, 1995; Tamez-Guerra *et al.*, 2000; El-Salamouny *et al.*, 2002 and Elnagar *et al.*, 2003).

The main goal of this study is to test several lignin products as natural UV protectants and their effect on the persistence of *Spodoptera littoralis* nucleopolyhedrovirus in comparison with other known and previously tested additives.

MATERIAL AND METHODS

Tested Virus:

Egyptian isolate of nucleopolyhedroviruses of the cotton leafworm, *Spodoptera littoralis* (Boisd.) was used in the present study. The virus was extracted from cadavers, highly purified and standardized using the method described by El-Salamouny *et al.*, (2003). The virus preparation suspended in Tris /HCl buffer pH 8. The virus concentration of the irradiated solution was 1000 fold higher than the estimated LC_{90}. Cittowett 0.025% was used to decrease the surface tension of the virus suspension.

Tested insect:

Newly hatched larvae of the cotton leafworm, *S. littoralis,* were used as test insects. The larvae were reared individually on a semi synthetic diet described by Hassani (2000). Egg-masses, larvae and pupae of the colony were reared at 28 °C, while the moths were kept at 25±2 °C for egg laying. The insect culture was kept under 60-70± 5% RH (relative humidity).

Tested additives:

Five additives were tested as UV protectants. Desulfonated lignin (DL), Lignosulfonate Alkali (LA), Magnesium Lignosulfonate (ML), Fluorescent brightener 28 (Tinopal LPW) (FB). DL and LA are from Sigma-Aldrich, ML from Borregard Deutschland and Nu-film (NF) from Andermatt Biocontrol, Switzerland. All the tested materials were dissolved in distilled water and added with an exact concentration to the virus suspension. A concentration of 10% was used for the screening of the lignin additives. Later on, a concentration of 1% was used to compare the best UV protectant to other tested additives.

UV irradiation tests:

Testing of the six materials was performed using the method described by Krieg *et al.* (1980) and Huber and Lüdcke (1996). 100 µl of *Spli*MNPV suspension either with or without additives was plated out in a thin layer on the glass surface of Petri-dishes (10 cm in diameter). The film was air-dried. Dry deposits of the virus suspension were irradiated for different exposure times (0, 1, 16, 32, 64, 96, 128 and 160 minutes) with a simulated source of UV light, using four Ultra- Vitalux lamps (OSRAM) at a distance of 160 cm. After irradiation, the treated virus deposit was resuspended in 10 ml of Tris/HCl buffer (pH 8). 5ml of the 10 were mixed with artificial diet and bioassayed against neonate larvae of the test insect (Huber, 1981 & El-Salamouny *et al.*, 2002). The bioassay plates were incubated at 26°C±2, 60-70% ±5 relative humidity and 16 hours of light and 8 hours of dark. Mortality due to infection was recorded after 12 days.

Absorption spectra of solutions:

Absorption spectra of solutions of all tested compounds were measured using a Spectrophotometer, UVIKON 922 (Kontron Instruments).

Statistical analysis:

The protection effect was estimated by virus survival half life (SHL) computed from the slope of the regression between the irradiation time and probit mortality (= log activity of the virus) (Finney, 1971). The formula for the calculation of SHL is: $T_{1/2}$= log 0.5 (S_1/S_2) where S_1= slope of concentration response line, obtained by testing five viral concentration of unirradiated *Spli*MNPV against *S. littoralis* neonate larvae and S_2= slope of UV irradiation time–virus activity line (Weber, 1984).

RESULTS

All UV-treated, lignin formulations of *Spli*MNPV were significantly more active against *S. littoralis* larvae than virus controls without additives. Both of desulfonated lignin and the lignin alkali preserved virus activity more than the magnesium lignosulfonate, when added in concentration of 10% to suspensions of *Spli*MNPV. The average slope value in case of virus alone treatment was 0.0153 and it decreased sharply to 0.005 and 0.0054 by the addition of desulfonated lignin and the lignin alkali, respectively. The obtained data showed that *Spli*MNPV lost its activity in short time, with a survival half-life value (SHL) of 100.9 minutes. Addition of desulfonated lignin (DL) and lignin alkali (LA) at the concentration of 10% prolonged the SHL to 312.4 and 323.7 min., respectively. Desulfonated lignin and lignin alkali seems more potent in virus protection than magnesium lignosulfonate (ML), a reference of previously tested lignin product (El-Salamouny *et al.*, 2002) where SHL value was 153 (only 1.6 fold) (Fig. 1 & Table 1). Addition of desulfonated lignin and lignin alkali at the concentration of 10% provided 3.1 fold improvement in virus persistence for both additives (Table 1).

Table 1: Influence of three lignin additives on the protection of SpliMNPV from the inactivation.

Treatment	Experiment 1		Experiment 2		Experiment 3		Average	
	Slope	SHL	Slope	SHL	Slope	SHL	Slope	SHL
Virus alone	0.015	101.3	0.013	116.9	0.018	84.4	0.153	100.9
V+DL 10%	0.005	304	0.004	380	0.006	253.3	0.005	312.4
Potency	(3.0)*		(3.25)		(3.0)		(3.08)	
V+ LA 10%	0.006	253.3	0.003	506.7	0.0072	211.1	0.005	323.7
Potency	(2.5)		(4.3)		(2.5)		(3.1)	
V+ML 10%	0.009	168.9	0.011	138.2	0.1	152	0.01	153
Potency	(1.7)		(1.2)		(1.8)		(1.6)	

* Between brackets = folds

Based on the obtained slope value as well as on the absorption spectra of the tested products, desulfonated lignin was chosen to compare with three other UV protectant additives at the level of 1% concentration. The protection rate of DL was close to that obtained with FB and provided a better protection than the Beberine (BB). For this test, the SHL value for the virus alone treatment of 126.7 minutes , was prolonged to 253.3, 217.1 and 168.9 minutes by addition of F. brightener LPW, desulfonated lignin and Beberine at a concentration of 1%, respectively. The obtained potency values were 2, 1.7, and1.3 fold, respectively. A high rate of protection was recorded if desulfonated lignin (DL) was added to F. brightener (both at the concentration of 1%), with a SHL value of 506.7 minutes (4 folds). No protection effect was detected in the case of Nu-Film since the survival half-life (SHL) of the virus with Nu-Film was very close to the virus alone treatment. (Fig.2).

Absorption spectra of the tested products:

All the tested compounds at the concentration 10% showed a high extinction rate in the UV range (Fig.3). The mixture of fluorescent brightener (1%) and desulfonated lignin showed the highest UV absorption, which was parallel to the obtained UV protection effect. When the concentration of all tested lignin additives was diluted, a reduced rate of absorption was detected. However, no reduction in absorption was obtained in case of F. brightener 28 when the same dilution rate was used (Fig. 4). In conclusion, desulfonated lignin and lignin alkali acts as a potent UV protection. The mechanism could be as a screening effect of the tested products.

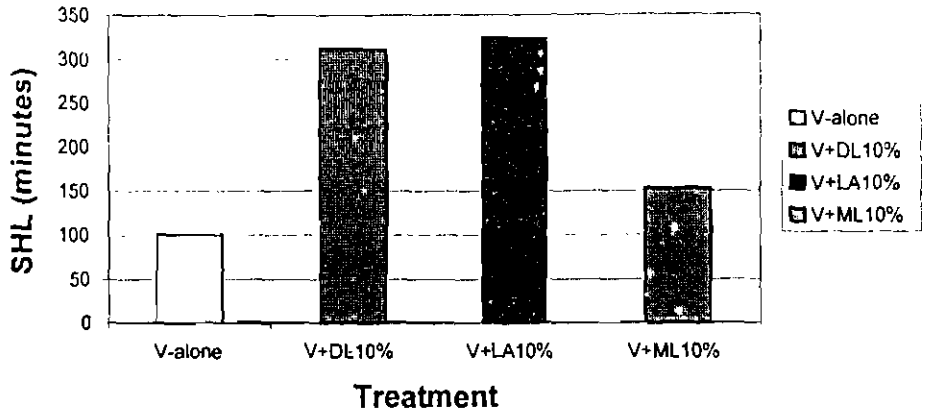

Fig.1: Effect of three Lignin additives on the persistence (survival half life (SHL50)) of *Spodoptera littoralis* MNPV.

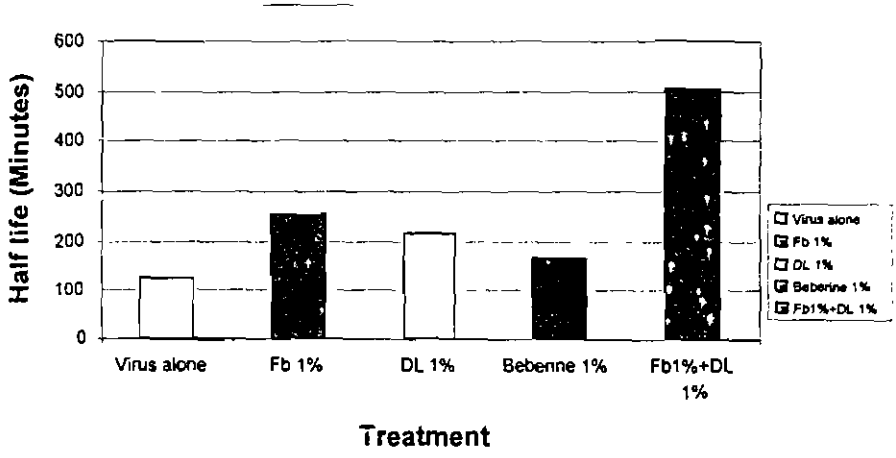

Fig.2: Comparison between Desulfonated Lignin and other additives as UV protectants.

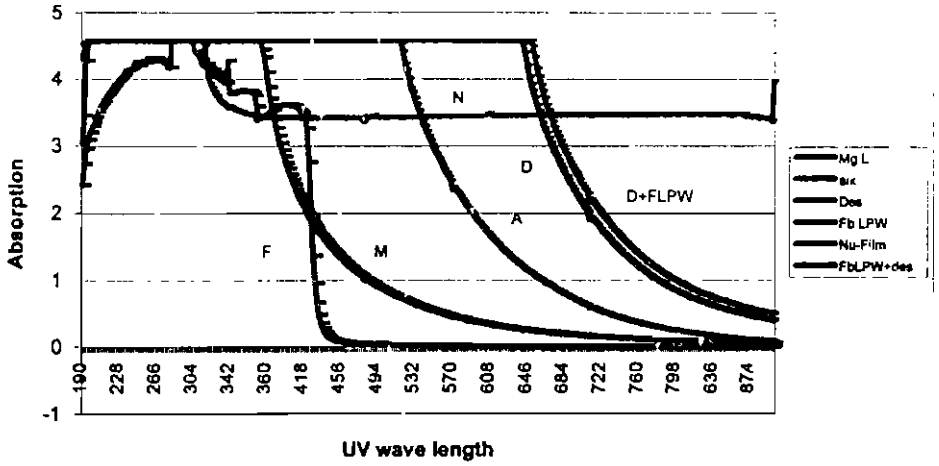

Fig.(3): Absorption rate of fluorescent brightener by different UV protectants at 1%.

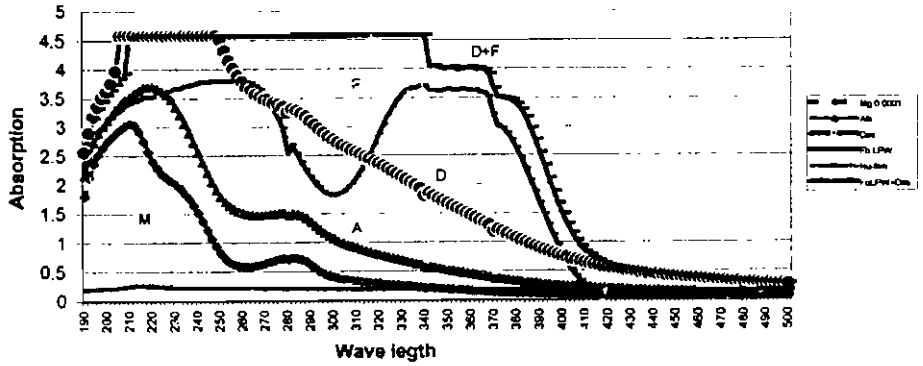

Fig. (4): Absorption rate of different UV protectants at the concentration of 0.01%.

DISCUSSION

Previous results of Elnagar and Abul Nasr (1980) confirm the fact that *Spli*MNPV can not be used on large scale application for the control of *S. littoralis*, unless a protectant agent against deterioration of virulence is added.

Several lignin products were tested as UV protectants in order to increase the resistance to solar degradation of baculovirus (El-Salamouny *et al.*, 2002, McGuire *et al.*, 2000 McGuire *et al.*2001 and Elnagar *et al.*, 2003). In the present study, two new lignin products, desulfonated lignin and lignin alkali prolonged the activity of *Spli*MNPV. Lignin derivatives could be used as effective UV protectants, which agrees with findings by El-Salamouny *et al.* (2002); Elnagar *et al.* (2003); McGuire *et al.* (2001), and Tamez-Guerra *et al.* (2000). Formulations with lignin had more insecticidal activity remaining after sunlight exposure than formulations without lignin (Tamez-Guerra *et al.*, 2000). More than 50% activity remained in formulations containing lignin, whereas unformulated virus retained less than 50% activity within 24 hrs after field application (McGuire *et al.*, 2001).

The absorption spectra of the tested products agreed with the obtained UV protection effect. The study shows no protectant effect of Nu-Film, which is used to improve the activity of *Bacillus thuringensis*, acting only as a sticker.

The present study explains the mechanism of the protection effect of the lignin products. There was no difference of the absorption rate in case of F. brightener between the tested concentrations 1% and 0.001%. The change in the absorption spectra by the reduction of the concentration of the DL and LA (1000 fold) explains that the protection effect at the high concentration (10%) was due to prevention of the UV light to penetrate. This could be due to the screening effect of the dark brown dye. This result is in line with previous record of the effect of several colored additives such as Congo red and carbon (Shapiro, 1989 & Jaques 1971).

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تأثير مركبات اللجنين كمواد حامية من الأشعة فوق البنفسجية لفيروس البوليهيدروسز النووى لدودة ورق القطن

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تم إختبار مادتين جديدتين من مشتقات اللجنين هما اليسلفوناتيد لجنين*desulfonated lignin* واللجنين ألكالى Lignin alkali كمواد حامية لفيروس دودة ورق القطن (*Spli*MNPV) من الأشعة فوق البنفسجية UV مقارنة ب لجنوسلفونات المغنيسيوم و العواكس الفلوروسنتية ٢٨ والبربرين والنوفيلم . أظهرت الدراسة تفوق مادتى desulfonated lignin و ال Lignin alkali كمواد طبيعية حامية للفيروس من الأشعة فوق البنفسجية لتحسين المستحضرات الفيروسية بحوالى ثلاث أضعاف الفيروس بمفرده. ميكانيكية الحماية يمكن أن تكون نتيجة للون الداكن للمواد والتى تمنع أشعة الUV من تنشيط الفيروس.

الدراسة توضح مدى قوة مادتى desulfonated lignin و ال Lignin alkali كإضافات حامية للباكلوفيروس وميكانيكية الحماية.