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EFFECT OF CERTAIN OPTICAL BRIGHTENERS ON THE SUSCEPTIBILITY OF *Spodoptera littoralis (BOISDUVAL)* (LEPIDOPTERA: NOCTUIDAE) TO A BACULOVIRUS: EFFECT ON SOME BIOLOGICAL ASPECTS

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ABSTRACT

The addition of Fluorescent brighteners at 0.1% concentration to *Spodoptera littoralis* nucleopolyhedrovirus (*Spli*MNPV) increased the susceptibility of *S. littoralis* larvae to virus. The highest rates of enhancement among the six tested optical brighteners were obtained in case of Blankophor BBH, Tinopal UNPA-GX and Blankophor HRS. LC_{50}'s values were sharply reduced from $1.9x10^4$ to $2.3x10^2$, $2.4x10^2$ and $4.94x10^2$ PIB's/ml diet by 82.6, 78.2 and 38.7 fold, respectively. Whereas, Blankophor RKH, Blankophor P167 and Blankophor BSU exhibited less effect than the previous three products.

Also, a reduction in the time required for killing 50% of the tested population (LT_{50}) was recorded with a value of 2.23 fold in case of Tinopal UNPA-GX. Effects on the larval weight, duration of larval stage, duration of pupal stage and the sex ratio of emerged moths were studied. The reduction in larval weight was correlated with a high rate of enhancement effect. The duration of the larval stage was longer in case of the brightener treatments especially with those effective products. No effect could be detected on the duration of the pupal stage and sex ratio. The present study suggests the potential use of Blankophor BBH and Tinopal UNPA-GX to improve the effect of *Spli*MNPV formulation.

Keywords: Baculovirus, Blankophor, Enhancement, Fluorescent brightener, nucleopolyhedrovirus, *Spodoptera littoralis*, Susceptibility, Enhancement, Tinopal.

INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered a key pest for several field and vegetable crops in Egypt (Hosny, 1980). A baculovirus such as nucleopolyhedrovirus or granulovirus is considered a promising biocontrol agent due to its safety, specificity and efficacy. Several efforts have been devoted to increase the susceptibility of insects to baculoviruses. Optical brighteners are widely used in detergent, paper, plastics and organic coating industries (Lanter, 1966). Following the discovery of the synergistic effect of Fluorescent brightener to baculovirus by Shapiro (1992), several studies aimed to increase the susceptibility of insects to baculoviruses. For example, the beet armyworm, *Spodoptera exigua* (Shapiro and Argauer, 2001), the rice armyworm *Mythimna separata* (Mukawa *et al.*, 2003), the common cutworm *Agrotis segetum* (El-Salamouny *et al.*, 2003), the celery looper, *Anagrapha falcifera* (Vail *et al.*, 1996), the black cutworm, *Agrotis ipsilon* (Boughton *et al.*, 2001& El-Salamouny *et al.*, 2001), the cotton leaf worm, *Spodoptera littoralis*

(El-Salamouny *et al.*,1997),the cotton bollworm, *Helicoverpa* (*Heliothis*) *zea* (Shapiro and Vaughn, 1995), Gypsy moth, *Lymantria dispar* (Shapiro and Dougherty, 1994). Several brighteners were tested to select the best synergistic effect for several insect hosts, *e.g.*, Blankophor BBH, Blankophor RKH, Blankophor P167 and Blankophor HRS enhanced the activity of *Choristoneura fumiferana* NPV whereas Blankophor BSB, Blankophor DML, Blankophor LPG and Blankophor BSU did not enhance the activity (Li and Otovos, 1999a). Also, Shapiro and Argauer (2001) reported that Blankophor BBH, Blankophor HRS, Blankophor P167, RKH reduced the LD_{50} of *Spodoptera exigua* MNPV whereas Blankophor BSU, DML and LPG had little effect. The LC_{50} values were reduced by 10.5 fold (Blankophor P167), 52.4 (Blankophor RKH), 87.3 (Tinopal LPW) and 131 fold (Blankophor BBH). Tinopal LPW enhances the susceptibility of *S. littoralis* larvae to *Spli*MNPV by a factor of 144 folds (El-Salamouny *et al.*, 1997). The mode of action of fluorescent brightener in relation to insect susceptibility has been proved by altering the peritrophic membrane (lines the whole midgut) (Wand & Granados, 2000) and El-Salamouny *et al.*, (2003). A side effect of using fluorescent brightener on the pollinators was recorded by reducing recruitment of insect to flowers and to locate rewards (Goulson *et al.*, 2000). Also, a reduction in growth of plants as a result of using F. brightener in the field was mentioned by Goulson *et al.*, (2003).

The purpose of present study is to test six fluorescent brightener products as synergistic factors in order to select the best enhancement effect in order to use it in virus formulations. Also, to determine their effects on some biological aspects of *S. littoralis* larvae.

MATERIAL AND METHODS

Test Insect:

Neonate larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) were used. The larvae were maintained on a semi synthetic diet described by Shorey and Hale (1965). Insects were maintained at 26±2°C and 65±5 R.H.

Virus:

A highly purified Egyptian isolate of *S. littoralis* nucleopolyhedrovirus (*Spli*MNPV) was used in present investigation. five concentrations for each treatment were used ranging from 10^3 to 10^5 for the virus alone treatment and from reduced 10^2 to 10^4 for the tested additives. The concentration was spaced by a factor of root of ten (?10= 3.162). *Spli*MNPV isolate was propagated in 5 days old *S. littoralis* larvae. The stock virus suspensions of virus were kept in Tris buffer, pH 8. Extraction of virus from the dead larvae in Tris/Hcl buffer was done by blending the dead larvae in a mortar. The obtained suspension was filtered through a cotton layer in between several layers of muslin cloth. The filtrate was centrifuged at 1000 rpm for 5 min. and the pellet was discarded. The obtained supernatant was centrifuged twice at 10000 rpm for 30 min and the pellet was collected. The virus pellet was

resuspended in Tris-Hcl buffer pH 8 and ultra centrifuged using Beckman centrifuge in sucrose gradient 40-70% W/W. The collected band was washed by centrifugation twice in distelled water and once in tris buffer at 10000 rpm for 30 min. Collected pellets contained the purified polyhedral inclusion bodies (PIB's) were stored in a small amount of Tris/Hcl buffer at -20°C, for further purification. The PIB were counted using a haemocytometer slide (Neubauer, Germany) depth 0.1*mm*, 1/400mm^2).

Synergistic additives:

The following six optical brighteners were provided by Dr. Martin Shapiro, Insect Biocontrol Laboratory, USDA, Maryland, USA:
Tinopal UNPA-GX (TUNPA-GX), Blankophor RKH (BRKH), Blankophor BBH (BBBH), Blankophor P167 (BP167), Blankophor HRS (BHRS), Blankophor BSU (BBSU).

Each F. brightener was further dissolved in distilled water and tested at the concentration of 0.1 % in the diet mixture.

Bioassay:

A standard diet-incorporation bioassay technique described by Huber (1981) was used for incorporating the tested additives with *S. littoralis* nucleopolyhedrovirus in a semi-synthetic diet. Standardization of the viral concentrations was based on the number of polyhedral inclusion bodies (PIB's)/ml of aqueous suspension. Five ml of the tested virus concentration as well as another 5 ml of the suspension of the tested additive were mixed with 40 ml of semi-synthetic diet at a diet temperature below 40°C. The virus-additive-diet mixture was then pored into a special bioassay plate (LICEFA, Bad-Salzuflen, Germany) (14x7x2cm, contains 50 cells). One newly hatched larva was placed into each cell. The plates were covered with tissue paper and glass plate fixed with rubber bands. Mortality due to virus infection was recorded daily up to 10 days.

Follow up for the biological aspects:

The larvae after finishing the food were tranferred to a new bioassay plate (with or without brightener until pupation, then transferred to 8 cm (in diameter) plastic cup with a plastic cover with small holes and followed up until emerging of the moths. Insects were maintained at 26±2°C and 65±5 RH.

Statistical analysis:

The LC_{50} and LT_{50} values were analyzed by probit analysis using the method described by Finney (1971). Estimation of the relative potencies of the treatments was calculated according to the changes in both values.

RESULTS

Effect of six fluorescent brighteners on the insect susceptibility:

Tinopal UNPA-GX, Blankophor RKH, Blankophor BBH, Blankophor P167, Blankophor HRS, and Blankophor BSU at the concentration of 0.1% have no insecticidal effect on *S. littoralis* larvae when administered orally. This was observed among the untreated and brightener-treated *S. littoralis* larvae.

Addition of Blankophor BBH, Tinopal UNPA-GX and Blankophor HRS to *Spodoptera littoralis* MNPV (*Spli*MNPV) reduced the concentration required for killing 50% of the tested larvae (=LC_{50}) from $1.9x10^4$ PIB's/ml diet to only $2.3x10^2$, $2.4x10^2$, $4.94x10^2$for the tested products, respectively. However, a very slight effect on the susceptibility of *S. littoralis* was detected in case of Blankophor RKH (LC_{50}= $6.7x10^3$). No synergistic effect could be detected in case of Blankophor P167 and Blankophor BSU products (Fig.1). The obtained potency values of 82.6, 78.2, 38.7 and 2.8 fold were obtained in case of Blankophor BBH, Tinopal UNPA-Gx, Blankophor HRS and Blankophor RKH, respectively.

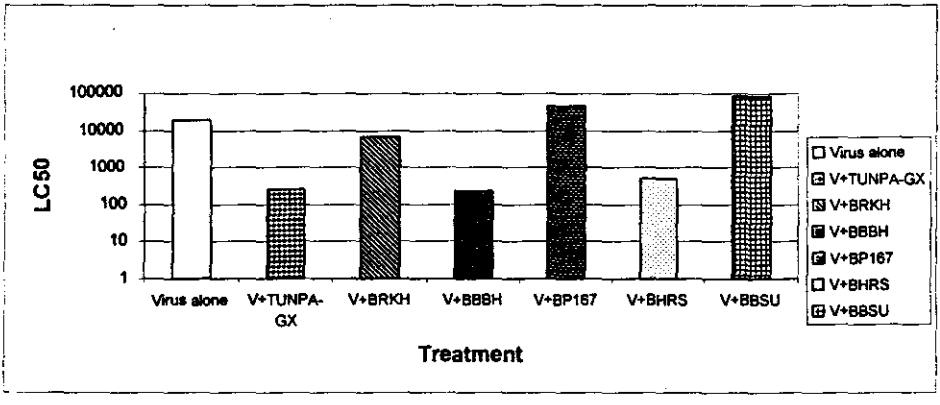

Fig. (1): Effect of six Fluorescent brightener as synergistic additives to increase the susceptibility of *S. littoralis* larvae to *Spli*MNPV.

Effect of tested products on the speed of larval mortality induced by virus:

The time required for 50% of the larvae to die from virosis(=LT_{50}) was determined for accumulated mortality from either the viral concentration alone or virus incorporated with the synergistic additives. LT_{50} values were influenced by *Spli*MNPV concentration, where it decreases by increasing virus concentration (Table.1).

At a fixed virus concentration for all treatments ($3.16x10^3$ PIB's/ ml diet), the LT_{50} value was reduced from 7.86 days for the virus alone treatment to 3.485, 4.199 and 6.995 days by addition of Tinopal UNPA-GX, Blankophor BBH and Blankophor HRS, respectively. This result suggests that *S. littoralis* larvae died more quickly when 0.1% concentration of F. brightener was

added to the tested virus. It was difficult to calculate the LT_{50} value due to the low rate of mortality. The highest obtained potency of speed of killing of 2.23 and 1.87 fold was detected in case of Tinopal UNPA-GX and Blankophor BBH, respectively (Table.2).

In conclusion, Tinopal UNPA-GX, Blankophor BBH, and Blankophor HRS showed the best effect.

Table (1): Effect of certain fluorescent brighteners on the LT_{50} s of *Spli*MNPV against *S. littoralis* larvae.

Treatment	LT_{50}	95% finducial limit (days)	
Virus alone		**From**	**To**
$3.1`6x10^3$	**7.860**	--	--
$1x10^4$	**5.137**	4.974	5.271
$3.16x10^4$	**5.612**	4.893	6.106
$1x10^5$	**4.988***	4.723	5.193
Tinopal UNPA-GX +V			
Tinopal UNPA-GX $+1x10^2$	**7.649**	6.878	8.370
Tinopal UNPA-GX $+3.16x10^2$	**7.820**	6.355	5.193
Tinopal UNPA-GX $+1x10^3$	**5.280***	5.022	5.491
Tinopal UNPA-GX $+3.16x10^3$	**3.485**	1.005	4.215
V+ Blankophor BBH			
Blankophor BBH $+1x10^2$	**7.544**	6.310	9.022
Blankophor BBH $+3.16x10^2$	**7.333**	6.598	8.077
Blankophor BBH $+1x10^3$	**5.839***	5.701	5.971
Blankophor BBH $+3.16x10^3$	**4.199**	3.546	4.591
Blankophor BBH $+1x10^4$	**4.358**	3.76	4.683
V+ Blankophor HRS			
Blankophor HRS $+1x10^2$	**7.835**	5.225	10.656
Blankophor HRS $+3.16x10^2$	**7.639**	6.892	8.441
Blankophor HRS $+1x10^3$	**6.550***	6.399	6.697
Blankophor HRS $+3.16x10^3$	**6.995**	6.010	7.909
Blankophor HRS $++1x10^4$	**4.676**	4.195	5.035

*= Gives about 90% mortality

Table (2): Potency values of the tested six F. brightener additives added to *Spli*MNPV and tested against *S. littoralis* larvae.

Treatment	Relative Potency (Folds)	
	Power of kill (LC_{50})	Speed of Kill (LT_{50})*
Virus+TUNPA-GX	78.21	2.225
Virus+Blankophor RKH	2.83	0.6
Virus+BBBH	82.58	1.871
Virus+Blankophor P167	4.11	1.446
Virus+Blankophor HRS	38.66	1.124
Virus+Blankophor BSU	0.23	1.362

*= Calculated at the concentration of $3.16x10^3$ PIB's/ ml diet.

Effect of tested products on some biological aspects:

Effect on the larval weight:

It was clearly noticed that the fluorescent brightener has reduced the weight of *S. littoralis* larvae compared to the untreated control. The average weight of the untreated larvae was 0.2 gram/ larva , which was highly decreased by the addition of Tinopal UNPA-GX and Blankophor BBH to 0.073 and 0.088 Gram/ larva, respectively. A slight reduction in the larval weight was recorded in case of Blankophor HRS and Blankophor RKH. No reduction in the larval weight was detected when Blankophor BSU and Blankophor RKH were tested. The decrease in the larval weight was found to be correlated to the synergistic effect compared to the untreated control (Fig. 2). In case of incorporation of virus($1x10^3$ PIB's/ ml diet) with the brightener treatment, the reduction in the larval weight was much higher than in F. brightener alone treatment. The average weight of larva was decreased from 0.159 in case of virus alone treatment to only 0.023, 0.066, 0.036 gram/ larva in case of adding the Tinopal UNPA-GX, Blankophor BBH and Blankophor HRS to the virus, respectively. A slight reduction in the larval weight was recorded in case of adding the Blankophor RKH and Blankophor P167 whereas, no effect could be detected in case of Blankophor BSU (Fig.2).

Effect on the duration of the larval stage:

The addition of F. brightener products prolonged the larval stage of *S. littoralis* than in the untreated control. The avarge duration of virus alone treatment was prolonged from 16.56 day in case of untreated control to 16.91, 16.94 and 16.94 days for Tinopal UNPA-GX, Blankophor BBH and Blankophor HRS, respectively. No difference could be detected between the avarage larval duration for either those maintained for continuous feeding on a diet containing brightener until pupation or those transferred after 12 days to a diet without brightener (Fig.3)

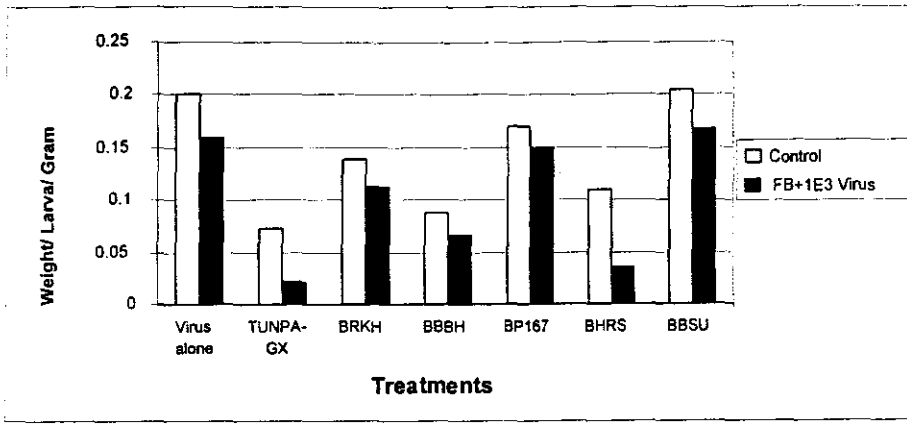

Fig. (2): Effect of certain F. brighteners on the larval weight of *S. littoralis*.

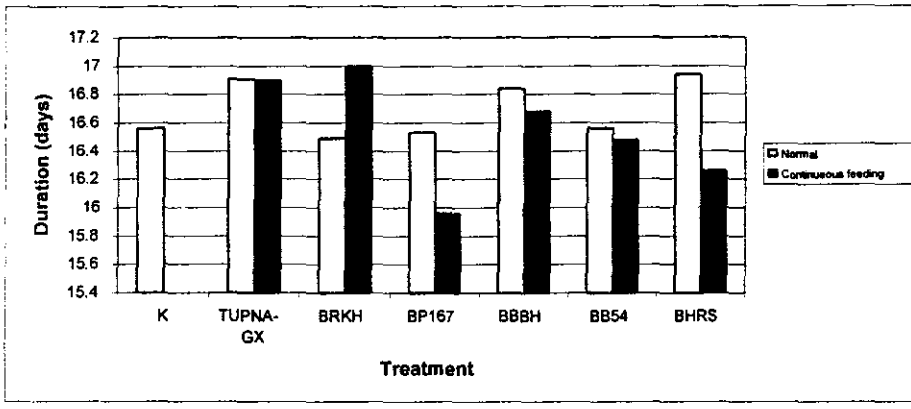

Fig. (3): Effect of certain Fluorescent brighteners on the duration of the larval stage of *S. littoralis*.

Effect on the duration of the pupal stage:

Data illustrated in Fig. (4). show that the pupation starts early in case of untreated control than in the brightener treatments (Fig. 4. a,b,c,d,e,f,g). In general, the duration of the pupal stage was much longer in case of the males (ranged from 11.41 to 12.08 day) than in the females (ranged from 10.5 to 11.5 day). No effect of fluorescent brightener on the duration of the pupal stage could be detected either in case of the male pupae or female ones (Fig.5).

Fig. 4. a. BBBH

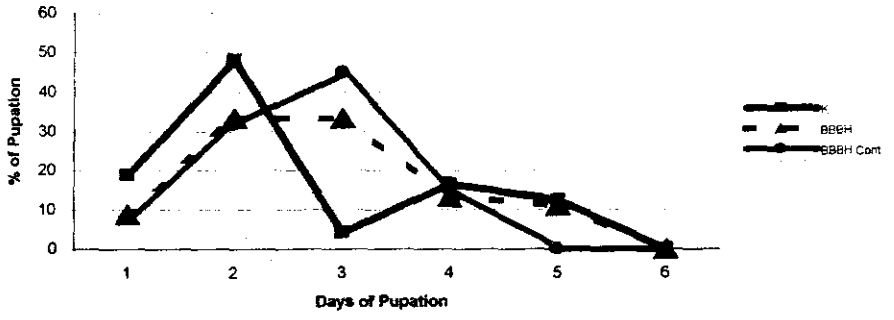

Fig. 4.b. Tinopal UPNA-GX

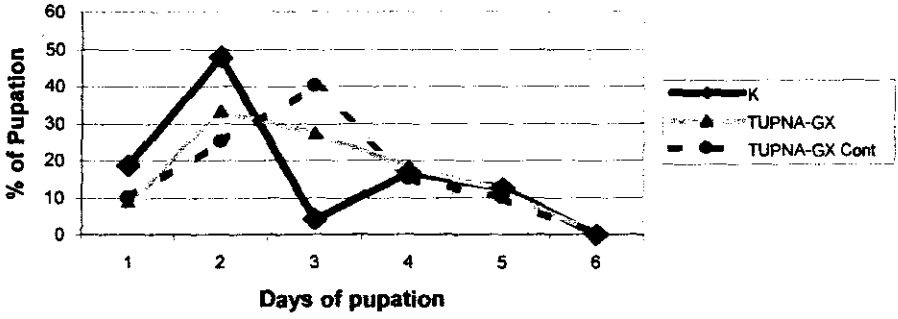

Fig. 4.c. BRKH

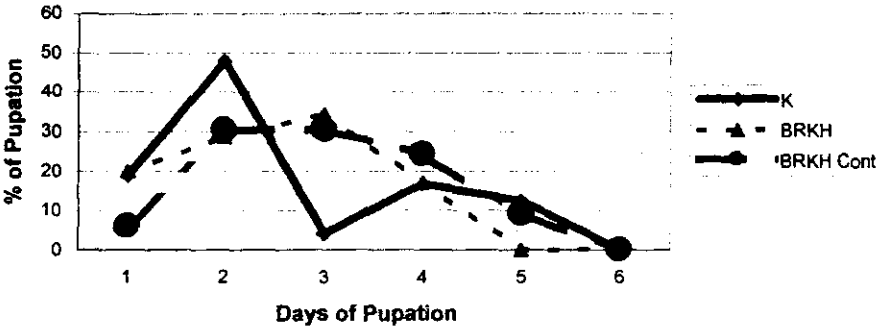

Fig. 4: Delay of the Pupation attributed to the effect of six F. brightener products.

Fig. 4.d. BHRS

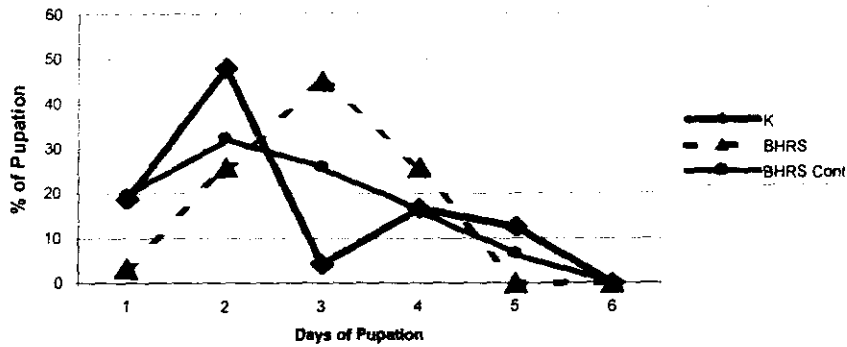

Fig .4.e. Blankophore P 167

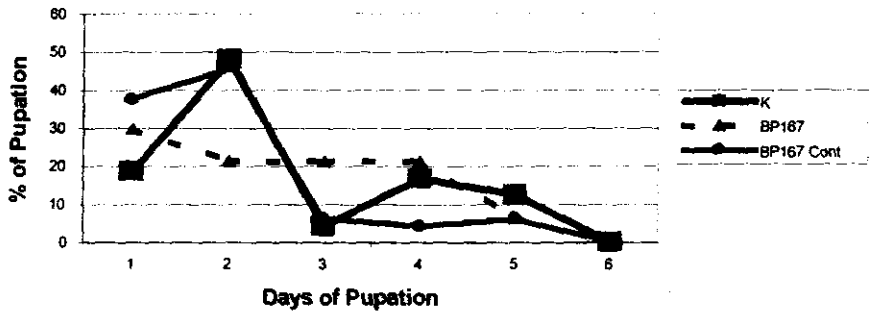

Fig. 4 .f. Blankophore BSU

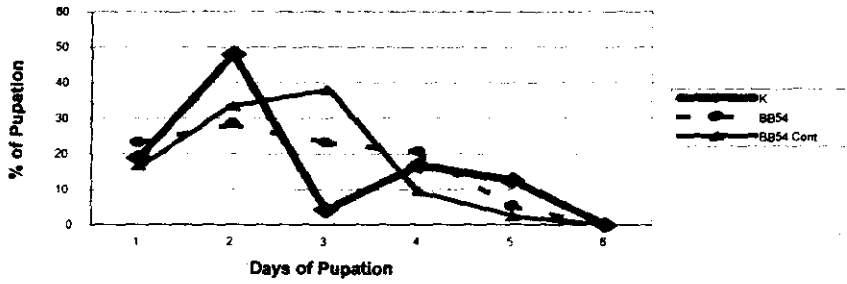

Fig.(4): Continue

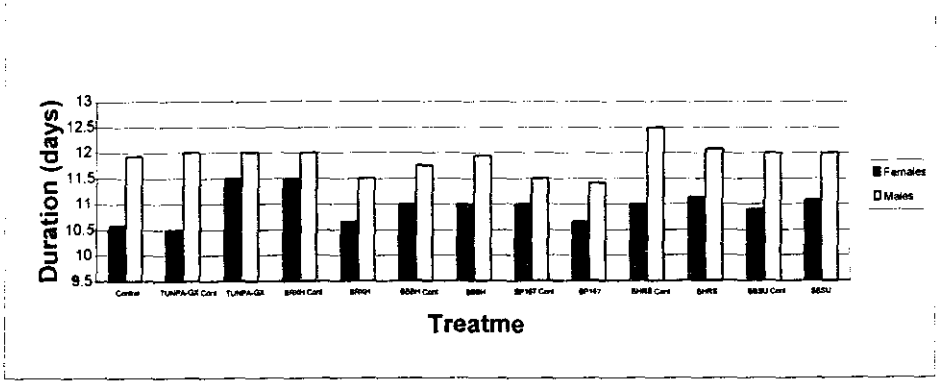

Fig. (5): No reduction in the pupal stage could be detected as a result of adding F. brighteners to *Spli*MNPV against *S. littoralis* larvae. Effect on the

Sex ratio of emerged moths:

The data presented in Fig. (6). showed that the number of emerged females was more than the emerged males except for only the Blankophor BBH which showed that the number of males was more (59%) than the females (41%).

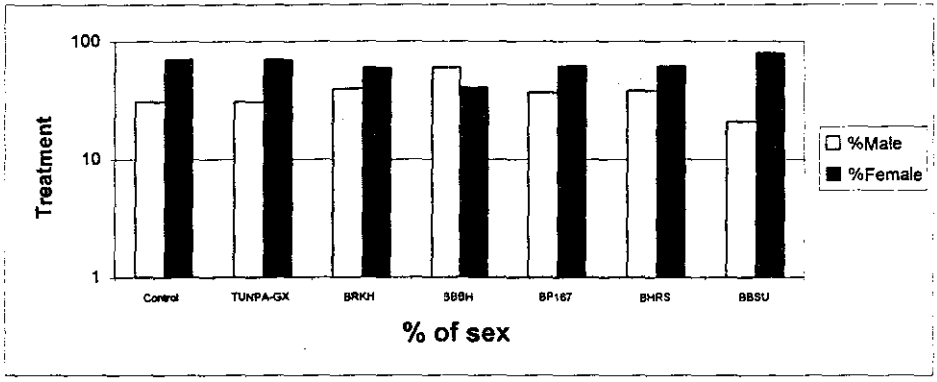

Fig.(6): Effect of certain F. brightener products on the sex ratio of *S. littoralis*.

DISCUSSION

Several records confirm the effect of Fluorescent brighteners as synergistic tool to increase the insect susceptibility to baculovirus (Shapiro and Vaughn 1995, Farrar and Ridgeway 1997, Shapiro and Argauer, 2001, El-Salamouny *et al.*, 2003). Screening of several brighteners to select suitable synergistic additives to increase the susceptibility of the insect was

previously studied by Li and Otovos (1999) for *Choristoneura fumiferana* and Shapiro and Argauer (2001) for *S. exigua*. In the present investigation, screening of several F. brighteners is made in order to find the most effective one for *S. littoralis*. Blankophor BBH showed an excellent enhancement factor which agree with that found by Farrar and Ridgeway, 1997, Li and Otovos, 1999 and Shapiro and Argauer 2001. Also, Tinopal UNPA-GX showed a high rate of synergism which agrees with that found by El-Salamouny *et al.*, (2003) and Mukawa *et al.*, (2003). However it was difficult to get a linear relation for Blankophor RKH, because this product was not soluble in water. Blankophor BSU did not enhance the activity of the tested virus which agree with that found by Li and Otovos (1999). Blankophor HRS and P167 had synergistic effect, as was obtained by Shapiro and Argauer (2001). The limited rate of enhancement (does not exceed 100%) may be explained in view of previous finding of El-Salamouny *et al.*, (1997) which showed a low rate of enhancement of the homologous virus than the heterologous ones. However, reducing of the LT_{50} value as a result of adding F. brightener to the virus agrees with that finding by Li and Otovos 1999a&b, Shapiro 2000 & Shapiro and Argauer 2001) where they reported a short LT_{50} values as a result of adding the f. brightener to the virus.

The different rate of synergism among the tested additives is due to the chemical composition and the binding effect. The differences in the relative ranking of the active fluorescent brightener might, however vary from host-virus system to another. For example, Blankophor HRS was most active against *S. exigua* system but was not active as Tinopal LPW, Blankophor BBH,Blankophor P167 and Blankophor RKH in the gypsy moth system.

Regarding the effect on some biological aspects of *S. littoralis* larvae, no previous results have mentioned a direct effect of the fluorescent brightener on biological aspects of the insects. The decrease in the larval weight agreed with that previously found by El-Salamouny *et al.*, (2003) on *Agrotis segetum* larvae when he reported a sharp decrease in the larval weight as a result of adding F. brightener. This effect could be due to the effect of these materials by altering the peritrophic membrane (PM) in the midgut. The present investigation showed that no effect took place on the duration of the pupal stage because the site of action of this material is the peritrophic membrane which is only in the larval midgut (Wang and Granados, 2000 and El-Salamouny *et al.*, 2003). The study open the door for more studies about the effect of synergistic additives on the biology of the test insects.

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تأثير إضافة مجموعة من العواكس الفلورسنتية على حساسية دودة ورق القطن لفيروس الباكلوفيروس: التأثير على بعض المظاهر البيولوجية.

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إن إضافة مادة العواكس الفلورسنتية (Fluorescent brightener 28) بتركيز ٠,١% إلى فيروس البوليهيدروسز ا لخاص بدودة ورق القطن عمل على زيادة قابلية اليرقات للإصابه بالفيروس. تم الحصول على أعلى معدل لتثبيط الفيروس بمركب Blankophor HRS, Tinopal UNPA-GX, Blankophor BBHعند المقارنة ببافي المركبات الأخرى. فقد قل التركيز السام النصفى بشدة من $1.9x10^4$ فى حالة إستخدام الفيروس بمفرده إلى $4.94x10^2$, $2.3x10^2$بوليهيدرا/مل بينة بمعدل تثبيطي بلغ ٨٢,٦و ٧٨,٢ و ٣٨,٧ ضعف لهذه المركبات على التوالى. بينما أظهرت مركبات Blankophor RKH, Blankophor P167 and Blankophor BSU معدل تثبيطى أقل عن الثلاث مركبات السابقة.

لقد أدى إضافة المركب Tinopal UNPA-GX إلى سرعة حدوث موت(LT_{50}) اليرقات بمعدل يصل إلى ٢,٢٣ ضعف.

تم دراسة تأثير إضافة هذه المركبات على بعض النواحى البيولوجية لدودة ورق القطن مثل التأثير على وزن اليرقات ومدة الطور اليرقى ومدة طور العذراء والنسبة الجنسية للفراشات الناتجة. أدى إستخدام مركبات العواكس الفلورسنتية إلى نقص فى وزن اليرقات عند إستخدام المادة بمفردها ونقص أكبر عند خلطها بالفيروس وكان النقص مرتبطا بالقدرة التثبيطية للمركب. أيضا لوحظ أن م دة طور اليرقة كان أطول فى حالة هذه المركبات المنشطة. لم يلاحظ أى تأثير على مدة طور العذراء أو على النسبة الجنسية للفراشات الناتجة.

الدراسة الحالية تبين أهمية إستخدام كل من مركبى Blankophor BBH and Tinopal UNPA-GX كإضافات لزيادة فعالية المستحضر الفيروسى لفيروس دودة ورق القطن*Spli*MNPV.