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Positive Effect of Fluorescent Brightener-28 and Neemazal T/S on the Activity of *Agrotis segetum* Granulovirus Tested Against *Agrotis ipsilon* (Hufn.) Larvae (Lepidoptera: Noctuidae)

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ABSTRACT

Fluorescent brightener-28 and Neemazal T/S (Neem) were used at different concentrations to increase the efficacy of *Agse*GV tested against *Agrotis ipsilon* (Hufn.) neonate larvae. F. brightener tested alone, did not result in any larval mortality whereas, the rate of larval mortality due to virus was increased with increasing concentrations of F. brightener additive, in comparison with virus alone treatment. The rate of enhancement of virus activity reached 1096.678 fold at 0.1% concentration. The calculated LT_{50} value decreased from 11.47 to 4.66 days at the concentration of $3.85x10^7$ capsule/ml diet when combined with 0.1% F. brightener. Neemazal T/S was added at three different concentrations (1,5 and 10, ppm) to *Agse*GV suspension and tested against *A. ipsilon* neonate larvae. Obtained results demonstrated that the Neem oil poorly enhanced the activity of *Agse*GV by reducing the LC_{50} value from $3.59x10^7$ capsule/ml diet for the virus alone treatment to only $7.11x10^6$ capsule/ ml diet for the virus + Neem (at 10 ppm) treatment (potency = 5.057 fold). The calculated LT_{50} value decreased from14.13 days for the virus alone treatment to 13.14, 10.56 and 7.2 days for the tested Neemazal concentrations, respectively. In conclusion, the combination of F. brightener or Neemazal T/S with the baculovirus, *Agse*GV may improve the activity of virus formulations.

Key Words: *Agrotis ipsilon,* baculovirus, cutworm, fluorescent brightener 28, granulovirus, Neemazal, synergism.

INTRODUCTION

Lepidopteran insects such as cutworms can be very wasteful feeders, destroying far more plant seedlings than they consume, and are also polyphagous (Bourner *et al.*, 1992). The black cutworm, *Agrotis ipsilon* (Hufnagel) is considered one of the major agriculture pests, not only in Egypt but also in many countries allover the world (El-Hemaesy, 1969). In Egypt, *A. ipsilon* attacks about 50 plant species; the larvae cause considerable damage to both winter and spring crop seedlings (Amin and Abdin, 1997 and El-Malki *et al.*, 1998).

Virus as biocontrol agents, particularly baculoviruses (BV's) have been shown to be highly effective against these insect pests, as well as being host specific and environmentally friendly. These viruses have been successfully used against a wide range of agricultural and forest pests (Entwistle and Evans, 1985 and Boughton *et al.*, 1999). *A. ipsilon* showed to be susceptible to several viruses (*A. segetum* NPV and *A. ipsilon* NPV) (Sherlock, 1983; Caballero *et al.*, 1987), and in India & U.S.A. (*A. ipsilon* NPV) (Santhanam and Kumaraswami, 1984 and Boughton *et al.*, 1999).

Several studies reported successful use of *A. segetum* granulosis virus (*Agse*GV) for the control of *A. segetum* and the closely related *A. ipsilon* (Shah *et al.*, 1979), *A. ipsilon* and *A. exclamationis* (Zethner, 1980, Zethner *et al.*, 1987, Caballero *et al.*, 1990 & 1991)

Several additives e.g., Neem extract have been used to increase efficacy of several baculoviruses such as nucleopolyhedroviruses. For example, it was evaluated when tested in combination with the *Lymantria dispar* (*Ld*MNPV) on artificial diet. The extract decreased the time required for viral kill of the larvae but did not decrease the concentration of virus (LC_{50}) require for kill (Shapiro *et al.*, 1994). When Neemazal-T was included in the virus mixtures (*SpliNPV*, *Spex*MNPV, *Auca*MNPV & *Agse*NPV) a dose dependent increase in potency was detected. The effect was greatest for *Spodoptera littoralis* NPV, in which the effectiveness of the virus was increased 2.9-fold relative to normal NPV inoculum (El-Salamouny *et al.*, 1997). Neem seed kernel extract (NSKE) at 2.5 % enhanced the activity of NPV at 10^2 PIB's against *Helicoverpa armigera* on cotton leaves (Murugan and Jeyabalan, 1998).

It is known that F. brighteners act as UV protectants (Shapiro, 1992), but they can also act as viral enhancers by decreasing the amount of virus needed for a 50 % effective lethal concentration (LC_{50}). Several baculoviruses have been enhanced by F. brighteners, *e.g., S. frugiperda* MNPV (*Sf*MNPV) (Hamm and Shapiro, 1992), *Ld*NPV and *Auca*MNPV (Dougherty *et al.*, 1996), *Ange*NPV (Fuxa and Richter, 1998), *Anagrapha falcifera* NPV (Vail *et al.*, 1996). Shapiro and Vaughn (1995) found that the addition of Tinopal

LPW reduced the LC_{50}s for *Heze*SNPV, *Anfa*NPV, *Hear*NPV, *Game*MNPV and *Auca*MNPV and significantly decreased the LT_{50}'s.

Susceptibility of the black cutworm, *A. ipsilon* was increased by addition of Fluorescent brightener-28 (Tinopal LPW) to baculoviruses, *Agse*MNPV, *Auca*MNPV and *Mabr*MNPV by the rate of 1806, 1040 and 336 fold when the concentration of 0.1% F. brightener was used for the tested viruses, respectively (El-Salamouny *et al.*, 2001). Also, Boughton *et al.*, (2001) reported a potential effect of F. brightener on the newly isolated *A. ipsilon* MNPV. El-Salamouny *et al.*, (2003) reported that *Agse*GV can be enhanced more than *Agse*NPV against *A. segetum* larvae; they attributed that to the smaller size of GV's to NPV's.

Addition of Tinopal LPW to the heterologous NPVs resulted in a reduction of LC_{50} and LT_{50} values by more than 35 % (Shapiro and Hamm, 1999). The LC_{50} values for heterologous NPV were reduced by 130 fold for both *Spex*MNPV and *Auca*MNPV and by 300 fold for *Anfa*NPV. Also,it increased the activity of *Hear*NPV and *Gm*MNPV. Tinopal LPW reduced the LT_{50}'s for all NPV's by 30-40 % (Shapiro, 2000).Tinopal LPW and Tinopal CBS-X were compared as enhancers for the *S. littoralis* NPV (*Spli*NPV) and GV (*Spli*GV) on third larval instar of *S. littoralis* (Varagas and Granados, 1998). Addition of Tinopal reduced the LD_{50} values but the mortality time was not significantly influenced. Tinopal enhances the median lethal dose (LD_{50}) of *Se*MNPV (Zou and Young, 1996 and Murillo *et al.*, 2001).

Washburn *et al.* (1998) and Wang and Granados (2000) demonstrated that calcoflour could inhibit peritrophic membrane (PM) formation in five tested lepidopteran species. This inhibition increased the larval susceptibility to baculovirus infection. Continuous inhibition resulted in retarded larval development and mortality.

Therefore, the aim of the present study was to evaluate the impact of certain additives, with a special reference to natural products (Neemazal T/S) as well as F. brightener as a safe chemical (used in wash powder and tooth paste), on increasing the activity of baculoviruses.

MATERIALS AND METHODS

Test insects:

Laboratory colony of the black cutworm, *A. ipsilon* was established using a semi-synthetic diet described by Shorey and Hale (1965) except the exclusion of formaldehyde from diet ingredients in maintaining test larvae.

Tested Virus:

Purified granulovirus, *A. segetum* GV (*Agse*GV) was obtained from the Institute for Biological Control, Federal Research Centre for Agriculture and Forestry (BBA), Heinrichstr. 243, D-64287, Darmstadt, Germany. The viral suspension was stocked in Tris buffer pH 8 and stored at-20°C. Serial dilutions of the stock virus suspension were made in Tris buffer pH 8.

Additives:

Neemazal-T/S (Trifolio, Germany) was further diluted in distilled water and tested at five concentrations of 1, 5, 10, 20 and 50 ppm.

Fluorescent brightener- 28:

The F. brightener- 28 (Tinopal LPW), Sigma, Aldrich was used at concentrations of 0.05, 0.075 and 0.1% and mixed with the virus-diet mixture. The F. brightener was further diluted in distilled water and tested in the diet mixture. The pH of the brightener dilution 1 % was pH 8.

Bioassay:

The diet-incorporation bioassay was used for testing synergistic effect of different additives on virus.

Diet incorporation bioassay:

Standardization was based on the number of (capsules (PIB's) /ml of aqueous suspension. Insects were maintained at 26±2°C and 65±5 R.H. and bioassay tests were performed using the semi-synthetic diet.

Five ml of virus (with or without additives), resuspended from each treated sample, were mixed with 45 ml of semi-synthetic diet at a diet temperature below 40°C. The contaminated diet was then distributed into special bioassay plates (LICEFA, Bad-Salzuflen, Germany). The plate, measuring 14x7x2 cm, contains 50 cells. One newly hatched larva was placed into each cell. The plates were covered with tissue paper and 14x7cm glass plate and fixed with rubber bands.

All treatments were incubated at 26±2°C and 65±5 R.H. for 16 days. Mortality due to virus infection was recorded every two days and up to 16 days. The larval mortality in the control was determined.

Statistical analysis:

The data of bioassay results were subjected to probit analysis using the method described by Finney (1971). The relative potencies of the treatments were calculated according to the changes in LC_{50} value.

RESULTS AND DISSCUSSIONS

Effect of different additives on increasing effectiveness of *Agrotis segetum* GV against *A. ipsilon* larvae:

Neemazal-T additive:

A preliminary bioassay test was conducted to determine the sub-lethal concentrations of Neemazal-T tested alone against neonate *A. ipsilon* larvae.The results showed the anti-moulting and anti-feedant effects of Neemazal-T, particularly at the high concentrations.

In case of incorporation of the Neemazal-T with the diet, the rate of larval mortality was 0, 4, 23.80, 100 and 100 % for the tested Neemazal-T concentrations: 1, 5, 10, 20 and 50 ppm, respectively.

Increase the efficacy of *Agse*GV against *A. ipsilon* test larvae by the Neemazal T/S:

Five different Neemazal T/S concentrations (0, 1, 5, 10, 20 and 50 ppm) were tested as additives to *Agse*GV. The obtained LC_{50} values were $3.59x10^7$, $3.32x10^7$, $1.51x10^7$ and $7.11x10^6$ capsule /ml diet for the tested *Agse*GV alone treatment, 1,5 and 10 ppm, respectively. The potency values were 1.081, 2.379 and 5.057 fold at the respective concentrations of the Neemazal T/S (Fig. 1).

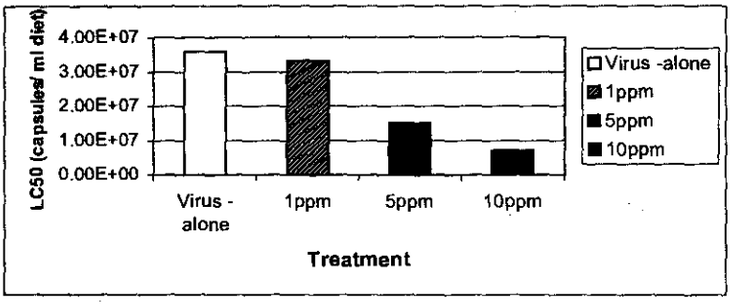

Fig. (1): Enhancement effect of Neemazal- T/S additive on infectivity of *A. segetum* GV against *A. ipsilon* neonate larvae.

At the highest GV concentration ($3.85x10^8$ capsule/ml diet), the LT_{50} value for the virus alone treatment was 5.4 days, while it was 7.5, 7.1, 6.8 and 6.5 days with Neem additive at the concentrations 1, 5, 10 and 20 ppm, respectively.

In case of the concentration of $1x10^8$ capsule/ml diet, the LT_{50} value for the virus alone treatment was 10.2 days, which decreased to 11.7, 10.4, 7.9 and 7.4 days with the Neem additive at the concentrations of 1,5,10,20 and 50 ppm, respectively (Fig.2).

Also, with the lowest concentration ($3.85x10^7$ capsule/ml diet) the estimated LT_{50} value for the tested virus alone treatment was 14.1 days, which decreased to 13.1, 10.6, 7.3 and 7.2 days with all abovementioned tested Neem additive concentrations, respectively. In conclusion, the estimated LT_{50} values for the tested virus alone treatment decreased with the gradual increase of Neem additive concentrations (Fig.2).

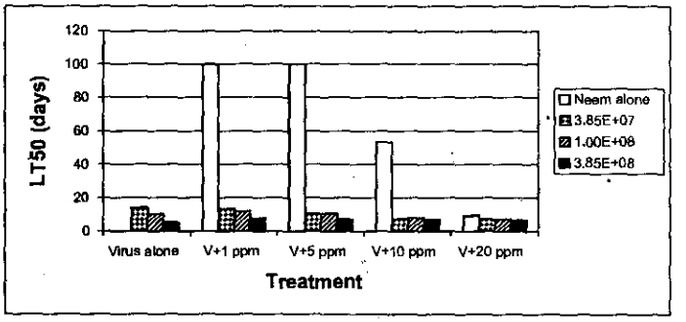

Fig. (2): Lethal median time (LT_{50}) values of *Agse*GV combined with different concentrations of Neemazal T/S against *Agrotis ipsilon* neonate larvae.

Increasing the efficacy of *Agse*GV against *A. ipsilon* test larvae by the Fluorescent brightener- 28 additive:

F. brightener- 28 (Tinopal LPW) additives was tested at 3 different concentrations (0.05, 0.075 and 0.1 % of diet). The results of diet incorporation bioassay revealed an enhancement effect at a concentration of 0.05 %. However, when F-brightener was tested alone, no larval mortality was obtained. The rate of larval mortality increased directly with the increase of F brightener- 28 concentration from 0.05 % to 0.075 and 0.1 % as compared to virus alone treatment.

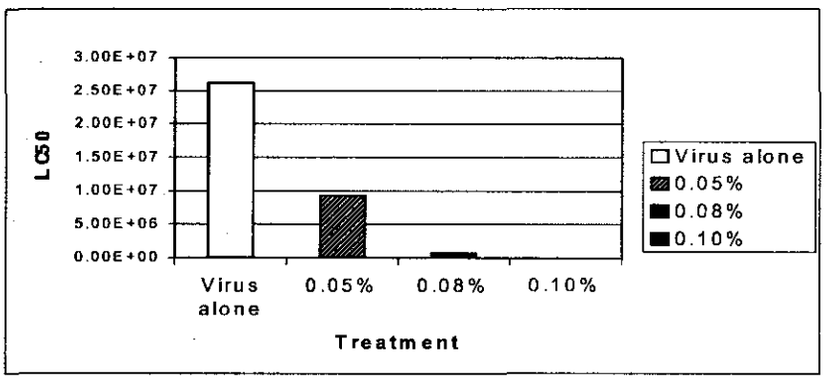

Fig. (3): Enhancement effect of F. brightener-28 additive on infectivity of *Agse*GV against *A. ipsilon* neonate larvae.

The LC_{50} value for the virus alone treatment was 2.62 x 10^7 capsule/ml diet. It decreased to 9.22 x 10^6, 6.21 x 10^5 and 2.39 x 10^4 capsule/ml diet at the tested F-brightener concentrations of 0.05, 0.075 and 0.1 %, respectively. The rates of enhancement (potency) were 2.84, 42.19 and 1096.67 fold, for the respective concentrations, and the slope values were 0.981, 0.718, 0.74 and 0.571, respectively (Fig.3).

Also, the LT_{50} value for the virus alone treatment was decreased with increasing the concentrations of *Agse*GV with F-brightener -28 in the diet (Fig.4).

The LT_{50} value for the virus alone treatment at 3.85x10^7 capsule/ml diet was 11.4 days, decreased to 8.03, 6.3 and 4.6 days with F-brightener additive at the concentrations of 0.05, 0.075 and 0.1 %, respectively. At the concentration 1 x 10^7 capsule/ml diets, the estimated LT_{50} value for the virus-alone treatment was decreased with the addition of the serial concentrations of F. brightener- 28 to 17.21, 9.56 and 6.21 days, respectively (Fig.4).

At the virus concentration of 3.85 x 10^6 capsule/ ml diet, the estimated LT_{50} values were 50.25, 24.25, 10.61 or 7.59 days for the virus alone treatment and that with F-brightener different concentrations, respectively (Fig.4).

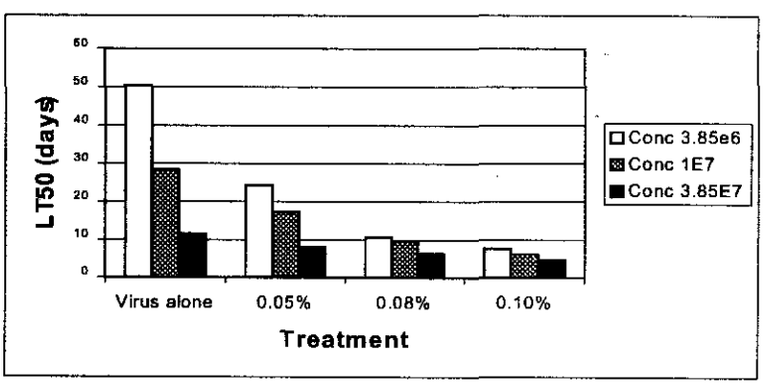

Fig. (4): Effect of different F. brightener- 28 additive concentrations on the LT_{50} values of *Agse*GV against *A. ipsilon* neonate larvae.

Synergistic effect:

Neem additive:

Neem seed kernel extract (NSKE) obtained from the Neem tree *Azadirachta indica* A. Juss (Meliaceae) is a demonstrated anti-feedant, insecticide and insect growth regulator against many insect species including

lepidopterans (Schmutterer, 1995). Evaluation of the combined efficacy of baculoviruses with Neem extract as an enhancing additive to GV virulence was previously reported.

In the present study, combination of Neem oil extract with *Agse*GV tested against *A. ipsilon* neonate larvae enhanced the activity of GV, measured by the decrease of the LC_{50} value from $3.59x10^{7}$ in the virus alone treatment to 7.11×10^{6} capsule/ml diet in the treatment of virus and Neem at 10 ppm (5.0567 fold). This result agrees with that of El-Salamouny *et al.* (1997). They found that Neemazal-T increased the infectivity of *Spli*NPV by 2.9 fold compared to the virus alone treatment.

The activity of NPV tested against *H. armigera* larvae was enhanced by the addition of NSKE (Neem seed kernel extract) at 2.5 % (Murugan and Jeyabalan, 1998). The combination of Neem oil at different concentrations (0.10-1.00 %) reduced the LC_{50} of *Spodoptera litura* NPV by 1.06 to 1.43 fold, respectively (Baskaran *et al.*, 1999). Present results also proved that the combination of neem oil with *Agse*GV decreased the LT_{50} value about 1.9 fold; from 14.1 days in the virus alone treatment to 7.2 days at virus concentration $3.85x10^{7}$ capsule/ml diet + Neem (10 ppm). The same result was observed with *Ld*NPV or *Spli*NPV (Shapiro *et al.*, 1994 and Baskaran *et al.*, 1999). The effect of Neem on larval weight in the present results was evident among the post-treatment survival *A.ipsilon* larvae. The observed reduction in larval weight (from 0.292 gm / larva in the control treatment and 0.243 gm / larva in the virus alone treatment to 0.003 gm /larva in the combined treatment) is probably due to the act of Neem as anti-feedent. This effect of Neem is well documented (Shapiro *et al.*, 1994; Cook *et al.*, 1996; Cook *et al.*, 1997 and Rabindra *et al.*, 1997). The mode of action of Neem is explained by its effect on the digestive enzyme activity and the biochemical composition in the midgut. One disadvantage of addition of Azadirachtin to viral formulation is fewer viruses produced and released into the environment (Cook *et al.*, 1996).

Fluorescent brightener-28:

Several reports proved that F. brightener-28 increases the efficacy of certain baculoviruses by decreasing LC_{50} and LT_{50} (Shapiro, 1992). In the present study, the addition of F. brightener-28 additive to the tested baculovirus, *Agse*GV has increased its virulence against *A. ipsilon* neonate larvae by reducing both the LC_{50} and LT_{50} values. These results are in agreement with those previously reported on the effect of F. brightener-28 as an enhancing additive to NPV virulence (Shapiro and Robertson 1992; Shapiro and Dougherty, 1994; Shapiro and Vaughan, 1995; Vail *et al.*, 1996; Zou and Young, 1996; Farrar and Ridgway, 1997; Shapiro, 2000; El-Salamouny *et al.*, 2003). It was also, observed that the rate of enhancement was higher with the heterologous system rather than with homologous one (Shapiro, 2000; El-Salamouny *et al.*, 2001). In the case of *Agse*GV, the LT_{50} was reduced from 11.47 to 4.66 days using the same additive. Similar results were reported by (Shapiro and Robertson, 1992; Hamm and Shapiro, 1992 and Zou and Young, 1996). Previous reports also mentioned that F. brightener-28 did not affect the tested insects (Shapiro, 1992). Dougherty *et al.* (1996) demonstrated that Tinopal LPW was effective only if it was presented with the virus at the time of ingestion. However, the combination of this additive with baculoviruses increases the rate of enhancement with increasing F. brightener concentration (Farrar and Ridgway, 1997). On the other hand, the rate of enhancement varied between different NPV's combined with F. brightener in the same host as concluded by Shapiro and Vaughn (1995). The addition of Tinopal LPW (1 %) to the heterologous NPV's reduced the LT_{50} (Shapiro and Hamm, 1999). Shapiro & Robertson, 1992; Adams *et al.*, 1994; Wang &Granados, 2000 and El-Salamouny *et al.*, (2003) suggested that selected brighteners inhibit or alter the chitinous peritrophic membrane (PM). Thus, greater numbers of virions penetrate the damaged PM, pass from the gut lumen into the heamocoel, and infect susceptible cells without a cycle of replication. It is worth mentioning that the effect of F. brightener-28 as an enhancement additive was tested for the first time with the *Agse*GV against the black cutworm, *A. ipsilon* in the present investigation.

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تأثير إيجابى لمركبات العواكس الفلوروسنتية و النيم على نشاط فيروس جرانيولو دودة اللفت القارضة (*AgseGV*) ضد يرقات حشرة الدودة القارضة السوداء

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أستخدمت مركبات كلا من العواكس الفلوروسنتية Fluorescent brightener 28 والنيم Neemazal T/S بتركيزات مختلفة لدراسة زيادة فعالية فيروس جرانيولو دودة اللفت القارضة *AgseGV* ضد اليرقات حديثة الفقس لحشرة الدودة القارضة السوداء *Agrotis ipsilon* Hb. · بينت النتائج أنه لم تعطى يرقات الدودة القارضة السوداء أى نسبة من الموت عند المعاملة بمادة العواكس الفلوروسنتية · بينما زاد معدل موت اليرقات بزيادة تركيز مادة العواكس الفلوروسنتية المضافة مقارنة بمعاملة الفيروس بمفرده· وكان أعلى معدل للزيادة التنشيطية هو١٠٩٦،٦٧٨ ضعف عند التركيز ٠،٠١% من المادة· كما نقص الوقت المميت النصفى من ١١،٤٧ الى ٤،٦٦ يوم عند تركيز الفيروس ١٠x٣،٨٥^{٢} كابسول /ملى من البيئة الغذائية وذلك عند التركيز ٠،٠١% من المادة· أضيف مركب Neemazal T/S الى فيروس *AgseGV* عند ثلاث تركيزات هى ١، ٥ و١٠ % وأختبر ضد يرقات الدودة القارضة السوداء حديثة الفقس· وقد أظهرت النتائج أن زيت النيم لم يؤد الى زيادة فى نشاط الفيروس كثيرا حيث أن قيمة التركيز المميت النصفى (LC_{50}) قد إنخفضت من $3.59x10^{7}$ فى حالة المعاملة الفيروسية المنفردة إلى فقط $7.11x10^{6}$ Capsule/ ml فى حالة معاملة الفيروس + النيم عند التركيز ١٠ ppm (= ٥،٠٥٧ ضعف)، كما نقص الوقت المميت النصفى من ١٤،١٣ يوم فى حالة الفيروس بمفرده الى ١٣،١٤، ١٠،٥٦ ، ٧،٠٢ يوم لتركيزات Neemazal T/S على التوالى· ويستنتج من ذلك أن إضافة مركب العواكس الفلوروسنتية أو النيم (Neem) لفيروسات الجرانيولو فيروس *AgseGV* يمكن أن يحسن من فعالية المستحضرات الفيروسية·