ACTIVATION OF STORED *SPODOPTERA LITTORALIS* NUCLEAR POLYHEDROSIS VIRUS (*SPLI*NPV) THROUGH THE ADDITION OF INORGANIC SULPHATE SALTS

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Abstract

In the present study, the potential of inorganic sulphate salts (copper sulphate and iron (II) sulphate) as triggers for activation of stored *Spli*NPV in *S. littoralis* 4th instar larvae was evaluated under laboratory conditions. Obtained results showed that the ability of sulfate salts to activate infections varied according to the type of tested chemical. Results also showed that copper sulphate and iron sulphate triggered lethal effect of polyhedrosis disease in larvae with range of 10.0-20.0%. In addition, results showed that observed larval mortality due to treatment with copper sulphate and iron (II) sulphate ranged from zero to 45%. This might be due to the intrinsic toxicity of the tested compounds. It was noticed that copper sulphate alone at different concentrations caused higher mortality percentage than iron (II) sulphate. It could be concluded that trace metals such as copper and iron can clearly modulate baculovirus induced disease, probably due to their different roles in insect immune functions. Further work is required to identify useful virus activation substances under field conditions.

Keywords: *Spli*NPV, chemical triggers, larval-mortality, copper sulphate, iron (II) sulphate.

INTRODUCTION

Because of their notable insecticidal properties, evident robustly host specificity and their outstanding biosafety characteristics, nuclear polyhedrosis viruses (NPVs) (Family: Baculoviridae) are being developed as biocontrol agents for lepidopteran pest insects (Eberle *et al*., 2012). The viral infection is induced when the target pest larvae ingest the virions that are occluded in polyhedral occlusion bodies (OBs) (Lua *et al*., 2003). Following infection, many infected insects would die in a period of several days. Although, there might be a part of treated insects survive treatment. This can be either because some individuals don't consume enough lethal OBs or because that the viral infection has not been initiated. The later may be due to inhibition of the viral infection by the host immune system (Marques and Carthew, 2007 and Pascual *et al*., 2012), or due to the virus adopting a low virulence non-lethal infection strategy (Sorrell *et al*. 2009; Virto *et al*., 2013; Moreno-García *et al*., 2014;

and Virto *et al*., 2016), or a combination of both. Early studies suggested that stressful conditions related to nutrient availability or the presence of other pathogens may trigger the activation of covert infections across many species of Lepidoptera (Podgwaite and Mazzone 1986). More recently, more systematic approaches have focused on rearing conditions such as high population densities (Opoku-Debrah *et al*., 2013), high relative humidity or ingestion of chemical compounds (Ilinykh *et al*., 2004). Additionally, a number of studies have reported the activation of covert infections following inoculation with viruses that naturally infect a different species of host insect, known as heterologous viruses (Kouassi *et al*., 2009).

The present study aims to evaluate the effect of the addition of two inorganic sulphate salts; Copper and Iron (II) sulphate, in different concentrations as chemical stressors that might trigger the activation of the stored SpliNPV infections in *Spodoptera littoralis* larvae, under laboratory conditions, with the purpose of estimating the value of this strategy in the control of *S. littoralis* populations on field crops.

MATERIALS AND METHODS

1. Insect cultures and virus stock:

A susceptible strain of *S. littoralis* was established from egg masses obtained from the Research Division of the cotton leaf worm, Plant Protection Research Institute, Dokki-Giza, Egypt. Newly hatched larvae were reared under laboratory conditions in an incubator at 26 ± 2º C, of 65 ± 10% R. H., and 8:16 L: D photoperiod at the microbial agent production unit, Plant Protection Research Institute, Dokki-Giza, Egypt.

The viral isolate of *Spli*NPV (Baculoviridae) was provided by viral insecticide production Laboratory (microbial agent production unit, Plant Protection Research Institute, Dokki-Giza, Egypt) in the form of a suspension in sterile distilled water, with a stock concentration of 2.4×10^{10} polyhedral inclusion bodies (PIB)/ml.

2. Purification of occlusion bodies (polyhedra) of *Spli*NPV:

Occlusion bodies were purified following the methods of Hunter-Fujita *et al*., (1998). Briefly, infected *S. littoralis* cadavers were collected and homogenized in 0.05M Tris, pH 7.5-7.8, 0.1 % sodium dodecyl sulfate (SDS) (2 ml buffer/g larva). The homogenate was filtered twice through a piece of muslin and cotton wool to eliminate the insect fragments. Filtrate was clarified three times by centrifuging at 5000 rpm for 3 min. The supernatant containing the polyhedra was centrifuged at 3000 rpm for 10 min under cooling (4ºC). The pellets of semi-purified polyhedra were re-suspended in Tris-SDS and centrifuged at 4000 rpm for 10 min under cooling. Finally, the pellets of

the purified polyhedra were re-suspended in sterile distilled water (1 ml H_2O/g viral precipitate), counted using a bacterial counting chamber under phase contrast microscopy at × 400, and stored at -20ºC until required.

3. Activation of *Spli*NPV infections under laboratory conditions:

For virus activation test, three replicates of 20 4th instar larvae were used. Three groups were designated. The first group was orally treated by leaf dipping technique with one of the following chemical compounds: 0.1, 0.5, 0.75, 1.0, and 2.0% (wt./vol.) copper sulphate and iron (II) sulphate (El-Gomhoria chemical and pharmaceutical company, Cairo, Egypt). The second group of larvae was treated with the previously stored viral isolate of *Spli*NPV at the concentration of 2.4×10^{10} polyhedral inclusion bodies (PIB)/100ml. The third group of larvae was treated with a mixture of used chemicals at different concentrations mixed with the used viral isolate at the ratio of 1/2:1/2. Identical treatments were performed using virus-free control insects.

4. Statistical analysis:

Mortality was recorded daily after 48 hours post treatment and cumulative larval mortality was determined at the end of the larval stage. Mortality percentage was corrected according to Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

1. Reactivation of *Spli*NPV by inorganic sulphate salts:

Results obtained from table (1) showed that 18.33% of treated 4th instar larvae of *Spodopter littoralis* responded to the used concentration of the previously stored *Spli*NPV. Results also showed that the larval mortality observed due to the addition of copper sulphate and iron (II) sulphate to the *Spli*NPV was enhanced to about 20.0% compared to NPV alone. Results also showed that addition of copper sulphate at 0.1, 0.5 and 0.75% to the NPV used concentration induced larval mortality of 15.0, 16.66, and 13.33%, respectively. Higher concentrations of copper sulphate induced either low or no mortality. Further, addition of iron (II) sulphate concentration of 0.1, 0.5, and 0.75% to used concentration of NPV resulted in 20.0, 13.33, and 10.0% larval mortality, respectively (table 1). Virus-killed larvae did not show the typical symptoms of polyhedrosis disease, specifically tissue liquefaction although larval mortality observed. In addition, results showed that observed larval mortality due to treatment with copper sulphate and iron (II) sulphate ranged from zero to 45%. This might be due to the intrinsic toxicity of the tested compounds (table 1). It was noticed that copper sulphate alone at different concentrations caused higher mortality percentage than iron (II) sulphate.

In the present study, we evaluated the potential of a selection of inorganic sulphate salts as triggers for activation of stored *Spli*NPV in *S. littoralis* larvae. The ability to activate infections varied according to the type of chemical being tested. Our results showed that copper sulphate and iron sulphate triggered lethal polyhedrosis disease in 10.0-20.0% of larvae. Trace elements such as copper and iron are essential for immune system function in insects (Popham *et al*., 2012a). Considering each of these metals in turn, virus infection significantly altered copper titres in the haemolymph of *Heliothis virescens*, but not in *Helicoverpa zea*, compared with those of healthy larvae (Popham *et al*., 2012a). Indeed, excess or deficiency of copper can strongly influence insect responses to infection. For example, Ilinykh *et al*. (2004) previously reported activation of *Lymantria dispar* multiple nucleopolyhedrovirus (*Ld*MNPV) in up to 18% of gypsy moth larvae that fed on diet containing 0.6% copper sulphate. In contrast, injection of *H. zea* larvae with a copper chelating compound increased the virulence of the homologous NPV (Washburn *et al*. 1996), possibly via its role at the active site of the phenoloxidase enzyme, a key component of the insect immune response to pathogens (Shelby and Popham, 2006). Despite the importance of iron availability in insects during infection by bacterial pathogens (Dunphy *et al*., 2002 and Watson *et al*., 2010), the role of this metal during baculovirus infection is poorly understood. Divalent and trivalent iron cations markedly reduced the pathogenicity of *Ld*MNPV OBs when administered simultaneously with OB inoculum (Shapiro, 2001). In a later detailed study, Popham *et al*. (2012b) demonstrated that dietary iron was retained in larval tissues and increased significantly in the plasma late in infection in *Heliothis virescens* larvae. In the present study, iron (II) sulphate was as effective as copper sulphate in *Spli*NPV activation, when used at the same concentrations. The examination of factors involved in virus activation allowed us to explore this as an alternative strategy for pest control. The possibility of triggering lethal diseases and initiating viral epizootics could improve the effectiveness and reduce the cost of baculovirus-based control methods and could also contribute to the design of alternative or complementary strategies of microbial control instead of, or in addition to, the inundative use of baculoviruses as insecticides. However, further work is required to identify useful virus activation substances under field conditions. We conclude that trace metals such as copper and iron can clearly modulate baculovirus induced disease, probably due to their different roles in insect immune functions.

Table 1. Percentage of virus-induced mortality and mortality due to other causes in virus-free *S. littoralis* larvae after treatment with copper sulphate and iron (III) sulphate at different concentrations under laboratory conditions

Treatment	Concentration	% Mortality of virus-free larvae (n)
Copper Sulphate ($CuSO_4$) +NPV	**0.10%**	15.00 (9)
	0.50%	16.66 (10)
	0.75%	13.33 (8)
	1.0%	3.33 (2)
	2.0%	0.00 (0)
Iron (II) Sulphate ($FeSO_4$) +NPV	**0.10%**	20.00 (12)
	0.50%	13.33 (8)
	0.75%	10.00 (6)
	1.0%	5.00 (3)
	2.0%	1.66 (1)
Copper Sulphate ($CuSO_4$)	**0.10%**	0.0 (0)
	0.50%	1.66 (1)
	0.75%	5.00 (3)
	1.0%	35.00 (21)
	2.0%	45.00 (27)
Iron (II) Sulphate ($FeSO_4$)	**0.10%**	0.0 (0)
	0.50%	0.0 (0)
	0.75%	1.66 (1)
	1.0%	3.33 (2)
	2.0%	5.00 (3)
NPV	**2.4×10^{10} (PIB)/100ml**	18.33 (11)
Control	**--**	0.0

REFERENCES

1. Abbott W. S. 1925. A method of computing the effectiveness of an insecticide. J. Eco. Entomol.; 18: 265-267.
2. Dunphy, G. B.; D. F. Niven; and J. S. Chadwick. 2002. Iron contributes to the antibacterial functions of the heamolymph of *Galleria mellonella*. J. Insect Physiol.; 48: 903-914.
3. Eberle, K. E.; J. A. Jehle; and J. Huber. 2012. Microbial control of crop pests using insect viruses. In: Integrated pest management: principles and practice. Ed. by Abrol, D. P. and Shankar, U. CAB International, Wallingford, UK, 281-298.
4. Hawtin, R. E.; T. Zarkowska; K. Arnold; C. J. Thomas; G. W. Gooday; L. A. King; J. A. Kuzio; and R. D. Possee. 1997. Liquefaction of *Autographa californica* nucleopolyhedrovirus infected insects is dependent on the integrity of virus encoded chitinase and cathepsin genes. Virol., 238: 243-253.
5. Hunter-Fujita, F. R.; P. F. Entwistle; H. F. Evans; and N. E. Crook. 1998. Insect Viruses and Pest Management. New York. John Wiley and Sons. 632 p.
6. Ilyinykh, A. V.; M. V. Shternshis; and S. V. Kuzminov. 2004. Exploration into a mechanism of transgenerational transmission of nucleopolyhedrovirus in *Lymantria dispar* L. in Western Siberia. BioControl, 49(4): 441-454.
7. Kouassi, L. N.; K. Tsuda; C. Goto; S. Mukawa; Y. Sakamaki; K. Kusigemati; and M. Nakamura. 2009. Prevalence of latent virus in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) and its activation by a heterologous virus. Appl. Entomol. Zool.; 44: 95-102.
8. Lua, L. H. L.; L. K. Nielsen; and S. Reid. 2003. Sensitivity of *Helicoverpa armigera* nucleopolyhedrovirus polyhedra to sodium dodecyl sulfate. Biol. Control, 26: 57-67.
9. Marques, J. T. and R. W. Carthew. 2007. A call to arms: coevolution of animal viruses and host innate immune responses. Trends Genet.; 23: 359-364.
10. Moreno-García, M.; R. Condé; R. Bello-Bedoy; and H. Lanz-Mendoza. 2014. The damage threshold hypothesis and the immune strategies of insects. Infect. Genet. Evol.; 24: 25-33.
11. Opoku-Debrah, J. K.; M. P. Hill; C. Knox; and S. D. Moore. 2013. Overcrowding of false codling moth, *Thaumatotibia leucotreta* (Meyrick) leads to the isolation of five new *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) isolates. J. Invert. Pathol.; 112: 219-228.

12. Pascual, L.; A. K. Jakubowska; J. M. Blanca; J. Cañizares; J. Ferré; G. Gloeckner; H. Vogel; and S. Herrero. 2012. The transcriptome of *Spodoptera exigua* larvae exposed to different types of microbes. Insect Biochem. Molec.; 42: 557-570.
13. Podgwaite, J. D. and H. M. Mazzone. 1986. Latency of insect viruses. Adv. Virus Res.; 31: 293-320.
14. Popham, H. J. R.; R. Sun; K. S. Shelby; and J. D. Robertson (2012a). Changes in trace metals in hemolymph of baculovirusinfected noctuid larvae. Biol. Trace Elem. Res.; 146: 325-334.
15. Popham, H. J. R.; R. Sun; K. S. Shelby; and J. D. Robertson. (2012b). Iron levels change in larval *Heliothis virescens* tissues following baculovirus infection. Biol. Trace Elem. Res.; 148: 356-362.
16. Shapiro, M. 2001. The effects of cations on the activity of the gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. J. Econ. Entomol.; 94: 1-6.
17. Shelby, K. S. and H. J. R. Popham. 2006. Plasma phenoloxidase of the larval tobacco budworm, *Heliothis virescens*, is virucidal. J. Insect Sci.; 6: 1-12.
18. Sorrell, I.; A. White; A. B. Pedersen; R. S. Hails; and M. Boots. 2009. The evolution of covert, silent infection as a parasite strategy. P. Roy. Soc. B-Biol. Sci.; 276: 2217-2226.
19. Virto, C.; D. Navarro; M. M. Tellez; R. Murillo; T. Williams; and P. Caballero. 2013. SeMNPV reactivation through stress factors in covertly infected *Spodoptera exigua*. IOBC-WPRS Bulletin, 90: 203-205.
20. Virto, C.; D. Navarro; M. M. Tellez; R. Murillo; T. Williams; and P. Caballero. 2016. Chemical and biological stress factors on the activation of nucleopolyhedrovirus infections in covertly infected *Spodoptera exigua*. J. App. Entomol.; 141(5): 384-392.
21. Washburn, J. O.; B. A. Kirkpatrick; E. Haas-Stapleton; and L. E. Volkman. 1998. Evidence that the stilbene-derived optical brightener M2R enhances *Autographa californica* M nucleopolyhedrovirus infection of *Trichoplusia ni* and *Heliothis virescens* by preventing sloughing of infected midgut epithelial cells. Biol. Control, 11: 58-69.
22. Watson, R. J.; P. Millichap; S. A. Joyce; S. Reynolds; and D. J. Clarke. 2010. The role of iron uptake in pathogenicity and symbiosis in *Photorhabdus luminescens* TT01. BMC Microbiol.; 10: 1-12.

استخدام بعض أملاح الكبريتات الغير عضوية لتنشيط الفيروس النووي لدودة ورق القطن

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هدفت الدراسة الحالية إلى تقييم فعالية بعض أملاح الكبريتات الغير عضوية (كبريتات النحاس، كبريتات الحديد) كعامل منشط للفيروس النووي لدودة ورق القطن. وقد أظهرت النتائج قدرة الأملاح المستخدمة لتنشيط كفاءة الفيروس تبعاً لنوع الملح المستخدم. أظهرت النتائج أن أملاح كبريتات النحاس وكبريتات الحديد زادت من فاعلية الفيروس كمسبب مرضي لليرقات بنسبة من ١٠-٢٠%. كما أظهرت النتائج أن المعاملة باستخدام الأملاح منفردة سببت نسب موت تتراوح من ٠-٤٥% وقد يرجع ذلك للسمية الفعلية للأملاح المستخدمة. وقد لوحظ أن استخدام كبريتات النحاس بتركيزات مختلفة سببت أعلى سمية مقارنة بكبريتات الحديد. وقد دلت النتائج على فاعلية أملاح النحاس والحديد لتنشيط كفاءة الفيروس كمسبب مرضي نتيجة لرد الفعل المناعي للحشرة المصابة. ومازالت بعض الأملاح تحتاج لمزيد من الدراسة لتحديد قدرتها على تنشيط كفاءة الفيروس فى ظروف حقلية.