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Effect of Individual and Combined Treatment with Azadirachtin and Spodoptera littoralis Multicapsid Nucleopolyhedrovirus (SpliMNPV, Baculoviridae) on the Egyptian Cotton Leafworm Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae)

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Abstract. The tetranortriterpenoid, azadirachtin, and the entomopathogenic virus, nucleopolyhedrovirus, are used as safe and new control measures for combating agricultural insect pests instead of the use of synthetic insecticides. They can be mixed together as an integrated pest management strategy. Thus, the current investigation was designed to determine the mortality, duration and weight gain of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae, and the yield of *Spodoptera littoralis* multicapsid nucleopolyhedrovirus (SpliMNPV) (Baculoviridae) when the fourth larval instars were treated individually with the LC_{50} of azadirachtin and of SpliMNPV, and in combination with each other using the LC_{25}, compared to non-treated larvae (control). The results obtained showed that combined treatment significantly enhanced the larval mortality by about 58.40 %, *i.e.* potentiation. Both individual and combined treatment significantly decreased the larval weight gain, whereas the larval duration was significantly increased, with the highest change in case of combined treatment. Azadirachtin–NPV mixture significantly decreased the viral yield (number of polyhedral inclusion bodies/g fresh larval body weight) by about 36.05 % compared to the individual treatment with the NPV. It can be concluded that although azadirachtin enhanced the pathogenicity (% larval kill) of SpliMNPV to *S. littoralis*, azadirachtin–SpliMNPV mixture is unlikely to be useful for the mass production of this viral isolate. Thus, these laboratory observations require validation in field studies under commercial growing conditions.

Key words: *Spodoptera littoralis*, azadirachtin, duration, weight gain, mortality, nucleopolyhedroviruses, viral yield.

Introduction

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is highly polyphagous infesting about 87 host plants including important field crops and various fruits and ornamental trees (TIESSEN, 2012).

Conventional pesticides, particularly organophosphorus and pyrethroid insecticides, are generally used for combating agricultural insect pests, but their indiscriminate usage may cause environmental pollution, toxic residual effect, development of pest resistance, negative impacts on non-target organisms and adverse effects on human and animal health (SUNDARARAJ, 1997). These problems forced to search for new control measures especially from plant sources, as plant-derived molecules are eco-friendly, bio-degradable, target specific and development of resistance by pests against them has not been reported so far (GAUTAM *et al.*, 2013). Today about 200 plants with insecticidal activities are known (SINGH *et al.*, 2001). Among these plants, the neem tree

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(*Azadirachta indica* A. Juss) (Meliaceae) has received a worldwide attention, where the active ingredient isolated from its seeds is a tetranortriterpenoid compound called azadirachtin, which acts as a potent insect growth regulator (REMBOLD *et al.*, 1982; KUBO & KLOCKE, 1982). The insecticidal activity of azadirachtin to nearly 550 insect species, including lepidopteran ones, was also reported (ABD EL-GHANY *et al.*, 2012; WONDAFRASH *et al.*, 2012; RADWAN & EL-SHIEKH, 2012; HUMMEL *et al.*, 2012).

On the other hand, nucleopolyhedrovirus (NPV) (Baculoviridae) is a natural microbial pathogen of Lepidoptera, which is an ideal tool in integrated pest management programs, highly specific to its host insects, safe to the environment, humans, other plants and natural enemies (YANG *et al.*, 2012). However, the narrow host range, slow speed of kill, large dose requirement and resistance development are some of the problems which have limited its use. These concerns have opened new arenas for the improvement of NPV formulations (MOSCARDI, 1999). In this context, the effect of NPV has been enhanced by combining with adjuvants like neem seed extracts (KUMAR *et al.*, 2008; BAJWA & AHMAD, 2012; WAKIL *et al.*, 2012; ZAMORA-AVILÉS *et al.*, 2013). Accordingly, there is a need to evaluate the species specific NPV either individually or in combination with synergists to understand its compatibility and efficiency.

Therefore, the objective of the present study is to elucidate the effect of azadirachtin and *Spodoptera littoralis* multicapsid nucleopolyhedrovirus (SpliMNPV), either alone or in combination with each other, on mortality, duration and weight gain of *S. littoralis* fourth larval instars, and on the yield of this viral isolate. The data may be helpful in formulating some control strategy for this pest.

Material and Methods

Insects. A susceptible strain of *S. littoralis* was established from egg masses obtained from Faculty of Agriculture, Cairo University, Egypt. Newly hatched larvae were transferred into plastic boxes (18×12×10 cm) containing about 0.5 cm thick semi-synthetic diet on which larvae were fed (SHOREY & HALE, 1965). The pupae were sexed and placed in sterilized containers (18×15×30 cm) for adult emergence. The containers were lined with tissue paper as an ovipositing stratum. Adult moths were fed on 10% sucrose solution. Insects were reared under laboratory conditions of 25–27ºC, 65–70% RH and 12h: 12h (L: D) photoperiod.

SpliMNPV and azadirachtin. The viral isolate SpliMNPV (Baculoviridae) was provided by Entomovirology Laboratory (Faculty of Agriculture, Cairo University, Egypt) in the form of a suspension in sterile distilled water, with a stock concentration of 3.4×10^{10} polyhedral inclusion bodies (PIB)/ml. This suspension was stored at -20ºC till use. Azadirachtin (96% purity) was obtained as a powder from Carl Roth GmbH + Co. Kg., and a stock solution (200 ppm) was prepared in sterile distilled water and stored at 4ºC till use (KUMAR *et al.*, 2008; WAKIL *et al.*, 2012; ZAMORA-AVILÉS *et al.*, 2013).

Purification of occlusion bodies (polyhedra) of SpliMNPV. Occlusion bodies were purified following the methods of HUNTER-FUJITA *et al.* (1998). 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, using a Beckman J2-21 MIE centrifuge and a 20 JA rotor. 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.

Mortality, development and growth of larvae, and viral yield. A preliminary

experiment was carried out to estimate the LC_{25} and LC_{50} values of SpliMNPV and azadirachtin against newly molted *S. littoralis* fourth larval instars, following the technique of treatment of the surface of semi - synthetic diet (ZAMORA-AVILÉS *et al.*, 2013), with minor modifications. Five viral concentrations (3.4×10^9, 3.4×10^8, 3.4×10^7, 3.4×10^6 and 3.4×10^5 PIB/ml) and six serial concentrations of azadirachtin (50, 25, 12.5, 6.25, 3.125 and 1.5625 ppm) were prepared. One ml of each concentration was poured onto the surface of the same semi - synthetic diet used for rearing, thoroughly swirled and air-dried. The treated diet was offered for 24 h to 4 h-previously starved fourth larval instars. Then, the larvae were fed on fresh untreated diet until pupation or death. The control experiment consisted of larvae fed on the same diet treated with distilled water only. Each concentration was repeated three times of 50 larvae each. The number of dead larvae was recorded. All the experiments were incubated at 25ºC. The same above procedures were also followed in case of studying the combined effect of SpliMNPV and azadirachtin, using a mixture of NPV and azadirachtin (LC_{25}: LC_{25}) (v/v).

The duration and weight gain of larvae treated alone with the LC_{50} of azadirachtin and of SpliMNPV, or in combination with the LC_{25} of azadirachtin and of SpliMNPV were recorded. In each treatment, larvae were individually weighed, SpliMNPV was purified from each larva and the number of PIB was counted following the technique described above. The concentration of PIB was expressed as the number of PIB/g fresh larval body weight. The experiment was repeated three times.

Statistical analysis. The percentage of mortality of treated larvae was corrected against that of non - treated ones (control) using Abbott's formula (ABBOTT, 1925) as follows: Corrected mortality (%) = (% Observed mortality - % Control mortality/100 - % Control mortality) 100. The corrected mortality was then subjected to propit analysis (FINNEY, 1971), from which the LC_{25} and LC_{50} were estimated. The correlation coefficient "*r* - value" was also determined for the dose – mortality relationship.

The interaction between azadirachtin and the virus, in relation to larval mortality, was differentiated according to the co-toxicity factor (MANSOUR *et al.*, 1966) as follows: Co - toxicity factor (%) = (%Observed mortality - %Expected mortality/%Expected mortality) 100, where a positive factor of 20 or more is considered as potentiation, a negative factor of 20 or more is considered as antagonism, while intermediate values (-20 and +20) indicate additive effect.

The toxicity bioassay was determined with POLO–PC software (LEORA SOFTWARE, 1987). Data were analyzed by one–way analysis of variance (ANOVA) using Costal Statistical Software (Cohort Software, Berkeley). When the ANOVA statistics were significant (at $P<0.05$), the means were compared by Duncan's multiple range test (SAS INSTITUTE INC., 2008).

Results

The bioassay test (Table 1) revealed that the mortality of the fourth larval instars of *S. littoralis* increased progressively with the increase of the viral concentrations (r = 0.995), with LC_{25} and LC_{50} values of 10.10×10^6 and 8.43×10^8 PIB/ml distilled water, respectively. The same pattern was also attained for the larval treatment with azadirachtin (r = 0.850), with LC_{25} and LC_{50} values of 9.95 and 23.15 ppm, respectively.

The results demonstrated (Fig. 1) show that the larval mortality (79.20 ± 3.85%) obtained due to the combined treatment with the LC_{25} of SpliMNPV and of azadirachtin was significantly enhanced ($P<0.05$) to about 36.05 % of that recorded as a result of treatment with the LC_{50} of the virus alone (50.23 ± 2.95%). Thus, the LC_{25} of SpliMNPV combined with that of azadirachtin became approximately ≥ the LC_{79}, instead of being theoretically equal to the LC_{50}. The estimated co - toxicity factor was 58.40%.

The weight gain of larvae treated alone or in combination with SpliMNPV and azadirachtin was significantly reduced ($P<0.05$) compared to the control. Combined

treatment reduced the larval weight by about 53.70 and 19.35% compared to the single treatment with the virus and azadirachtin, respectively (Table 2).

In the present study, single and combined treatment of *S. littoralis* larvae with azadirachtin and NPV significantly increased (P<0.05) the larval duration compared to the control, with the highest magnitude in case of combined treatment (Table 2).

Combining azadirachtin with SpliMNPV resulted in a significant decrease (P<0.05) in the viral yield by about 36.05% compared to the infection with the virus alone. The decrease in the viral yield was concomitant with the decrease in the larval weight gain (Table 2).

Table 1. Susceptibility of *Spodoptera littoralis* fourth larval instars to the entomopathogenic virus SpliMNPV and azadirachtin.

Treatment	LC_{50}	95% Fiducial limits		LC_{25}	95% Fiducial limits		Slope	χ^2 (d.f.)
		Lower	Upper		Lower	Upper		
SpliMNPV	8.43×10^8 (PIB/ml)	4.22×10^8 (PIB/ml)	2.53×10^9 (PIB/ml)	10.10×10^6 (PIB/ml)	5.05×10^6 (PIB/ml)	3.03×10^7 (PIB/ml)	0.3510	$\chi^2_{(3)}$ = 3.45
Azadirachtin	23.15 (ppm)	9.26 (ppm)	45.84 (ppm)	9.95 (ppm)	4.70 (ppm)	13.02 (ppm)	0.8906	$\chi^2_{(4)}$ = 5.23

Data are mens of three replicates of 50 larvae each (Total n = 150 larvae).

Table 2. Weight gain and duration of *Spodoptera littoralis* fourth larval instars treated alone with the entomopathogenic virus SpliMNPV and azadirachtin and in combination with each other.

Treatment	Larval weight gain ± SE (mg)	Larval duration ± SE (days)	Viral yield ± SE (PIB/ml)
Control	0.93 ± 0.10^a	10.35 ± 0.03^a	-
SpliMNPV	0.54 ± 0.09^b	12.45 ± 0.04^b	$3.44\times10^5 \pm 0.60\times10^{5a}$
Azadirachtin	0.31 ± 0.02^c	15.48 ± 0.34^c	-
Azadirachtin+ SpliMNPV	0.25 ± 0.01^d	18.24 ± 0.25^d	$2.20\times10^5 \pm 0.80\times10^{5b}$

Data are mens of three replicates of 50 larvae each. Figures followed by different letters were statistically significant (P<0.05) using one - way analysis of varianve (ANOVA). PIB: Polyhedral inclusion bodies.

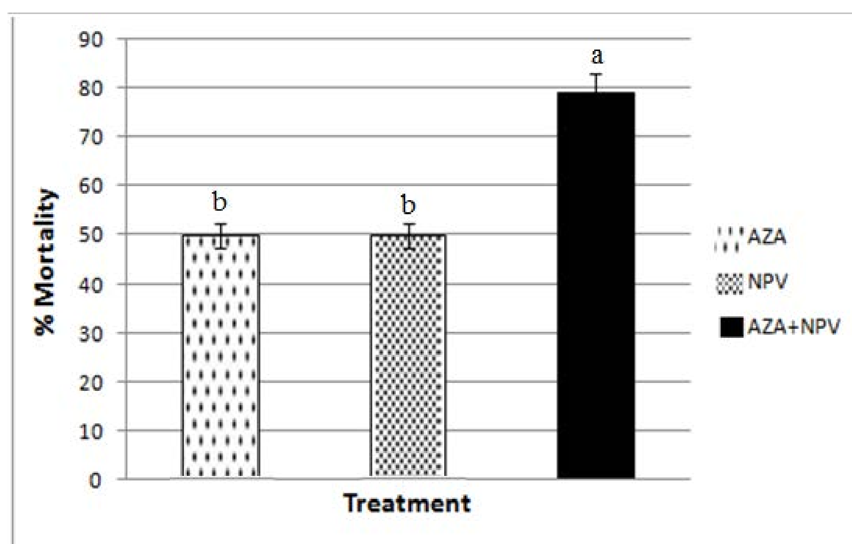

Fig. 1. Mortality of *Spodoptera littoralis* fourth larval instars treated alone with the LC_{50} of the entomopathogenic virus SpliMNPV (8.43×10^8 PIB/ml) and the LC_{50} of azadirachtin (23.15 ppm), and the combined treatment with the LC_{25} of SpliMNPV (10.10×10^6 PIB/ml) and the LC_{25} of azadirachtin (9.95 ppm). Graph bar represents the standard error (SE) of the mean of three replicates; 50 larvae each. Columns with different letters are significantly different from each other (P< 0.05) using one-way analysis of variance (ANOVA). AZA: Azadirachtin; NPV: SpliMNPV; AZA + NPV: Azadirachtin combined with SpliMNPV.

Discussion

The present study may help in better management of *S. littoralis* larvae in future using SpliMNPV and azadirachtin. The LC_{50} value of SpliMNPV against *S. littoralis* larvae (8.43× 10^8 PIB/ml reached) in the present investigation was considered higher than that reported for other NPVs against several lepidopteran species (MANGOLER, 1974; JAMES & ARTHUR, 1992). KRISHNAYYA *et al.* (2000) reported that the LC_{50} value of ingested azadirachtin against the third larval instars of *Spodoptera litura* was 110.20 ppm. Thus, based on the LC_{50} level, it appears that the fourth larval instars of *S. littoralis* fed on azadirachtin in the current study, with LC_{50} of 23.15 ppm, were about five times as susceptible as the third larval instars of *S. litura*.

The co-toxicity factor (58.40%) attained in this study indicates that interaction between azadirachtin and SpliMNPV was potentiation, based on the formula suggested by MANSOUR *et al.* (1966). Similarly, enhancement of the virulence of NPV after combination with azadirachtin has been reported by other authors (KOPPENHÖFER & KAYA, 2000; KUMAR *et al.*, 2008; WAKIL *et al.* 2012; ZAMORA-AVILÉS *et al.* 2013). The potentiation of the infectivity of NPV after combination with azadirachtin may be attributed to the damage of the mid gut secretory cells and the peritrophic membrane, with a concomitant decrease in the activities of the digestive enzymes, thereby facilitating the easy penetration of the active viral bodies and proliferation for subsequent pathogenic effects (KUMAR, 1998; BAJWA & AHMAD, 2012; ZAMORA-AVILÉS *et al.*, 2013).

The decrease in larval weight gain of *S. littoralis* treated either alone or in combination with azadirachtin and SpliMNPV is in accordance with the results obtained for the larvae of *S. littoralis* (ABD EL-GHANY *et al.*, 2012), *Spodoptera frugiperda* (ZAMORA-AVILÉS *et al.*, 2013) and *Helicoverpa armigera* (WONDAFRASH *et al.*, 2012; WAKIL *et al.*, 2012) fed on azadirachtin and NPV alone or in combination with each other. SCHMUTTERER (1988) reported that azadirachtin and azadirachtin containing neem seed extracts acts as anti - feedant to insects. This may be due to the direct action of azadirachtin on the centers that control feeding and metabolism (BARNBY & KLOCKE, 1987). KUMAR *et al.* (2008) attributed the decrease in larval weight gain in *H. armigera* treated alone or in combination with NPV and azadirachtin to low food consumption and utilization in terms of the efficiency of conversion of the ingested and digested food into biomass. Moreover, it has been concluded that reduced growth, as a consequence of sub-lethal treatments, may be the result of diversion of host energy from metabolism and growth to combat or support the pathogen (ROTHMAN & MYERS, 1996) or hormonal changes induced by the pathogen (PARK *et al.*, 1993). Reduced larval weight gain is important from the practical standpoint because it might negatively impact pest population dynamics in the subsequent generations (ZAMORA-AVILÉS *et al.*, 2013).

In contrast to the results obtained for the larval weight gain, treatment with NPV or azadirachtin, either separately or in combination with each other, decreased the larval duration. These results are in consistence with those attained for *H. armigera* (KUMAR *et al.*, 2008) and *S. frugiperda* (ZAMORA-AVILÉS *et al.*, 2013) treated alone or in combination with azadirachtin and NPV. The molting delay which results from treatment with azadirachtin was attributed to the inhibition of ecdysteroid production (MALCZEWSKA *et al.*, 1988). On the other hand, baculovirus blocks molting and interferes with normal development as it causes weak expression of the ecdysteroid UDP - glucosyltransferase gene which encodes the enzyme ecdysteroid UDP - glucosyltransferase. This enzyme catalyzes the transfer of glucose from UDP-glucose to ecdysteroids which are insect molting hormone (REILLY, 1995). Moreover, failure of pupation, as a result of viral infection, was due to maintenance of high level of circulating juvenile hormone (SUBRAMANYAM & RAMAKRISHNAN, 1981).

SpliMNPV yield decreased due to combining with azadirachtin. Similar results were also reported by other authors (COOK

et al., 1996; ZAMORA-AVILÉS *et al*., 2013). These results can be interpreted by the suggestion of SHAPIRO *et al*. (1986) who suggested that NPV growth and yield are highly dependent on cell growth, thus NPV yield depends on the rate of larval weight gain during infection rather than on the initial or final weight.

Conclusions

It appears that azadirachtin is a valuable component for the formulation of SpliMNPV, by mixing at the ratio of LC_{25}: LC_{25}, especially in inundative release programmes, as it synergized the viral pathogenicity, in terms of larval mortality. Nevertheless, this mixture is unlikely to be useful for the mass production of this viral isolate. Thus, future studies on field efficacy under commercial growing conditions are required to demonstrate that the observed laboratory potentiation of azadirachtin–SpliMNPV mixture will be translated to improved control of *S. littoralis* in the field.

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References

ABBOTT W.S. 1925. A method of computing the effectiveness of an insecticide. - *Journal of Economic Entomology*, 18: 265-267.

ABD EL-GHANY M.A., M. FARAG, H. YOUSEF, M.H.M. AHMED, S. EL-BADRAWY, A.A.H. EL-RAHMAN. 2012. Insecticidal activity of *Melia azedarach* L. triterpenoids against *Spodoptera littoralis* (Boisd.). - *Journal of American Sciences*, 8: 661-667.

BAJWA A.A., A. AHMAD. 2012. Potential applications of Neem based products as biopesticides. - *Health*, 3: 116-120.

BARNBY M.A., J.A. KLOCKE. 1987. Effects of azadirachtin on the nutrition and development of the tobacco budworm, *Heliothis virescens* (Fabr.) (Noctuidae). - *Journal of Insect Physiology*, 32: 69-75.

COOK S.P., R.E. WEBB, K.W. THORPE. 1996. Potential enhancement of the gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus with the triterpene azadirachtin. – *Environmental Entomology*, 25: 1209-1214.

FINNEY D.J. 1971. *Probit Analysis*, third ed. London. Cambridge University Press. 333 p.

GAUTAM K., P. KUMAR, S. POONIA. 2013. Larvicidal and GC – MS analysis of flavonoids of *Vitex negundo* and *Andrographis paniculata* against two vector mosquitoes *Anopheles stephensi* and *Aedes aegypti*. - *Journal of Vector Borne Diseases*, 50: 171 – 178.

HUMMEL H.E., D. F HEIN, H. SCHMUTTERER. 2012. The coming of age of azadirachtin and related tetranortriterpenoids. - *Journal of Pesticides*, 5: 82-87.

HUNTER-FUJITA F.R., P.F. ENTWISTLE, H.F. EVANS, N.E. CROOK. 1998. *Insect Viruses and Pest Management*. New York. John Wiley and Sons. 632 p.

JAMES R.F., R.R. ARTHUR. 1992. Virulence and multigeneration passage of a nuclear polyhedrosis virus selected for an increased rate of vertical transmission. - *Biological Control*, 2: 171-175.

KOPPENHÖFER A.M., H.K. KAYA. 2000. Interaction of a nuclear polyhedrovirus with azadirachtin and imidacloprid. - *Journal of Invertebrate Pathology*, 75: 84-86.

KRISHNAYYA P.V., M.S. SUNDER, P.A. RAO. 2000. Evaluation of lethal concentrations of azadirachtin based proprietary formulation for larvicidal and ovicidal effects of *Spodoptera litura* (Fabr.). - *Journal of Entomological Research*, 24: 147-150.

KUBO I., J.A. KLOCKE. 1982. Short communication: Azadirachtin, insect ecdysis inhibitor. - *Agricultural and Biological Chemistry*, 46: 1951-1953.

KUMAR N.S. 1998. Combined Effect of Nuclear Polyhedrosis Virus and Azadirachtin on the Feeding, Growth, Development, Biochemical and Histopathological Changes of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). (Ph. D. thesis). Bharathiar University, India.

KUMAR N.S., K. MURUGAN, W. ZHANG. 2008. Additive interaction of *Helicoverpa armigera* nucleopoly-hedrovirus and azadirachtin. - *BioControl*, 53: 869-880.

LEORA SOFTWARE. 1987. *POLO – PC: A User's Guideline to Probit or Logit 786 Analysis*. LeOra Software.

MANGOLER A. 1974. Bioassay of nuclear polyhedrosisvirus of the gypsy moth, *Prothetria dispar*. - *Journal of Invertebrate Pathology*, 23: 190-196.

MALCZEWSKA M., D.B. GELMAN, B. CYMBOROWSKI. 1988. Effects of azadirachtin on development, juvenile hormone and ecdysteroid titres in chilled *Galleria mellonella* larvae. - *Journal of Insect Physiology*, 34: 725-732.

MANSOUR N.A., M.E ELDEFRAWI., A. TAPPOZADA, M. ZIED. 1966. Toxicological studies on the Egyptian cotton leafworm *Prodenia litura* F. VII. Potentiation and antagonism of órgano-phosphorus and carbamates. - *Journal of Economic Entomology*, 59: 307-311.

MOSCARDI F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. - *Annual Review of Entomology*, 44: 257-289.

PARK E.J., J.P. BURAND, C.M. YIN. 1993. The effect of baculovirus infection on ecdysteroid titer in gypsy moth larvae (*Lymantria dispar*). - *Journal of Insect Physiology*, 39: 791-796.

RADWAN O.A., Y.W.A. EL-SHIEKH. 2012. Degradation of neem oil 90% EC (Azadirachtin) under storage conditions and its insecticidal activity against cotton leafworm, *Spodoptera littoralis*. - *Researcher*, 4: 77-83.

REILLY D.R. 1995. Baculovirus-encoded ecdysteroid UDP-glucosyltransferases. - *Insect Biochemistry and Molecular Biology*, 25: 541-550.

REMBOLD H., G.K. SHARMA, T. GZOPPELT, H. SCHMUTTERER. 1982. Azadirachtin: A potent insect growth regulator of plant origin. – *Journal of Applied Entomology*, 93: 12-17.

ROTHMAN L.D., J.H. MYERS. 1996. Debilitating effect of viral diseases on host Lepidoptera. - *Journal of Invertebrate Pathology*, 67: 1-10.

SAS INSTITUTE INC. 2008. *SAS / STAT® 9.2 User's Guide*. Cary, NC. SAS Institute Inc.

SCHMUTTERER H. 1988. Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. - *Journal of Insect Physiology*, 34: 713-719.

SHAPIRO M., J.L. ROBERTSON, R.A. BELL. 1986. Quantitative and qualitative differences in gypsy moth nuclear polyhedrosis virus produced in different-aged larvae. - *Journal of Economic Entomology*, 79: 1174-1177.

SHOREY H.H., R.L. HALE. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. - *Journal of Economic Entomology*, 58: 522-524.

SINGH R., B. SINGH, R.A. VERMA. 2001. Efficiency of different indigenous plant products as grain protectant against *Callosobruchus chinensis* L. on pea. - *Indian Journal of Entomology*, 63: 179-181.

SUBRAMANYAM B., N. RAMAKRISHNAN. 1981. Influence of a baculovirus infection on molting and food consumption by *Spodoptera litura*. - *Journal of Invertebrate Pathology*, 38: 161-168.

SUNDARARAJ R. 1997. Evaluation of neem seed oil and some insecticides against the babul whitefly *Acaudaleyrodes rachipora* (Singh) (Aleyrodidae: Homoptera) on *Acacia senegal* seedlings. - *Pestology*, 21: 34-37.

TIESSEN S. 2012. Indiana's most unwanted invasive plant pests, Indiana Co-operative Agricultural Pest Surveys Program (CAPS), Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval). [http://www.extension.entm.purdue.edu/CAPS/pestInfo/egyptLeafworm.htm]. Accessed: 2.7. 2012.

WAKIL W., M.U. GHAZANFAR, F. NASIR, M. ABDUL QAYYUM, M. TAHIR. 2012. Insecticidal efficacy of *Azadirachta indica*, nucleopolyhedrovirus and chlorantraniliprole singly or combined against field populations of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). - *Chilean Journal of Agricultural Research*, 72: 53-61.

WONDAFRASH M., E. GETU, G. TEREFE. 2012. Neem, *Azadirachta indica* (A. Juss) extracts negatively influenced growth and development of African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). - *Academic Journal of Entomology*, 5: 22-27.

YANG M.M., M.L. LI, Y. ZHANG, Y.Z. WANG, L.J. QU, Q.H. WANG, J.Y. DING. 2012. Baculoviruses and insect pests control in China. - *African Journal of Microbiology Research*, 6: 214-218.

ZAMORA-AVILÉS N., J. ALONSO-VARGAS, S. PINEDA, J. ISAAC-FIGUEROA, P. LOBIT, M. MARTÍNEZ-CASTILLO. 2013. Effects of a nucleopolyhedrovirus in mixtures with azadirachtin on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae and viral occlusion body production. - *Biocontrol Science andTechnology*, 23: 521-534.

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