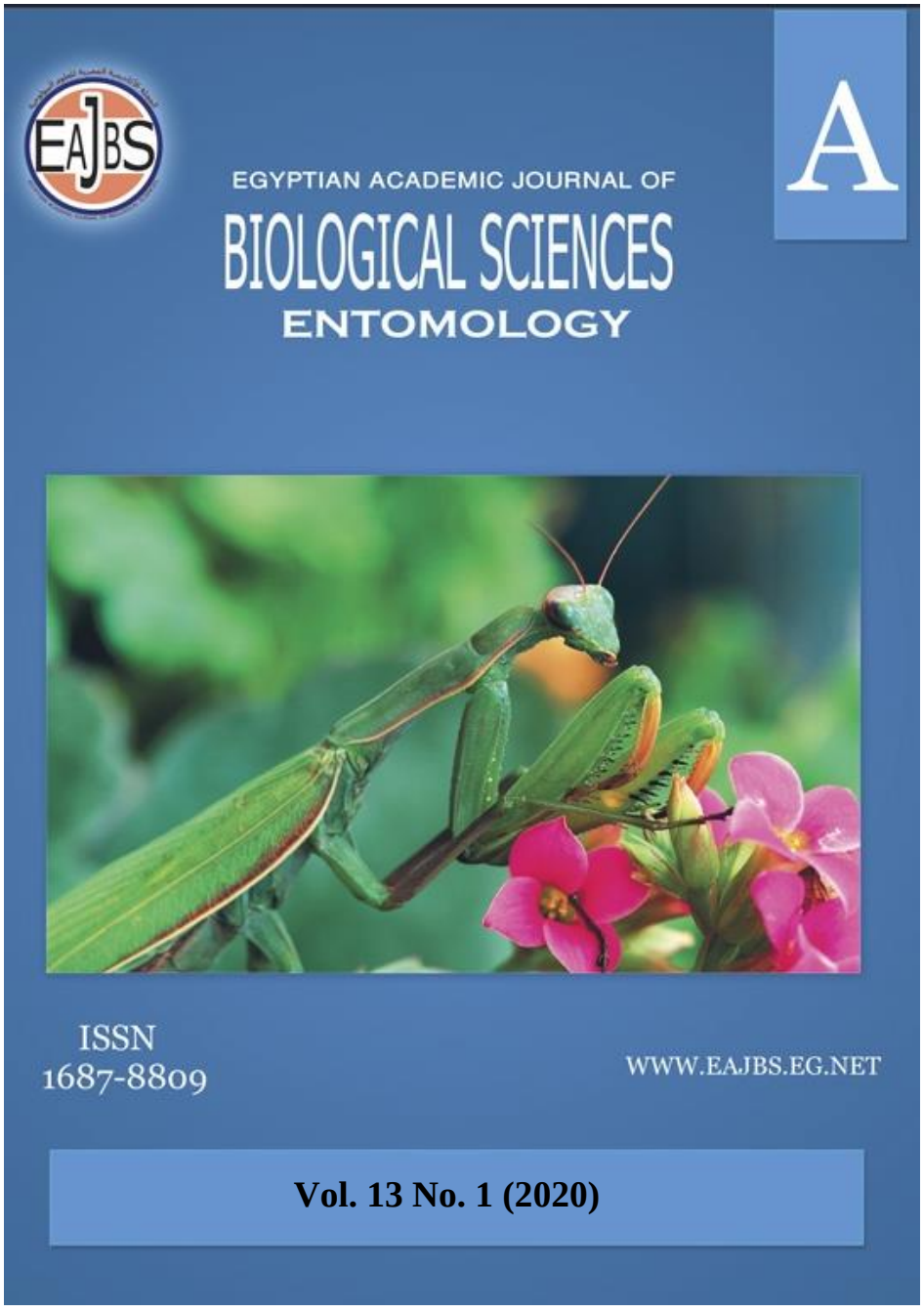

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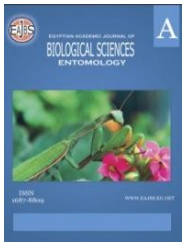

Enhancing the Efficacy of Certain Spray-Dried Baculovirus (*Agip*MNPV) against Cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae)

Atef M. M. Sayed[1], Robert W. Behle[2], and Ghada N. El-Masry[1]

1- Plant Protection Research Institute, Agricultural Research Center, Dokki, Egypt.

2- United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Research, Crop Bioprotection Research Unit, Peoria, Illinois, USA.

Email: atef.mahmoud1@gmail.com

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ABSTRACT

The insecticidal efficacy of various baculovirus formulations against the cutworm *Agrotis ipsilon* was determined. The treatments consisted of molasses from suagrcane mixing with each of wheat germ, biochar, talc, chitosan, silica gel, pyrolysis bio-oilaqueous phase, pyrolysis bio-oilorganic phase, calcium carbonate, cornflour, calcium cassinate, aluminium potasium sulphate, Diatomaceous earth, dextrin, lignin PC 1307, soy screen, blankphor, skim milk powder, potassium cassinate, magnsium chloride, date molasses and pregelatinized starch. All applied in spray-dried at 2.2 x 10^9 occlusion body(OB) mL^{-1}. The bioassay of each spray-dried formulation using the droplet feeding method by the blue solution containing 2% sugar and 0.1% blue dye. The resulting suspensions should all contain 0.377 x 10^6 OB mL^{-1}, represent the LD_{70} for the unformulated virus. Exposure newly hatched larvae of cutworms and transfer to individual diet cups for each treatment and incubated at 28^{o}C in dark incubator for 7 days. The obtained results revealed that satisfactory control of the pest of 100% larval mortality compared to untreated control was achieved with the formulations containing molasses mixing with talc, silica gel, calcium carbonate, cornflour, calcium cassinate, diatomaceous earch, soy screen, and dextrin. However, the formulations containing chitosan, pyrolysis bio-oilaqueous phase and pyrolysis bio-oilorganic phase gave the lowest mortalities as 12.2, 11.1 and 6.7%, respectively. These findings is an attempt to provides an interesting alternative developed biopesticide formulations made with natural ingredients that could improve the efficacy and persistence of virus-based biopesticides.

INTRODUCTION

Baculoviruses (family: *Baculoviridae;* genus: *Nucleopolyhedroviruses*) are a highly diverse group of circular double-stranded DNA genome viruses and present a seemingly good alternative to broad-spectrum insecticides attributed to their efficacy, specificity, and safety to humans and other non-target organisms (Ikeda *et al*., 2015; and Herniou *et al*., 2011). Due to their high virulence and narrow host range, baculoviruses have been developed as environmentally sound microbial pesticides for pest management in agriculture,

horticulture and forestry settings around the world (Kabaluk et al., 2010; Szewczyk et al., 2009; and Erlandson, 2008).

Two different types of virions were formed during the infection cycle of the baculoviruses. Virions that received their membrane from the plasma membrane of the host cell the cell are called budded viruses (BV), it contains only a single nucleocapsid. Occlusion Derived Virions (ODV) are assembled within the host cell. It embedded within a crystalline protein matrix that forms occlusion bodies (OB). Furthermore, baculoviruses are divided into two morphological groups according to their OB morphology. The OBs of granuloviruses (GV) (genus *Betabaculovirus*) contain only one virion and are smaller than those of the nucleopolyhedroviruses (NPV) (genera *Alpha-*, *Gamma-* and *Deltabaculovirus*), which contain numerous ODVs (Herniou et al., 2011). The OB is the infectious stage of the virus and is important in spreading the virus between hosts (Cory and Myers, 2003).

The cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuiidae) is a seriously devastating agricultural insect pest of field crops, vegetables, ornamental plants, golf course and sports fields worldwide. Cutworms spend most of their life cycle underground or close to the soil surface where feed on seedlings, stems, and other parts of plants. *A. ipsilon* causes severe crop damages in infested plant fields varies from 20-80% due to its various host range, hidden lifestyle, feeding behavior, prolonged egg-laying, and its ability for long-distance migration. More than 80% of the losses occur after reaching the fourth instar of larvae, which cuts several plants overnight (Binning et al., 2015, Liu et al., 2015). It is difficult to detect, especially late larval stages that stay within the soil. So, will be complicated their control by spray applications of insecticides and remains inadequate for the control of this pest because of its larval hiding behavior during the daylight hours and the resistance to most of the chemicals (Brosi &Potteri, 2010). Therefore, the negative impact of the chemicals has led researchers to search for new control strategies.

Hence, alternative more consumer-friendly and environmentally safe biocontrol agents are needed for controlling this pest.

The baculovirus, *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (*Agip*MNPV) was isolated and characterized successfully from larvae of the cutworm. Recently, it used as a potential alternative microbial agent for suppression of *A. ipsilon* (Harrison, 2009; Prater *et al*., 2006; Bourner and Cory, 2004; and Behle, 2017).

Enhancing the efficacy of baculovirus by adding some ingredients which increased infection and improved pest control. The aim of the formulation is to combine the advantageous effects of formulation ingredients without compromising on cost, efficacy, and protection from adverse environmental factors (Behle *et al*., 2006). In addition to improving the speed of kill, efficacy, host range, and persistence applied research on the formulation of BV remains one of the most important routes to BV product improvement (Behle and Birthisel, 2014). Adjuvant may need to be incorporated to inhibit microbial contamination as well as protect the baculovirus from adverse environmental factors such as temperature, leaf surface exudates, sunlight, wind, and rain, thereby improving their persistence, helping to maintain their insecticidal activity, enhance storage stability and maximize application efficiency (Behle *et al*., 2011; Lasa *et al*., 2009). Additives that enhance the active ingredient such as flour, oil, and sugar often act as phagostimulants and have other enhancing properties such as facilitating adhesion of the virus to plants. These can be used as carriers or encapsulating agents to spray-dry the active ingredients (Tamez-Guerra *et al.*, 2002)

Spray-drying has been used successfully to produce powders of nucleopolyhedroviruses, and this enables them to withstand spray-drying conditions (Arhurs and Lacey, 2004). This process has the advantage that it may kill contaminating microorganisms in the product (Jones and Burges, 1997). Dry formulations are easier to

handle and store than liquid formulations due to their reduced weight and package size (Seaman, 1990).

Efforts have been ongoing to develop strains of baculoviruses with greater potency or other attributes to decrease the cost of their use through a lower cost of production or application. This research reported an attempt aimed to determine the bioinsecticidal activity of various spray-dried formulations with the baculovirus *Agip*MNPV for managing *Agrotis ipsilon.*

MATERIALS AND METHODS

Rearing of Experimental Insect:

A population of the cutworm *Agrotis ipsilon* has been provided by Dr. Mortada (Cutworm Research Department, Plant protection Research, Dokki, Egypt) and maintained on Southland cutworm commercial insect diet (Southland Products, Inc., Lake Village, AR) at Plant Protection Laboratory, Ismailia Agricultural Research Station, Ismailia, Egypt, where it was established for several generations as described by Behle 2017.

Adult moths were kept for two weeks in groups of about 20 individuals in transparent plastic cylinders (20 cm diameter, 25 cm height) that were faced inside with rough-surfaced paper tissues. Insect eggs were collected three times a week by replacing the paper tissues; they were incubated at 25°C for several days until hatching. Thirty neonates were transferred onto an artificial diet of soy-wheat germ diet (Frontier Scientific Service-USA) in individual cups to fill a plastic tray of 30 cups. Each cup containing 3 mL artificial diet. Larvae were kept in an incubator at 25°C with a 16/8 h light/dark photoperiod until they reached the pupal stage. Pupae were collected and incubated at 25 °C until the imagoes hatched.

Baculovirus Preparation:

Agrotis ipsilon multicapsid nucleopolyhedrovirus (*Agip*MNPV) stock was provided by Dr. Robert Behle (USDA-ARS-National Center for Agricultural Utilization Research, Crop Bioprotection Research Unit, Peoria, IL. USA). This stock was produced by a single amplification passage through *A. ipsilon* larvae and was purified by a series of centrifugation and washing steps. *Agip*MNPV polyhedral occlusion bodies (OBs) were enumerated by a Neubauer bright-line hemocytometer (Fisher, Pittsburg, PA) with a phase-contrast microscope.

For producing a fresh virus, five larvae were transferred onto chunks of Southland diet per cup to fill a tray of 30 cups and incubated for 7 days at 27 ºC. A total of 30 trays of Southland diet, by dispensing a thin layer of diet in each cup. Cups were prepared and treated with 100 µL virus suspension per cup at a concentration of 10^6OB mL^{-1}. When larvae reached 7 days incubation, only one larva was transferred into each treated cup and covered with a lid. Then, the treated larvae were incubated for 7 days at 28ºC. Dead larvae were harvested for OB purification. Polyhedra extracted and purification as described previously by Behle, 2017; and Boughton *et al*., 2001. Sodium azide was added to polyhedral preparations to a final concentration of 0.02% to prevent bacterial growth, and virus stocks were stored at 4ºC. The cadavers were thoroughly homogenized in 0.5% sodium dodecyl sulfate (SDS) using homogenizer. The resulting suspension was filtered through three layers of gauze that were washed with additional volumes of 0.5% SDS. The OBs were pelleted at centrifuge of 750 *g* for 10 min and then washed three times by re-suspending the pellet twice in 0.1% SDS and once in 0.5 M NaCl. The OBs were then centrifuged again at 750 *g* for 10 min and resuspended in an appropriate volume of deionized water (dH2O). For OB purification, the homogenate was centrifuged at 5000 *g* for 10 min and the pellet was re-suspended in 0.5% SDS. The OB suspension was pelleted, then washed in 0.5 M NaCl,

pelleted again and finally re-suspended in a smaller volume of dH2O. All OB purification stock were stored at -20°C until used.

Formulation Preparation:

Twenty-three ingredients were made with the baculovirus *Agip*MNPV. Materials for each formulation as shown in Tables 1& 2 were mixed in water, then the mixture was spray-dried. Molasses-based spray-dried formulations were selected and prepared to determine to screen the optimal ingredients for insecticidal activity of baculovirus by a dosage-response droplet-feeding assay.

Twenty-three spray-dried formulations ingredients were made with molasse (Kroger Stores Inc., Bentonville AR, USA) included as follow: Calcium carbonate; Magnsium chloride; Potassium caseinate; Aluminium potassium sulfate dodecahydrate ($AlKO_8S_2$ 12 H_2O); Pregelatinized starch; corn flour; silica gel (SiO_2, high-purity grade, pore size 60 Å, 200 mesh particle size); Chitosan (Crab shell, ≥85% deacetylated), crab-shell chitosan solution (1%, wt/vol) of chitosan was prepared by dissolving 10 g of chitosan in 1% aqueous solution of the acetic acids under continuous stirring at 60°C for overnight to improve the solubility of chitosan and then followed by centrifugation at 5000 rpm, 20°C for 25 min to separated not dissolved chitosan particles, the pH values of the chitosan solutions was adjusted to 4.0 and by adding 0.1 *M* hudroxide sodium for pH adjusted to 6.8. The previous compounds were purchased from Sigma-Aldrich (Chemical Co., St Louis, Mo, USA).

The other compounds were wheat germ (Great Value, Walmart Stores Inc., Bentonville AR, USA); Non-fat instant dry milk (skim milk powder); Talc (Hydrous magnesium silicate, Fisher Scientific, 1 Reagent Lane, Fair Lawn, NJ); Diatomaceous earth (HYFLO, Celite Corp., Lompoc, CA, USA); Soy screen (10%, composite, 0.486:100 soy screen: starch ratio, ca:32.7 soy, NCAUR sample 19238-330 powder);

Biochar (Biochar Options LCC, Hartland WI, USA), the procedure of preparing the biochar sample suspensions was identical to the described by Wang et al., 2013, the pHs of the biochar suspensions were adjusted to 6.8 and other suspension was buffered by adding $NaHCO_3$ 16.8 g to pH adjusted to 6.8.; The used bio-oils (aquous and organic phases) was obtained from Robert. A. Moreau and Charles A. Mullen (Sustainable Biofuels and Co-Products Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane Wyndmoor PA 19038);

Lignin (PC-1307, Westvaco, Charleston Heights, SC) which dissolved in water at 10% w/w. Then, virus stock was added to the lignin solution (50mL) followed by adding slowly calcium chloride dihydrate (1 g $CaCl_2$ $2H_2O$ diluted in 60 ml water) while stirring vigorously in a blender to creating the dryer feedstock, after the lignin dissolved, the pH was adjusted to 9.41;

The optical brightener blankophor (Bayer, Houston, TX), 4g in 50 mL followed by added slowly solution of 1g $CaCl_2$ $2H_2O$ in 30 mL to creating the dryer feedstock.

For all the previous prepared formulations, the pH was adjusted for each. The virus concentration per gram of solids is a calculated value based on the count of the technical virus and the amounts of the added ingredients as shown in Tables 1&2.

Table 1. List of ingredients used for spray-dried formulations of *Agip*MNPV tested against *Agrotis ipsilon*

Formulations / Ingredients	Wheat germ	Talc	Silica	Calcium carbonate	Corn flour	Calcium cassinate	Aluminium Potassium sulphate	Magnsium Chloride	Lignin PC 1307	Blankphore	Date molasse
Molasse (sugarcane)	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g
Wheat germ	5 g							5 g			
Talc		5 g									
Silica gel			5 g								
Calcium carbonate				5 g							
Corn flour					5 g						
Calcium cassinate						5 g					
Aluminium Potassium sulphate							5 g				
Magnsium Chloride								0.25			
Lignin PC 1307									50 ml of 10% solution		
Blankphor										4g in 50 mL (added slowly 1g $CaCl_2$ in 30 ml	
Date molasse											5 g
Total weight	6.25 g	6.25 g	6.25 g	6.25 g	6.25 g	6.25 g	6.25 g	6.25 g	6.25 g	6.50 g	6.25 g
Unformulated virus @1.05 x 10 ^{9} OB/mL	4.3 mL										
Final volume at 5% solids	125 mL										
Expected virus load OB/g	2.2 x 10 9 OB/g										
pH	7.37	7.02	6.06	8.47	5.31	6.1	3.18	9.41	10.09	7.53	6.41

Table 2. List of ingredients used for spray-dried formulation of *Agip*MNPV tested against *Agrotis ipsilon.*

Formulations / Ingredients	Biochar	Chitosan	Bio-oil (organic phase)	Bio-oil (aqueous phase)	Pre-gelatinized starch	DE	Soy Screen	Dextrin	SKM	Potassium cassinate	Chitosan	Biochar
Molasse (sugarcane)	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g	1.25 g
Biochar (125 mL)+ 200 mL $NaHCO_3$	21 g											
Chitosan + 50 mL NaOH (0.1 *M*)		1.75 g										
Bio-oil (pyrolysis organic phase)			5 g									
Bio-oil (pyrolysis aqueous phase)				5 g								
Pregelatinized starch					5 g							
Diatomaceous earth (DE)						5 g						
Soy Screen							5 g					
Dextrin								5 g				
Skim milk powder (SKM)									5 g			
Potassium cassinate										5 g		
Chitosan											1%	
Biochar + 20 mL potassium phatlate 0.05M, buffer 4.0												5 g
Expected total weight	22.25 g	3.0 g	6.25 g	6.25 g	6.25 g	6.25 g	6.25 g	6.25 g	6.25 g	6.25 g	2.25 g	6.25 g
Unformulated virus @1.05 x 10 ^{9} Ob/ml	4.3 mL											
Final volume at 5% solids	325 mL	120 mL	125 mL									
Expected virus load OB/g	2.2 x 10 ^{9} OB/g											
pH	8.76	4.00	3.51	4.75	6.05	8.04	5.86	6.03	7.17	6.8	6.22	6.41

Spray Drying Formulations:

Each spray-dried molasse formulation was made to contain 2.2_x 10^9 OB mL^{-1}. Evaluating the spray drying of *Agip*MNPV formulations in the final product based on the OB count provided with the virus stock and the weight of ingredients added to the formulation. The first step in mixing the dryer feedstock for the prepared twenty-three ingredients was to determine the amount of product to be able to calculate the amount of water (125 mL) required to provide a final spray-dryer feedstock consisting of/5% (w/v) solids.

All spray-dried formulations of the feedstock suspensions (125 mL) with a concentration of 2.2 x/10^9 OB mL^{-1} were dried in a Niro atomizer portable spray dryer (Niro, Columbia, MD, USA). Spray-drying conditions were inlet temperature of 130-135°C, the outlet temperature of 60-70°C, 4.5 heat setting, air pressure of 5.6 kg/cm2, 5% solids (w/v) feedstock concentration and 20 mL min^{-1} feed rate. Each suspension was continually agitated using a magnetic stirrer during spray-drying to ensure that the active ingredient

remained evenly suspended. The percentage of powder recovery was calculated based on the amounts of dry ingredients used in the feedstock. The water activity (a_w) of these suspensions was measured using the AquaLab series 4TEV (Decagon Devices, Inc., Pullman, WA, USA) equilibrated at 25 °C. The resulting dried powder was expected to contain 2.2 x/10^9 OBs g^{-1}. Fresh preparations were made within 2 weeks of use.

Bioassay:

The activity of spray-dried formulations prepared with virus cutworm (*Agip*MNPV) at 2.2 x 10^9 occlusion bodies (OB) mL^{-1} was performed using a modified droplet feeding method described by Hughes et al., 1986. Each spray-dried formulation ingredient sample was mixed into 10 mL of a feeding blue solution containing 2% (w/w) sucrose, and 0.1% blue dye (w/w) FD&C Blue 1 (Noveon Hilton Davis, Cincinnati, OH). Then, sonicate at 40% for 5 seconds by probe sonicator. The resulting suspensions should contain 0.377 x 10^6 OB mL^{-1} which represents the lethal dose (LD_{70}) for the unformulated virus that based on the results was obtained by Behle, 2017.

After mixing, about 60 small drops from each sample were placed in individual plastic 50-mm Petri dishes. Five Petri dishes per treatment were employed. 15 neonate *A. ipsilon* were placed in each dish to feed on the drops and Petri dishes were capped to reduce evaporation of the droplets. After feeding for about 5 min, six larvae with blue stained intestines from each dish were transferred to individual cups containing 3 mL artificial diet to fill a tray of 30 cups representing a replicate. Three replicates for each treatment and incubated for 7 days at 28^{o}C in a dark Conviron I24 L incubator (Controlled Environments, Inc., Asheville, NC). After incubation, the numbers of live and virus-killed larvae were counted to calculate the mortality percentage for each treatment. Dead larvae that were not symptomatic for virus infection were omitted from statistical analysis.

Data Analysis:

All experiments were conducted using a completely randomized design. All experiments were repeated three times on different dates using different insect cohorts. Viral formulations made with different ingredients were subjected to analysis of variance (ANOVA) and treatment means were separated using Tukey's statistic test at *P* 0.05. All analyses were performed by using software Biostat (2009).

RESULTS AND DISCUSSION

Spray-dried formulations of AgipMNPV made with molasse had greatly significant different levels of activity effect ($F_{22, 206}$=217.17, P<0.0001) based on the ingredients added to the formulation. Statistical analysis of the results indicated that satisfactory control of the pest of 100% larval mortality compared to untreated control was significantly achieved with the formulations containing molasse mixing with talc, silica, calcium carbonate, cornflour, calcium cassinate, diatomaceous earch, soy screen, and dextrin. Also, wheat germ, date molasse, skim milk powder and potassium cassinate gave 92.08, 91.25, 94.18 and 93.75%, respectively as shown in Table 3 and Figure 1.

Mortality of larvae exposed to formulation made with biochar (with buffer at 4.0), magnsium chloride, aluminium potasium sulphate, blankphor and pregelatinized starch gave 91.53, 90.74, 90.37, 89.71 and 86.25%, respectively. Whereas, the formulation made with lignin PC 1307 and biochar with $NaHCO_3$ gave the significant mortalities tended to be intermediate 78.89 and 63.52%, respectively. However, the formulations containing chitosan, chitosan with NaOH (0.1 *M*), pyrolysis bio-oilaqoues phase, and pyrolysis bio-oilorganic phase gave the lowest significant mortalities as 27.78, 12.21, 11.12 and 6.70%, respectively.

The products of spray-dried containing diatomaceous earth (DE), dextrin, skim milk powder (SKM) and biochar (with buffer adjusted pH at 6.8) were greater recovery percentages of 91.61, 81.85, 78.34 and 74.62 % than other tested compounds. On the other hand, water activity (a_w) of the viral formulated samples was measured at 25 ºC with an electronic meter and ranged from 0.013 to 0.307 in equilibrium with 1.3-30.7 % relative humidity (Table 3).

Table 3. Recovery, water activity (a_w) of the spray-dried formulation ingredients made with baculovirus *Agip*MNPV and mortality percentage (mean ± se) of *Agrotis ipsilon* exposed to the formulated virus.

No.	Ingrdeients	Spray-dried formulation ingrdeients			Mortality
		Recovery		Water activity (a_w) at 25 °C	Percentage (mean ± se)*
		weight (g)	%		
1	Wheat germ	2.06	32.96	0.248	92.08 ± 4.89
2	Talc	4.38	70.08	0.284	100.00 ± 0.00
3	Silica gel	4.04	64.64	0.252	100.00 ± 0.00
4	Calcium carbonate	3.79	60.64	0.307	100.00 ± 0.00
5	Corn flour	3.38	54.08	0.263	100.00 ± 0.00
6	Calcium cassinate	2.25	36.00	0.216	100.00 ± 0.00
7	Aluminium Potassium sulphate	1.22	19.52	0.241	90.37 ± 3.09
8	Magnsium Chloride	2.22	35.52	0.227	90.74 ± 8.54
9	Lignin PC 1307	3.49	55.84	0.256	78.89 ± 7.64
10	Blankphor	1.73	26.62	0.162	89.73 ± 4.69
11	Date molasses	1.73	27.66	0.170	91.25 ± 9.07
12	Biochar (125 mL)+ 200 mL $NaHCO_3$	16.79	74.62	0.250	63.52 ± 7.92
13	Chitosan + 50 mL NaOH (0.1 *M*)	2.10	70.00	0.24	12.21 ± 2.89
14	Bio-oil (pyrolysis organic phase)	0.17	2.72	0.013	6.70 ± 3.63
15	Bio-oil (pyrolysis aqueous phase)	0.06	0.96	0.017	11.12 ± 3.39
16	Pregelatinized starch	4.25	67.95	0.190	86.25 ± 15.48
17	Diatomaceous earth (DE)	5.73	91.61	0.210	100.00 ± 0.00
18	Soy Screen	2.78	44.44	0.230	100.00 ± 0.00
19	Dextrin	5.12	81.85	0.210	100.00 ± 0.00
20	Skim milk powder (SKM)	4.90	78.34	0.210	94.18 ± 2.42
21	Potassium cassinate	3.05	48.76	0.290	93.75 ± 2.89
22	Chitosan	1.50	66.67	0.290	27.78 ± 13.23
23	Biochar + 20 mL potassium phatlate 0.05M, buffer 4.0	2.80	44.44	0.230	91.85 ± 3.65

* Analysis of variance (F) = 120.72; degree of freedom (df) = 22, 206; *Probability* (*P*) < 0.0001; critical value for comparison = 2.26; and standard error for comparison = 6.2.

The cutworm, *A. ipsilon* is a pest causing damage to a variety of plants. A recently discovered baculovirus has the potential to be developed as a microbial-based biological pesticide to provide targeted control of this insect pest. In an effort to develop this baculovirus as a biological pesticide, experiments were conducted to evaluate the insecticidal activity of different viral ingredient formulations against cutworm larvae.

Formulation ingredients that enhance the active ingredient such as sugar, starch, lignin, and flour provide more than one benefit to biological pesticides. It has been acted as a phagostimulant, facilitating adhesion of the virus to plants, it is thought to play a role in protecting the occlusion body when it dries on the crop, and improved field performance and storage stability of a baculovirus. These can be used as carriers or encapsulating agents to spray-dry the active ingredients (Tamez-Guerra *et al*. 2002).

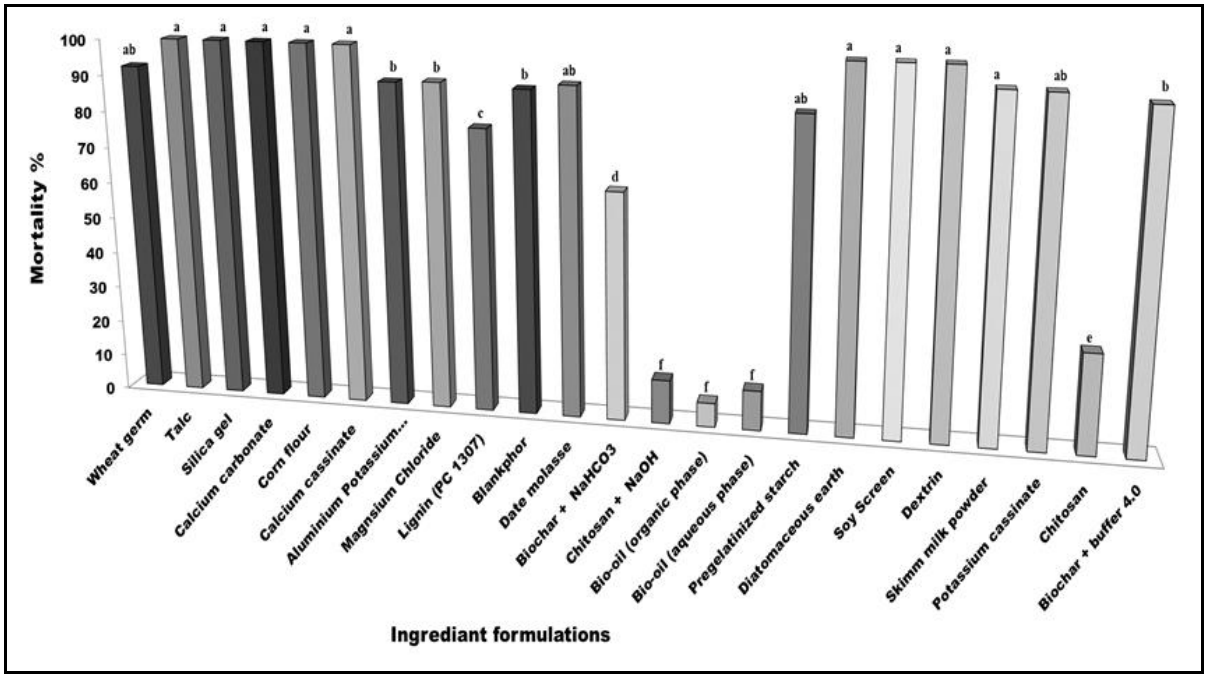

Fig. 1. Percentage mortality of different formulation ingredients for baculovirus *Agip*MNPV against cut worm, *Agrotis ipsilon*

Means followed by the same letter are not significantly different (LSD) ($F = 120.72$; df = 22, 206; $P < 0.0001$; critical value for comparison = 2.26; standard error for comparison = 6.2).

Various compounds have been reported as a potential adjuvant for inclusion in baculovirus formulations to overcome some of the inherent biological limitations, act as feeding stimulants and provide UV protection. Among these compounds optical brighteners, chitinase, boric acid, gums, sugar, pregelatinized flour, lignin, starch, talc, ground maize cob, maize oil and botanical extracts have received some interest as an attempt to enhance the efficacy of baculoviruses, protect from environmental factors that may inactivate it; increased viral persistence, and by accelerating the development of the disease (Mascarin & Delalibera 2012; and Behle *et al*., 2006).

Water activity is defined as the ratio of the water vapor pressure of a material to the vapor pressure of pure water at the same temperature (Robertson, 2006). It is a straightforward measure of water available for chemical and biological reactions and, therefore, a meaningful parameter in studies with dehydrated microorganisms. The unformulated virus suspension contained a higher microbial load throughout the accelerated storage period than the formulated samples. Although the formulated samples may contain more nutrients for contaminating bacteria, the free water content in the unformulated virus suspension may be higher, whereas the formulated suspensions would have contained molecularly bound water (Burges and Jones, 1998). Microorganisms rely on water activity of above 0.6 in their environment to reproduce (Esse *et al*., 2004). The water activity of the suspensions was expected to be lower in the formulated suspensions and this could be the reason for the higher microbial load in the unformulated suspension. These studies identified key storage conditions that improved storage stability and will facilitate the commercial development of viral-based bioinsecticides. The production of baculovirus OBs in sufficient quantity for commercialization is often labor-intensive. Better control possibly could be sustainably improved by selecting for *Agip*MNPV strains having higher virulence, or by formulating the virus with synergists or performance-enhancing adjuvants. Since large-scale commercial production of baculovirus biocontrol agents is mainly require large insect rearing facilities as well as techniques for infection of larvae, OB purification, and

formulation. Behle (2017) reported that the development of this baculovirus is considering as an additional tool for the integrated control of the cutworm. However, other challenges of the development of baculovirus biopesticides concern the production, safety, and stability of baculoviruses in the field. The present findings open up new issues on the compatibility of these ingredients so that a viable viral formulation can be developed with enhanced efficacy.

Conclusion

As a result, the differences in insecticidal activities from the present work showed that talc, silica, calcium carbonate, cornflour, calcium cassinate, diatomaceous earth, soy screen, dextrin. wheat germ, date molassese plus wheat germ, pregelatinized starch, potassium cassinate, potasium aluminium, blankphor, magnsium chloride, and biochar (with buffer 4.0) formulations trended to provide greater mortality of exposed insects compared with other formulations and can be used to enhancing the production baculovirus formulation biopesticide for cutworm, *A. ipsilon* control.

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ARABIC SUMMARY

تحسين فاعلية بعض مستحضرات الرش الفيروسية المجففة *Agip*MNPV ضد الدودة القارضة *Agrotis ipsilon* (Lepidoptera: Noctuidae)

عاطف محمود محمد سيد[١]- روبرت بيهال[٢] – غادة نبيل عبد الرحمن المصري[١]

١- معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقي - مصر
٢- وزارة الزراعة الامريكية - الخدمات البحثية الزراعية - المركز الدولي لاستخدام البحوث الزراعية- الولايات المتحدة الامريكية

تم تقدير فعالية النشاط الابادي لمستجضرات فيروسية عديدة ضد الدودة القارصة السوداء. تتكون هذه المستحضرات من خلط مولاس قصب السكر مع كل من جنين القمح، البيوشار، التلك، كيتوزان، سيلكاجيل، الزيوت الحيوية) المائي والعضوي) الناتجة من الانحلال الحراري، كربونات الكالسيوم، دقيق الذرة، كاسينات الكالسيوم، كبريتات الالومنيوم والبوتاسيوم، الدياتوميت، لجنين PC 1307، صويا سكرين، بلانكفور، مسحوق الحليب الخالي من الدسم، كاسينات البوتاسيوم، كلوريد الماغنسيوم، دبس التمر، والنشا الجيلاتينية . تم تجفيف جميع المكونات عند تركيز ٢,٢ × ١٠^{٩} / مل جسم محتواه(OB) . تم التقييم الحيوي لكل مستحضر مجفف بطريقة تغذية القطيرات بواسطة محلول أزرق الذي يحتوي على ٢٪ السكر و ٠,١ ٪ صبغة زرقاء. يحتوي جميع المستحضرات المتحصل عليها علي ٠,٣٧٧ ×١٠^{٦} OB / مل ، وتمثل الجرعة القاتلة ٧٠ ٪ من الافراد المعاملة للفيروس غير المستحضر. تم تعرض اليرقات الحديثة الفقس ونقلها منفرده في أكواب البينة ثم وضعها بالحضان علي درجة حرارة ٢٨ ^{o}م في الظلام لمدة ٧ أيام. بينت النتائج التي تم الحصول عليها أنه قد تحقق مكافحة الآفة بنسبة ١٠٠٪ موت لليرقات باستخدام المستحضرات التي تشتمل على مخلوط المولاس مع التلك، السيليكا جيل، كربونات الكالسيوم، دقيق الذرة، كاسينات الكالسيوم، الدياتوميت ، الصويا سكرين والديكسترين. ومع ذلك، فقد أعطت المستحضرات التي تحتوي على الكيتوزان، والانحلال الحراري للزيوت الحيوية بالمرحلة المائية والعضوية أدنى معدل للموت بنسبة ٦,٧ ، ١١,١ ، ١٢,٢ ٪ على الترتيب. تعتبر هذه النتائج هي محاولة لتوفير بدائل مثيرة للاهتمام يمكن أن تؤدي إلي تطوير مستحضرات مبيدات حيوية من مكونات طبيعية لزيادة تحسين فعالية وثبات المبيدات الحيوية القائمة علي الفيروس.