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Propolis Isolates Gathered from Different Regions in Egypt Affects Baculovirus-Uv Protection

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ABSTRACT

Three natural propolis derived from three different Egyptian regions (Alexandria, Dakahleya and Giza) containing different groups of antioxidants were tested as UV- protectants additives of *Spodoptera littoralis* nucleopolyhedro virus (*Spli*NPV) suspension, to prolong the virus activity. *Theubromo cacao* additive was tested as previously documented material that gives good protection. Screening results were based on bioassays, four main parameters were calculated Originally Activity Remaining (OAR) %, LIT_{50} (Median Lethal Inactivation Time), Potency and Reduction % of the virus after exposure to UV source. This investigation had three steps, two at the laboratory and the last one was at the field. Field evaluation was during June to August 2015 on tomato plants at eastern farm of Faculty of Agriculture, Cairo University. Propolis additives reserved virus activity for 30.788, 46.407, 75.325 LIT_{50} Hours for Alexandria, Dakahleya and Giza isolates; respectively. *Theubromo cacao* additive gave 58.011 LIT_{50} value while the virus alone treatment was only 14.479 hour at the field. The obtained result demonstrated that certain propolis isolates gave better protection than cacao which may lead in the future to analyze the groups of antioxidants or flavonoids responsible for this protection.

Keywords: *Antioxidants, baculovirus activity, field-biocontrol application, Propolis, Spodoptera littoralis NPV, virus protection.*

INTRODUCTION

Baculovirus, considered promising microbial agents for the biological control of lepidopterous pests (EPPO, 2015). *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is one of the most destructive pests in Africa, Asia, and Europe (El-Aswad *et al.,* 2003) the use of insecticides in agriculture field causes biological imbalance and resistant problems (Bulmer *et al.,* 2009; Yadav 2010). Exposure to sunlight causes decrease of infectivity of baculovirus (Elnagar 1982; Ignoffo *et al.,* 1989 and Jones et al., 1993). Many trials have been examined to protect baculoviruses most of them based on testing artificial materials (Shapiro and Bell, 1982, Shapiro, 1989; Ramakrishnan and Chaudhary, 1991; Ignoffo and Garcia, 1994; Shapiro and Dougherty, 1994, Arivudainambi *et al*., 2000, Reddy and Divakar, 2001, Cisneros et *al.,* 2002; Baskaran, 2007, and Kamaldeep & Battu, 2008). Protection based on natural products also well documented either Lignin (Jacques, 1977, Tamez-Guerra *et al.,* 2000; El Salamouny *et al.,* 2002; Elnagar *et al.,* 2003), Vitamins (Murahabaskaran *et al.,* 2000), Antioxidants (Zarin and Eglite, 1985, Deotale *et al.,* 2003, Deotale *et al*., 2007), Other antioxidants derived from plants (Shapiro *et al*.,2008, El Salamouny *et al.,* 2009, El Helaly *et al*., 2009 El Helaly 2013 and El Helaly *et al*., 2013) Flavonoids and rich groups of antioxidants found in natural derived materials may play the main role in baculovirus protection. (El Helaly 2016) Propolis is a natural resin and sap made by trees. Propolis has a long history of being used as treatment of various diseases, foods and biocosmetics (Burdock, 1998). In addition propolis contains phenol compounds, terpenoids, aromatic and aliphatic acids (Marcucci, 1995). The chemical composition of propolis is variable (Park et al., 2004 and Christov et al., 2005).Thus lead to evaluated three natural propolis derived from three different Egyptian regions (Alexandria, Dakahleya and Giza) for their suitability as UV protectants to *Spli*NPV under laboratory and field conditions in Egypt.

MATERIALS AND METHODS

Insect colony

A laboratory colony of the cotton leaf worm, *Spodoptera littoralis* (Boisd.), was established on a semi synthetic diet of Shorey and Hale (1965).

Virus inoculum

A Local isolate of *Spodoptera littoralis* nuclepolyhedrovirus (*Spli*NPV) originally isolated in Egypt by Abul Nasr (1956).

Propolis:

The propolis samples were collected from Giza, Mansoura and Alex provinces.

Propolis Samples preparation:

Twenty grams of each province were taken, extracted with 50 ml of 10% ethanol at room temperature (26°C ±2). This act was done three times with 24 hours interval between each time. The alcoholic extracts were evaporated under vacuum at 50 °C till dryness, this method was according to Matsuno, 1997. The resulted extracted matter differ from provenance to another as follows it gave 400 mg dry weight for Mansoura, 480 mg dry weight for Alexandria and 210 mg /w for Giza . five grams of each dry propolis material was soaked in 50 ml distilled water for 24 hours to get a final stock of 10% then blended and the filtrate extract was added to virus inoculums according to the method described by Shapiro *et al.* (2008).The final mixture used in the UV-irradiation tests was adjusted to get a final additive/virus mixture of final 1% and 5% concentration additive and PIB's/ of *Spli*NPV enough to cause 90% mortality (LC_{90}) as a control treatment

Laboratory Irradiation test

First and second steps of screening trials, sunlight UV was simulated (SUV) using a set of four UV lamps (Ultra-Vitalux, OSRAM, Germany) (Huber and Ludcke, 1996). Virus with or without additives resembling 200 fold LC_{90} were spread inside a Petri dish. After air drying, the dishes with the virus film on surface were exposed to the UV irradiation sources. For the first screening trial, the periods of exposure were 30, 60,

120, 180, 240 and 300 minutes extended to 600 minutes for the second screening. The virus after irradiation was re-suspended in 10 ml distilled water and kept refrigerated in a glass tube until bioassay. Laboratory bioassay was performed using the diet surface contamination technique described by, Cisneros *et al.* (2002). Two ml of collected PIBs suspension were spread on surface of 50 ml semi-artificial diet poured in a special bioassay plate divided to 50 cells. Untreated control by using either distilled water or additive only was used as a negative control. Neonate larva was confined each to a well of a microbial plate covered by two layers of tissue paper and glass cover fixed with rubber band. Virus without additives (in distilled water) was used for comparison. The plates were incubated at 26±2ºC and 60±5% R.H. under the laboratory conditions. Bioassay experiment was examined (Fritsch and Huber, 1985)

Field Experiment

An experimental area about 1/8 feddan (1 feddan = 4200 m2)of tomato, located at the Agriculture Experimental Station, Faculty of Agriculture, Cairo University, Giza Governorate was selected and one small scale open field test was set up. One concentration of tested additives was prepared (5% w/v) and kept in the fridge till spraying. At the time of field application, the virus and tested additive were thoroughly mixed together and the measured volume was transferred into a hand sprayer. Virus suspension treatments were applied separately to tomato foliage using one liter hand sprayer. Leaves were randomly collected from treated / untreated pants at 0, 0.41, 1, 2, 4, and 7 days post application and kept individually. Each leaf was placed into a glass bottle, on which 10 neonate larvae were allowed to feed for 48 hr. before transferred daily to fresh leaves from the same treatment. Larval mortality was recorded until day 14. Virus persistence was calculated Bioassay tests were repeated in five replicates with 10 larvae per treatment (Shapiro *et al.,* 2008)

Statistical analysis

Concentration-mortality regressions were calculated to determine the effectiveness of tested material as UV additives for the *Spli*NPV. Slope and LC_{50s} values were calculated according to the method described by Finney (1971).The potential of the material to prolong the virus persistence as described by (Muro and Paul, 1985).

RESULTS

The theory of this investigation was built in three progressive steps, preliminary step where virus either alone or mixed with additives were exposed to virus for only five hours at 1% concentration. Followed by the second step, which gave clearer image of the real effect of these additives, it was at the same concentration but for longer periods seven and ten hours, then the final field step. Preliminary bioassay Figure (1) showed that activity of *Spli*NPV without additive was lost soon after treatment. The calculated lethal inactivation time for 50% of the tested *S. littoralis* neonate larvae was 46.791 minutes this activity increased to 342.703, 886.368, and 595.155 and 793.521 minutes by adding Alex propolis, Dakahleya propolis, Giza propolis and Cacao; Respectively.

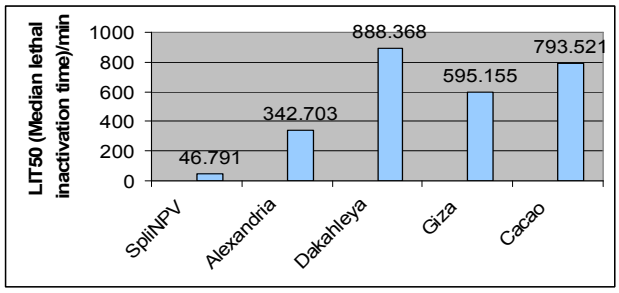

Figure (1) LIT_{50} (Median lethal inactivation time) among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or Cacao at 1% concentration, all exposed to different UV irradiation periods.

Virus mixed with Alex propolis gave the lowest potency folds 7.324 (Figure 2) followed by virus mixed with Giza propolis isolate, cacao and Dakahleya propolis isolate where they gave 12.719, 16.985 and 18.943 potency folds; respectively. Besides, the original activity remaining (OAR %) after five hours (Figure 3), showed that the virus mixed with Dakahleya isolate gave 73.13% followed by virus mixed with cacao, Giza propolis isolate and Alex propolis isolate where they gave 69.85, 56.92 and 50.00 %; Respectively while Virus alone treatment gave only 18.223 % OAR.

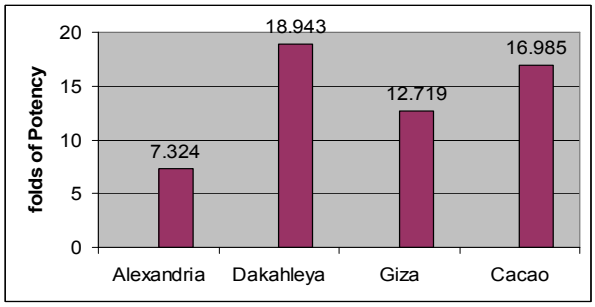

Figure (2) Potency among *S littoralis* neonate larvae treated with *Spli*NPV in combination with propolis isolates or Cacao at 1% concentration, all exposed to different UV irradiation periods maximum 5 hours.

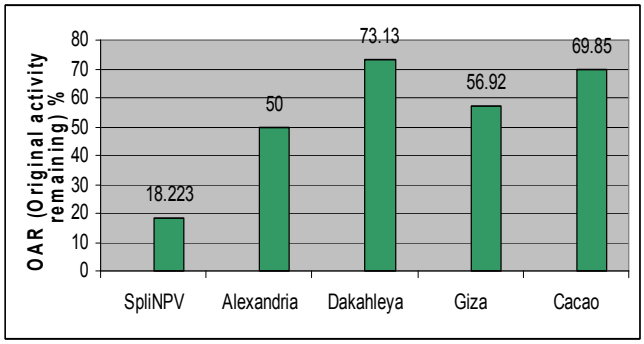

Figure (3) OAR (Original activity remaining) among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or Cacao at 1% concentration, all exposed to five hours UV irradiation period.

Different irradiation periods were 30, 60, 180 and 300 min gave percentages of mortality 52.41, 47.52, 53.69 and 17.69 with virus alone treatment wile it gave 84.89, 76.30, 64.51, and 49.59 % when virus mixed with Alex propolis isolates (Table 1) these percentages increased when Virus mixed with propolis from Dakahleya Gov., and propolis from Giza and Cacao where it gave 98.36, 85.08 and 98.76 30 min post application, 88.75, 82.30 and 93.44 % with 60 min application, 82.85, 71.37 and 88.211 % with 180 min and finally 71.07, 56.45 and 68.42 % 300 min post application, respectively. as it is known the reduction % has adverse effect with prolonging the viral activity, Table (2) showed that reduction in viral activity was 79.22%, while it decreased to 49.59, 42.725 with virus mixed with propolis from Alex and Giza; respectively the lowest reduction found to be with virus mixed with Dakahleya isolate and virus mixed with cacao where it gave 26.1 and 29.53 %; respectively.

Second stage showed that under prolonged period of UV application virus mixed with cacao gave 14.93 LIT_{50} hours while it gave 12.63, 11.275 and 7.392 for virus mixed with propolis derived from Dakahleya, propolis derived from Giza and virus mixed with Alexandrian propolis. Virus alone treatment gave 1.026 only LIT_{50}. (Fig 4)

Table (1) Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or Cacao at 1% concentration, all exposed to different UV irradiation periods.

Irradiation exposure period (min)	Mortality % among larvae tested with				
	*Spli*NPV alone	NPV+ The indicated additives			
		Alexandria Propolis	Dakahleya Propolis	Giza Propolis	Cacao
Zero time	96.91 (235/244)	99.18 (242/244)	97.17 (241/248)	99.17 (240/242)	97.95 (240/245)
30	52.41 (130/248)	84.89 (208/245)	98.36 (240/244)	85.08 (211/248)	98.76 (239/242)
60	47.52 (115/242)	76.30 (190/249)	88.75 (221/249)	82.30 (200/243)	93.44 (228/244)
180	53.69 (80/149)	64.51 (160/248)	82.85 (203/245)	71.37 (177/248)	88.211 (217/246)
300	17.69 (43/243)	49.59 (121/244)	71.07 (172/242)	56.45 (140/248)	68.42 (68.24)
Control	0.00 (0/244)	0.00 (0/243)	0.00 (172/242)	00.14 (1/243)	0.00 (0/248)

Table (2) Average rates of reduction among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or Cacao at 1% concentration, all exposed to different UV irradiation periods.

Irradiation exposure period (min)	Mortality % among larvae tested with				
	*Spli*NPV alone	NPV+ The indicated additives			
		Alexandria Propolis	Dakahleya Propolis	Giza Propolis	Cacao
30	44.5	14.29	------	14.09	------
60	49.39	22.88	8.42	16.87	4.01
180	43.22	34.67	14.32	27.8	9.739
300	79.22	49.59	26.1	42.72	29.53

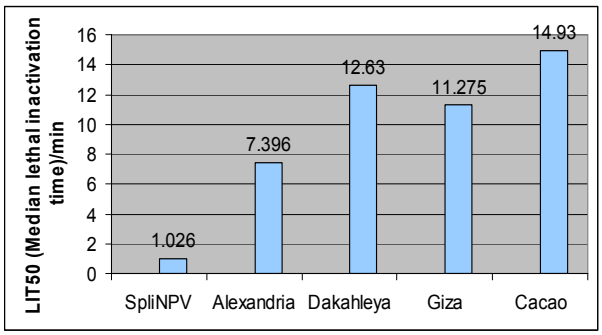

Figure (4) LIT_{50} (Median lethal inactivation time) among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or cacao at 1% concentration, all exposed to different UV irradiation periods.

The mortality percentage represented in Table (3) and average of reduction in Table (4) indicated that virus alone treatment gave 28.08, 14.78, 6.08, and 12.16 mortality percentage 3, 5, 7 and 10 hours, these percentages increased with virus mixed with Alexandrian propolis to 75.69, 58.75, 52.73 and 41.49 and to 87.24, 84.72, 62.83 and 59.58 % with Virus mixed with Dakahleya isolate, 76.02, 65.98, 61.22 and 52.02 % when virus mixed with propolis derived from Giza Gov. Virus mixed with cacao gave 85.81, 81.81, 68.70 and 60.81dead % at these periods of applications. Reduction % 10 hours post application gave lowest % when virus mixed with cacao and virus mixed with propolis derived from Dakahleya with 39.19 and 39.07%; respectively, these percentages get higher with virus samples mixed with propolis from Giga and Alexandria provenance where they gave 43.87 and 55.84% while virus alone treatment gave the highest reduction percentage 80.5%.

Table (3) Average rates of mortality in virus activity expressed in mortality rates among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or cacao at 1% concentration, all exposed to different UV irradiation periods.

Irradiation Exposure periods (Hours)	Mortality % among larvae tested with				
	*Spli*NPV alone	NPV + the indicated additives			
		Alexandria Propolis	Dakahleya Propolis	Giza Propolis	Cacao
Zero time	92.66 (139/150)	97.33 (146/150)	98.65 (146/150)	95.89 (147/149)	100.00 (148/148)
3	28.08 (41/146)	75.69 (109/144)	87.24 (130/149)	76.02 (111/146)	85.81 (127/148)
5	14.78 (21/142)	58.75 (87/148)	84.72 (122/144)	65.98 (97/147)	81.81 (117/143)
7	6.08 (9/148)	52.73 (77/146)	62.83 (93/148)	61.22 (90/147)	68.70 (101/147)
10	12.16 (18/148)	41.49 (61/147)	59.58 (87/146)	52.02 (77/148)	60.81 (90/148)
Control	0.00 (0/149)	0.00 (0/149)	0.00 (0/149)	0.00 (0/144)	0.00 (0/148)

Potency and OAR parameters gave the same trend where it gave in ascending potency folds as follows: 14.551, 12.3, 10.989 and 7.204 with virus mixed with all of cacao, Dakahleya propolis, Giza propolis and Alexandrian propolis; respectively as it

demonstrated in Figs (5 and 6) the OAR% was only 13.12 hours while cacao prolonged virus activity to 60.81, and it was prolonged to 60.39, 54.24 and 42.62 % with Dakahleya propolis, Giza propolis and Alexandria propolis additives.

Table (4) Average rates of reduction in virus activity expressed in mortality rates among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or cacao at 1% concentration, all exposed to different UV irradiation periods.

Irradiation Exposure periods (Hours)	Mortality % among larvae tested with NPV + the indicated additives				
	*Spli*NPV alone	Alexandria Propolis	Dakahleya Propolis	Giza Propolis	Cacao
3	64.58	21.64	11.41	19.87	14.19
5	77.88	38.55	13.93	29.91	18.19
7	86.58	44.6	35.82	34.67	31.3
10	80.5	55.84	39.07	43.87	39.19

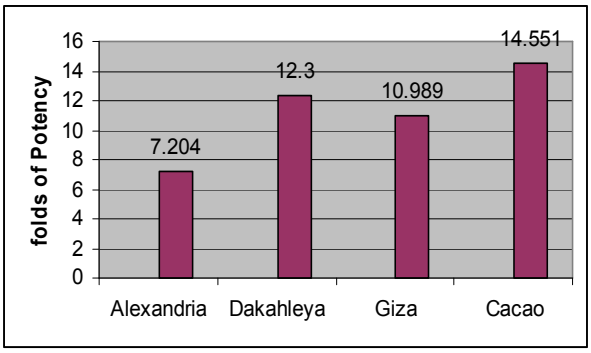

Figure (5) Potency among *S littoralis* neonate larvae treated with *Spli*NPV in combination with propolis isolates or cacao at 1% concentration, all exposed to different UV irradiation periods

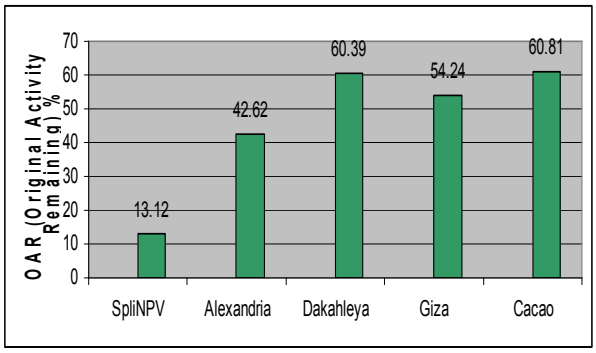

Figure (6) OAR (Original Activity Remaining) among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or cacao at 1% concentration, all exposed to different UV irradiation periods

Both first and second steps showed fluctuations between cacao and propolis additives but all of them preserved the virus much more than virus alone treatment. Third step was field step, it showed (table 5) that 48 hours later virus alone treatment gave 6% only, while it gave 82.97, 67.34, 54.00 and 42.00% with cacao Dakahleya propolis, Giza propolis and Alexandria propolis additives.

Table (5) Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or Cacao at 5 % concentration, all exposed to different natural sunlight irradiation periods.

Irradiation exposure period (hour)	Mortality % among larvae tested with NPV+ The indicated additives				
	*Spli*NPV alone	Alexandria Propolis	Dakahleya Propolis	Giza Propolis	Cacao
Zero time	98.00 (49/50)	98.00 (49/50)	97.87 (46/47)	100.00 (50/50)	96.00 (48/50)
10	60.00 (30/50)	96.00 (48/50)	89.13 (41/46)	95.65 (44/46)	94.00 (47/50)
24	42.00 (21/50)	78.72 (37/47)	88.00 (44/50)	82.97 (39/47)	80.00 (40/50)
48	6.00 (3/50)	42.00 (21/50)	67.34 (33/49)	54.00 (27/50)	82.97 (39/47)
96	2.00 (1/50)	22.00 (11/50)	42.00 (21/50)	40.42 (19/47)	56.25 (27/48)
168	2.00 (1/50)	14.00 (7/50)	23.40 (11/47)	18.00 (9/50)	26.53 (13/49)
Control	0.00 (0/50)	0.00 (0/50)	0.00 (0/50)	0.00 (0/50)	0.00 (0/49)

Table (6) Average rates of reduction in virus activity expressed in mortality rates among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or cacao at 1% concentration, all exposed to different UV irradiation periods.

Irradiation Exposure periods (Hours)	Mortality % among larvae tested with NPV + the indicated additives				
	*Spli*NPV alone	Alexandria Propolis	Dakahleya Propolis	Giza Propolis	Cacao
3	64.58	21.64	11.41	19.87	14.19
5	77.88	38.55	13.93	29.91	18.19
7	86.58	44.6	35.82	34.67	31.3
10	80.5	55.84	39.07	43.87	39.19

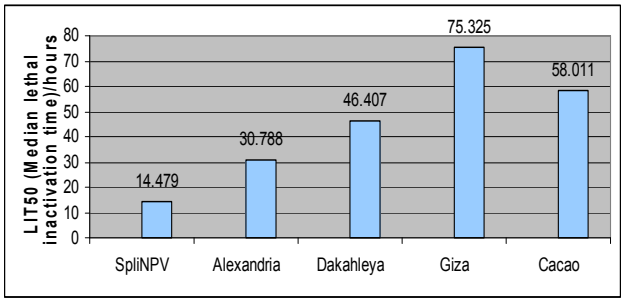

Figure (7) LIT50 (Median lethal inactivation time) among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or Cacao at 5 % concentration, all exposed to different natural sunlight irradiation periods.

The median lethal inactivation time showed that 75.325 with Giza propolis additive followed by 58.001, 40.407, 30.788 hours with cacao, Dakahleya and Alexandrian propolis Additives while virus alone treatment preserved viral activity for only 14.479 hour (Fig 7). Potency folds cleared that Propolis additives from Giza give the highest preservation to the virus with 5.202; respectively followed by cacao additive with 4.006 fold and propolis additive derived from Dakahleya and Alexandria with 3.205 and 2.126 folds; respectively.

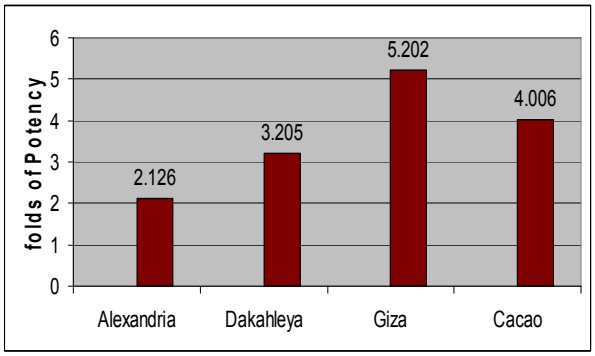

Figure (8) Potency among *S littoralis* neonate larvae treated with *Spli*NPV in combination with propolis isolates or Cacao at 5 % concentration, all exposed to different natural sunlight irradiation periods.

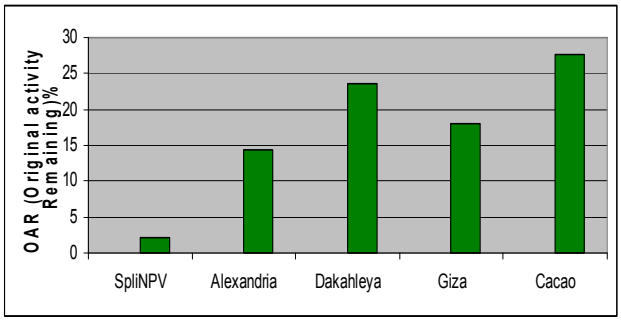

Figure (9) OAR (Original activity Remaining) among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with propolis isolates or Cacao at 5 % concentration, all exposed to 168 hour at natural sunlight irradiation periods.

The trend of OAR % 168 hours later was the same where it gave in ascending order 27.63, 23.5, 18.00 and 14.28 % with cacao, Dakahleya propolis, Giza propolis and Alex propolis additives; respectively while the OAR% for Virus alone was only 2.04.

DISCUSSION

Previous studies demonstrated that plant extracts increase baculovirus persistence (Shapiro et *al*., 2007a, b; Shapiro *et al.,* 2008 and El Salamouny *et al.,* 2009, Deotale et al., 2007 Hong et al, 1996; Mahajan and Sharma 2004 and Nautiyal and Venkataraman 2005.) Cacco toke place as a good protectants under both laboratory and field conditions, (El-Helaly *et al*., 2009 , El-Helaly 2013) Moringa and Rice bran (El-Helaly *et al.,* 2013) This work is the first record to use propolis in Baculovirus protection. Propolis found to be rich in Caffic acid (Schimdt and Buchmann, 1992). Flavonoids, cinnamic acid (Age, 1994) antioxidants (Matsuno, 1997) and possess antioxidant activities (Banskota *et al*, 2000). It has been suggested that activities of propolis depend mainly on the presence of flavonoids (Havsteen, 1983) The obtained result indicated that propolis isolates gathered from different regions protect virus in different manner and that Giza isolate found to give better protection than cacao. This investigation leads to further focusing on flavonoids different groups directly and its role in protection.

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عزلات البروبوليز المتحصل عليها من مناطق مختلفة فى مصر تؤثر على حماية الفيروسات العصوية المغلفة من الأشعة فوق البنفسجية

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تم إختبار ثلاث عزلات من البروبوليز المتحصل عليها من ثلاث مناطق فى مصر (الإسكندرية، الدقهلية و الجيزة) و هى محتواه على مجاميع مختلفة من مضادات الأكسدة كمواد مضافة حامية من الأشعة فوق البنفسجية لمخاليط فيروس دودة ورق القطن البوليهدروزى النووى لإطالة كفاءة الفيروس . نبات *Theubromo cacao* المضاف تم إختباره حيث سبق توثيقه مسبقا كمادة تعطى حماية جيدة. نتائج الفحص كانت مبنية على التقدير الحيوى، أربعة معايير رئيسية تم حسابهاهى نسبة المتبقى الحقيقى للكفاءة OAR%، الوقت النصفى لفقد الكفاءة، Potency ، ونسبة الإختزال للفيروس بعد التعرض لمصدر للأشعة فوق البنفسجية. هذا البحث كان له ثلاث خطوات، إثنان فى المعمل و الأخيرة فى الحقل. التقييم الحقلى كان خلال يونيو إلى أغسطس لعام ٢٠١٥ على نباتات الطماطم فى المزرعة الشرقية بكلية الزراعة جامعة القاهرة. البوبوليز المضاف حفظ كفاءة الفيروس ل ٣٠.٧٨٨، ٤٦.٤٠٧، ٧٥.٣٢٥ ساعة LIT_{50} بالنسبة لعزلات إسكندرية، الدقهلية و الجيزة على الترتيب. نبات*Theubromo cacao* المضاف أعطى ٥٨.٠١١ قيمة LIT_{50}، بينما معاملة الفيروس منفردا كان فقط ١٤.٤٧٩ ساعة بالحقل. النتائج المتحصل عليها تشير إلى أن عزلات بروبوليز معينة تعطى حماية أفضل من الكاكاو و الذى قد يؤدى فى المستقب لتحليل مجاميع مضادات الأكسدة أو الفلافونويد المسؤلة عن الحماية.

كلمات مفتاحية : مضادات الأكسدة، كفاءة الفيروسات العصوية المغلفة، تطبيقات مكافحة حيوية حقليا، بروبوليز، فيروس دودة ورق القطن البوليهدروزى النووى، حماية الفيروس.