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Mixture of Natural Antioxidants can Improve *Spodoptera littoralis* (Boisd.) Nucleopolyhedrovirus, (Baculoviridae) Stabilty Under Egyptian Field Conditions

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ABSTRACT

Four promising natural plants previously proved to prolong the activity of *Spodoptera littoralis* (Boisd.) nuclepolyhedrovirus (*Spli*NPV) suspension under Egyptian field conditions, examined alone or in combination with virus in six mixtures. Screening results were based on bioassay. Different parameters were investigated to prove the value of protection, % of mortality, reduction %, LIT_{50}, Potency and Original Activity Remaining (OAR).DPPH assay was investigated for all treatments. The obtained results showed that, virus mixed with cacao + green coffee treatment gave the highest LIT_{50} (203.474 hours) while scored only (20.172 hours) with virus alone treatment. Original activity remaining indicated that *Spli*NPV mixed with both cacao + green coffee treatment preserved 100% of its activity for five days while it gave only 1.04 % for *Spli*NPV alone treatment and gave 41.06, 31.17, 17.65 and 42.56 with cacao, green coffee, moringa and both cacao + moringa additives; respectively five days post investigation. This investigation suggests that mixing natural derived antioxidants provides good protection to baculovirus at Egyptian sunny conditions.

Keywords*: antioxidants, DPPH, OAR, nuclepolyhedrovirus, ultraviolet, Spodoptera littoralis NPV.*

INTRODUCTION

The Biological control method is an important component of integrated pest management IPM. Baculoviruses biopesticides have many advantages as tool in IPM, (Ravensberg, 2011). However, baculoviruses, like others biopesticides, present some difficulties, such as short field stability (Mills and Kean, 2010; Regnault-Roger, 2012). More than 60 baculovirus bio-pesticides have been used to control diverse insect pest all around the world (Moscardi, 1999). The treated areas increased up to more than two million hectares in 2010-2012 (Yang *et al.,* 2012). Antioxidants derived from natural plants proved to elongate virus stability last decade. The first record of the use of plant extracts to increase the persistence of insect viruses was by Shapiro *et al.* (2007a, b). Both of the green tea and black tea were reported to be UV protective additive to the beet armyworm nuclepolyhedrovirus (Shapiro *et al.,* 2008 and El Salamouny *et al.,* 2009) Mango leaf extract (Deotale *et al*., 2007) Cacao, green coffee, green and red cabbage (El-Helaly *et al.,* 2009 and El-Helaly *et al.,* 2013) Moringa and Rice bran (El-Helaly, 2013).

The present study was aimed to evaluate in both laboratory and field tests the role of addition the mixtures of the most promising previous additives Moringa, green tea, green coffee cacao and used *Spli*MNPV alone against *S. littoralis*.

MATERIALS AND METHODS

Test Insect

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) established on the semi-synthetic diet described by Shorey and Hale (1965) used in the present investigation.

Virus inocula

Spodoptera littoralis (Boisd.) multiple embedded nuclepolyhedrovirus (*Spli*MNPV), Egyptian isolate.

UV-Protective additives

Four different plant-derived materials (Moringa, green tea, green coffee and cacao) were evaluated as UV-protective additives to *Spli*MNPV suspension. Alone or in mixtures as follows (cacao + moringa, cacao + green coffee, cacao + green tea, moringa + green coffee, moringa + grean tea and green coffee + green tea) One gram of each dry plant material was soaked in 100 ml distilled water for 24 hours then blended and filtrated through three layers of muslin and the filtrate was the stock additive to the tested virus concentration ($1x\ 10^9$ PIB's/ ml) to prepare a final concentration of 1% of the material additive according to the method described by Shapiro *et al.,* (2008) for additives alone, for mixtures half gram of each additive was soaked.

Simulated UV radiation in the sunlight and bioassay

Sunlight UV was simulated using UV lamps according to (Huber and Ludcke, 1996). 50 µl was spread inside a Petri dish (10 cm in diameter), virus film were exposed to the tested irradiation source, after exposures of virus treatment to UV irradiation. The polyhedra deposits in the Petri-dish were resuspended in 10 ml distilled water for use in bioassay tests. Two mls of collected PIB's suspension were applied on the surface of 50 ml semi-artificial diet. An un-treated control treated, with only distilled water. Neonate test larvae, of each treatment, were allowed to feed on the treated diet surface till pupation. Mortality recorded up to 14 days post application (Fritsch and Huber, 1985). Treated insects were laboratory maintained at 25± 2 ºC and 65± 5 R. H.

Field experiment

The promising additives resulted from laboratory tests were further evaluated under outdoors field conditions. For this purpose, about 1/3 feddan of tomatoes (1 feddan = 4200 m^2) was used and one small scale field test was set up to confirm laboratory results. The virus inocula *Spli*NPV was adjusted to 10^8 OBs/ml (=LC_{90-95}). At the time of field application, the virus and tested additives were mixed together and transferred into a hand sprayer. Virus suspension treatments were applied separately to tomato foliage using half liter hand sprayer. Untreated leaves and virus treated leaves were randomly collected at 0, 1, 2, 4, 7, and 10 days post application and kept individually in plastic bags at room

temperature until tested. Each leaf was placed into a glass bottle, on which 10 neonate larvae were allowed to feed for 24 hr. before transferred daily to fresh leaves from the same treatment. Larval mortality was recorded daily until day 14. (Shapiro *et al*., 2008)

DPPH assay

The measurement of the DPPH radical scavenging activity was performed according to Brand-Williams *et al*., 1995. The scavenging activity percentage was determined according to Mensor *et al.,* 2001.

Statistical analysis

Concentration-mortality regressions were calculated to determine the effectiveness of tested material as UV protective additives for the *Spli*MNPV. Slope and LC_{50s} values were calculated according to the method described by Finney (1971). Original activity remaining percentages (OARs %) were determined for each treatment according to Muro and Paul, (1985) in which NPV- caused larval mortality post UV exposure were divided by NPV- caused larval mortality pre UV exposure and multiplied by 100. for DPPH The experiment was done in triplicate for each substance. The results were expressed as percentage decrease with respect to control values and compared by one-way ANOVA and Turkey's test. A difference was considered statistically significant if $p<0.05$.

RESULTS

Screening results revealed loosing of more than 80 % of virus alone treatment activity five hours post application, where it gave 48.00, 33.33, 25.00, 14.00 mortality % after 30, 60, 180, 300; respectively. (Table 1, Fig 1) while other treatments preserved the virus activity *Spli*NPV mixed with cacao, grean tea, grean coffee and moringa treatments gave 49.00, 28.12, 39.39, 40.20 mortality %; respectively. mixing natural antioxidants increase the mortality percentage when *Spli*NPV was mixed with cacao + green coffee, cacao + moringa, moringa+ green coffee and moringa+ green tea where it increased the mortality % to 62.10, 55.67, 45.83 and 40.00; respectively, mixing *Spli*NPV with cacao + green tea or with green coffee + green tea gave 30.20 or 23.65 respectively.

Table (1) Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to different UV irradiation periods.

Irradiation exposure period (hours)	Mortality % among larvae tested with *Spli*NPV alone or mixed the indicated additives at 1%										
	***Spli*NPV alone**	**cacao**	**Green tea**	**green coffee**	**moringa**	**cacao+ moringa**	**cacao + green coffee**	**cacao+ green tea**	**Moringa + green coffee**	**moringa+ grean tea**	**Green coffee + green tea**
Zero time	99.00 (99/100)	93.93 (93/99)	98.96 (96/97)	93.87 (92/98)	98.00 (98/100)	93.93 (93/99)	99.00 (99/100)	100.00 (98/98)	92.00 (92/100)	93.00 (93/100)	96.00 (96/100)
0.5	48.00 (48/100)	93.75 (90/96)	98.96 (71/100)	72.44 (71/98)	85.41 (82/96)	93.00 (93/100)	100.00 (98/98)	71.00 (71/100)	89.69 (87/97)	76.00 (76/100)	69.00 (69/100)
1	33.33 (32/96)	80.00 (80/100)	61.85 (60/97)	69.00 (69/100)	72.16 (70/97)	90.00 (90/100)	97.00 (97/100)	66.00 (66/100)	79.38 (77/97)	70.00 (70/100)	58.76 (57/97)
3	25.00 (25/100)	71.00 (71/100)	39.00 (39/100)	43.00 (43/100)	64.58 (62/96)	76.04 (73/96)	82.29 (79/96)	31.00 (31/100)	68.00 (68/100)	61.85 (60/97)	39.00 (39/100)
5	14.00 (14/100)	49.00 (49/100)	28.12 (27/96)	39.39 (39/99)	40.20 (39/97)	55.67 (54/97)	62.10 (59/95)	30.20 (29/96)	45.83 (44/96)	40.00 (40/100)	23.65 (22/93)
Control*	0.00 (0/100)	0.00 (0/100)	0.00 (0/99)	0.00 (0/99)	0.00 (0/100)	1.03 (1/97)	0.00 (0/97)	0.00 (0/96)	0.00 (0/95)	0.00 (0/96)	0.00 (0/100)
LIT_{50}	70.561	411.241	321.455	412.122	390.247	496.231	851.231	439.211	532.177	536.211	211.11

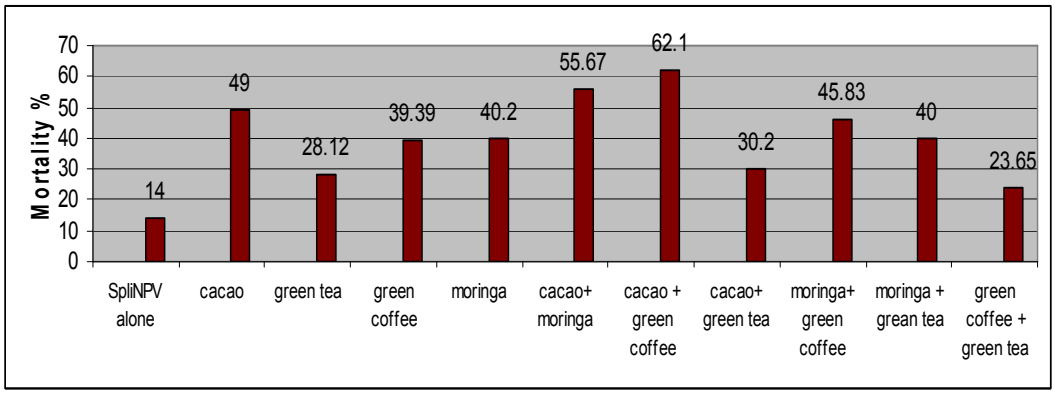

Fig (1) Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixtures, all exposed to 300 min UV irradiation periods.

The obtained results represented in (Table 2 and Fig 2) showed that virus alone treatment gave the highest rates in reduction where it gave 85.00% while it gave 44.93, 70.85, 54.48, 57.8, 38.26, 36.9, 69.8, 46.17, 53.00 and 72.35 % with cacao, green tea, green coffee, moringa, both of cacao + moringa, cacao + green coffee, cacao + green tea, moringa + green coffee, moringa + green coffee and green coffee + green tea; respectively 300 min post application. The highest LIT_{50} found when that virus mixed with cacao + green coffee, where its activity lasts for 851.231 min (14.18 Hours) while it lasts for only 70.561 min (1.17 hours) with virus alone treatment.

Table (2) Average rates of reduction among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to different UV irradiation periods.

Irradiation exposure period (hours)	Mortality % among larvae tested with *Spli*NPV alone or mixed the indicated additives										
	*Spli*NPV alone	cacao	green tea	green coffee	moringa	cacao+ moringa	cacao + green coffee	cacao+ green tea	moringa+ green coffee	moringa + grean tea	green coffee + green tea
Zero time	99.00	93.93	98.96	93.87	98.00	93.93	99.00	100.00	92.00	93.00	96.00
0.5	51.00	0.18	0.00	21.43	12.59	0.93	---------	29.00	2.31	17.00	27.00
1	65.67	13.93	37.11	24.87	28.84	3.93	2.00	34.00	12.62	23.00	37.24
3	74.00	22.93	59.96	50.87	33.42	17.89	16.71	69.00	24.00	31.15	57.00
5	85.00	44.93	70.84	54.48	57.8	38.26	36.9	69.8	46.17	53.00	72.35

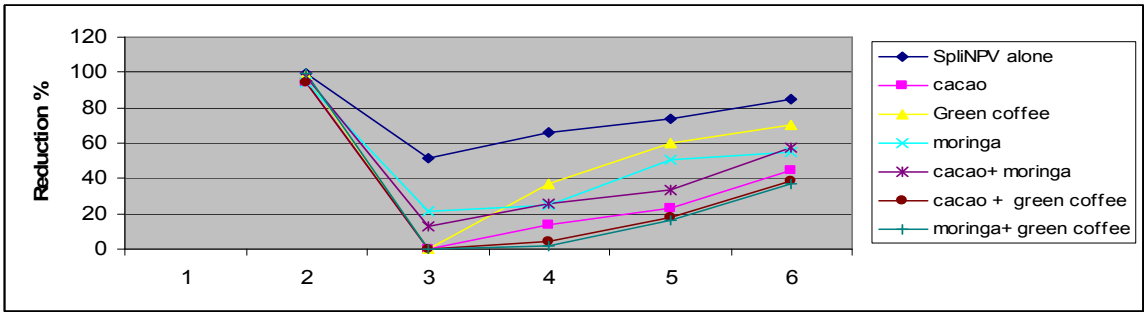

Fig (2) Average rates of reduction among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to different UV irradiation periods.

Original activity remaining for *Spli*NPV alone treatment gave 48.48, 33.66, 25.25, 14.14 % half hour, one, three and five hours post application; respectively, while it increased to 99.80, 85.16, 75.58 and 52.16 % when *Spli*NPV mixed with cacao and it gave 100.00, 62.50, 39.40 and 28.41 when *Spli*NPV mixed with green tea, 77.17, 73.50, 45.80 and 41.96 % when *Spli*NPV mixed with green coffee, 87.15, 73.63, 65.89 and 41.02 % when *Spli*NPV mixed with moringa, 99.00, 95.81, 80.95 and 59.26 % when *Spli*NPV mixed with cacao+ moringa, 100.00, 97.97, 83.12 and 62.72 % when *Spli*NPV mixed with cacao + green coffee, 71.00, 66.00, 31.00 and 30.20 % when *Spli*NPV mixed with cacao + green tea, 97.78, 86.28, 73.91 and 49.81 % when *Spli*NPV mixed with moringa + green coffee, 81.72, 75.26, 66.50 and 43.01% when *Spli*NPV mixed with moringa + green tea, Finally it gave 71.87, 61.20, 40.62 and 24.63 % when *Spli*NPV mixed with green coffee + green tea (Table 3, Fig 3).

Table (3) Average rates of Original Activity Remaining among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to different UV irradiation periods.

Irradiation exposure period (hours)	Mortality % among larvae tested with *Spli*NPV alone or mixed the indicated additives										
	*Spli*NPV alone	cacao	green tea	green coffee	moringa	cacao+ moringa	cacao + green coffee	cacao+ green tea	moringa+ green coffee	moringa + grean tea	green coffee + green tea
Zero time	99.00	93.93	98.96	93.87	98.00	93.93	99.00	100.00	92.00	93.00	96.00
0.5	48.48	99.80	100.00	77.17	87.15	99.00	100.00	71.00	97.78	81.72	71.87
1	33.66	85.16	62.50	73.50	73.63	95.81	97.97	66.00	86.28	75.26	61.20
3	25.25	75.58	39.40	45.80	65.89	80.95	83.12	31.00	73.91	66.50	40.62
5	14.14	52.16	28.41	41.96	41.02	59.26	62.72	30.20	49.81	43.01	24.63

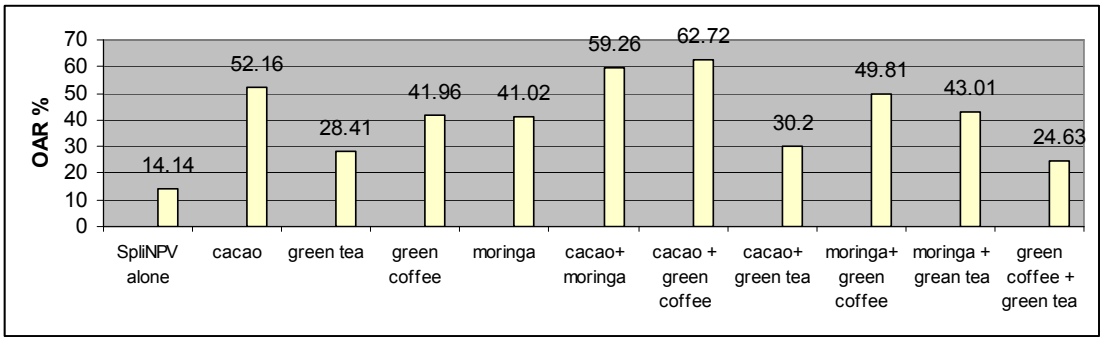

Fig (3) Average rates of Original Activity Remaining among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to 300 min post UV irradiation.

Another bioassay was done with higher concentration 5% of all additives and with elongated periods of investigation which reached 10 hours. Besides the treatments that included grean tea at the previous step was removed from this and further step of evaluation because it gave the lowest protection of *Spli*NPV. mortality % with *Spli*NPV alone treatment gave 36.73, 24.74, 22.44 and 11.57 % 3, 5, 7 and 10 hours post exposure to the synthetic UV, mortality % increased to 93.87, 80.80. 70.00 and 57.00 % when *Spli*NPV mixed with cacao, 92.78, 80.61, 69.00 and 53.00 % when *Spli*NPV mixed with green coffee, 85.56, 72.44, 68.68 and 43.00 % when *Spli*NPV mixed with moringa, 97.95, 90.90, 85.56 and 59.37 % when

*Spli*NPV mixed with cacao + moringa, 100.00, 95.87, 90.62 and 69.47 % when *Spli*NPV mixed with cacao + green coffee, 89.79, 77.77, 71.13 and 51.04% when *Spli*NPV mixed with both moringa + green coffee. (Table 4, Fig 4) The highest LIT_{50} found when that virus mixed with cacao + green coffee, where its activity lasts for 15.299 hours while it lasts for only 0.57 hour with virus alone treatment. Reduction % was the lowest when *Spli*NPV mixed with cacao + grean coffee or cacao + moringa where it gave only 26.53 and 39.59 %; respectively. When *Spli*NPV mixed with cacao, grean coffee or moringa alone the reduction of virus activity reached 41.89, 47.00 and 57.00 %; respectively while it gave 48.96 when *Spli*NPV mixed with both moringa + green coffee, *Spli*NPV alone treatment scored the highest degree of reduction where it gave 87.41% (Table 5 & Fig 5).

Table (4) Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to different further elongated UV irradiation periods.

Irradiation exposure period (hours)	Mortality % among larvae tested with *Spli*NPV alone or mixed the indicated additives at 5% total concentration						
	*Spli*NPV alone	cacao	Green coffee	moringa	cacao+ moringa	cacao + green coffee	moringa+ green coffee
Zero time	98.98 (98/99)	98.98 (98/99)	100.00 (96/96)	100.00 (96/96)	98.96 (96/97)	96.00 (96/100)	100.00 (96/96)
3	36.73 (36/98)	93.87 (92/98)	92.78 (90/97)	85.56 (83/97)	97.95 (96/98)	100.00 (98/98)	89.79 (88/98)
5	24.74 (24/97)	80.80 (80/99)	80.61 (79/98)	72.44 (71/98)	90.90 (90/99)	95.87 (93/97)	77.77 (77/99)
7	22.44 (22/98)	70.00 (70/100)	69.00 (69/100)	68.68 (68/99)	85.56 (83/97)	90.62 (87/96)	71.13 (69/97)
10	11.57 (11/95)	57.00 (57/100)	53.00 (53/100)	43.00 (43/100)	59.37 (57/96)	69.47 (66/95)	51.04 (49/96)
Control DW	0.00 (0/92)	0.00 (0/98)	0.00 (0/100)	0.00 (0/99)	0.00 (0/96)	0.00 (0/97)	0.00 (0/97)
LIT_{50}	0.57	11.438	10.112	9.425	12.316	15.299	10.911

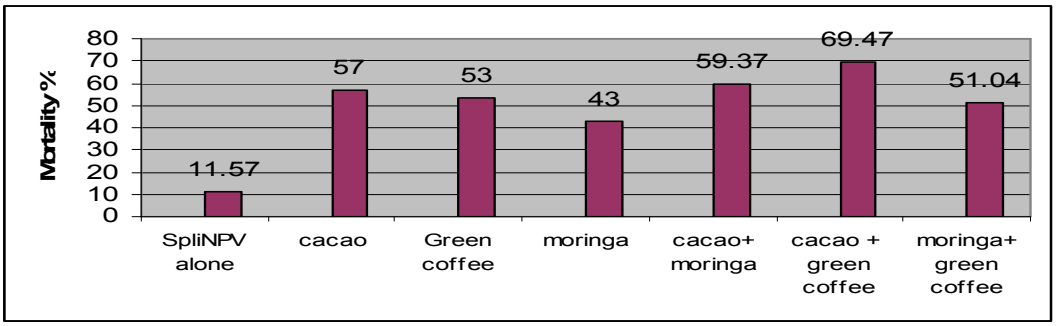

Fig (4) Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixtures, all exposed to 10 hours UV irradiation periods.

Table (5) Average rates of reduction among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to different further elongated UV irradiation periods.

Irradiation exposure period (hours)	Mortality % among larvae tested with *Spli*NPV alone or mixed the indicated additives						
	*Spli*NPV alone	cacao	Green coffee	moringa	cacao+ moringa	cacao + green coffee	Moringa + green coffee
Zero time	98.98	98.98	100.00	100.00	98.96	96.00	100.00
3	62.52	5.11	7.22	14.44	1.01	------	10.21
5	74.24	18.18	19.39	27.56	8.06	0.13	33.33
7	76.54	28.98	31.00	31.32	13.4	5.38	28.87
10	87.41	41.98	47.00	57.00	39.59	26.53	48.96

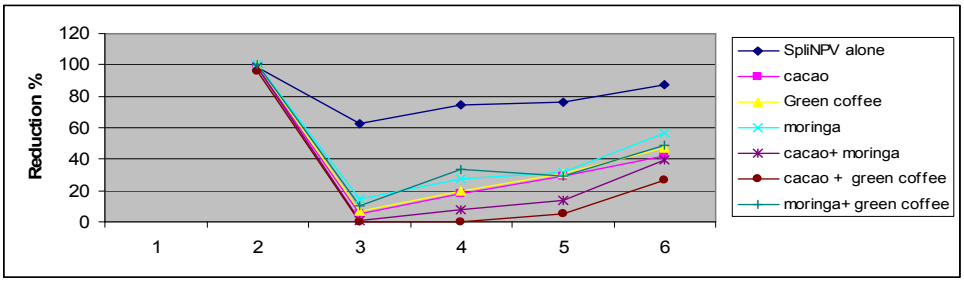

Fig (5) Average rates of reduction among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to different prolonged UV irradiation periods.

The OAR % also gave the same trend of mortality % as showed that in (Table6 &Fig 6) where *Spli*NPV alone treatment scored 11.68 %/10 hr. post irradiation to artificial sun light while it gave 57.58,

53.00, 43.00, 59.99, 72.36 and 51.04 when *Spli*NPV mixed with cacao, green coffee, moringa or with both cacao + moringa, cacao + green coffee and moringa + moringa + green coffee. The last screening test was evaluation of additives under Egyptian sunny conditions, where *Spli*NPV alone treatment found to lose almost all its activity 48, 96 and 168 hours post irradiation where it scored 7.14, 1.03 and 1.04% of mortality only while *Spli*NPV mixed with both green coffee and green tea treatment single out with 98.96 mortality % 168 hours post irradiation and gave almost complete protection. *Spli*NPV mixed with both cacao + moringa gave high mortality % (82.47) 96 hour post irradiation while cacao, green coffee and moringa additives gave when they mixed with *Spli*NPV 81.81, 78.12 and 74.22 mortality % 96 hours post irradiation.(table 7 & Fig 7).

Table (6) Average rates of Original Activity Remaining among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to different prolonged irradiation periods.

Irradiation exposure period (hours)	Mortality % among larvae tested with *Spli*NPV alone or mixed the indicated additives						
	*Spli*NPV alone	cacao	Green coffee	moringa	cacao+ moringa	cacao + green coffee	moringa+ green coffee
Zero time	98.98	98.98	100.00	100.00	98.96	96.00	100.00
3	36.74	94.83	92.78	85.56	98.97	100.00	89.79
5	24.99	81.63	80.61	72.44	91.85	99.86	77.77
7	22.67	70.72	69.00	68.68	86.45	94.39	71.13
10	11.68	57.58	53.00	43.00	59.99	72.36	51.04

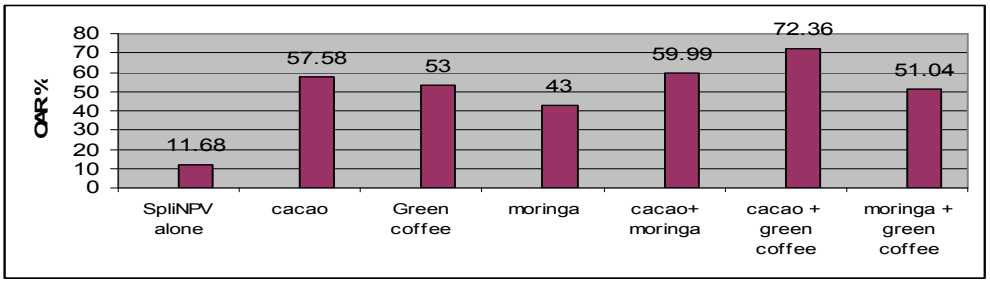

Fig (6) Average rates of Original Activity Remaining among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to 10 hr. post UV irradiation.

Table (7) Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to natural sunlight UV different irradiation periods.

Irradiation exposure period (hours)	Mortality % among larvae tested with *Spli*NPV alone or mixed the indicated additives at 5% total concentration					
	*Spli*NPV alone	cacao	Green coffee	moringa	cacao + green coffee	cacao+ moringa
Zero time	100.00 (99/99)	96.90 (94/97)	95.87 (93/97)	96.87 (93/96)	96.96 (96/99)	95.87 (93/97)
10	89.00 (89/100)	100.00 (98/98)	95.78 (91/95)	100.00 (96/96)	97.97 (97/99)	98.96 (96/97)
24	40.40 (40/99)	100.00 (96/96)	100.00 (96/96)	100.00 (97/97)	98.96 (96/97)	90.62 (87/96)
48	7.14 (7/98)	98.95 (95/96)	100.00 (95/95)	98.97 (97/98)	100.00 (97/97)	90.62 (87/96)
96	1.03 (1/97)	81.81 (81/99)	78.12 (75/96)	74.22 (72/97)	82.65 (81/98)	82.47 (80/97)
168	1.04 (1/96)	39.79 (39/98)	29.89 (29/97)	17.7 (17/96)	98.96 (96/97)	40.81 (40/98)
Control DW	0.00 (0/96)	0.00 (0/98)	0.00 (0/98)	0.00 (0/96)	0.00 (0/97)	0.00 (0/96)
LIT_{50}	20.172	159.681	139.631	125.709	203.474	193.360

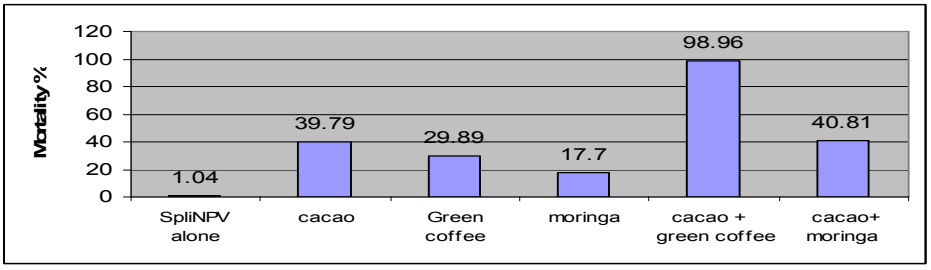

Fig (7) Average rates of mortality among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixtures, all exposed to 168 hours UV irradiation periods.

Reduction in virus activity was almost 100% 96 hours post irradiation where it gave 98.03 % , it scored very low rates with all additives 15.09, 17.75, 22.65, 14.31 and 13.00 % of reduction with cacao, green coffee, moringa, cacao + green coffee and cacao + moringa additives; respectively 96 hours post irradiation. (Table 8) Virus mixed with cacao + green coffee treatment gave the highest LIT_{50} value, where its activity lasts for 203.474 hours while it lasts for only 20.172 hours with virus alone treatment.

Original activity remaining showed that *Spli*NPV mixed with both cacao + green coffee treatment preserved 100% of its activity for five days while it gave only 1.04 % for *Spli*NPV alone treatment and gave 41.06, 31.17, 17.65 and 42.56 with cacao, green coffee, moringa and both cacao + moringa additives; respectively five days post investigation.(Table9&Fig 8)

DPPH assay

Antioxidant activity was the highest with both cacao + green coffee (85.3%) while it gave 51.03, 41.19, 33.65 and 77.56 with cacao, green coffee, moringa and both cacao + moringa additives; respectively.

Table (8) Average rates of reduction among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to natural sunlight UV different irradiation periods.

Irradiation exposure period (hours)	Mortality % among larvae tested with *Spli*NPV alone or mixed the indicated additives					
	*Spli*NPV alone	cacao	Green coffee	moringa	cacao + green coffee	cacao+ moringa
Zero time	100.00	96.90	95.87	96.87	96.96	95.87
10	11.00	-----	-----	-----	-----	-----
24	59.60	-----	-----	-----	-----	-----
48	92.86	-----	-----	-----	-----	-----
96	98.03	15.09	17.75	22.65	14.31	13.00

Table (9) Average rates of Original Activity Remaining among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to natural sunlight UV different irradiation periods.

Irradiation exposure period (hours)	Mortality % among larvae tested with *Spli*NPV alone or mixed the indicated additives					
	*Spli*NPV alone	cacao	Green coffee	moringa	cacao + green coffee	cacao+ moringa
Zero time	100.00	96.90	95.87	96.87	96.96	95.87
10	89.00	100.00	99.9	100.00	100.00	100.00
24	40.40	100.00	100.00	100.00	100.00	94.52
48	7.14	100.00	100.00	100.00	100.00	94.52
96	1.03	84.42	81.48	76.61	85.24	86.02
168	1.04	41.06	31.17	17.65	100.00	42.56

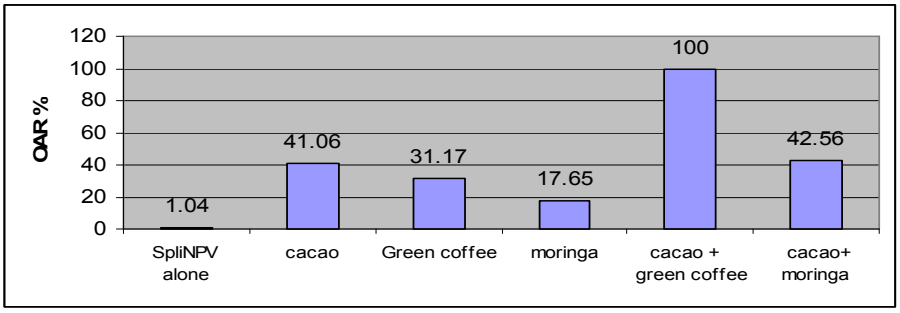

Fig (8) Average rates of Original Activity Remaining among *S littoralis* neonate larvae treated with *Spli*NPV either alone or in combination with different antioxidants or their mixture, all exposed to 168 hours post natural sunlight UV .

DISSCUTION

Baculoviruses are the major group of arthropod viruses (Herniou and Jehle, 2007). They are considered as important biological control agents (Szewczyk *et al.,* 2009), and have been used at forestry since 1900s as biological control agents, biotechnology (Contreras-Gómez *et al.,* 2014). 500 species are known of the baculovirus have been isolated from the order Lepidoptera (Possee *et al.,* 1997; Szewczyk *et al.,* 2006). As it is will known Baculoviruses, present some shortage in field stability (Stewart *et al*., 1991). It is will proved that plant extract proved to prolong baculovirus stability against Sun light, even artificial or natural sources (Deotale *et al*., 2007; Shapiro *et al.* 2007a, b; Shapiro *et al.,* 2008; El Salamouny *et al.,* 2009; El-Helaly *et al.,* 2009 and El-Helaly, 2013; El-Helaly *et al.,* 2013) This article provides an over view of the role of natural antioxidants and their mixtures in baculovirus protection under both natural and artificial antioxidants. It should be noted that antioxidant activity of food extracts can be determined using a (stable free radical s scavengers: galvinoxyl, diphenyl-b-picr ylhydrazyl [DPPH] (Nakatani 2003; Brown and Kelly 2007; Chen 2008; Iacopini *et al.,* 2008). These antioxidant compounds include flavonoids, phenolic acids, carotenoids, and tocopherols that can inhibit Fe3+/AA-induced oxidation, scavenges free radicals, and act as reductions (Khanduja 2003; Ozsoy *et al.,* 2009).mixing natural plants could increase oxidative stress in order to accumulative effect of antioxidants groups, both bioassay results and DPPH results proved that mixing cacao with green coffee gave highest protection to baculovirus in field and the biggest read of free radicals. This work suggest that this work should followed by studying the direct effect of different flavonoids on protection toward applicable formulation in the field.

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خلـط مضـادات الأكسـدة الطبيعيـة يمكـن أن يحسـن بقائيـة فيـروس دودة ورق القطـن البوليهـدروزيس النـووى (Baculoviridae) تحت ظروف الحقل المصرية

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أربعة نباتات طبيعية سبق و أثبتت أنها واعدة لإطالة كفاءة فيروس حشرة دودة ورق القطن البوليهدروزيس النووى تحت الظروف المشمسة المصرية. تم أختبارها منفردة أو مخلوطة مع الفيروس فى صورة أربعة مخاليط. نتائج الفحص مبنية على التقدير الحيوى، تم إستخدام دلائل مختافة لإثبات قيمة الحماية المتحصل عليها، نسبة الموت، الإختزال، الوقت اللازم للاحتفاظ بنصف الكفاءة، القدرة الفعلية و نسبة الكفاءة الحقيقية المتبقية . تم تقدير الـ (DPPH) لكل المعاملات. مخلوط الفيروس مع كل من الكاكاو + القهوة الخضراء أعطى أعلى بقائية LIT_{50} (٢٠٣.٤٧٤ ساعة) بينما سجلت فقط (٢٠.١٧٢ ساعة) مع معاملة الفيروس منفردا. الكفاءة الحقيقية المتبقية أظهرت أن مخلوط الفيروس مع كل من الكاكاو + القهوة الخضراء حفظ ١٠٠% من كفاءة الفيروس لمدة خمس أيام بينما أعطى فقط ١.٠٤% للفيروس منفردا و أعطى ٤١.٠٦، ٣١.١٧، ١٧.٦٥ و ٤٢.٥٦ مع الكاكاو، القهوة الخضراء، المورينجا و كل من الكاكاو + المورينجا، على الترتيب خمسة أيام بعد إجراء الإختبار. هذا البحث يقترح أن خلط مضادات الأكسدة المشتقة من نباتات طبيعية توفر حماية جيدة للفيروسات العصوية المغلفة تحت الظروف المشمسة المصرية .