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THE EFFECTIVENESS OF SPINOSAD AND NEEM EXTRACT AGAINST *Spodoptera littoralis* (BOISD.) AND *Spodoptera exigua* (HUBNER): EXPLORING POSSIBILITIES TO ENHANCE THE BIO-PESTICIDE PERSISTENCE WITH NATURAL UV PROTECTANTS UNDER FIELD-SUNLIGHT CONDITIONS OF SAUDI ARABIA

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Ultraviolet (UV) sunlight is considered to be the core factor reducing efficacy of bio-pesticides especially under the sunny-field conditions. The effectiveness and persistence of two bio-pesticides neem plant extract and spinosad were evaluated under the simulated and field conditions against the cotton army worm, *Spodoptera littoralis* (Boisd.) and the beet armyworm *Spodoptera exigua* (Hubner). Our findings indicate that persistence of both the bio-pesticides was significantly reduced at 21 day post-application when tested against first instar larvae of the *S. exigua*. However, the use of natural UV protectants henna and clove, screened in the present study had conserved the effectiveness of both bio-pesticides at 21-day post treatment against the neonate larvae of *S. exigua*. In contrast, when the bio-pesticides were applied against neonate larvae of *S. littoralis*, only spinosad formulated with green tea and clove was found effective at 1 day post-application with 86% and 55% mortality, respectively. When these pesticides were applied against second instar larvae, again spinosad blended with clove was showing mortality of about 50% at 3 days of application, whereas all other formulations caused very low mortality. Thus, the present data conclusively demonstrate that the bio-pesticides persistence could be enhanced by using extracts of henna and clove and that their application could be effective only to newly infested crops with neonate larvae.
Keywords: Bio-pesticides, natural additives, *Spodoptera exigua*, *Spodoptera littoralis*, UV protectants.

INTRODUCTION

The proliferation of environmental pollution and development of insect resistance awareness against chemical insecticides as well as *Bacillus thuringiensis*, the entomopathogenic bacteria, has led to a growing interest in the use of bio-pesticides like neem extract, baculoviruses, and spinosad in crop protection. These bio-pesticides are not only effective, but also safe, eco-friendly, and have narrow species spectra (Summer *et al*., 1975; Burges *et al*., 1980). These advantages support bio-pesticides to be the promising alternatives to reduce the dependency on harmful chemical insecticides. Bio-pesticides have been successfully tested against several insect pests and several commercial products, are readily available in the market, predominantly in USA and Europe (Villaverde *et al*., 2016; Butt *et al*., 1999).

In 1990, the bio-pesticide, SpinTor® based on the active ingredients spinocyn A and spinocyn D was discovered by Mertz and Yao (1990) from the fermentation of the soil Actinomycetes bacterium (*Saccharopolyspora spinosa*). Spinosad is an insect control agent that provides high efficacy against numerous lepidopteran larval stages infesting various crops. It is primarily composed of two active components, spinosyn A and spinosyn D. It has low toxicity to the beneficial insects (DeAmicis *et al*., 1997; Kirst *et al*., 1992) but is rapidly degraded in the environment (Yano *et al*., 2002). Moreover, the azadirachtin, the protein isolated from neem extract, has a wide spectrum against insect pests. Its application has caused a significant reduction in the weight of gypsy moth larvae that was probably because of decline in the host feeding (Cook *et al*., 1997). Liang *et al*. (2003) evaluated three commercial insecticides, Agroneem™, Ecozin™, and Neemix™ in the laboratory against diamond back moth (*Plutella xylostella*). All these products exhibited significant anti-feedant effect. The larvae on treated leaves quickly stopped feeding and dropped off from the leaves, resulting in no or minimal damage to the treated cabbage leaves. The weight, length, and diameter of the larvae that fed on neem

treated leaves were significantly smaller, 0.012–0.016 mg/ larva, 13.5–14.8 mm, and 2.0–2.5 mm, respectively compared with those fed on water-treated leaves (0.058 mg/larva, 30.2 mm in length, and 4.8 mm in diameter). It is documented that the exposure of azadirachtin under sunlight caused a rapid decreasing of anti-feeding activity in neonate larvae of fall armyworm (*Spodoptera frugiperda*) as compared to unexposed azadirachtin (Stokes and Redfern, 1982). Also, it is reported that azadirachtin and its related compounds are very sensitive to sunlight and degrade rapidly with half-lives of 11.3 hours and 5.5 hours for azadirachtin A and azadirachtin B, respectively, while the limonoids, a group of phytochemicals isolated from citrus that is used as insecticide, degraded only after a few minutes under sunlight conditions (Caboni *et al*., 2006).

Both armyworms have a high economic impact because they damage several crops in Saudi Arabia. These armyworms are known to damage plants belonging to 44 different families including grasses, legumes, crucifers, and deciduous fruits of highly economic importance. In North Africa, they damage many vegetable crops, while in Egypt they are the major pests of cotton. In Southern Europe, such as in Sicily these insects are considered as important pest commercial flowering plants vegetables in the glasshouses (Vaamonde, 2009). These armyworms are highly polyphagous pests and are widely distributed in the temperate regions and Mediterranean countries including Gulf countries such as Saudi Arabia (Al-Kherb, 2014). The control of these armyworms usually involves the excessive use of chemical pesticides which has developed the resistance and resurgence in these insect pests. The excessive use of the chemicals also provides biological accumulation and biological magnification to the environment and non-target species.

Currently, the organic farming is being promoted as an environmentally friendly approach in most of the developed countries. Unfortunately in developing nations, very little or no attention has been paid to these issues. Since, the bio-pesticides are sensitive to ultraviolet (UV) sunlight they need to be protected for their effectiveness and longtime persistence in the environment. Formulations of the bio-pesticides using natural plant extracts as UV protectants is very promising to protect the bio-pesticides persistence from the adverse sunny conditions. In the present study, we tested spinosad and neem extract along with natural additives as UV protectants against cotton armyworm, *S. littoralis* (Noctuidae: Lepidoptera) and beet armyworm, *S. exigua* (Noctuidae: Lepidoptera) as a model for organic farming in Saudi Arabia.

MATERIALS AND METHODS

***Insects mass rearing*:** The adults of cotton armyworm and beet army worm were collected from vegetable farms in Alamariyah, Riyadh Province, Saudi Arabia by using a light trap. The adults were then kept in the plastic jars provided with sugar solution soaked-cotton as food resource, and opaque papers as egg laying substrate. The adults were maintained in growth chamber (Steridium, Australia) at 25°C, 65% RH, and 15:9 light and dark. The eggs were collected and surface sterilized using 1% chlorox solution (v/v). The eggs were then washed in the running tap water and incubated in the growth chamber. The hatched larvae were then transferred into white bean artificial diets (Shorey and Hale 1965) in plastic cups (50 mm diameter and 80mm height). until reached to the 4th instar, when they were maintained individually on the artificial diet until pupation. The pupae were collected, surface sterilized with chlorox solution (1%) and washed in the running tap water. The pupae, 20-25 individuals, were then placed in the plastic jars until adult emergence. After emergence, the adults were fed on sugar solution and opaque papers were placed inside the jars for oviposition. In this experiment, the newly hatched (24 h old) and second instar larvae were used.

***Bio-pesticides and natural additives preparations*:** The bio-pesticides used in these experiments were the Tracer (48% spinocyn) (Dow AgroSciences, UK) and NeemAZAl (1% azadirachta A) (Trifolio-M GmbH, Germany) as spinosad and neem extract resources, respectively. These bio-pesticides were purchased from Al Rasheed Trading Comp. Riyadh Saudi Arabia. Ten plant extracts: henna leave, dates fruit, clove flower, lemon fruit, red grape fruit, green tea leave, kiwi fruit, pomegranate fruit without skin, olive fruit, and red beetroot were evaluated as UV protectants.

The natural additives were prepared following methods described by El Salamouny *et al*. (2009 a, b) and Shapiro *et al.* (2008). The each of air dried plant extract was grinded into powder using commercial blender. Concentrations of 1%, 2%, and 10% (w/v) of each extract were made by soaking the powder in autoclaved distilled water for overnight. After that, the solution was filtered by using double muslin cloth. The solutions were kept cool at 4ºC until further used.

***Preliminary experiments*:** Preliminary bioassays were conducted to determine the lethal concentration causing 95% mortality (LC_{95}) of the tested bio-pesticides against both insect species at 1st and 2nd larval instar. The serial concentrations of 0, 0.5, 1.0, 2.0, 4.0, and 6.0 ppm of spinosad and 0, 10, 12, 14, 16, and 18 ppm of neem extract were made by diluting the measured volume of stock solution into sterilized distilled water and tested against the two insects. A 1mL of each solution was homogenously spread onto 50 mL artificial diet surface that was placed in a bioassay plate (50mm by 10mm). After spread, the bioassay cell was fixed to separate the diet into 50 cells (10mm by 10mm each). One larva per cell was placed gently using camel brush and allowed to feed on the surface contaminated diet. Thus, each treatment was using 50 larvae and repeated for three replicates. Treatment using sterilized distillate water was used as a control bio-pesticides treatment. The larval mortality was recorded daily until 10 days after application. On the last

observation day, the weight of the survived larvae was measured to observe the sub lethal effects of each treatment.

***Bio-pesticides persistence evaluation under simulated sunlight by using ultraviolet B lights*:** In order to get the baseline data of bio-pesticides persistence, the irradiation tests were done under ultraviolet B light (UVB) in the laboratory. A 40µL of 10X LC_{95} of each bio-pesticide was homogenously spread onto glass petri dish (55mm diameter 15mm height). The bio-pesticides were exposed to the UVB for 0, 1, 2, 3, 4, and 5 hours. Each treatment was replicated three times. After the irradiation, the dry deposit material of each treatment was re-suspended in 4mL distilled water. Then, its effectiveness was tested against 1 day old larvae of *S. exigua*. The bioassay procedure and replicate was the same as described in the preliminary experiment.

***Bio-pesticides persistence evaluation under sunlight*:** In order to get the baseline data of bio-pesticides persistence under sunlight, the irradiation tests were done in summer during 29 May – 5 June 2014. A 40µl of 10X LC_{95} of each bio-pesticide was homogenously spread onto glass petri dish (55mm diameter 15mm height). After that, the bio-pesticides were exposed under direct sunlight for 0, 1, 2, 3, 4, 5, 6, and 7 days. Each treatment included three replicates. After irradiation, the dry deposit material of each treatment was re-suspended in 4mL distilled water. Then its pathogenicity was tested against 1 day old 1st larval instar of *S. exigua*. The bioassay procedure and number of replicates was the same as explained in previous bioassays.

***Preliminary bioassay of plant natural additive as UV protectants for the bio-pesticides under UV B lights*:** To evaluate the protection activity of natural additives and select the best two plant extracts, bio-pesticides were blended with the additives. Above mentioned additives (2% v/v) were used for making 10X LC_{95} solutions. A 40µl of 10X LC_{95} in 2% additives of each bio-pesticide was homogenously spread onto glass petri dish (55mm diameter 15mm height). After that, the bio-pesticides were exposed under UV B for 0 and 5 hours. As the controls; sterilized distillate water, bio-pesticide without additive (bio-pesticides alone), without exposed to UVB, and bio-pesticide alone exposed under UVB were used. Each treatment was using three replicates. After 5 hours exposure, the dry deposit material of each treatment was re-suspended in 4mL distilled water. Then its effectiveness was tested against 1 day old 1st larval instar of *S. exigua*. The bioassay procedure and replicate was the same to that of previous experiments.

***Semi-field bioassay of natural UV protectants ability to enhance the bio-pesticides persistence against armyworms*:** Semi field tests were performed under the sunny conditions in Saudi Arabia on cabbage plants. Six weeks prior to the experiment, 1000 cabbage seeds were grown in the green house at Educational Agricultural Farm, Aldirab, Riyadh Saudi Arabia. Each seed was sown in a small pot with organic peat moss (50 mm diameter 40 mm height). Two weeks old seedlings, having five leaves each, were transplanted in the field at a distance of 250 mm by 500 mm between plants. Each plant was provided with a drip water irrigation system. The cabbage plants were used for experiments after four weeks of transplantation.

Prior to the semi-field bioassay, each bio-pesticide was blended with each of clove and henna extracts, the best two additives identified from the preliminary screening, and the green tea extract (as positive control). The concentrations of 100X LC_{95} of the bio-pesticides were diluted into 10% plant extracts and 1% (v/v) (at final concentration) Triton X-100 was added as a surfactant (Bio Basic Inc., CA) to have 500mL volume of 10XLC_{95} at the final concentration. The solution was then poured into a 1,000 mL hand sprayer for the application. The bio-pesticides application was made by homogenously spraying seven cabbage leaves per plant. Three cabbage plants were used as a replicate for each treatment. Since we had seven treated cabbage leaves per plant, thus seven different intervals of sun light exposure were applied: 0, 1, 3, 5, 7, 14, and 21 days after the application. After the respective exposure, one leave was taken, placed in a zip plastic bag, and stored at -20^{0}C until further use in the bioassay.

Before the bioassay, a frozen leaf disc sample of each treatment was taken (30 mm diameter) then put onto a 10mm thick solid bacteriological agar (1% w/v) provided in the plastic cup (50mm diameter 80mm height). The leave discs were incubated at the room temperature for about 1 hour until the leaves reach the ambient temperature. After that, each leaf disc was introduced with 20 individuals of 1-day-old larvae. For the control treatments, the cabbage leaves sprayed with water and exposed for 21 days under sun light were used. The larvae were allowed to feed on the leaf disc for 24 hours, and then transferred on the artificial diet provided in 50 cells bioassay plate. The mortality recorded daily until the day 10 of the application. A further experiment using clove extract that was the best extract in, this experiment, was used against both *S. littoralis* and *S. exigua* at second instar stage. The procedures were the same as described in the 1st larval instar experiment.

***Statistical analysis*:** The experiments were performed using complete randomized design. One way ANOVA was performed to estimate the effect of treatments on the insect mortality, and to evaluate their sub-lethal effect on the larval weight. The estimation of lethal concentrations (LC) was done using probit analysis based on Finney (1971). Meanwhile, the t-test was done to compare the effects of two bio-pesticides to the insect mortality. Two factorial analyses were done to study the effects of exposure times and the bio-pesticide formulations on the mortality. All the statistical analyses were carried out using SPSS 13.0 (SPPS Inc., 2010).

RESULTS AND DISCUSSION

This study explores the effectiveness of bio-pesticides against two armyworm species (*S. littoralis* and *S. exigua*), the highly damaging pests of clover and many vegetable crops in Saudi Arabia. It focuses on the possibilities to enhance their persistence with natural plant extracts as UV protectants under simulated and field conditions. The mortality (%) of the both armyworm species treated with bio-pesticides in the laboratory is given in Table 1. The results revealed that both species have different susceptibility response to the bio-pesticides, spinosad and neem extract. First instar larvae of the cotton armyworm were more susceptible showing ~99% mortality at 4 ppm and 12 ppm for spinosad and neem extract, respectively, than second instars. In contrast, the second instar larvae showed high susceptibility to the spinosad where a 100% mortality was observed at the lowest concentration (1 ppm), whereas the neem extract had shown only sub lethal effect. In case of beet armyworm, the results indicated that both bio-pesticides have lethal effect on both 1st and 2nd instar larvae. The application of 1 ppm and 4 ppm of spinosad concentrations was required to kill more than 95% of 1st and 2nd instar larvae, respectively whereas, in neem extract a concentration of 18 ppm was required to kill about 85% and 95% of 1st and 2nd instar beet armyworm larvae, respectively. Spinosad and neem extract have been widely used to control other insect pests. Spinosad has also been used to control the early larval instars of tomato borer, *Tuta absoluta* (Lepidoptera: Gelechiidae) where the 1st larval instar was reported to be the most susceptible stage (Hashemitassuji *et al*., 2015). Meanwhile, neem extract and spinosad combinations have also shown the satisfactory results when applied to control rice planthopper. These bio-pesticides were much effective to control the planthopper on the day of application whereas the activity significantly decreased after seven days of treatment (Ahmad *et al*., 2015).

Data on the toxicity of bio-pesticides against both armyworms are presented in Table 2. Spinosad was effective to kill the larvae within 24 hours of application. LC_{95} values of spinosad at 1-d post treatment against 1st and 2nd instars of cotton armyworm and beet armyworm were 10.26, 14.28, 5.56, and 13.47 ppm, respectively whereas, slow killing action was observed in the neem extract treatments. Significant mortality of the tested insects was recorded on 5–7 days post-

Table 1. The mortality percentage (means±SE) of *S. littoralis* and *S. exigua* after 10 days of spinosad and neem extract applications under laboratory conditions.

Bio-pesticides	*S. littoralis*		*S. exigua*	
	1st instar	2nd instar	1st instar	2nd instar
Spinosad	**Mortality (%)**			
Control H_2O	2.65±0.67a	1.33±1.33a	6.00±3.06a	5.00±3.0a
0.5 ppm	33.85±10.45b	95.38±1.71b	84.77±1.75b	65.78±2.99b
1.0 ppm	47.68±6.24b	100±0c	97.33±1.33c	76.48±9.89bc
2.0 ppm	94.21±2.82c	100±0c	97.98±1.15c	85.92±1.96cd
4.0 ppm	99.33±0.67c	100±0c	98.67±0.67c	95.31±0.65d
6.0 ppm	100±0c	100±0c	100±0c	98.01±0.01d
Neem Extract				
Control H_2O	9.33±3.53a	0.0±0.0	4.81±0.61a	4.00±2.31a
10 ppm	94.00±3.06b	4.67 ±4.67	58.00±4.16b	56.67±8.35b
12 ppm	99.35±0.65b	4.49 ±3.61	76.00±2.17bc	86.00±4.0c
14 ppm	98.67±0.67b	2.34 ±1.97	78.00±13.11bc	92.00±3.46c
16 ppm	99.33±0.67b	2.70 ±1.17	80.59±4.47bc	92.00±3.46c
18 ppm	100±0b	3.03 ±1.85	85.33±2.91c	95.33±1.33c

Means in the same column followed by different letters were significantly different at α: 0.05.

Table 2. The lethal concentrations of spinosad and neem extract against the 1st and 2nd larval instars of *S. littoralis* and *S. exigua*.

Bio-pesticides	Spinosad				Neem Extract			
Insects	*S. littoralis*[a]		*S. exigua*[a]		*S. littoralis*[b]		*S. exigua*[b]	
Larval stage	1st instar	2nd instar	1st instar	2nd instar	1st instar	2nd instar	1st instar	2nd instar
LC_{50} (ppm)	2.19	6.81	1.40	7.18	8.58	Sub-lethal	0.71	6.39
LC_{95} (ppm)	10.26	14.28	5.56	13.47	21.03	Sub-lethal	6.84	13.76

[a]The lethal concentration was calculated on day 1 post- treatment. [b]The lethal concentration was calculated on day 7 post-treatment.

Table 3. Sub lethal effects (means ±SE) of spinosad and neem extracts as measured by weight in *S. littoralis* and *S. exigua* at ten days post application.

Bio-pesticides	*S. littoralis*		*S. exigua*	
	1st instar	2nd instar	1st instar	2nd instar
Spinosad				
Control H_2O	1.42 ±0.09c	3.41 ±0.13a	0.20 ±0.03b	1.0 2± 0.09b
0.5 ppm	0.85 ±0.17b	2.79 ±0.30a	0.001 ±0.00a	0.46 ±0.21ab
1.0 ppm	0.65 ±0.03b	na	0.001 ±0.00a	0.75 ±0.26ab
2.0 ppm	0.08 ±0.04a	na	0.01 ±0.008a	0.57 ±0.19ab
4.0 ppm	0.0±8a	na	0.01 ±0.004a	0.18 ±0.00a
6.0 ppm	na	na	na	
Neem Extract				
Control H_2O	1.69 ±0.09d	3.28 ±0.11c	0.159 ±0.02b	0.99 ±0.13b
10 ppm	1.00 ±0.08c	2.83 ±0.29c	0.008 ±0.001a	0.84 ±0.08b
12 ppm	0.57 ±0.06b	2.19 ±0.04b	0.014 ±0.002a	0.11 ±0.06a
14 ppm	0.54 ±0.11b	2.02 ±0.19b	0.015 ±0.005a	0.03 ±0.009a
16 ppm	0.22 ±0.08a	1.36 ±0.06a	0.009 ±0.000a	0.03± 0.01a
18 ppm	0.16 ±0.02a	1.06 ±0.20a	0.010 ±0.004a	0.01 ±0.008a

Means in the same column followed by different letters were significantly different at α: 0.05; [na] No available data as all insects were dead.

applications for both the 1st and 2nd instar larvae. The neem extract has shown only sublethal effect on cotton armyworm during 2nd larval instar. The estimated LC_{95} of neem extract against 1st instar larvae of cotton armyworm, and 1st and 2nd instar larvae of beet army worm was 21.03, 6.8, and 13.7 ppm, respectively.

The sublethal effects of spinosad and neem extract against as indicated by reduction in larval weight of both armyworms are shown in Table 3. Both bio-pesticides have shown significant sublethal effect on the treated larvae as compared to the control (water). The increased concentration of the bio-pesticides application decreased the larval weights. The spinosad treatments at the highest concentration (4 ppm) decreased the larval weight up to 94% when compared to H_2O treated larvae. While in the neem extract treatments, the larval weight was decreased up to 98%.

The bio-pesticides were degraded when exposed to ultraviolet light. The effect of UVB light on the efficacy of spinosad and neem against 1st instar larvae of beet armyworm under lab conditions is shown in Figure 1. When exposed to UVB light for 2 hrs, the efficacy of spinosad and neem extract decreased up to 50%. The effectiveness of bio-pesticides under field-sunlight conditions of Saudi Arabia is demonstrated in Figure 2. Both the treatments showed 70% efficiency bio-pesticides at 7 days of sunlight exposure. This longer persistency under sunlight conditions as compared to lab results (Fig. 1) can particularly be explained by the fact that UVB from the sunlight was significantly lower than that of UVB light treatments in the lab. It is known that ultraviolet lights oxidize and degrade the protein (Bandyopadhyay *et al*., 1999).

Efforts were made to enhance the bio-pesticides persistence by using plant extracts as UV protectants. The effect of plant extracts on the bio-pesticides efficiency (persistence) after an exposure of 5 hrs to UVB are shown in Figure 3. The results reveal that clove, henna, dates, pomegranate, green tea, and kiwi extracts preserved the activity of bio-pesticides when exposed to UVB light. However, under field conditions only clove, henna, and green tea extracts which gave the best results in the lab were tested on cabbage plants.

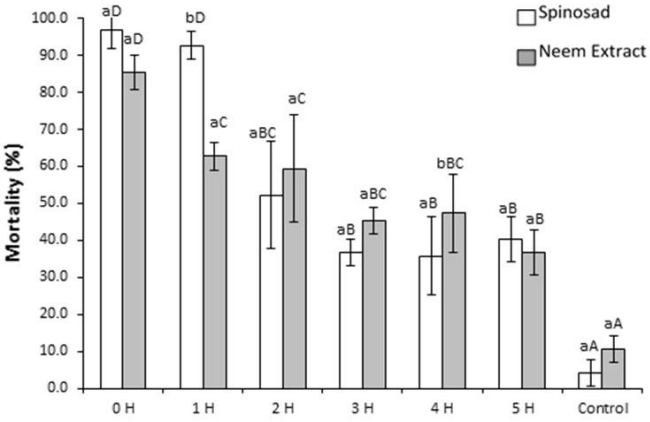

Figure 1. The effect of UVB exposure on the bio-pesticides efficiency against the 1st instar larvae of *S. exigua*. Columns with same small letter in the same treatment are not significantly different at α: 0.05 (based on the t-test analysis), while columns with same capital letters between the treatments are not significantly different at α: 0.05 (based on the LSD).

Mortality (%) of the beet armyworm 1st instar larvae when allowed to feed on the cabbage leaves treated with bio-pesticides blended with plant extracts under field- sunlight conditions are given in Table 4. Our data indicate that the

Table 4. Mortality percentage (means ±SE) of the 1st instar larvae of *S. exigua* in response to spinosad and neem extract applications formulated with henna, green tea and clove extracts used as natural UV protectants on the cabbage plants under field-sunlight conditions.

Exposure	Neem Extract				Spinosad			
(day)	Alone	N±HN	N±GT	N±CV	Alone	S±HN	S±GT	S±CV
0^{ns}	95.00±5.00cA	100±0.0bA	96.67±1.67bA	90.00±5.00bcA	100±0.0cA	95.00±2.89cB	100±0.0cA	100±0.0cA
1^{ns}	95.00±2.89cA	100±0.0bA	95.0±2.89bA	93.33±2.89bcdA	95.00±2.89bcA	96.67±3.33cA	96.67±1.67cA	100±0.0cA
3^{ns}	90.00±2.89bcA	96.67±3.33bA	98.33±1.67bA	98.33±1.67cdA	90.00±2.89bcA	95.00±2.89cA	93.33±4.41bcA	88.00±6.01bA
5^{ns}	91.67±4.41bcA	91.67±6.01bA	100±0.0bA	88.33±1.67bA	90.00±2.89bcA	98.33±1.67cA	83.33±6.67bcA	91.67±4.41bcA
7$^{*)}$	98.33±1.67cA	98.33±1.67bA	96.67±1.67bA	98.33±1.67cdA	88.33±7.26bcA	83.33±6.01bA	83.33±6.67bcA	95.00±2.89bcA
14$^{*)}$	98.33±1.67cA	98.33±1.67bA	96.67±1.67bA	100±0.0dA	85.00±5.00bA	91.67±4.41bcA	78.33±12.02bA	93.33±1.67bcA
21^{ns}	85.00±2.89bA	98.33±1.67bB	93.33±6.67bB	93.33±6.67bcdB	88.33±4.41bcA	96.67±1.67cC	76.67±4.41bB	95.00±2.89bcB
H_2O	1.67±1.67a	1.67±1.67a	1.67±1.67a	1.67±1.67a	1.67±1.67a	1.67±1.67a	1.67±1.67a	1.67±1.67a
Additive alone	-	3.42±1.71a	3.42±1.71a	3.42±1.71a	-	3.42±1.71a	3.42±1.71a	3.42±1.71a

Means in the same column followed by the same small letter are not significantly different at α: 0.05; Means in the same row followed by the same capital letter are not significantly different at α: 0.05; $^{ns)}$ No significant difference in mortality (%) of respective exposure between the neem extract and spinosad formulations at α: 0.05; $^{*)}$ There was a significant difference in mortality (%) of respective exposure between the neem extract and spinosad formulations at α: 0.05

sunny conditions decreased effectiveness of both bio-pesticides. When neem extract alone was applied, the efficiency was dropped significantly (10%) from 95% to 85% at 21 days of exposure under field-sunlight conditions (F= 180.68; P< 0.001). In contrast, where the natural additives henna, green tea, and clove were blended with the treatments, the drop in efficacy was non-significant and was only 1.7 %, 3.3%, and 3.3%, respectively whereas in spinosad alone efficacy decreased to 12% on 21st days post application, while in case of henna extract no decrease was observed. In case of clove extract added application, only a 5% decrease in the spinosad efficacy was observed at 21 day post application was recorded. An unexpected significant decrease of 23% (F= 155.94; P< 0.001) was observed in case of green tea treated-spinosad applications and this effect is unexplainable.

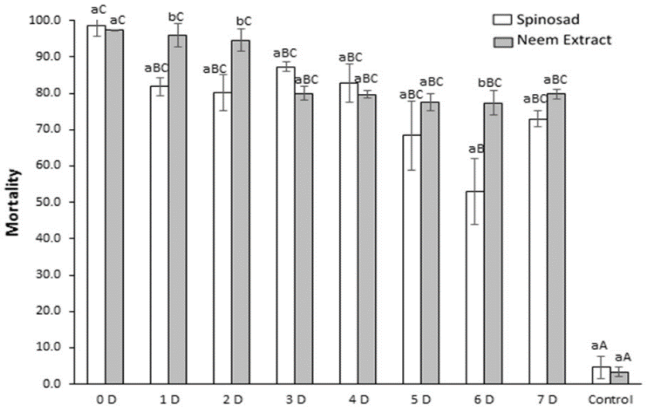

Figure 2. The effect of sunlight exposure on the bio-pesticides efficiency against the 1st instar larvae of *S. exigua.* Columns with same small letters in the same treatment are not significantly different at α: 0.05 (based on the t-test analysis), while columns with same capital letters between the treatments are not significantly different at α: 0.05 (based on the LSD).

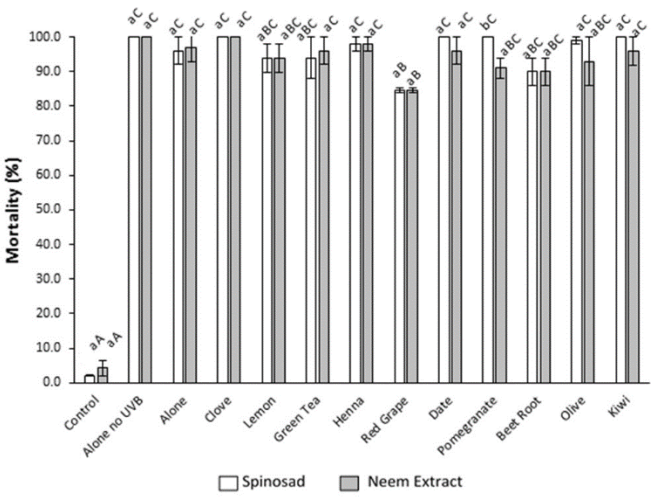

Figure 3. The effect of UVB exposure for a period of 5 hours on the bio-pesticides efficiency after treated with natural UV protectants (means ±SE) against the 1st instar larvae of *S. exigua* under laboratory conditions. Columns with same small letters in the same treatment are not significantly different at α: 0.05 (based on the t-test analysis), while columns with same capital letters between the treatments are not significantly different at α: 0.05 (based on the LSD)

Decrease in mortality clearly demonstrate a significant difference between UV-protectants-treated and untreated bio-pesticides (Table 4). Overall, the addition of henna and clove extracts as UV protectants in the neem extract and spinosad caused significant mortalities as compared to the treatments without the UV protectants bio-pesticides. Moreover, no significant differences in the mortality (%) of the neonate beet

armyworm larvae on 21 days post application were observed in the neem extract and spinosad formulations.

The effectiveness of natural additive-biopesticide formulations against 1st larval instar of cotton armyworm are shown in Table 5. In contrast to the efficacy against the beet armyworm (Table 4), the addition of natural plant extracts in neem extract have not shown any significant increase in its persistency, since all the treatments only present sublethal effect (<50% mortality). In spinosad treatments, the efficacy of spinosad alone at 1 day post exposure under field-sunlight conditions, spinosad-henna, spinosad-green tea, and spinosad-clove was decreased as much as 70, 76, 5, and 31%, respectively. This implies that green tea and clove enhance the spinosad efficacy, although only for 1 day after application. There was significant difference between the mortality in neem extract and spinosad treatments. Mortality in the spinosad treatments was higher than that of neem extract treatments, especially in the treatments at 1-3 days under field-sunlight conditions. In general, the data also revealed that neonate larvae of the cotton armyworm are probably more resistance as compared to those of beet armyworm.

The efficiency of neem extract and spinosad after blending with natural UV protectants is given in Table 6. The results indicated that neem extract formulations have only sublethal effects since the highest mortality observed was only 13-16% whereas in case of spinosad formulations, the data showed that at least clove extract enhanced its efficiency up to ~ 50% until 3 days post application. No significant differences were found in mortality (%) between insect species. The spinosad showed good control of the *S. littoralis* strain collected from cotton plantations in Turkey under laboratory conditions (Aydin and Gurkan, 2006). Spinosad is also found to be

Table 5. Mortality percentage (means ±SE) of the 1st instar larvae of *S. littoralis* in response to spinosad and neem extract applications formulated with henna, green tea and clove extracts used as natural UV protectants on the cabbage plants under field-sunlight conditions.

Exposure (day)	Neem Extract				Spinosad			
	Alone	N+HN	N+GT	N+CV	Alone	S+HN	S+GT	S+CV
0*)	24.07±8.71abA	37.07±8.27dA	41.27±27.28aA	7.37±2.22bcA	91.67±1.67cA	95.00±2.89cA	91.67±6.01cA	86.67±6.67bA
1*)	37.50±4.33abB	25.70±8.32cdBC	13.70±1.30aAB	6.83±1.83bcA	21.56±8.21abA	18.87±6.53abA	86.67±8.81cB	55.00±15.00aB
3*)	22.23±6.19abB	10.00±2.89abcAB	11.93±3.47aAB	1.77±1.77abA	18.33±7.64abA	32.07±6.53bA	33.33±10.93abA	26.10±5.63aA
5ns	12.47±6.29aA	24.23±3.69bcdA	20.27±2.66aA	12.03±1.52cA	18.70±1.88abA	28.70±8.16abA	23.33±6.01abA	25.00±2.86aA
7*)	3.33±3.33aA	8.87±1.94abA	11.67±6.01aA	12.90±2.37cA	25.00±5.00bA	18.26±10.97abA	26.30±8.50abA	28.33±8.33aA
14ns	25.7±7.4abAB	6.27±6.27aABB	35.86±14.69a	0.0±0.0aA	10.00±2.88abA	18.67±4.36abA	43.77±15.36bA	23.33±18.56aA
21ns	56.67±14.81cC	11.67±1.67abcA	43.37±2.26aBC	27.70±2.91dAB	18.33±6.67abAB	16.93±1.55abA	26.27±6.87abAB	36.20±4.77aB
H_2O	8.33±1.67a	8.33±1.67ab	8.33±1.67a	8.33±1.67c	8.33±1.67a	8.33±1.67ab	8.33±1.67a	8.33±1.67a
Additive alone	-	7.10±4.34a	17.27±4.69a	12.53±2.53c	-	3.42±1.71a	3.42±1.71a	16.60±7.31a

Means in the same column followed by the same small letter are not significantly different at α: 0.05; Means in the same row followed by the same capital letter are not significantly different at α: 0.05; ns) No significant difference in mortality (%) of respective exposure between the neem extract and spinosad formulations at α: 0.05; *) There was a significant difference in mortality (%) of respective exposure between the neem extract and spinosad formulations at α: 0.05.

Table 6. Mortality percentage (means ±SE) of the 2nd instar larvae of *S. exigua* and *S. littoralis* in response to spinosad and neem extract applications formulated with clove extract used as natural UV protectant on the cabbage plants under field-sunlight conditions.

Exposure (day)	*S. exigua*				*S. littoralis*			
	Neem Extract		Spinosad		Neem Extract		Spinosad	
	Alone	N±CV	Alone	T±CV	Alone	N±CV	Alone	T±CV
0ns	13.51±1.49dA	5.18±2.89aA	85.0±5.77bB	83.33±6.01dB	15.47±3.31cA	16.84±4.28aA	85.0±10.41bB	90.00±7.64cB
1ns	0.00±0aA	17.37±12.70aA	8.51±4.45aA	53.33±16.67cdB	5.0±2.89abA	37.05±14.67bAB	6.67±4.41aA	60.00±21.79bcB
3ns	3.33±1.67abA	15.00±7.64aAB	1.75±1.75aA	50.00±25.16bcdB	8.33±4.41bA	5.0±5.0aA	3.70±3.70aA	43.33±19.22abB
5ns	3.33±3.33abA	8.33±6.01aA	6.93±4.66aA	8.33±3.33abA	0.0±0.0aA	0.0±0.0aA	8.33±3.33aAB	20.00±7.64abB
7ns	5.00±2.89abcA	0±0.0aA	0±0.0aA	16.67±9.28bcA	3.72±1.87abA	0.0±0.0aA	8.33±3.33aA	26.67±21,86abA
14ns	10.0±2.89cdA	0±0.0aA	3.51±3.51aA	15.0±15.0abcA	0.0±0.0aA	0.0±0.0aA	1.67±1.67aA	15.0±7.64aB
21ns	8.69±1.58bcdA	0±0.0aA	1.67±1.67aA	23.33±15.89abcA	0.0±0.0aA	1.67±1.67aA	1.67±1.67aA	13.33±6.01aB
H_2O	0±0.0a	0±0.0a	0±0.0a	0±0.0a	1.67±1.67ab	1.67±1.67a	1.67±1.67a	1.67±1.67a
Additive alone	-	0±0.0a	-	0±0.0a	-	3.42±1.71a	-	3.42±1.71a

Means in the same column followed by the same small letter are not significantly different at α: 0.05; Means in the same row followed by the same capital letters are not significantly different at α: 0.05; ns) No significant difference in mortality (%) of respective exposure between the neem extract and spinosad formulations at α: 0.05.

effective in controlling *S. littoralis* under field conditions on tomato plantation (El-Helaly and Bendary, 2015).

The natural additives tested in the present study were selected based on the hypothesis of their high content of antioxidant. The antioxidants decrease the oxidation of bio-pesticides caused by reactive oxidative species (ROS). Clove and henna extracts contain phenolic compounds such as tannins and flavonoid. The presence of polyphenolic compounds in the formulation absorb the UV light, thus could protect from oxidation (Shapiro *et al*., 2009a; Musa and Gasmelseed, 2012). This protection from UV light may also be because of its content of Apigenin (Svobodová *et al*., 2003; Shan *et al*., 2005; Chaudhary *et al*., 2010).

***Conclusion*:** The present findings conclusively demonstrate that the blended bio-pesticides (with natural UV protectants) were more promising to be applied under Saudi sunny-field conditions since these additives could keep the persistency at least up to 21 days, although their application has been more effective only for neonate than later larval stages. However, among the three natural UV protectants (henna, green tea and clove extracts) used in the field, the clove has shown a slightly better performance than the others. Further screening of natural products as UV protections should be extensively done to find more economical products to improve the efficacy of bio-pesticides.

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