Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 60(1) (2022), 191 – 198 **https://assjm.journals.ekb.eg**

Effect of Some Storage Conditions on Emamectin Benzoate Formulation and Its Activity against *Spodoptera Littoralis*.

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Abstract

The present study was carried out to investigate the potential activity of Speedo WG 5.7% emamectin benzoate formulation (A.I) at different accelerated storage conditions to predict the stability of the formulation under storage at 54 ºC ,effect of UV rays and effect sun light in initial and after 3 , 14 , 21 and 35 days of exposure**.** Moreover to evaluate its toxicity against 4th cotton leaf worm *Spodoptera littoralis.* Our results indicated that the stability of active ingredient was 5.66% in initial before exposure to different storage conditions.In the treatment of temperature at 54 ºC the stabilities were 5.50 , 4.71 , 3.93 , and 3.55 % after exposure to ultraviolet rays they were 5.46 , 5.05, 4.22 , and 3.73 %, while after exposure to sunlight were5.03 , 4.94 , 3.78 ,and 3.42% after 3-14 -21-35 days , respectively. Storage for a period of 45 days, was decomposed about 50%(TD50) of the active ingredient in the formulation at 54ºC reached to 48.47% ,to 53.57%under ultraviolet rays and under direct sunlight was 51.43 %. The toxicity results showed that the LC_{50s} of Speedo formulation recorded14.957mg/l at initial, and 35.097, 71.040, 160.307, 429.257mg/l after 54 ºC temperature,22.580, 36.329, 59.431, 91.568 mg/l after exposure to UV and(51.935, 90.601, 233.491, 2432.3) mg/l after the treatments of the exposures to direct sunlight.

Keywords: Emamectin benzoate,Speedo,*Spodoptera littoralis*, temperature.

Introduction

Emamectin benzoate, a new insecticide, was derived from the avermectin family with advanced thermal stability,more water solubility and a wider range of insecticidal activity of avermectin. **(Zhu*et al.,* 2011).** Avermectin family of this group of insecticides is effective in very small quantities and often decomposes quickly; resulting in lower exposure and largely avoiding the pollution problems caused by traditional pesticides and could be used safely as a component of Integrated Pest Management (IPM) programs.Besides to the economic importance of cotton leaf worm, *S. littoralis* as one of the most dangerous harms insect pests on field's crops, greenhouse or open field not only in Egypt but also on the most countries **(Abdu-Allah *et al.,* 2009).** This insect has not any diapauses in Egypt. Consequence cotton leaf worm can be adapted in different areas in Egypt and can tolerate wide range of temperature. It is recorded from 3 to 46ºC in night winter and day summer. The use of insecticides is still the main method for controlling the cotton leaf worm. Macrolactone insecticides are known as microbial bioinsecticides, derived from actinomycetes bacterium species **(Copping & Menn, 2000; Putter *et al.,* 2000).** Emamectin benzoate is the second generation of spinosad and abamectin.Emamectin benzoate is a modified fermentation result of the soil microorganism, *Streptomyces avermitilis.*Avermectin insecticides effect on the insect nervous system increases the flow of chloride ion at the neuromuscular junction in the nervous system, owing to this effect, the arthropods start stop feeding and irreversible paralysis **(Ishaaya *et al*,.2002).**The objective of this study to evaluate the effect of three environmental factors temperature, ultra violet rays and direct sunlight on the formulation stability and toxicity of emamectin benzoate against cotton leaf worm.

Materials and Methods

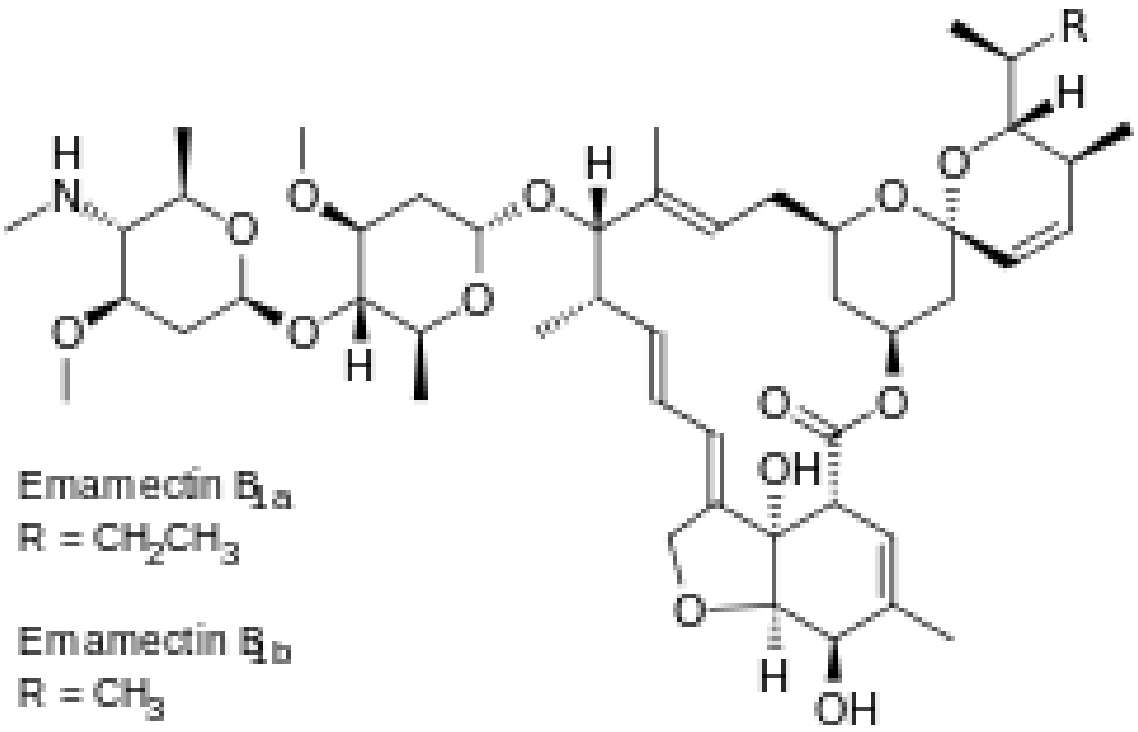
Pesticide used

Speedo formulation (5.7%) was provided (Shoura Company)

Chemical formula: $C_{49}H_{75}NO_{13}$

Moucler weight: Emamectin B1a benzoate: 1008.3.

Emamectin B1b benzoate: 994.2. Spectra.



1- Insect rearing

Egg masses of cotton leaf worm, *Spodoptera littoralis* were taken from Pest Rearing Department in Central Agricultural Pesticides Laboratory (CAPL). New hatched larvae were transferred to clean glass jars covered with muslin, held in position with rubber bands. They were fed on castor bean leaves, *Ricinus communis* at 27±2℃ and 65± 5% RH **(El-Defrawi *et al*., 1964).** Then, full grown larvae were moved to plastic bowls**,** placed upon saw dust to absorb the extra humidity, provided with new plant leaves and pupae were daily collected, sexed and kept in paper cups on moistened saw dust and covered with muslin. Full-grown pupae of both sexes were transferred to adult rearing cages previously described by **Sallam (1969)**

3-Chemical analysis:

The active ingredient percent of emamectin benzoate was determined before and after different storage conditions (hot storage at 54ºC, under UV rays, and direct sun light) after 3, 14, 21, and 35 days exposure by liquid chromatography.

4-Reagents:

Deionized water LC grade, methanol / acetonitrile LC grade and emamectin benzoate were used.

5-Standard solution preparation:

Ten mg (related to purity of 100%) from emamectin benzoate standard insecticide was weighted into 25 ml volumetric flask. After dilution with suitable solvent as methanol LC grade to the mark, the resulting solution has to be checked well to prepare standard solution.

6-Sample solution preparation:

Take weight from Speedo formulation equivalent to the concentration of reference standard material. Then, dilute to the mark with the same standard solution solvent methanol LC grade and mixing well. This is the sample solution.

7-Calibration:

Inject emamectin benzoate standard solution into high-performance liquid chromatography (HPLC) column. Ensure reproducibility of injections to obtain emamectin benzoate retention time. Ensure linearity of standard injections with serial dilution. Using practice ensure baseline.

8-Determination the stability of emamectin benzoate (speedo 5.7% WG) under storage conditions:

Emamectin benzoate 5.7% WG was firstly stored at 54± 2 ºC ,to evaluate the effect of temperature, storage formulation 20g were placed in the beaker and spread it without using pressure, in a smooth even layer of constant thickness. . A disk was placed on the surface of the sample in beaker then the beaker stored

in an oven at 54 C˚± 2 defined periods of time 3, 14, 21, 35 days, at the end of each period, the beaker removed from the oven, the disk was taken off, and then the beaker was set in a desiccator and allowed to cool at room temperature **Method: MT46, CIPAC F (1995)**.Then, exposure to ultraviolet rays,UV Japan lamp with specifications (G13T8 tube, 30 W, 254 nm) was put in a tightly locked wooden box connected to an electrical source, placed the insecticides lamps directly with distance about 10 centimeters below the source of light inside the box for3 , 14 , 21 and 35 days, and to direct sunlight insecticide samples were maintained under sunlight, samples at a dominating temperature ranged between (32 and 38C̊), for the defined periods of time 3, 14, 21, 35 days were put in Petri dishes, **Soliman(1994); Shokr (1997)and Barakate *et al* (1999)**. The time required for breaking down half of the active ingredient (TD50) was also estimated. Then, the decomposition rates of emamectin benzoate 5.7% WG samples were determined by HPLC .All samples were assessed three times during the experiment period and the mean was taken. Chromatographic conditions were showed in Table (1).

Solvent (A) was methanol90% Solvent (B) was 10%Acetonitrile LC grade; solvents composition and temperature play a principal role in the separated process by inducing the interaction between sample and absorbent. Reagent with ultrasonic degassing filter, column temperature 25 ºC, injection volume was 5 µL,column: Luna C 18 reversed phase column. Amount of emamectin benzoate was determined by comparison to external standard solution. All reagents were HPLC grade (**Baohua 2014**).

Table 1. Conditions of HPLC for analysis of emamectin benzoate

Insecticide	Solvent system 100%	Flow Rate ml/min	Wave length	LOD ng
Emamectin benzoate	**A90%/ B 10%**	**1 ml//min**	**197 nm**	**0.03**

9-HPLC Instrument

The type of chromatographic HPLC system model Agilent Technologies 1100 series with quaternary pump, Chromatographic C18 stainless steel column (25 cm length, 4.6 mm inner diameter and 4.0 µm particles) and UV detector was employed.

10-Bioassay techniques:

Different concentrations of tested insecticide were prepared and tested against the 4th instar larvae by dipping technique. Castor bean leaves were dipped for about 20 seconds in each concentration. The leaves were left for air dryness and offered to the larvae for 24 hours. The influence of toxicological effect on cotton leaf worm by bioassay test to determine LC_{50} and LC_{90} was implemented the mortality percentages were, calculated and corrected for mortalities by:**Abbott´s formula (1925).**

Corrected mortality = $\frac{X-Y}{Y}$ X 100

Where: X= % mortality in treatment.

Y=% mortality in control.

Statistical analysis:

Probit analysis was used to calculate the LC_{50} and LC_{90}, The corrected percentage of mortality was used to calculate the LC_{50} values according to **(Finney1971)** using software (321958) package Ldp lines analysis version 1.0. Toxicity index was calculated by followed equation:

Toxicity index= $\frac{\text{lc50 of the most effective sample}}{\text{lc50 of the sample}}$ x 100

Results and Discussion

The degradation % of emamectin benzoate was determined by High Performance Liquid Chromatography (HPLC).

1-Effect of storage conditions on decomposition % of emamectin benzoate.

Data in table (2) showed that the stability of the active ingredient of emamectin benzoate was 5.66% at initial before the exposure to the storage conditions. However, after exposure the compound to five storage periods (3, 14, 21,35 and45 days) the stability decreased and recorded 5.50, 4.71,3.93,3.55 and 2.76% at54± 2 ºC, 5.46,5.06,4.22,3.73 and 3.05% after exposure to ultra violet rays and 5.03,4.94,3.78,3.42 and 2.93% under exposure to direct sun light, respectively , the decomposition percent of the compound increased with increasing the exposure periods to reach 3.5,17.3,31.0,37.7 and 48.87% 54± 2 ºC,it was 4.20,11.22,25.96,34.56 and 53.57% after exposure to ultra violet rays and reached to 11.75,13.33,33.68,40.0and 51.43% under exposure to direct sunlight

These results are in agreement with (**Thompson *et al*. 2000)** and **(Shang *et al*.2013)** who reported that the primary way to degradation of spinosad and emamectin benzoate is photo degradation. That emamectin benzoate formulationis less stable than other avermectins.

Results indicated that the time required to break-down half of the standard material was estimated, after approximately 45 days under the three storage conditions at a temperature 54 ºC, and exposure to ultraviolet rays, and to direct sunlight.

Storage for a period of 45 days, decomposed about 50 %(TD50) of the active ingredient formulation under different storage conditions. At 54 ºC it was as 48.47%, and after exposure to ultraviolet rays was53.57% while exposure to direct sunlight recorded 51.43 %.

Table 2. Active ingredient and decomposition percent of emamectin benzoate at different storage conditions

Time of exposure in days	EMAMECTIN BENZOATE **5.7%**					
	Temperature54 ºC		Ultra-violet rays **(UV)**		Direct sunlight	
	a.i %	**Decom %**	**a.i %**	**Decom %**	**a.i %**	**Decom %**
Initial	5.66	0	5.66	0	5.66	0
3	5.50	3.5	5.46	4.2	5.03	11.75
14	4.71	17.3	5.06	11.22	4.94	13.33
21	3.93	31.0	4.22	25.96	3.78	33.68
35	3.55	37.7	3.73	34.56	3.42	40
45	2.763	48.47	3.054	53.57	2.93	51.43

*a.i = active ingredient *Decom. = Decompsotion

2-Bioassays and determination of lethal concentrations:

The toxicity effect of emamectin benzoate at different storage conditions for 3, 14, 21, 35 days at 54±2°C, under ultra violet and storage under direct sunlight against the 4th Instar larvae of *Spodoptera littoralis* were given in Table (3) and Figures (1, 2 and 3).

The bioassay tests showed a reduction in toxicity, respectively after storage at different storage conditions, the values of LC_{50s} after storage at 54 ºC were 35.1, 71.0, 160.3, 429.3 mg/l, and after exposure to ultraviolet rays were 22.6, 36.3, 59.4, 91.6 mg/l and after storage under direct sunlight were 51.9, 90.6, 233.5, 2432.3 mg/l.

These results are in agreement with **Abdu-Allah, (2017)** who indicated that the effect of post treatment temperature on the toxicity of four avermectin insecticides against *Spodoptera littoralis*, that the stability of avermectins could be properly evaluated prior to registration as these products.

Table 3. The toxicity effect of emamectin benzoate at different storage conditions

Conditions	**Time of exposure in days**	**LC_{50}mg/l**	**LC_{90}mg/l**	**X^2**	**Slope ±SE**	**Toxicity index**
Initial	**0**	**14.957**	**65.175**	**0.645**	**2.005±0.28**	**100.00**
Temperature	**3**	**35.097**	**129.35**	**0.018**	**2.262±0.39**	**42.61**
54 ºC	**14**	**71.040**	**283.014**	**0.189**	**2.134±0.59**	**21.05**
	21	**160.307**	**895.925**	**0.138**	**1.714±0.66**	**9.330**
	35	**429.257**	**3101.33**	**0.051**	**1.492±0.84**	**3.484**
UV rays	**3**	**22.580**	**106.886**	**0.811**	**1.898±0.29**	**66.24**
	14	**36.329**	**211.558**	**0.548**	**1.674±0.28**	**41.17**
	21	**59.431**	**349.817**	**0.419**	**1.664±0.38**	**25.16**
	35	**91.568**	**583.902**	**0.032**	**1.592±0.58**	**16.33**
Sunlight	**3**	**51.935**	**359.27**	**0.286**	**1.525±0.38**	**28.79**
	14	**90.601**	**376.70**	**0.143**	**2.070±0.46**	**16.50**
	21	**233.491**	**1167.04**	**0.100**	**1.834±0.79**	**6.405**
	35	**2432.3**	**65718.5**	**0.017**	**0.895±0.86**	**0.615**

Data in Figure (1)showed that the LC_{50}s values of emamectin benzoate were increased gradually from 14.957 for initial to35.1, 71.0, 160.3, 429.3 mg/l,after storage at 54 ºCfor 3, 14, 21, 35 days

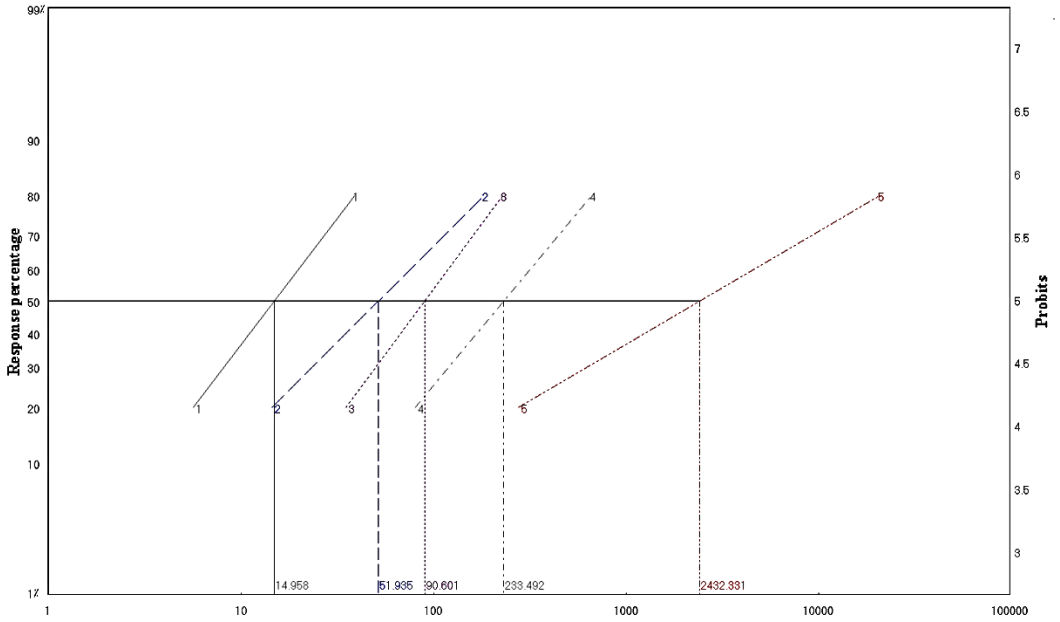


Figure (1): shows the relation between log insecticide conc. Of emamectin benzoate and the mortality of 4th instar of *S. littoralis* at54±2°C

Data in Figure (2) showed that the LC_{50}s values of emamectin benzoate were increased from 14.957 for initial to22.6, 36.3, 59.4, 91.6 mg/l after exposure to ultraviolet for 3, 14, 21, 35 days

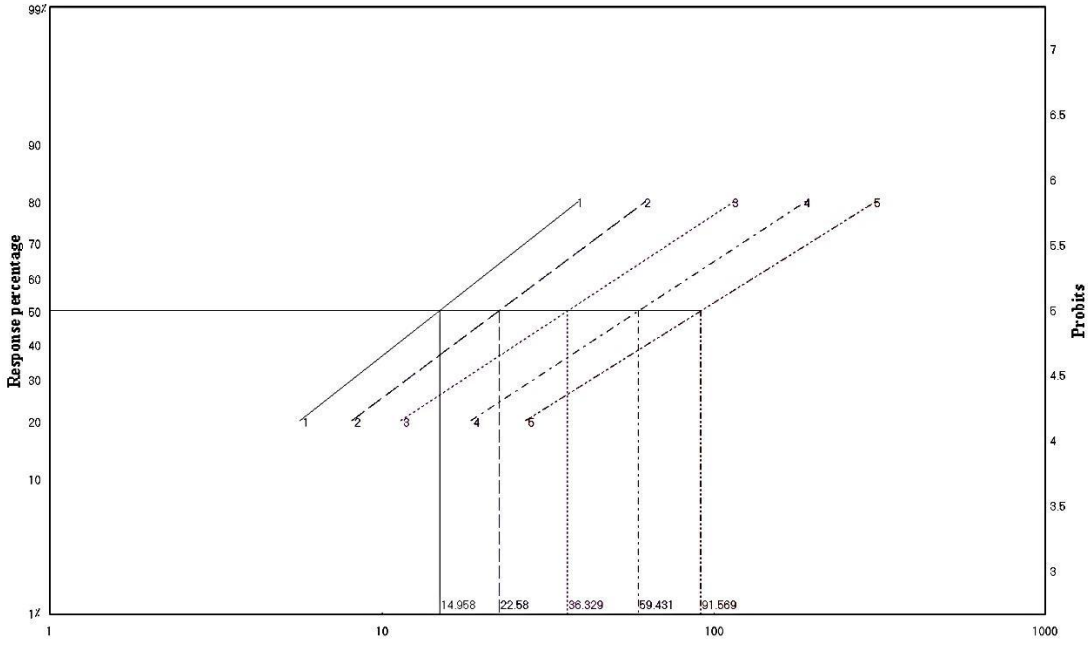


Figure (2): shows the relation between log insecticide conc. Of emamectin benzoate and the mortality of 4th instar of *S. littoralis* before and after exposure to UV rays

Data in Figure (3) showed that the LC_{50}s values of emamectin benzoate were increased from 14.957 for initial to51.9, 90.6, 233.5, and 2432.3 after exposure to direct sunlight for 3, 14, 21, 35 days

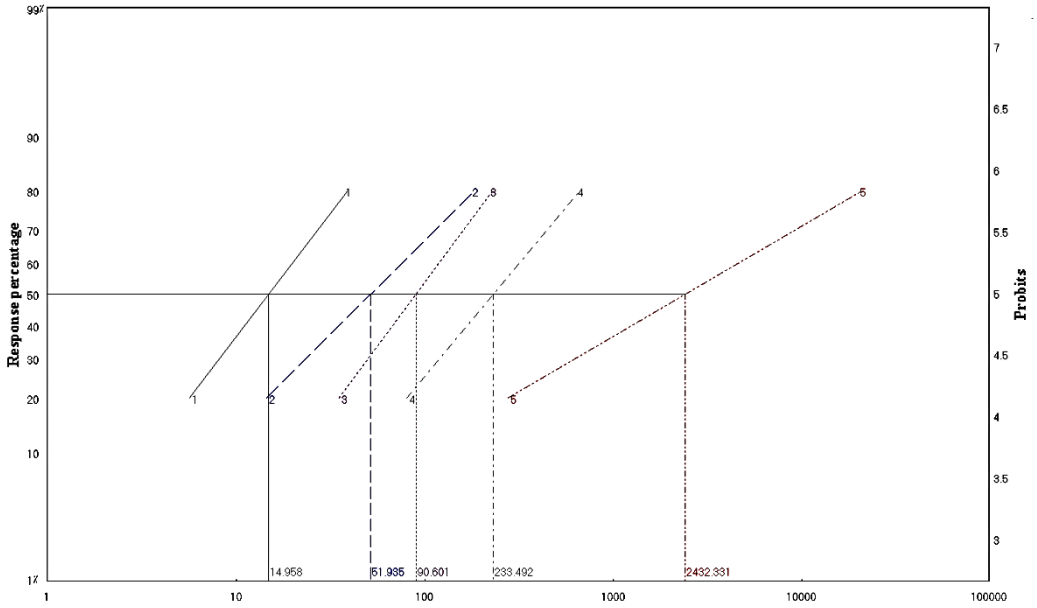


Figure (3): shows the relation between log insecticide conc. Of emamectin benzoate and the mortality of 4th instar of *S. littoralis* before and after exposure to sun light

From the previous results, we conclude that the most influencing factor on the toxicity of the pesticide is exposure to direct sunlight, followed by exposure to high temperatures54 ºC and then exposure to ultraviolet rays.

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تأثير بعض ظروف التخزين على مستحضر إيمامكتين بنزوات وفعاليته ضد دودة ورق القطن

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اجريت هذه الدراسة لقياس مدي فعالية مستحضر سبيدو 5.7 WG٪ إيمامكتين بنزوات في ظروف تخزين مختلفة لتحديد مدي ثباته تحت التخزين عند 54 درجة مئوية ، وتأثير الأشعة فوق البنفسجية وضوء الشمس في البداية وبعد 3 ، 14 و 21 و 35 يومًا من التعرض. علاوة على ذلك تم تقييم دراسات السمية ضد دودة ورق القطن *Spodoptera littoralis*. أشارت نتائجنا إلى أن ثبات المادة الفعالة كانت 5.66٪ في البداية قبل التعرض لظروف تخزين مختلفة وعند درجة حرارة 54 درجة مئوية كانت 5.50 ، 4.71 ، 3.93 ، 3.55٪ وبعد التعرض للأشعة فوق البنفسجية 5.46 ، 5.05 ، 4.22، و 3.73٪ ، بينما بعد التعرض لأشعة الشمس 5.03 ، 4.94 ، 3.78 ، 3.42٪ بعد 3-14 -21-35 يوم على التوالي.وكان التخزين لمدة 45 يومًا ، تحطم حوالي 50٪ (TD50) من المادة الفعالة وفي ظل ظروف تخزين مختلفة عند 54 درجة مئوية كان التحطم 48.47٪ – وبعد التعرض للأشعة فوق البنفسجية 53.57٪ والتعرض لأشعة الشمس المباشرة سجل51.43٪ بمرور الوقت المتبقي من بنزوات إيمامكتين. أظهرت النتائج أن التركيز المميت النصفي لتركيبة سبيدو سجل 14.957 mg/l في البداية ، 35.097 ، 71.040 ، 160.307 ، 429.257 mg/l بعدالتعرض لدرجة حرارة 54 مئوية ، 22.580 ، 36.329 ، 59.431 ، 91.568 mg/l بعد التعرض للأشعة فوق البنفسجية و 51.935 ، 90.601 ، 233.491 ، 2432.3 mg/l بعد التعرض لأشعة الشمس المباشرة.