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Research Article

Influence of Environmental Factors on Stability and Bioactivity of Certain Bioinsecticides Against *Spodoptera littoralis* (Boisd)

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Abstract

Background and Objective: The biological efficacy of bioinsecticides gradually decreases with time especially if these compounds are subjected to environmental factors (e.g., temperature, UV, sunlight). Also the activity can be altered if not stored properly. The objectives of this work were to examine the influence of temperature, light, types of water and shelf storage on the stability of some formulations of two bioinsecticides and also to assess the bioactivity of these bioinsecticides against cotton leaf worm. **Materials and Methods:** The active ingredients of formulations of Tracer (spinosad 24% SC), Radiant (spinetoram 12% SC), Proclaim and Broact (emamectin benzoate 5% SG) were determined using HPLC post exposure of the compounds to several environmental factors, i.e. temperature, UV and sunlight and storage for 2 years under ambient condition. Parallel a bioassay test was carried out to assess the biological activity of each bioinsecticide against neonate and the 2nd larvae instar of the cotton leafworm *Spodoptera littoralis*. Data were subjected to statistical analysis using LD-P line version 1.0. **Results:** The results revealed that the loss (%) of all tested bioinsecticides after storage for 2 years was above the permissible limits of FAO specifications. In accordance with this trend, the bioassay tests showed a reduction in toxicity value (LC_{50} s) for neonate and 2nd instar larvae of cotton leaf worm. Moreover, samples stored under direct sunlight and UV their biological activity were reduced to half values of their LC_{50} . Photolysis of aqueous solutions for bioinsecticides reduced the half-life especially in the case of emamectin benzoate formulation. **Conclusion:** Results indicated that stability of bioinsecticides could be properly evaluated prior to submission for registration as these products showed less stability under storage at ambient conditions. Also the decision makers will take these results into considerations and examine such products case by case.

Key words: Spinosad, emamectin benzoate, bioactivity, photolysis, cotton leaf worm, shelf life, temperature

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biopesticides have been defined as a certain type of pesticides that are derived from natural materials like plants, bacteria, fungi, virus and certain materials¹. In addition to, plant-incorporated protectants (PIPs) belong to the biopesticides group. Biopesticides are usually inherently less toxic than conventional pesticides, which affect only on target pest without any hazard to animals and humans. This group of pesticides is effective in very small quantities and often decompose quickly, resulting in lower exposure and largely avoiding the pollution problems caused by conventional pesticides and could be used safely as a component of Integrated Pest Management (IPM) programs².

The global market rates for the use of biopesticides in various forms, are increasing continuously, it held about 5% of the total crop protection market at approximately 3\$ billion in value worldwide and it's expected the market to reach more than 4.5\$ billion or more than 7% of the total crop protection market³ in 2023. However, biopesticides are less stable under environmental conditions. For example, spinosad and emamectin benzoate are biologically derived insecticides produced via fermentation and both compounds have a macromolecule with several chemically active groups causing their stability under normal conditions is expected to be lower than traditional pesticides. Consequently, these compounds are less stable under environmental conditions e.g., UV, sunlight, pH and temperature etc^{4,5}.

Earlier studies reported that degradation of spinosad in the environment occurs through a combination of routes, primarily photo and microbial degradation to its natural components. Hydrolysis does not contribute significantly to the degradation process⁶. Another study⁷ reported that photo degradation is the major route of spinosad dissipation. Similarly, emamectin benzoate degraded rapidly under sunlight, Half-life for emamectin benzoate is less than 3 h as a thin film under sunlight and less than half-day in water under sunlight^{8,9}. It is well known that products based on natural molecules tend to be less stable than synthetic compounds¹⁰.

Very little information regarding the effect of normal environmental conditions on stability and activity of bioinsecticides under current local conditions is known. So, in this work, the efficacy of the both tested bioinsecticides commonly used in controlling cotton leaf worm under normal field conditions was investigated. The stability and thereby, the toxicity of these compounds are substantially influenced

by temperature, sunlight and water source type. Moreover, storage and shelf-life studies of bioinsecticides are limiting factors determining their performance. Therefore, the objectives of the present study were to examine the influence of temperature, light, types of water and shelf storage on the stability of some formulated bioinsecticides and also to assess the bioactivity of these bioinsecticides against cotton leafworm.

MATERIALS AND METHODS

This research was carried out at the Faculty of Agriculture, Cairo University with cooperation with the Central Agriculture Pesticide Laboratory, Agriculture Research Center, Egypt during 2015-2017.

Bioinsecticides: Two formulations represent spinosyns family were used in this study. Tracer (Spinosad 24% SC) and Radiant (Spinetoram 12% SC) were given kindly from Dow Agro. Sciences, Egypt. Emamectin benzoate two formulations were used (Proclaim 5% SG and Broact 5% SG) which produced by Syngenta Agro. Sciences and Hebei Veyong Bio-Chemical Co., Ltd., respectively and obtained kindly from Central of Agriculture Pesticides Laboratory (CAPL), Agriculture Research Center, Egypt.

Insect rearing and bioassays

Insect source: Cotton leafworm larvae were obtained from the laboratory colony of *Spodoptera littoralis* (Boisd). The insect has been reared in the laboratory for several years in the laboratory of pesticide center-faculty of agriculture, Cairo University without exposure to any insecticides as described by previous study¹¹. Larvae were reared on fresh castor bean leaves at $26 \pm 1^\circ\text{C}$, $75 \pm 5\%$ RH. The pupae and adults were transferred to suitable cages as planned for mating and egg laying. Emerged moths were fed with a 10% sugar solution.

Bioassays: Bioassays were carried out using the leaf dipping technique¹². Five serial concentrations calculated as active ingredient ranging from 10-0.025 mg L⁻¹ (ppm) for spinosad, 1-0.03 mg L⁻¹ (ppm) for spinetoram and 0.05-0.0003 mg L⁻¹ (ppm) for emamectin benzoate of each tested compound for the neonates and 2nd larvae instar were fed on the castor bean leaves immersed in aqueous solution for each bioinsecticide concentration for 20 sec with gentle agitation and then allowed to dry under an air flow. Twenty five larvae for each replicate were placed in glass jar and fed on treated

leaves. Four replicates for each concentration were used. Leaves dipped in water served as control. All glass jars were kept under $25 \pm 1^\circ\text{C}$, relative humidity of 65%. After 24 h of exposure, castor leaves treated with bioinsecticides concentrations were removed and fresh non-treated leaves were administrated successively for 3 days. Mortality was recorded at 24 and 96 h post-treatment and corrected for natural mortality by Abbott formula¹³.

Statistical analysis: Probit analysis was used to estimate the LC_{50} and LC_{90} of each compound. Data were corrected for mortality from control by Abbott¹³. The corrected percentage of mortality was used to calculate the LC_{50} values according to Finney¹⁴ using software 321958 package Ldp lines analysis version 1.0. Toxicity index was calculated according to formula given below¹⁵:

$$\text{Toxicity index} = \frac{\text{LC}_{50} \text{ of the most effective sample}}{\text{LC}_{50} \text{ of the sample}} \times 100$$

Physical studies

Storage tests: The formulated samples of each bioinsecticide were stored and the accelerated hot storage, shelf storage and storage in outdoor tests were conducted according to CIPAC¹⁶.

Standard reference: Ten milligram from the analytical grade of each bioinsecticide (Spinosad, Spinetoram and Emamectin benzoate) was weighed, transferred to 25 mL volumetric flask and dissolved in methanol to volume. Serial concentrations were prepared through diluting stock solution for each compound and used to establish reference standard curve. Suitable weights from commercial formulation of Tracer 24 and 12% w/v from Radiant and Proclaim and Broact 5% SG w/w were taken as each weight contained equivalent active ingredient. Each sample was dissolved in methanol and transferred to 25 mL volumetric flask.

Aqueous photolysis: The photodegradation rates of each bioinsecticide in aqueous solution were measured according to Ahmed *et al.*¹⁷ and Adak and Mukherjee¹⁸ with some modification. As three different sources of water (Nile, ground and drain water) collected from Al Bureejat Village (Beheira Governorate) were used in this test. The physical and chemical properties for each water type was determined and presented in Table 1 according to Lico *et al.*¹⁹ and Rice *et al.*²⁰. Aqueous solution from each tested commercial bioinsecticides was prepared in each source of water.

Samples were prepared by taking 20 mL water containing 1500 μg (active ingredients) in clear bottle then exposed to direct sunlight for 1, 2, 4, 24, 72 and 120 h. Average temperature ranged between 32 and 38 $^\circ\text{C}$. At the end of the exposure, samples were transferred to separatory funnel and extracted three times with 50 mL of dichloromethane. The combined dichloromethane was dried over anhydrous sodium sulphate, evaporated at near dryness on a rotary evaporator under vacuum and analyzed by the identical method. For recovery test, untreated samples of each water type were spiked with known amount of each bioinsecticide. Samples were passed through the entire process of extraction, analysis as previously described, percentage of recovery was calculated (Table 2).

UV light: To study the effect of UV light exposure on the stability of the tested bioinsecticide, a thin film method was conducted in glass Petri dishes (external diameter of 9 cm). A solution of each bioinsecticide was prepared in methanol according to Das and Mukherjee²¹ and Mate *et al.*²² with some modification. As 1 mL of methanol containing 1500 μg (active ingredients) was placed in glass Petri dishes, Solvent (methanol) was allowed to evaporate at room temperature. Petridishes were exposed to UV light (G13T8 tube, 30 W, 254 nm) for 1, 2, 4 and 8 h. Residues of the exposed tested bioinsecticide were dissolved quantitatively in HPLC acetonitrile (analytical grade) transferred to glass stopper test tubes and the residues were determined by HPLC.

Determination of active ingredient: The operating analysis conditions by HPLC of the three tested bioinsecticides were presented in Table 3. The conditions of analysis for the active ingredient spinosad and spinetoram according to West²³ and emamectin benzoate according to Kottiappan and Anandham²⁴ with some modification. The column used for spinosyns was C18 reversed phase $250 \times 4.6 \text{ mm} \times 5 \mu\text{m}$ and for emamectin benzoate was C18 reversed phase $150 \times 4.6 \text{ mm} \times 5 \mu\text{m}$. The mobile phase was acetonitrile: Water for both tested pesticides. The UV lamp was diode array with wavelength ranges from 197-225 nm.

Kinetic study: To study the rate of degradation of the tested bioinsecticide to calculate the half-life time ($T_{1/2}$), the following equation according to Moye *et al.*²⁵ was used:

Table1: Physiochemical properties of water

Parameter (s)	Nile water	Ground water	Drain water
pH*	7.17	7.33	7.41
Conductivity Ms*	463	775	787
Salinity (%)*	0.2	0.4	0.4
Total dissolved solids (mg L ⁻¹)*	219	370	378
Elements (ppb)**			
Cr	N.D	N.D	N.D
Co	N.D	25	37
Cu	N.D	N.D	N.D
Fe	N.D	N.D	N.D
Mn	41	49	48
Ni	33	40	45
Zn	N.D	35	N.D
Sn	29	33	42
Cd	N.D	N.D	N.D
Pb	N.D	N.D	N.D
Sb	0.9	0.88	0.97
As	N.D	N.D	N.D

*Determination was conducting according to Lico *et al.*¹⁹, **Elements determination were carried out by using method of Rice *et al.*²⁰

Table 2: Recovery of bioinsecticide from different sources of water

Type of water	Nile water		Ground water		Drain water	
	ppm found	Recovery (%)	ppm found	Recovery (%)	ppm found	Recovery (%)
Tracer 1.5	1.45	96.66	1.41	94	1.4	93.33
Radiant 1.5	1.46	97.33	1.42	94.87	1.39	92.66
Proclaim 1.5	1.37	91.33	1.36	90.66	1.29	86.00
Broact1.5	1.36	90.4	1.33	88.66	1.3	86.66

Table 3: Conditions of analysis for spinosad, spinetoram and emamectin benzoate

Bioinsecticide conditions	Spinosad+Spinetoram	Emamectin benzoate
HPLC	Agilent technologies 1200 series	Agilent technologies 1100 series
Detector	Ultra violet detector	UV detector
Column (Stationary phase)	C18 (250×4.6 mm×5 µm)	C18 (150×4.6 mm×5 µm)
Wave length	197 nm	225 nm
Mobile phase	Acetonitrile:Water (90:10 v/v)	Acetonitrile: Water (90:10 v/v)
Flow rate	1.3 mL min ⁻¹	1.0 mL min ⁻¹
Retention time	1.3-1.8 min	1.8-2.6 min
Injection volume	5 µL	5 µL

$$\frac{T1}{2} = \frac{0.693}{K}$$

Where:

K = 1/Tx.ln a/bx

T = Time in days or hours

RESULTS AND DISCUSSION

Effect of storage under accelerated hot storage and shelf

life: The effect of storage of spinosad, spinetoram and emamectin benzoate formulations at 72±2°C for 3 days, 54±2°C for 14 days, 35±2°C for 12 weeks and storage for 2 years as shelf life were shown in Table 4 and 5. The results indicated that spinosad and spinetoram tolerate storage for short periods under different temperatures. In contrast, loss

(%) of both bioinsecticides was increased when stored for long periods. While, after 3 days storage of spinosad (Tracer) and spinetoram (Radiant) at 72°C, the loss (%) was 2.50 and 2.23%, respectively and was 2.46 and 0.52% after storage of both bioinsecticides for 14 days at 54°C, respectively. However, the loss (%) of spinosad and spinetoram after 12 weeks of storage at 35°C were 5.22 and 5.76%, respectively. Similarly, emamectin benzoate tolerated storage for short periods but the percentage of loss was increased when the storage periods prolonged, where the loss (%) after 3 days at 72°C and 14 days at 54°C for Proclaim were 4.70 and 3.27%, respectively. As well, the loss (%) was increased after 12 weeks at 35°C for Proclaim was 10.43%. The other formulation of emamectin benzoate (Broact) was less stable when stored for either short time or for a long time, the loss (%) after 3 days at 72°C, 14 days at 54°C and 12 weeks at 35°C was 7.55,

Table 4: Thermostability of tested bioinsecticides under accelerated hot storage

Bioinsecticides	Spinosyns				Emamectin benzoate			
	Trace		Radiant		Proclaim		Broact	
	Content (w/v, %)	Loss (%)	Content (w/v, %)	Loss (%)	Content (w/w, %)	Loss (%)	Content (w/w, %)	Loss (%)
Temperature/time								
Zero time	23.58	0	11.64	0	4.89	0	4.90	0
72±2°C-3 days	22.99	2.50	11.38	2.23	4.66	4.70	4.53	7.55
54±2°C-14 days	23.00	2.46	11.58	0.52	4.73	3.27	4.65	5.10
35±2°C-12 weeks	22.35	5.22	10.97	5.76	4.38	10.43	4.18	14.69

Table 5: Effect of storage at ambient conditions for two years on stability of the tested bioinsecticides

Bioinsecticides	Spinosyns				Emamectin benzoate			
	Tracer		Radiant		Proclaim		Broact	
	Content (w/v, %)	Loss (%)	Content (w/v, %)	Loss (%)	Content (w/w, %)	Loss (%)	Content (w/w, %)	Loss (%)
Time								
Zero time	23.63	0	11.69	0	4.89	0	4.90	0
3 months	23.50	0.55	11.64	0.43	4.79	2.04	4.75	3.06
6 months	23.21	1.81	11.56	1.11	4.60	5.93	4.65	5.10
24 months (2 years)	21.73	8.04	10.92	6.43	3.59	26.58	3.46	29.38

5.10 and 14.69%, respectively. According to FAO²⁶ specifications and evaluations, the permissible loss at the stored insecticide must be less than 5% of the active ingredient. This means that spinosyn sample stored at 35°C for 12 weeks deteriorated to the unexpected level. In addition, results showed that emamectin benzoate formulations were less stable than spinosyn compounds as presented in Table 4 and 5. Obtained, results clearly showed that the rate of loss of spinosad, spinetoram and emamectin benzoate formulations under investigation was influenced by changes in temperature degrees and duration of storage. Data in Table 5, showed that the deterioration of bioinsecticide samples is proportionally increased with time elapsed and this was clear in the case of the two emamectin benzoate formulations. Results showed that the tested bioinsecticides are degradable under normal condition. However, the percentage of loss of the active ingredients in the formulation was higher than the permissible limit set by FAO²⁶. Therefore, the decision maker should take shelf life studies of the bioinsecticides in considerations when as required data when bioinsecticide is registered. The results agree with Gupta and Dikshit⁴ and Villaverde *et al.*⁵ who reported that biopesticide shelf life is often low and therefore difficult to achieve a viable product after one or two years under ambient conditions.

Effect of storage under direct sunlight on stability of bioinsecticides: Results in Table 6 showed that the loss of stored formulations under direct sunlight in their packages for 2, 7 and 15 days proportionally increased after exposure to

direct sunlight, as the loss was 10.71, 14.20, 39.79 and 45.81% after exposure to direct sunlight for 15 days for Tracer, Radiant, Proclaim and Broact, respectively. The obtained results agree with Thompson *et al.*²⁷ and Shang *et al.*²⁸ who reported that the primary route to degradation of spinosad and emamectin benzoate is photodegradation. It is observable from the data presented in Table 4 and 5 that emamectin benzoate formulations are less stable than spinosad and spinetoram formulations.

Bioassays and determination of lethal concentrations:

The toxic effects of spinosyns and emamectin benzoate at different storage conditions including; storage in the oven at 35±2°C for 12 weeks and that storage under direct sunlight for 2 days against the neonate and the 2nd Instar larvae of *Spodoptera littoralis* were given in Table 7 and 8.

Bioinsecticide toxicity varied from one formulation to another. The bioassay tests showed a reduction in toxicity value (LC_{50S}) to 50 and 40% for Tracer and Radiant, respectively after storage at 35±2°C for 12 weeks. Also, toxicity of the two formulations of emamectin benzoate decreased to ~80% for neonate and 2nd larvae. The same trend of results was obtained of both bioinsecticides (spinosyns and emamectin benzoate) at zero time, storing at 35±2°C for 12 weeks and 2 days under sunlight, as their activities on the 2nd instar larvae of *S. littoralis* were assayed (Table 8). Data in Table 4 and 6 revealed that the degradation rate not only affected by active ingredients but also affected by their activity against the test insect. Studies on the activity of these bioinsecticides after degradation are not available.

Table 6: Photolysis of tested bioinsecticides under direct sunlight

Bioinsecticides	Spinosyns				Emamectin benzoate			
	Tracer		Radiant		Proclaim		Broact	
	Content (w/v, %)	Loss (%)	Content (w/v, %)	Loss (%)	Content (w/w, %)	Loss (%)	Content (w/w, %)	Loss (%)
Time								
Zero time	23.63	0	11.69	0	4.70	0	4.65	0
2 days	23.21	1.78	11.38	2.65	3.79	19.36	3.83	17.63
7 days	22.27	5.76	11.24	3.85	3.55	24.47	3.54	23.87
15 days	21.10	10.71	10.03	14.20	2.83	39.79	2.52	45.81

Table 7: Activity of the tested bioinsecticides against neonate larvae of *Spodoptera littoralis* at different storage conditions

Bioinsecticides	Conditions	LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ^2	Slope±SE	Toxicity index
Tracer	0	0.253	18.295	1.56	0.581±0.06	100.00
	1	0.521	40.477	2.26	0.829±0.08	48.56
	2	0.864	64.13	5.59	0.579±0.06	29.28
Radiant	0	0.074	0.547	1.72	1.474±0.15	100.00
	1	0.122	0.73	6.00	1.621±0.15	60.66
	2	0.159	1.324	0.38	1.394±0.15	46.54
Proclaim	0	0.0012	0.0082	1.71	1.549±0.16	100.00
	1	0.0052	0.095	4.46	1.017±0.10	23.08
	2	0.011	1.504	2.33	0.596±0.10	10.91
Broact	0	0.0005	0.025	8.92	0.764±0.09	100.00
	1	0.0033	0.078	3.08	0.935±0.10	15.15
	2	0.014	0.317	5.12	0.955±0.11	3.57

0: Zero time, 1: Storage at 35±2°C for 12 weeks, 2: Storage under direct sunlight for 2 days

Table 8: Activity of the tested bioinsecticides against 2nd larvae of *Spodoptera littoralis* at different storage conditions

Bioinsecticides	conditions	LC ₅₀ (ppm)	LC ₉₀ (ppm)	χ^2	Slope±SE	Toxicity index
Tracer	0	0.713	13.831	0.68	0.995±0.09	100.00
	1	0.846	71.756	2.25	0.665±0.08	84.279
	2	1.115	86.761	3.02	1.637±0.15	63.946
Radiant	0	0.08	0.897	8.37	1.218±0.11	100.00
	1	0.12	0.778	4.21	1.581±0.21	66.667
	2	0.139	1.631	3.03	1.197±0.14	57.554
Proclaim	0	0.0012	0.016	2.94	1.128±0.14	100.00
	1	0.0068	0.056	3.43	1.393±0.13	17.647
	2	0.0077	0.288	1.62	0.816±0.11	15.584
Broact	0	0.0012	0.009	6.47	1.440±0.15	100.00
	1	0.0073	0.092	5.83	1.168±0.12	16.438
	2	0.0098	0.101	0.27	1.267±0.11	12.245

0: Zero time, 1: Storage at 35±2°C for 12 weeks, 2: Storage under direct sunlight for 2 days

Generally, the toxicity of these bioinsecticides are very high if not exposed to the factors that influence their stability²⁹⁻³².

Effect of photolysis in water: Most biopesticide formulations are sold in concentrated form and have to be diluted in water before they can be applied. Stability of aqueous solution of bioinsecticides depends upon the source of water and the exposure period to sunlight. In this study, three sources of water were used (river Nile, ground and drain water). Naturally, the various sources of water are different in their physiochemical properties and this may reflect on the stability of the tested bioinsecticides. Data in Table 9 showed that the degradation of aqueous solutions of the tested bioinsecticides was rapidly occurred in the presence of sunlight, as half-lives for Tracer and Radiant in Nile, ground and drain water were

about 1 day. While half-lives of Proclaim and Broact (emamectin benzoate) were shorter than those in spinosyns where half-lives were less than half day. These results agree with Cleveland *et al.*⁷, Mushtaq *et al.*⁹, Mushtaq *et al.*¹⁷, Liu and Li³³ and Shimokawatoko *et al.*³⁴, as they reported that the half-life of spinosad is less than one day and about one to two days for spinetoram and the half-life of emamectin benzoate was less than half-day. As also Halley *et al.*⁸ found the half-life of emamectin benzoate as a thin film was 3 h when exposed to direct sunlight. However, amount of samples kept away from sunlight for 5 days were not changed and remained almost with no change.

Photodegradation under UV light: The effect of the exposure of Tracer (spinosad), Radiant (spinetoram), Proclaim and Broact (emamectin benzoate) to UV light was set up in

Table 9: Photolytic rate and half-life of the tested bioinsecticide in different water types exposed to sunlight

Bioinsecticides	Water source								
	Nile water			Ground water			Drain water		
	K	R ²	t _{1/2} h	K	R ²	t _{1/2} h	K	R ²	t _{1/2} h
Tracer	0.03460	0.93	20.03	0.03238	0.92	21.41	0.04219	0.93	16.43
Radiant	0.02507	0.97	27.65	0.02559	0.94	27.09	0.02752	0.95	25.19
Proclaim	0.09665	0.85	7.17	0.08907	0.88	7.78	0.10104	0.90	6.86
Broact	0.10399	0.89	6.67	0.10223	0.91	6.78	0.10931	0.87	6.34

K: Degradation rate, R²: Determination coefficient, t_{1/2}: The time required to decrease the concentration of pesticide residue to half

Table 10: Photolytic rate and half-life of the tested bioinsecticide exposed to UV light

Bioinsecticides	K	R ²	t _{1/2} h
Tracer	0.22783	0.91	3.04
Radiant	0.08733	0.98	7.94
Proclaim	0.24081	0.95	2.88
Broact	0.22396	0.98	3.09

K: Degradation rate, R²: Determination coefficient, t_{1/2}: The time required to decrease the concentration of pesticide residue to half

Table 10. The results indicated that the tested bioinsecticides degraded rapidly under UV light like sunlight. The half-life values were 3.04, 7.94, 2.88 and 3.09 h for Tracer, Radiant, Proclaim and Broact, respectively. It is clear from the present study that bioinsecticides (spinosyns and emamectin benzoate) were vulnerable to degraded under UV light under laboratory conditions. These results agree with Mushtaq *et al.*⁹, Adak and Mukherjee¹⁸ and Zhu *et al.*³⁵ who reported that a half-life of spinosad under UV and sunlight exposure was 1.65 and 5.24 h, respectively. Whereas, the half-life of emamectin benzoate was less than 3 h. However, it was also found that time required to reach half-life increased with the increase of the bioinsecticide concentration.

CONCLUSION

Results indicated that stability of bioinsecticides could be properly evaluated prior to submission for registration as these products showed less stability under storage at ambient conditions. Also, data draw the attention that the type of water used for insecticides application considerably influenced their stability and thus their effectiveness. Hence, the source of water should be selected prudently as it could deteriorate the active ingredients contained in each insecticide formulation. Moreover, the tested bioinsecticides should be stored in proper conditions in order to conserve their bioactivity. Furthermore, the farmers and pest control applicators could get benefit from obtained data as care should be taken when application of these bioinsecticides under filed conditions, particularly temperature and sunlight. Finally further research is needed to have a better insight of

mitigation the influence of unfavorable environmental factors on bioinsecticides in order to sustain their performance and extend their shelf life time.

SIGNIFICANCE STATEMENT

The current study discovered that the shelf life of the two tested bioinsecticides is relatively short and less than two years. In contrast, the regulations for pesticide registration in Egypt require that pesticides must have two years shelf storage stability. Therefore, comprehensive studies on submitted compounds for registration are needed for each compound case by case.

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