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Effect of storage temperature conditions on the stability and biological effectiveness of insecticides formulation

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Abstract:

The present work was carried out to study the insecticide formulations for all chlorpyrifos 48 %EC, spinosad 24 % SC, and methomy l 90 % SP through storage at 54ºC for 21 days. The toxicity of three insecticides (Chlorpyrifos, spinosad, and methomyl) against the 4th larval instar of the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) at different exposure times. The LC50values chlorpyrifos were 0.62 , 0.62, 0.527 , 0. 380 and the LC50 values Methomyl were 279.44, 279.44, 259.9, and 189.0 after 0,7 14 and 21 days respectively storage period at 54C. The stability of cholrpyrifos, spinosad, and methomyl active ingredients were 48.62, 24.61, and 91.61% before storage for all cholrpyrifos , spinosad, and methomyl and become after storage at the end of the experiment were 38.79,22.01 and 80.91% for 21dyes storage respectively. The loss experiment percentage was 20.79, 10.50, and 11.68 when the cholrpyrifos , spinosad, and methomyl compound previously were stored at 54 ºC for 21 days. On the other hand, it was demonstrated that chlorpyrifos and methomyl had high acute toxicity against cotton leafworms. spinosad negatively affected the longevity and fecundity of the pest.

Introduction

The cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is the most destructive pest of tomato and cotton In Egypt. The cotton leafworm feeds on most plant parts including stem, leave, flower buds, flower heads, and fruits at different larval development stages (Amin and Gergis, 2006).

Spinosad is a bioinsecticide based on the fermentation product of the soil bacterium Saccharopolyspora spinosa (Sparks *et al*., 1998). This pesticide has two unique modes of action, acting primarily on the insect nervous system at the nicotinic acetylcholine receptor, and exhibiting activity at the GABA receptor (Watson, 2001).

Chlorpyrifos exerts its toxic action by inhibiting cholinesterases (ChE) enzymes of the nervous system, (Ware and Whitacred, 2004). The insecticide (Methomyl), is a broad-spectrum insecticide that belongs to the carbamate family of pesticides. It is

used for foliar treatment of vegetable, fruit, and field crops, cotton, commercial ornamentals, and in and around poultry houses and dairies (Alam, 1996). According to Gosselin (1984), it is a very toxic and hazardous compound and a pollutant causing environmental concerns because of its high solubility in water.

The present work aims to study the insecticides formulations for all chlorpyrifos 48 %EC, spinosad 24 % SC, and methomy l 90 % SP through storage at 54ºC for 21 days and studying of the toxicity of three compounds (Chlorpyrifos, spinosad and methomyl) against the 4th larval instar of the cotton leafworm at different exposure times.

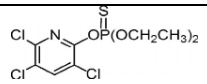
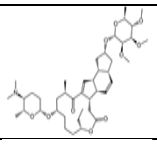
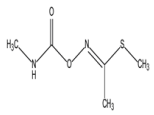
Materials and methods

1. Insect used:

Test insect a laboratory strain of the cotton leafworm, *S. littoralis* was reared in the laboratory on castor bean leaves under laboratory conditions of 26 ± 2°C and 65 ± 5% RH. (El-Defrawi *et al*., 1964). The culture of the cotton leafworm was initiated from freshly collected egg masses supplied from the Division of Cotton Leafworm, Central Agricultural Pesticide Lab, Dokki, Giza, Egypt.

2. Source of pesticides: Center Agric. Pesticides Laboratory
(Table 1).

Table (1): Pesticide formulation types, the active ingredient, structure and its impurities.

Treed name	Formulation types	Active ingredient	Structure	Impurities
Bestban 48 %	EC	Cholrpyrifos		**Sulfotep** O,O,O',O'-tetraethyl dithiopyrophosphate,)
Treser 24 %	SC	Spinosad		UND
Lanet 90 %	Sp	Methomyl		UND

3. Bioassay tests:

The efficiency of the tested insecticides; chlorpyrifos, spinosad, and Methomyl, were assessed against the 2nd instar larvae of *S. littoralis. Serial* concentrations of each tested insecticide were prepared using water. Disks (9 cm. diameter) of castor bean leaves were dipped in the tested concentrations for 10 seconds then left to dry and offered to larvae, which starved for 4- 6 hours before treatment **(**Merdan, 1968). Each treatment was replicated 3 times (10 larvae per). Control disks were dipped in water only. The larvae were allowed to feed on treated leaves for 24 hr., transferred to fresh untreated ones. The LC50, LC90, and slope values of the tested compounds were calculated using Finney's equation (Finney, 1971), through a software computer program.

4. Sample preparation for tested pesticides:

Accurately weighed sufficient samples formulation equivalent to 10 mg of standard in a different 25 ml volumetric flask for each sample, and slowly mixed with methanol and the volume was completed with methanol.

5. Storage conditions:

The tested chlorpyrifos, sapinsad, and methomyl formulation

were stored at 54ºC for 14 days according to FAO (2002, 2004, and 2006), respectively. During the storage period, samples were taken at 0, 3,7, 14, and 21 days to determine the active ingredient, and there impurities content for the tested formulations under testes.

6. Determination of active ingredient Gas-Liquid Chromatography (GLC):

Determination of cholrpyrifos by GLC analysis compared with the standard used cholrpyrifos has carried out GLC model Agilent Technologies, the column with flame ionization detector (FID).

7. Conditions:

Injection Temperature: 225 °C
Detector Temperature: 300 °C
Oven Temperature: 130 °C for 5 min.,

8. Determination of product degradation of cholrpyrifos and methomyl by GC /Ms:

Apparatus Agilent B, 5977 AMSD gas chromatography equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capacity Colum (30 m x 0.025 mm HP -5 0.25 microm 60 to 325/325 ºC). Samples were injected under the following condition; Helium was used as carrier gas at approximately 1 ml /min, pulsed split mode, split ratio (10:1) split-flow 10 ml /min. The solvent delay was 4 min and the injection size were 1 UL. Oven temperature program, %0 ºC for O<5 min, the 10 /min ramp to 190 ºC followed by a 10 ºC /min ramp to 210 ºC for 1 min followed by a 10 ºC /min ramp to 300 ºC and held for 2 min (total run time followed by an injection temperature was set at 280 ºC. Wiley mass spectral data was used in the identification of the Separated peaks

9. Determination of spinosad and methomyl by HPLC:

The active ingredient for spinosad and methomyl were evaluated by HPLC. A revise phase high-performance liquid chromatographic was used for quantitative analysis. Agilent technologies 1200 series HPLC in student equipped with degasser , quaternary pump, photodiode array detector connected with injection system and computer (Model vectra was used for analysis. The stationary phase consisted of lichrosphere on Rp-8 packed stainless steel column 15cm x4.6mm id .

Results and discussion

1. Toxicity of chlorpyrifos, spinosad, and methomyl and biological insecticides different storage periods on larvae *Spodoptera littoralis* .

***Serial*:**

The results presented in Table (2) show the toxicity of three insecticides (Chlorpyrifos, spinosad, and methomyl,) against the 4th larval instar of the cotton leafworm, S. littoralis at different exposure times. This experiment was conducted at laboratory conditions of 26°C and 65 ± 5% RH. Among the tested insecticides, chlorpyrifos was the most effective compound followed by chlorpyrifos, spinosad Methomyl the least effective one after 7, 14, and 21 days of exposure storage 54°C.

The results indicated that there was a positive relationship between the time post-treatment chlorpyrifos and methomyl. The LC50 values chlorpyrifos were 0.62, 0.62, 0.527, 0. 380 and the LC50 values. Methomyl was 279.44, 279.44, 259.9, and 189.0 after treatment 0,7, 14 and 21 days, respectively, storage period at 54°C. These obtained results were in agreement with Awad *et al.* (2019). Results obtained from acute toxicity assays of the tested insecticides showed that chlorpyrifos, spinosad, and methomyl were the most toxic chemicals against cotton bollworms. Similar results were found by Aslam *et al.* (2004), who have reported that

chlorpyrifos was the most effective insecticide for controlling cotton bollworm among tested in 192 insecticides. Nirmal and Manjit (2008) reported that the LC50 values of spinosad and chlorpyrifos were 0.04 and 0.66, respectively. Differences in LC50 values of these two studies would be due to differences in the duration of exposure to the insecticide and larval instar. Therefore, according to our results and the mentioned studies, spinosad and chlorpyrifos have a high potential for controlling cotton leafworm. Chlorpyrifos had the highest slope among the test insecticides. The high slope indicates that a slight increase in insecticide concentration will lead to high mortality compared with the other insecticides. However, the higher slope indicates that there will be increased selection pressure on the population. Thus, there will be a greater risk of selection of resistant individuals compared with the other insecticides especially in cases of continued use of the same insecticide.

Table (2): Toxicity of chlorpyrifos, spinosad and methomyl and biological insecticides a different storage periods larvae of *Spodoptera littoralis* . *Serial*.

Insecticides	Storage period (Days)	Slope	SE	LC_{50}	LC_{90}
Cholrpyrifos	0	0.669	0.308	0.62	279.44
	7	0.669	0.308	0.62	279.44
	14	0.309	0.589	0.527	259.0
	21	0.380	0.479	0. 380	189.0
Spinosad	0	0.212	0.9571	19973,8	25.6
	7	0.212	0.9571	19973,8	25.6
	14	0.156	1.261	112675.94	14.91
	21	0.196	1.044	12341.11	13.59
Methomyl	0	0.753	0.122	0.163	30.30
	7	0.631	0.235	0.1981	25.11
	14	0.540	0.278	0.148	24.11
	21	0.680	0.288	0.123	19.746

0 = initial storage
Slope = toxicity line intercept
SE = Standard Error of the slope

On the other hand, it was demonstrated that chlorpyrifos and methomyl had high acute toxicity against cotton leafworm. Spinosad negatively affected the longevity and fecundity of the pest. These impacts are very important for practical management of the pest, because these effects may lead to the reduction of the pest population to a lower level even under economic injury levels. Therefore, the authors propose that both lethal and sub-lethal effects of the insecticides should be considered in developing a pest management program.

2. Effect of storage at 54 ºC on the active ingredient (a.i) of cholrpyrifos , spinosad, and methomyl formulation:

The present data in Table (3) illustrated that the stability of cholrpyrifos, spinosad, and Methomyl active ingredients were 48.62, 24.61, and 91.61 % before storage for all Cholrpyrifos, spinosad, and Methomyl and become after storage at the end of the experiment were 38.79, 22.01 and 80.91% for 21 dyes storage, respectively. The loss experiment percentage was 20.79, 10.50, and 11.68 % when the cholrpyrifos , spinosad, and methomyl compound previously were stored at 54 ºC for 21 days.

According to the international organization WHO specification (Storage at 54 ºC for 3 days and FAO specification storage at 54 C for 14 days. In general, increasing temperature and period of exposure of increasing the rate of degradation of insecticides Emara *et al.* (2009). Pesticides can be degraded by photolysis, hydrolysis, oxidation and reduction, metabolism (plant, animals, or microbes), temperature and pH, FAO/IAEA (2009) . Also, the impurities of chlorpyrifos 48 % EC before exposure 54 º-C were 0.32 before storage and become after storage 0.73, 1.61 ,2.23, 2.82 and 3.11 were stored at 54 ºC for 0,1,3, 7,14 and 21 days respectively. , this results in disagreement with FAO maximum impurity of 3mg/kg in cholrpyrifos.

Table (3): Effect of Storage at 54 ºC on active ingredient (a.i) of cholrpyrifos , spinosad and methomyl formulation.

Storage period (days)	Cholrpyrifos 48 %			Spinosad % 24%		Methomyl 90%	
	Active ingredient (a.i)	Loss	Sulfotep g/kg Cholrpyrifos	(a.i)	Loss	Active ingredient (a.i)	Loss %
0	48.62	00	0.32	24.61	00	91.61	00
1	48.50	0.24	0.73	24.31	1.22	91.00	0.66
3	48.00	1.27	1.61	23.50	4.51	90.02	1.73
7	47.62	2.06	2.23	22.80	7.35	88.50	3.39
14	46.50	4.36	2.82	22.25	9.58	85.11	4.42
21	38.51	20.79	3.11	22.01	10.56	80.91	11.68

0* one hour before exposure to storage

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