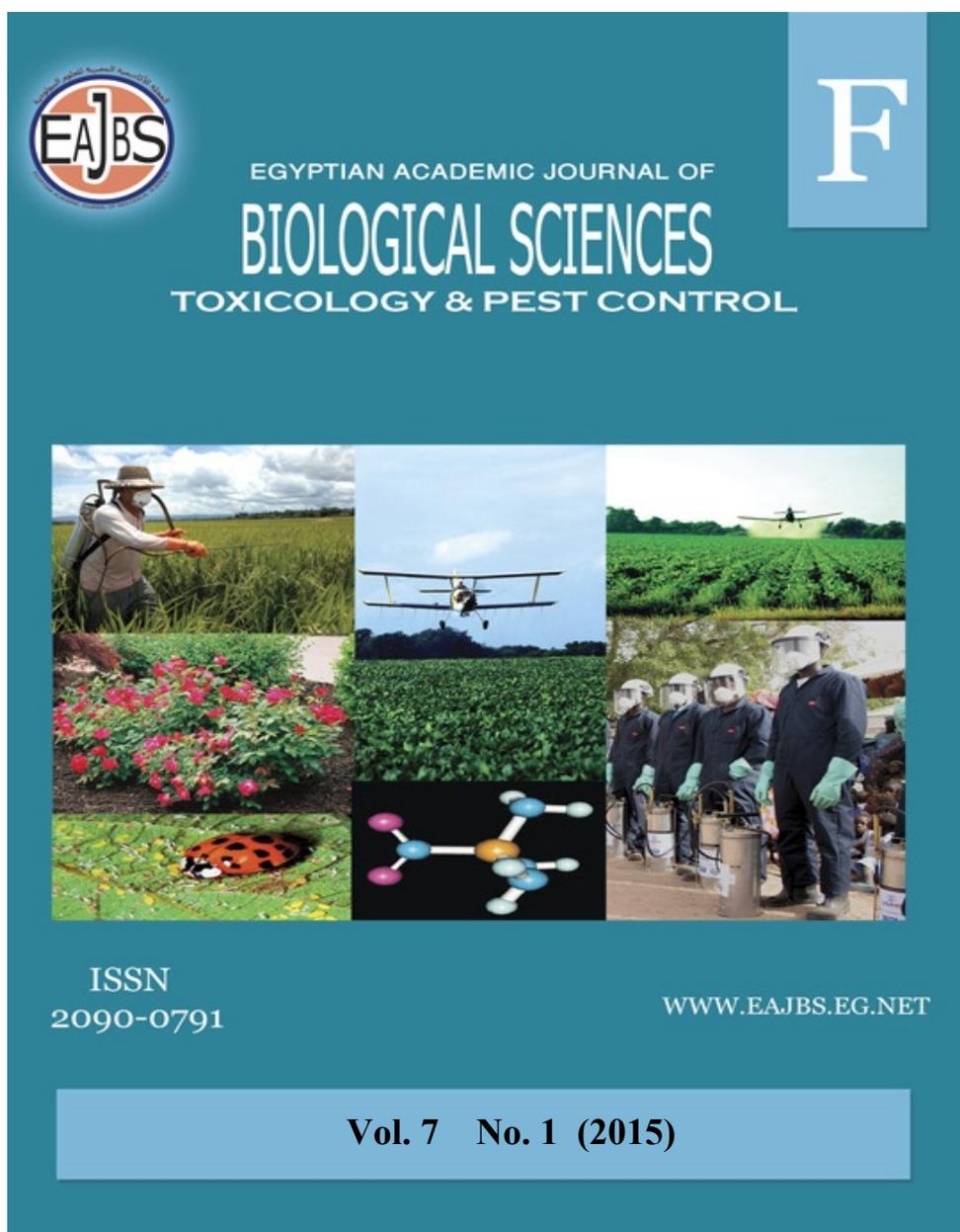


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Signs on The Persistence And Effectiveness of Some Novel Insecticides Against *Spodoptera littoralis* (Boisduval) on Different Host Plants

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ABSTRACT

The persistence of pyridalyl, emamectin-benzoate, spinetoram, hexaflumuron, and chlorpyrifos on cotton (*Gossypium barbadense*, var. Giza 86) and castor bean (*Ricinus communis*) foliage under field conditions, via bio-determination of median lethal time (Lt_{50}) values, and their efficacy against the 4th instar larvae of *Spodoptera littoralis* (Boisd.) laboratory strain were investigated. The local and systemic activities and fastness against washing with water of the tested insecticides on cotton and castor bean were studied as well. Pyridalyl residues were significantly the most persistent on castor bean and cotton plants with Lt_{50} values of 14.91 and 9.93 days, respectively. On the contrary, spinetoram had the least persistent residues on castor bean and cotton recording Lt_{50} of 0.20 and 1.06 days, respectively. With exception of pyridalyl, the tested insecticides were significantly more persistent on cotton than on castor bean plants. Pyridalyl and emamectin-benzoate proved to be the most effective against the 4th instar larvae of *S. littoralis* that fed on treated cotton plants. When the larvae were fed on treated castor bean plants, pyridalyl and hexaflumuron resulted in the superior percentages of larval mortality. The insecticides implicated in this study did not demonstrate any appreciable systemic activities in cotton or castor bean plants against the 4th instar larvae of *S. littoralis*, although they possessed strong local activities. The initial deposits of chlorpyrifos, pyridalyl, hexaflumuron, and emamectin-benzoate were significantly more stable on sprayed castor bean plants than on sprayed cotton plants versus elimination and washing with water. The initial deposit of spinetoram was completely unstable on sprayed cotton or castor bean plants. These results indicated that the kind of the treated host plant is very effective factor in toxicological properties of the tested insecticide and this could be useful in bio assay experiments design.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered one of the most destructive and economic pests of cotton, vegetables, ornamentals and other field crops (Kandil *et al.*, 2003).

It is widely distributed throughout Africa, Mediterranean Europe and several parts of Asia (Azab *et al.*, 2001). It is active all year round in Egypt without a hibernation period forming seven generations per year with three generations of them on cotton (Temerak, 2007), causing economic damages resulting from feeding on the leaves, fruiting points, flower buds and, occasionally, on green bolls. Application of insecticides to control *S. littoralis* is unavoidable procedure especially when outbreak occurs or its population density exceeds the economic threshold. Unfortunately, the intensive and unwise application of broad-spectrum insecticides against *S. littoralis*, over the past 25 years, has led to development of its resistance to many registered toxicants for its control and caused serious problems in the environment components (Abo-Elghar *et al.*, 2005; Aydin and Gürkan, 2006). Therefore, alternative materials that are effective against this pest, safe to humans, environmental friendly and compatible with integrated pest management practices are needed. In this scenario, using new types of insecticides that originated from natural agents or disrupt the physiological processes of the targeted pests could be useful as alternatives for conventional insecticides (Thompson *et al.*, 2000; Smagghe *et al.*, 2003). Among these new and promising insecticides are pyridalyl, emamectin-benzoate and spinetoram which characterized with their new and/or unique modes of action, have the potential for crop protection against economic pests and low toxicity to environment components and natural enemies (Foster *et al.*, 2003; Michaud and Grant, 2003; Sakamoto *et al.*, 2005).

Emamectin-benzoate, a novel macrocyclic lactone insecticide, is a semi synthetic derivative of the naturally occurring avermectin molecules (Ioriatti *et al.*, 2009), it acts by binding to GABA

and H-Glutamate receptors in the nervous system causing chloride ion flux in the neuromuscular junction (Fanigliulo and Sacchetti, 2008). Thus, treated insect larvae stop feeding, become irreversibly paralyzed, and die in 2-4 days post treatment. Spinetoram is the second generation of Spinosyns, it offers increased efficacy over a wide range of insects with a similar environmental and toxicological profile to its parent compound, spinosad, with a higher residual activity (Sparks *et al.*, 2008). Spinetoram causes excitation of the insect nervous system by altering the function of nicotine and GABA-gated ion channels in special binding sites (Crouse and Sparks, 1998). Pyridalyl is a novel synthetic insecticide of Sumitomo Chemical Co. Ltd with contact and ingestion toxic effect against lepidopteran and Thysanopteran pests (Isayama *et al.*, 2005). It has different biochemical mode of action from that of existing insecticides. Pyridalyl inhibits cellular protein synthesis in insect cells and inhibits mitochondrial respiration causing decrease in ATP concentration in the cell (Sakamoto *et al.*, 2012).

The present work was undertaken to study the persistence of pyridalyl, emamectin-benzoate, spinetoram, hexaflumuron and chlorpyrifos on cotton and castor bean plants under field conditions via determination of median lethal time (Lt_{50}). Also their efficacy against 4th instar larvae of *S. littoralis*, their local and systemic activity and their fastness against washing with water were studied as well.

MATERIALS AND METHODS

Insects

A laboratory strain of *S. littoralis* was obtained from Plant Protection Research Institute, ARC, Giza, Egypt. This strain was reared at Sakha Agricultural Research Station for 20 generations, away from any insecticidal

contaminations, under constant conditions of $25 \pm 2C^{\circ}$ and 65 ± 5 RH. The larvae were fed on fresh leaves of castor bean, *Ricinus communis*, as described by El-Defrawi *et al.* (1964). The newly moulted 4th instar larvae were used in all experiments of this study.

Insecticides

The commercial formulations of pyridalyl (Pleo 50% EC, Sumitomo Chemical Co. Ltd, Japan), emamectin-benzoate (Proclaim 5% SG, Syngenta Agrosiences, Switzerland), spinetoram (Radiant 12% SC, Dow Agrosiences, UK), hexaflumuron (Cameron 10% EC, Shandong Tianfeng Biotechnology Co. Ltd, China) and chlorpyrifos (Dursban 48% EC, Dow Agrosiences, USA) were obtained from their respective manufacturers.

Persistence and effectiveness of the tested insecticides on cotton and castor bean plants against *S. littoralis* larvae under field conditions

The experiments were conducted at the farm of Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt. Cotton seeds var. Giza 86 were sown on April 15th 2014 in the experimental area, which divided into plots 42m² each. The recommended agricultural practices were applied in the experimental area without using any insecticides. Castor bean seeds were sown around the cotton area at the same sowing date of cotton. Unplanted belts (three meters width) were left as barriers between plots to avoid contamination with drifts. Six treatments (the five insecticides and the control) were arranged in this area in a randomized complete block design with four replications. Application of the tested insecticides was done on July 30th 2014 on cotton and castor bean under field conditions of $38 \pm 2C^{\circ}$ and 75 ± 5 RH at day, during the experiment period. Tap water was used in diluting of the tested insecticides at their field recommended rates and a Knapsack sprayer (CP₃) equipped with one nozzle

was used in application of the insecticides. The final volume of spray solution represented 476 liters per hectare.

Under constant laboratory conditions of $25 \pm 2C^{\circ}$, 65 ± 5 RH and 14:10, L: D, photoperiod, ten newly moulted 4th instar larvae of *S. littoralis* were put in a 500 ml plastic pot and covered with a clean piece of muslin cloth, representing one replication. Ten replications were made for each treatment at each date of feeding. The sprayed leaves of cotton or castor bean were picked up after one h from spray (zero time), and then after 1,3,5,7,10 and 14 days post spray and transferred directly to the laboratory for feeding the tested larvae for 24h and then replaced with untreated ones. Number of dead larvae was recorded after 48 h of feeding in case of all tested insecticides with exception of hexaflumuron, where the number of dead larvae was recorded after 72h of feeding and the percentages of mortality were estimated. The larva was considered dead if no movement was observed when it was touched with a small brush.

Local and systemic activities of the tested insecticides

The method described by Inbar *et al.* (2001) was adopted with slight modifications, where 50 cotton plants and 25 castor bean plants of two months age were used in this experiment. The upper half (the top) of each cotton or castor bean plant was closely covered with polyethylene bags to avoid contamination with sprayed insecticides. The lower half (the bottom) of selected cotton or castor bean plants was sprayed with the tested insecticides at their recommended rates using handheld sprayer. Ten cotton and five castor bean plants were individualized for each insecticide. One h after spray, the polyethylene bags were removed. 24 h after spray, the unsprayed leaves, from the top of treated cotton or castor bean

plants (to determine systemic activity), and sprayed leaves, from the bottom of treated cotton or castor bean plants (to determine local activity) were picked up. The sampled leaves were transferred to the laboratory for feeding the tested newly moulted 4th instar larvae of *S. littoralis* with the same method that described previously. The percentages of larval mortality were estimated and displayed.

Fastness of the tested insecticides against washing with water.

In this experiment, five cotton plants and three castor bean plants of three months age were sprayed with one of the tested insecticides at their recommended rates using a handheld sprayer. One h after spray, the upper and lower surfaces of leaves of the treated plants were washed carefully to run-off using tap water with aid of Knapsack sprayer (CP₃) in continues work for one h. The treated plants were subsequently left to complete dryness. Then, cotton or castor bean leaves were sampled and transferred to the laboratory for feeding the newly moulted 4th instar larvae of *S. littoralis* with the same method that described previously. The percentages of larval mortality were estimated and displayed.

Statistical analysis

Mortality data were corrected for mortality in control by Abbott's formula, and analyzed by probit analysis (Finney, 1971) using POLO- PC software (Le Ora Software, 1987) for time-mortality regression lines. Differences were considered significant based upon non-overlapping of 95% confidence limits. Corrected mortality percentages were subjected to one-way ANOVA and Duncan's Multiple Range Test was used to determine significant differences between means (P= 0.05) using CoStat system for Windows, Version 6.311.

RESULTS AND DISCUSSION

Field persistence of the tested insecticides on cotton and castor bean plants

Persistence of pyridalyl, emamectin benzoate, spinetoram, hexaflumuron and chlorpyrifos on cotton and castor bean plants under field conditions, expressed in median lethal time (Lt₅₀) and the corresponding confidence limits and slope values are presented in Table 1. It is obvious that pyridalyl was the most persistent on cotton plants followed by hexaflumuron and emamectin-benzoate with Lt₅₀ values of 9.93, 9.18 and 8.69 days, respectively.

Table 1: Persistence of the tested insecticides (Median lethal time, Lt₅₀) on the foliage of cotton and castor bean plants under field conditions against *Spodoptera littoralis* 4th instar larvae.

Insecticide	Treated plant	Conc.* mgAI/L	Lt ₅₀ (days)	95% Confidence limits	Slope value	X ²
Pyridalyl	Cotton	250	9.93	8.91– 11.26	2.749 ± 0.269	3.199
	Castor bean	250	14.91	12.08—29.94	2.004 ± 0.271	11.155
Emamectin-benzoate	Cotton	15	8.69	7.85 – 9.94	3.515 ± 0.403	5.418
	Castor bean	15	5.66	4.32 – 7.10	1.704 ± 0.158	1.599
Spinetoram	Cotton	24	1.06	0.79 – 1.35	1.118± 0.086	3.349
	Castor bean	24	0.20	0.05 – 0.40	0.482± 0.114	0.818
Hexaflumuron	Cotton	100	9.18	7.16 – 13.98	1.809 ± 0.288	3.467
	Castor bean	100	8.01	7.38 – 8.73	3.456 ± 0.307	1.725
Chlorpyrifos	Cotton	2400	5.14	4.10 – 6.17	3.669 ± 0.281	16.382
	Castor bean	2400	2.48	1.93 – 3.19	1.043 ± 0.260	1.435

* The used field recommended rate expressed in mg A.I. per L

On the contrary, spinetoram significantly showed the shortest persistence period on cotton plants

translated in Lt₅₀ value of 1.06 days only. In case of castor bean plants, also pyridalyl had significantly the superior

persistent activity with Lt_{50} value of 14.91 days; spinetoram and chlorpyrifos gave the inferior persistent periods indicated with Lt_{50} values of 0.20 and 2.48 days, respectively. From these results it is clear evident that, pyridalyl was most persistent on castor bean than on cotton plants. Whereas, the other tested insecticides showed the contrast, where they were more persistent on cotton than on castor bean plants.

The persistence of pesticides on treated plants depends upon many factors such as physiochemical properties of the pesticide, environmental factors and surface chemistry of the treated plants. Many pesticides are significantly more persistent on kiwifruit than on other fruit crops (Holland *et al.*, 1984). In this respect, Spinosyns had half-lives of 10-20 days on kiwifruit foliage compared to several days on the surfaces of other plant species (Mc Donald *et al.*, 1998). The results of the current study coincided with that of other studies. El-Barkey *et al.* (2008) found that spinetoram had

short persistence period on cotton under field conditions against *S. littoralis* larvae. Emamectin-benzoate was more persistent than spinetoram either on cotton or on castor bean plants and the two insecticides were more persistent on cotton comparing to castor bean (Abdu-Allah, 2010). El-Dewy (2013) reported that pyridalyl was more persistent than emamectin-benzoate on cotton plants with Lt_{50} values of 7.74 and 5.59 days, respectively against the 4th instar larvae of *S. littoralis*.

Efficiency of the tested insecticides against 4th instar larvae of *S. littoralis*

Data presented in Table 2 indicated that, when the 4th instar larvae of *S. littoralis* were fed on cotton treated plants, pyridalyl proved to be the most effective followed by emamectin-benzoate and hexaflumuron recording 100, 100, and 96% larval mortality after zero time of spray and 70.33, 65.00, and 58.17% mean of larval mortality through 14 days post spray, respectively.

Table 2: Efficiency of the tested insecticides against the 4th instar larvae of *Spodoptera littoralis*, laboratory strain fed on treated cotton or castor bean plants.

Insecticide	Treated plant	Conc.* mgAl per L.	% Larval mortality \pm SD after indicated days of spray						Average of larval mortality
			Zero time	3 days	5 days	7 days	10 days	14 days	
Pyridalyl	Cotton	250	100 \pm 0.00	94 \pm 1.096	81 \pm 1.095	62 \pm 2.775	51 \pm 1.00	34 \pm 2.074	70.33 \pm 1.534 ab
	Castor bean	250	100 \pm 0.00	95 \pm 0.548	85 \pm 1.140	80 \pm 0.707	71 \pm 2.828	42 \pm 1.304	78.83 \pm 2.132 a
Emamectin-benzoate	Cotton	15	100 \pm 0.00	92 \pm 0.837	87 \pm 0.837	60 \pm 2.449	41 \pm 2.449	10 \pm 0.707	65.00 \pm 0.867 bc
	Castor bean	15	98 \pm 0.447	79 \pm 0.707	66 \pm 1.140	62 \pm 2.168	27 \pm 1.304	2 \pm 0.447	56.17 \pm 2.697 cd
Spinetoram	Cotton	24	88 \pm 1.985	32 \pm 1.483	23 \pm 0.707	17 \pm 1.342	18 \pm 2.049	8 \pm 0.837	31.00 \pm 1.624 e
	Castor bean	24	56 \pm 1.140	26 \pm 1.517	9 \pm 1.342	4 \pm 0.548	6 \pm 0.548	0 \pm 0.00	16.83 \pm 0.935 f
Hexaflumuron	Cotton	100	96 \pm 1.788	86 \pm 1.140	71 \pm 1.414	55 \pm 1.949	28 \pm 2.387	13 \pm 1.304	58.17 \pm 1.112 c
	Castor bean	100	100 \pm 0.00	92 \pm 1.304	81 \pm 1.414	54 \pm 1.517	39 \pm 3.033	20 \pm 1.581	64.33 \pm 1.038 bc
Chlorpyrifos	Cotton	2400	100 \pm 0.00	90 \pm 1.414	42 \pm 2.864	31 \pm 1.924	18 \pm 1.304	4 \pm 0.548	47.50 \pm 1.827 d
	Castor bean	2400	100 \pm 0.00	70 \pm 1.225	15 \pm 2.236	8 \pm 1.304	3 \pm 0.447	0 \pm 0.00	32.67 \pm 0.734 e

* The used field recommended rate expressed in mg A.I. per L

In the same column, figures followed by the same letters are not significantly differed by Duncan's Multiple Range Test, P = 0.05

When the larvae were fed on treated castor bean plants, pyridalyl was significantly the most effective followed by hexaflumuron and emamectin-benzoate causing 100, 100 and 98% larval mortality after zero time of spray and 78.83, 64.33 and 56.17% mean of larval mortality through 14 days post spray, respectively. It is clear that pyridalyl and hexaflumuron were more effective when they were applied on castor bean than on cotton plants. On the other side, emamectin-benzoate was more effective in case of cotton treated plants than in case of castor bean treated ones. The conventional insecticide, chlorpyrifos, resulting in 100% larval mortality after zero time of spraying cotton or castor bean plants. While, its activity declined sharply and significantly from the 5th day post spray recording 47.50 and 32.67 % mean of larval mortality in case of treated cotton and castor bean plants, respectively. Spinosyn compound, spinetoram, exhibited the inferior activity against 4th instar larvae of *S. littoralis* translated in 88 and 56% larval mortality after zero time of spray, and 31.00 and 16.83% mean of larval mortality in case of treated cotton and castor bean, respectively. These results substantiated the abovementioned high persistence of pyridalyl, emamectin-benzoate and hexaflumuron on cotton and castor bean plants. Cook *et al.* (2004) found that

pyridalyl and emamectin-benzoate controlled *S. exigua* infestation up to 10 days after treatment of cotton field plants. Emamectin-benzoate proved to be more efficient than spinosad (the first generation of spinetoram) against *S. litura* larvae (Ahmad *et al.*, 2006). Abdu-Allah (2010) stated that emamectin-benzoate was more effective than spinetoram against *S. littoralis* larvae. Moreover, he confirmed that emamectin-benzoate and spinetoram were more potent in controlling *S. littoralis* on cotton treated plants than on castor bean treated ones. In this respect, El-Dewy (2013) reported that pyridalyl and emamectin-benzoate both gave 100% initial effect and 54.0 and 42.13% residual effect, respectively toward *S. littoralis* larvae on cotton plants.

Local and systemic activities of the tested insecticides.

The results of local and systemic activities of the tested insecticides in cotton and castor bean plants against 4th instar larvae of *S. littoralis* are illustrated in Figure 1. The systemic activities were determined 24h after spray, in order to give sufficient time to the tested compounds to establish their systemic properties via movement in plant sap from lower-treated leaves of the plant to

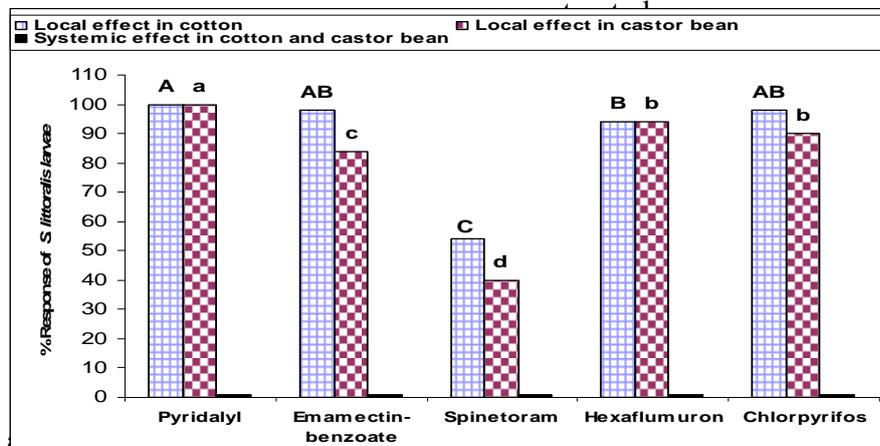


Fig. 1: Local and systemic activities of the tested insecticides against the 4th instar larvae of *S. littoralis*. The same letters from the same case above the columns means insignificant differences by Duncan's Multiple Range Test, P = 0.05.

In case of cotton treated plants, pyridalyl had the highest local activity causing 100% mortality in the 4th instar larvae of *S. littoralis* followed by emamectin-benzoate (98%), chlorpyrifos (98%), hexaflumuron (94%) and spinetoram (54%) larval mortality. In case of castor bean treated plants, pyridalyl found to have the superior local activity, 100% mortality, against 4th instar larvae of *S. littoralis* followed by hexaflumuron (94%), chlorpyrifos (90%) and emamectin-benzoate (84%) larval mortality. In this respect, spinetoram possessed the inferior local effect translated in 40% larval mortality. Also, the results of this experiment clearly indicated that all the tested insecticides did not demonstrate any real systemic activity in cotton or castor bean plants. As a result of that, all tested insecticides recorded zero percent mortality in the 4th instar larvae of *S. littoralis* after feeding on upper-untreated leaves of cotton or castor bean.

Fastness of the tested insecticides against washing with water

The effect of washing with water on the stability of the initial deposits of the tested insecticides on sprayed cotton and castor bean plants, measured with the mortality percentage occurred in *S.*

littoralis 4th instar larvae after feeding on sprayed then washed leaves is presented in Fig. 2. It is obvious that the tested insecticides proved to be more stable on castor bean than on cotton foliage after exposure to washing with water. Furthermore, it has been observed that chlorpyrifos was significantly the most stable on castor bean and cotton, comparing to the other tested insecticides, causing 98% and 88% larval mortality, respectively. Pyridalyl, hexaflumuron and emamectin-benzoate showed less stability against washing with water translated in 88, 70 and 64% larval mortality in case of castor bean and 66, 52 and 18% larval mortality in case of cotton, respectively. The bio-insecticide, spinetoram, was unstable and completely washed with water where it did not leave any appreciable residues on cotton or castor bean foliages recording 0% larval mortality. These results could be explained with the diversity of the tested insecticides in their affinity to adsorption into epicuticular waxes and deeper tissues of sprayed cotton and castor bean plants, thereby the initial deposits of the compounds withstand elimination and washing with water from sprayed foliage with different degrees.

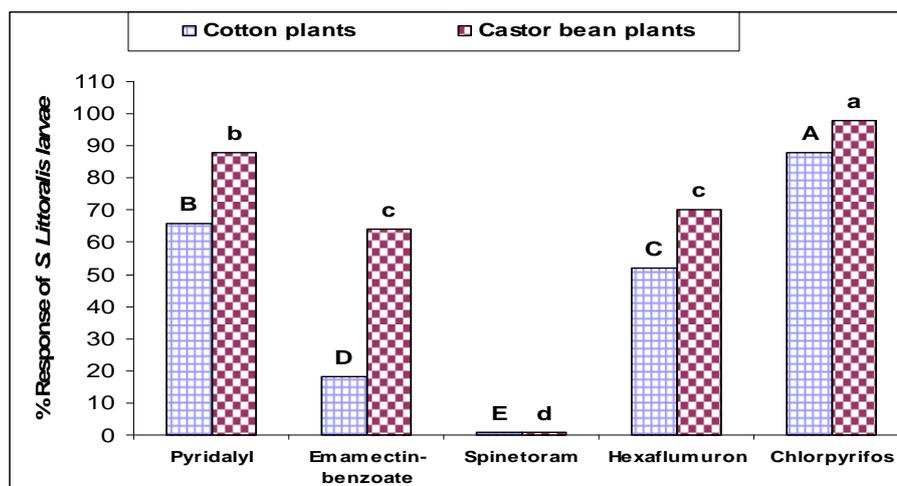


Fig. 2: Fastness of the tested insecticides on cotton and castor bean plants against washing with water measured with the mortality percentage occurred in *S. littoralis* 4th instar larvae. The same letters from the same case above the columns means insignificant differences by Duncan's Multiple Range Test, P = 0.05.

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ARABIC SUMMERY

دلائل على ثبات وفاعلية بعض المبيدات الحديثة ضد دودة ورق القطن على العوائل النباتية المختلفة

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تمت دراسة ثبات مركبات بيريداليل، إيمامكتين بنزوات، اسبنتورام، هكسافلوميرون، كلوربيريفوس على نباتات القطن صنف جيزه 86 ونباتات الخروج تحت الظروف الحقلية وذلك من خلال التقدير الحيوي للوقت اللازم لقتل 50% من التعداد (Lt_{50}) وكذلك فاعلية هذه المركبات ضد يرقات العمر الرابع للسلالة المعملية لدودة ورق القطن. كذلك تمت دراسة النشاط الموضعي والجهازي لهذه المبيدات وثباتها ضد الغسيل بالماء من على السطح المعامل لنباتات القطن والخروج. أظهرت النتائج أن مبيد بيريداليل كان معنويًا الأكثر ثباتًا على نباتات الخروج والقطن المعاملة به مسجلًا قيم Lt_{50} هي 14,91، 9,93 يوم على الترتيب. على العكس من ذلك كان مبيد اسبنتورام الأقل ثباتًا على الخروج والقطن مسجلًا قيم Lt_{50} هي 1,06، 0,20 يوم على الترتيب. باستثناء مركب بيريداليل فإن كل المركبات المختبره كانت معنويًا أكثر ثباتًا على القطن عن الخروج. عند تغذية يرقات العمر الرابع لدودة ورق القطن على نباتات القطن المعاملة بالمبيدات المختبره كان مبيد بيريداليل وإيمامكتين بنزوات هما الأكثر فاعلية. بينما عند تغذية اليرقات على نباتات الخروج المعاملة كان مبيد بيريداليل و هكسافلوميرون هما الأكثر فاعلية. لم يثبت أي من المبيدات المختبره في هذه الدراسة أي نشاط جهازي يمكن تقديره في نباتات القطن أو الخروج المعاملة بها علي الرغم من أنها أظهرت نشاط موضعي قوي على كلا النباتين ضد دودة ورق القطن. كان المترسب الأولي لمبيدات كلوربيريفوس، بيريداليل، هكسافلوميرون، إيمامكتين بنزوات معنويًا أكثر ثباتًا على نباتات الخروج المعاملة ضد الإزاحة و الغسيل بالماء عنه علي نباتات القطن المعاملة. كان المترسب الأولي لمبيد اسبنتورام غير ثابت تمامًا ضد الغسيل بالماء من على كل من نباتات القطن والخروج المعاملة به. توضح هذه النتائج أن نوع العائل النباتي المعامل يعتبر عامل مؤثر جدا في تحديد الخواص التوكسيكولوجية للمبيدات المختبره، وهذا ربما يكون مفيدا في تصميم تجارب التقييم الحيوي.