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EFFECT OF TEBUFENOZIDE AND DIFLUBENZURON ON SOME BIOLOGICAL ASPECTS OF COTTON LEAFWORM *Spodoptera littoralis* (BOISD.) COMPARISON WITH CHLORPYRIFOS

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ABSTRACT

During summer season, 2014 an experiment was carried out at El-Gemmeiza Agricultural Research Station Gharbia Governorate to evaluate the efficiency and latent effect of chlorpyrifos, tebufenozide, and diflubenzuron on some biological aspects of cotton leafworm *Spodoptera littoralis* (Boisd.). The efficiency and latent effect proved that these insecticides had high efficiency compared to the control treatment especially for the 4th instar larvae followed with 5th instar and 6th instar ones. Tebufenozide was the most effective compound compared with the other two compounds in suppressing the insect development, while chlorpyrifos recorded the least toxic effect on 6th instar larvae after 15 days from treatment. Tebufenozide affected the development of *S. littoralis* pupation where, zero days of larval feedings treatment recorded zero pupae formation of the 4th, 5th and 6th instar larvae. The highest mortality of pupae was recorded at the 5th day after treatments followed by the 10th day after treatments and finally at 15th day after treatments, for Tebufenozide, diflubenzuron and chlorpyrifos for all instars larvae,respectively.Tebufenozide recorded the highest mortality of pupae. The least moths emergence was recorded with tebufenozide in the 5th day after treatment with no significant differences with diflubenzuron for the 4th instar larvae. The moths emergence increased gradually for the three tested compounds from the 4th to the 5th and the highest in case of the 6th instar larvae. Results showed that all treatments succeeded in reducing the alive instar larvae , high mortality of pupae and less number of moths emergence. Successful results happened to be more promising for the 4th instar larvae than the 5th and 6th instar larvae.
Keywords: *Spodoptera littoralis*, instar larvae, pupae, Tebufenozide, Diflubenzuron, Chlorpyrifos

INTRODUCTION

Cotton (*G. barbadense* L.) belongs to the family *Malvaceae*, is attacked by more than 46 different pests in 32 different countries. The cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae), is an important pest of cotton in Southern Europe, Africa and the Middle East (Hosny *et al.* 1986). The larvae feed mainly on leaves and stems and can seriously retard growth or reduce production of the cotton crop. It is extremely polyphagous, and always appropriate to inflict excessive damage *(*Amin and Salam, 2003). The most important groups of chemical insecticides used on cotton are pyrethroids and organophosphates, which classified by the World Health Organization (WHO) as 'moderately hazardous'. Organophosphate compounds are generally acutely toxic, it inhibits the enzyme cholinesterase which have severe effects on the central nervous system and death can result from severe poisoning. Chlorpyrifos one of the organophosphate compounds act as a cholinesterase inhibitor is non-systemic insecticide with contact, stomach, and respiratory action. It is used in the control of Coleoptera, Diptera, Homoptera and Lepidoptera in soil or on foliage in over 100 crops (Clegg and van Gemert, 1999). But the intensive use of broad spectrum insecticides against *S. littoralis* has led to develop resistance to many pesticides for its control (Aydin and Gu¨ rkan, 2006). These have caused economic losses in the short term and, in the long term and that lead for searching to another tool of control. Thus, insect growth regulators (IGR's) differ widely from the commonly used insecticides, as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system (Oberlander, *et al.* 1997). Diflubenzuron (IGR) is used at a rate of 50-150 g/ha for control of a wide range of leaf-eating insect pests in cotton, soyabeans, citrus, tea, vegetables and mushrooms. Also, tebufenozide (mimic) belongs to a class of (IGRs), bisacylhydrazine ecdysteroid agonists, mimicking the natural insect moulting hormone 20-hydroxyecdysone (20-E) (Dhadialla *et al.* 1998 ; Oberlander and Silhacek 1998). It is used to control of lepidopteran larvae, generally at 0.06-0.3 lb/a. IGRs is non-systemic with contact and stomach action. Their comprehensive effects and high selectivity as well as lower toxicity to non-target animals and the environment, provide new tools for integrated pest management (Tunaz and Uygun 2004 and Huang, *et al.* 2008).

So, the objective of this research was to assess the effect of the organophosphate compound chlorpyrifos and the two IGRs compounds on the control of 4th ,5th and 6th instar larvae of the cotton leafworm *S. littoralis***.**

MATERIALS AND METHODS

1- Laboratory strain of the cotton leaf worm s.littoralis:

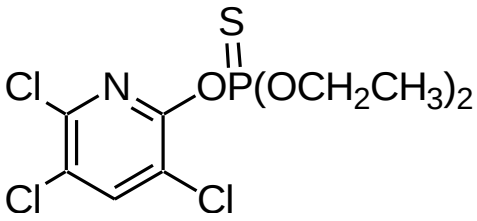
A laboratory strain of the cotton leafworm *S.littoralis* was used in this study to investigate of the insecticide organophosphate (chlorpyrifos) and the two IGR compounds, diflubenzuron and tebufenozide. Egg masses of the cotton leafworm were obtained from the rearing of laboratory colon in Sakha Agricultural Research Station, Kafer El-Sheikh Governorate. The egg masses were maintained on castor leaves *Ricinous communis* inside glass container until hatching. The hatched larvae were transferred by paintbrush into disinfected glass containers, covered with muslin secured with rubber bands and fed daily with fresh castor leaves, as described by El-Defrawi *et al.* 1964 till reaching the proper instar larvae stage needed for this research.

An area of 400 m^2 was planted with cotton variety Giza 86 in the El-Gemmeza Research Station farm, divided into 4 blocks a 100 m^2 each and treated with the three respected compounds in addition to untreated one using a knapsack sprayer provided with one nozzle delivering 200littre water/feddan. Cotton leaves were collected from both treated and untreated ones for larval feeding.

For each of the three tested compounds and control to evaluate the mortality number of the respective larvae (4^{th}, 5^{th} and 6^{th} instar larvae) due to larval feeding on treated cotton leaves. The efficiency of Chlorpyrifos and the IGR compounds on the respected instar larvae of cotton leafworm was estimated by feeding all the three target groups of larvae with treated cotton leaves for 48 hours (beside the untreated control). Treated cotton leaves were then removed and fresh untreated ones were provided. The first treatment, the respected instar larvae were fed on the treated cotton leaves immediately after compounds applications, the second treatment the respected instar larvae/ fed on the treated cotton leaves after five days from compounds applications while the third one was after ten days and the fourth one was after fifteen days from applications. Five replicates (each of 10 larvae) in glass jars (30X25 diameter) covered with muslin and maintained under room temperature were used for each compound. In addition ten larvae fed on fresh untreated leaves were used as control. The larvae of cotton leafworm were monitored daily to record the number of the alive ones in open feeding till pupation. Also, after complete pupation, the pupae were examined daily to find out the pupation percentages, the mortality percentages of the remaining ones and the percentages of moth emergence. The pupae resulted from all larval stages traeted with different toxicants were placed in glass jars separately for each of the different larval stages and different treatments in addition to the untreated one. The keeping jars were monitor daily in expecting the adults, then the adults confined in another glass jar containing a cotton pad soaked with 20% sugar solution as a food.

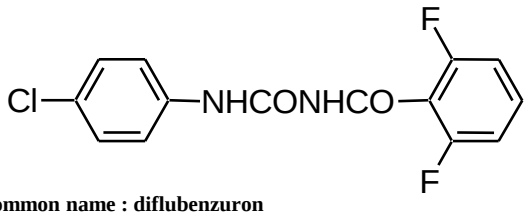
2- Insecticides used:

Chlorpyrifos



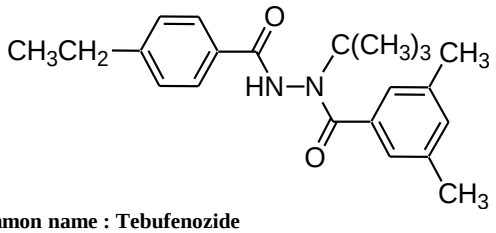
- Common name : Chlorpyrifos
-Chemical Abstracts name *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate.
- Rate of application : 1 litre/fed.

Diflubenzuron (250) Insecticide IRAC 15 ; benzoylurea



- Common name : diflubenzuron
-Chemical Abstracts name:*N*-[[(4-chlorophenyl) amino] carbonyl] -2,6-difluorobenzamide
-Molecular weight: 310.7
-Molecular formula :C114 H9 CLF2 N2 O2
- rate of application: 400 ml/ fed.

Tebufenozide (762) insecticide IRAC 18; diacylhylrazine



- Common name : Tebufenozide
-Chemical abstracts name: 3,5-dimethylbenzoic acid 1-(1,1-dimethylethyl)-2-(4-ethylbenzoyl) hydrazide
-Molecular weight: 352.5
-Molecular formula :O 22 H28 N2 O2
- rate of application : 350 ml/ fed.

For all experiments, mortality values were corrected according to Abbot's formula (1925), however possible. Duncan's multiple range test, Duncan (1955) at 5% level was used to compare the means of different treatments at the end of the larval stages using MINITAB program.

RESULTS AND DISCUSSION

Table (1) showed the mortality percentages of the 4^{th}, 5^{th} and 6^{th} instar larvae of *S. littoralis* following feeding on cotton leaves treated with tested compounds, where complete mortality occurred at zero days after treatment for the 4^{th} instar larvae to all tested compounds. The 5^{th} and the 6^{th} instar larvae at zero day after treatments recorded mortality of 94, 90 and 91%; 76, 70 and 62% for tebufenozide, diflubenzuron and chlorpyrifos; respectively. It was obviously noticed that the mortality number of instar larvae decreased gradually as the older larval stages it may have more to lerant the tested compounds than the younger ones. Also, chlorpyrifos differ significantly throw the four treatments for the three larval stages and with the two IGR compounds. Mortality of percentages of larvae that fed on treated cotton leaves at after treatment ware the 5^{th} day ranged between 95, 85, and 70; 78, 70 and 65; and 64, 60 and 52% for tebufenozide, diflubenzuron and chlorpyrifos; for the three larval stages; respectively. Similar results recorded for the 3^{rd} and 4^{th} treatments (10^{th} and 15^{th} day of to applecation) for all tested

compounds and throw all larval stages. Tebufenozide gave the best results and highest mortality percentages compared with diflubenzuron and chlorpyrifos throw all larval stages. Chlorpyrifos treatment, reduced mortality % of larvae for all treatments and throw all larval stages ,this degradation may be rely on the low persistence of the compound throw the long time of cotton leaves applications and also, due to the higher tolerance of the 5th and 6th instar larvae. Comparing the efficiency of the three tested compounds Tebufenozide came in the first rank followed by diflubenzuron and chlorpyrifos was the third one. Also, treating the younger instar larvae gave higher mortality and more promising than the older ones throw all treatments. The control treatment % mortality were 0, 2 and 3% which differ significantly with other tested compounds for all treatments and for all instar larval stages.

Table (2) ippus trated the effect of larval feeding with the tested compounds on the percentages of *S. littoralis* pupation development where, at zero days larval feedings treatment recorded zero pupae formation to the 4th, 5th and 6th instar larvae. 6th instar larvae recorded highest number of pupae formation at the 5th , 10th and 15th days treatments compared with the 5th and 4th instar larvae ones and this results may be return to the high tolerant of the 6th instar larvae more than the 5th and the 4th instar ones. The chemical compound chlorpyrifos recorded highest numbers of pupae formations throw all treatments and same trends for the 5th and 6th instar larvae. No significant difference was recorded for the number of pupae formations between the chemical compound and the control throw all treatments for the 4th , 5th and 6th instar larvae and this results may be return to the limited latent effect of the chemical compound. Tebufenozide compound was the most effective one compared with diflubenzuron and chlorpyrifos which recorded only 44.2 pupae at the 5th days after treatment in the 4th instar larvae which happened to be the least number of pupae formations throw all the 5th and 6th instar larvae.

Table (1). Mortality percentages of the 4th, 5th and 6th instar larvae of *S. littoralis* following feeding with Tebufenozide, Diflubenzuron and Chlorpyrifos on cotton leaves

Treatments	Rate of application L/fed.	Mortality Percentages 4th instar larvae					5th instar larvae					6th instar larvae					Total Mean
		0 days	5 days	10 days	15 day	Mean	0 days	5 days	10 days	15 day	Mean	0 days	5 days	10 days	15 day	Mean	
Tebufenozide	0.350 L/f	100	95	69	52	79 a	94	78	45	39	64 a	76	64	34	14	47 a	63.33 a
Diflubenzuron	0.400 L/f	100	85	57	39	70.25 b	90	70	42	33	58.75 b	70	60	24	10	46a	58.33 b
Chlorpyrifos	1 litre/f	100	70	40	15	56.25 c	91	65	18	10	46 c	62	52	12	5	32.75b	45 b
Control	———	3	3		2	2 d	2	3	0	2	1.75 d	3	2	0	2	1.75 c	1.83 c

Table (2) . Effect of larval treatment with Tebufenozide, Diflubenzuron and Chlorpyrifos on the percentages of *S. littoralis* pupation development

Treatments	Rate of application L/fed	Percent pupation 4th instar larvae					5th instar larvae					6th instar larvae					Total Mean
		0 days	5 days	10 days	15 day	Mean	0 days	5 days	10 days	15 day	Mean	0 days	5 days	10 days	15 day	Mean	
Tebufenozide	0.350 L/f	---	44.2	61	66	42.8 d	--	74	77	86.4	59.35 d	--	87.8	89.7	88.5	66.5b	56.22 b
Diflubenzuron	0.400 L/f	--	53.2	65.6	74	48.2 c	--	78	82	88.6	62.15 c	---	89.6	89.7	89.6	67.23 b	59.19 b
Chlorpyrifos	1 litre/f	--	88.4	89	92.8	67.55 b	--	91.6	92.5	92.7	69.2 b	--	92.5	92.5	93.4	69.6 b	68.78 b
Control	———	94.5	93.6	93	93.3	93.6 a	95.5	92.5	93.6	93.6	93.8 a	93.5	92.6	93	93.5	93.15 b	93.52 a

The latent effect of the tested compounds on mortality of *S. littoralis* pupae following feeding of the 4th, 5th and 6th instar larvae was illustrated in table (3). Highest mortality of pupae was recorded at the 5th day after treatments followed by the 10 day and at last at the 15 day, for tebufenozide, diflubenzuron and chlorpyrifos; and for all instars larvae; respectively. Tebufenozide recorded the highest mortality number of pupae (20.6, 20.3 and 20.2%) at the 5 days after treatments with recorded the 4th , 5th and 6th instars larvae respectively. So, the same trend was at the 10 th and 15 th days after treatments with the 4th ,5th and 6th instars larvae respectively. No significant differences was recorded for pupae mortality with the Tebufenozide at each of 5 th days, 10 th day and 15 th days with the 4th , 5th and 6th instars larvae, respectively. Diflubenzuron came in the 2nd rank where the mortality number of pupae was (16.8, 14.4, and 10.2) , (15, 13 and 8) and (15.4, 15.3 and 7) the 4th , 5th and 6th instars larvae; respectively. Finally chlorpyrifos came in the third one. Comparing the efficiency of the three tested compounds on larvae and the latent effect on pupae it was noticed that the efficacy of the three tested compounds reduced to great extent on pupae. Also, the chlorpyrifos has very limited latent effect on pupae than on larvae. There were no available number of pupae formation zero time after treatment in case of 4th instars larvae as previously mentioned from at (Table 1) which recorded zero alive larvae. Also, zero alive pupae was recorded with the three tested compounds for the 5th and 6th instars larvae treatments. High significant differences were recorded between tebufenozide, diflubenzuron and the control treatments.

Table (4) illustrate the effect of larval treatment with the tested compounds on moth emergence of *S. littoralis*, where the highest moths emergence(91.3)was recorded at the 15th day after treatment with chlorpyrifos for the 6th instar larvae with no significant

differences with 5th and 4th instar larvaeat the15th days after treatmen, and with the control ones. The least moths emergence 42.5 was recorded for tebufenozide at the 5th day treatment with no significant differences with diflubenzuron for the 4th instar larvae. The moths emergence increased gradually for the three tested compounds from the 4th to the 5th and the highest in the 6th instar larvae and this result may be return to the long time of compounds applications that led to the degradation of the tested compounds and also, to the high tolerant of the 6th instar larvae more than the 5th and 4th ones. No available pupae was found at the zero time after treatments for all compounds and throw all instar larvae as previously mentioned from table (3).

Table (3) .Mortality percentages of *S. littoralis* pupae following feeding the 4th ,5th and 6th instar larvae with Tebufenozide, Diflubenzuron and Chlorpyrifos on cotton leaves

Treatments	Rate of application L/fed	Mortality Percent of pupae 4th instar larvae					5th instar larvae					6th instar larvae					Total Mean
		0 days	5 days	10 days	15 day	Mean	0 days	5 days	10 days	15 day	Mean	0 days	5 days	10 days	15 day	Mean	
Tebufenozide	0.350 L/f	--	20.6	15	12	11.9 a	--	20.3	14.5	9	10.95 a	--	20.2	10.3	8	9.6 a	10.82 a
Diflubenzuron	0.400 L/f	--	16.8	14.4	10.2	10.35 b	--	15	13	8	9 b	--	15.4	15.3	7	9.4 a	9.58 a
Chlorpyrifos	1 litre/f	--	12	10	8	7.5 c	--	10	5	3	4.5 c	--	9	5	2	4 b	5.33 b
Control	———	1	3	2	2	2 d	1	3	2	1	1.75 d	3	3	2	1	2.5 c	2.08 c

Table (4) . Effect of larval treatment with Tebufenozide, Diflubenzuron and Chlorpyrifos on moth emergence of *S. littoralis*

Treatments	Rate of application L/fed	Moth emergence % 4th instar larvae					5th instar larvae					6th instar larvae					Total Mean
		0 days	5 days	10 days	15 day	Mean	0 days	5 days	10 days	15 day	Mean	0 days	5 days	10 days	15 day	Mean	
Tebufenozide	0.350 L/f	--	42.5	44.2	49.4	34.03 d	---	55	58.6	71.6	46.55 d	--	72.2	79.1	79.4	57.67 d	46.08 c
Diflubenzuron	0.400 L/f	--	44.4	47.5	53.2	36.28 c	--	58	63.4	76.2	49.3 c	--	78.5	80.4	82.6	60.38 c	48.65 c
Chlorpyrifos	1 litre/f	--	81.8	82.4	86.4	62.65 b	--	88	88.4	88.4	66.2 b	--	88.2	89.6	91.3	67.28 b	65.38 b
Control	———	93	93	94.5	92	93.13 a	94	92	92.9	93.6	93.13 a	95.4	94	91.6	92.8	93.45 a	93.24 a

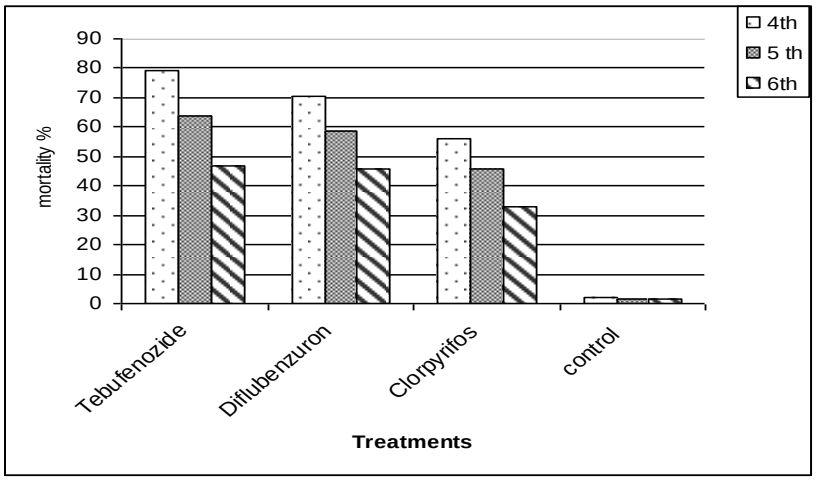


Fig (1) Mortality percentages of the 4th , 5th and 6th instar larvae of *S. littoralis* following feeding with Tebufenozide, Diflubenzuron and Chlorpyrifos on cotton leaves

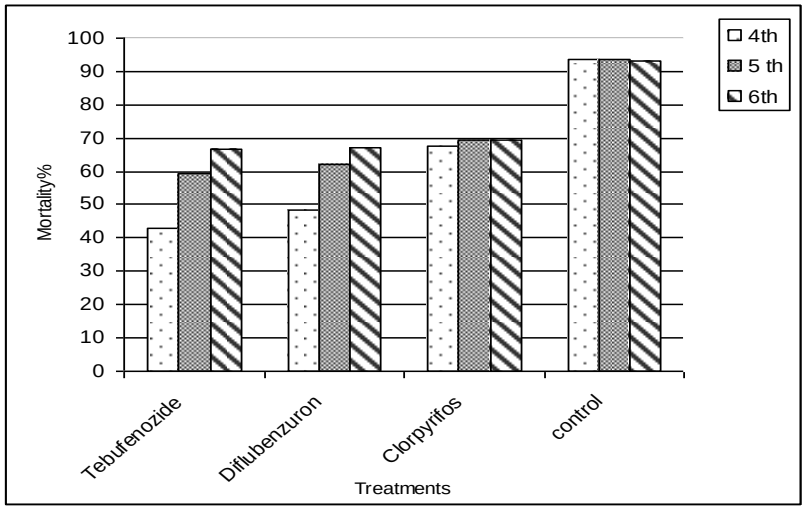


Fig (2).Effect of larval treatment with Tebufenozide, Diflubenzuron and Chlorpyrifos on the percentages of *S. littoralis* pupation development

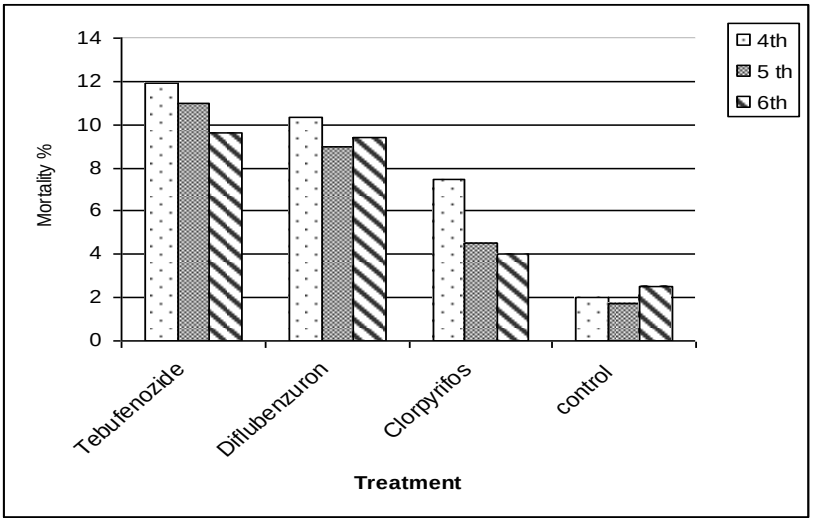


Fig (3) Mortality percentages of *S. littoralis* pupae following feeding the 4th ,5th and 6th instar larvae with Tebufenozide, Diflubenzuron and Chlorpyrifos on cotton leaves

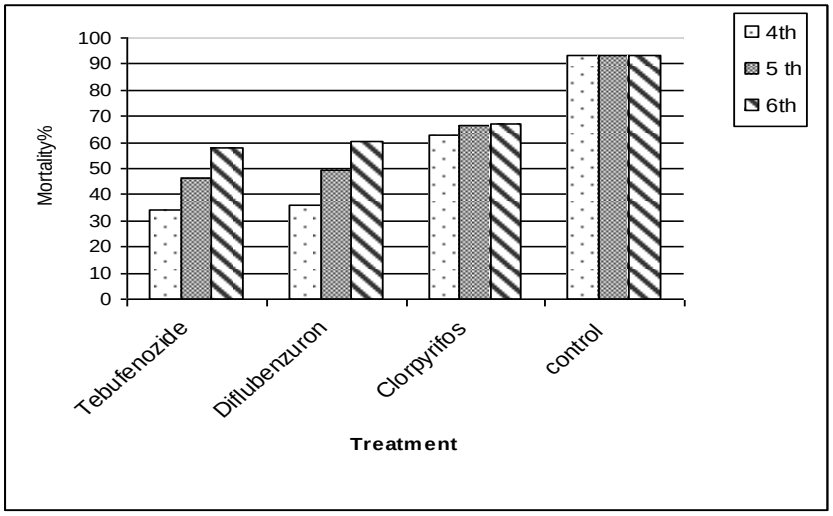


Fig (4) Effect of larval treatment with Tebufenozide, Diflubenzuron and Chlorpyrifos on moth emergence of *S. littoralis*

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تـأثير مركبـى تيبيوفينوزيـد و ديفلوبنزيـورون علـى بعض المظـاهر البيولوجيـة لـدودة ورق القطن مقارنـة بمركـب كلوروبيريفوس .

المدثر عبد العظيم وهبه

معهد بحوث وقاية النباتات - مركز البحوث الزراعية

تـم تنفيـذ هذا البحـث خـلال صـيف ٢٠١٤ فـى محطـة البحـوث الزراعيـة بـالجميزة محافظـة الغربيـة وذلـك بغـرض تقييم الكفـاءة والاثـر البـاقى لمركبـات كلوروبيريفوس (مبيد الفوسفورى) وكـلا من مركبـى تيبيوفينوزيـد و ديفلوبنزيـورون وهمـا من منظمـات النمو الحشرية علـى بعض الجوانـب الحيوية لدودة ورق القطن، ولقد وجد ان هذه المركبات الثلاثة كـان لهـا تـأثير كبيـر فـى خفض تعداد اليرقـات خاصـة فـى العمر اليرقـى الرابـع يليـة العمر اليرقى الخامس ثم السادس، ولقد وجد ان مركب تيبيوفينوزيد كان الاكثر فاعلية فى خفض تعداد الآفة ،وان مركب كلوروبيريفوس كان الاقل فـى الفاعليـة فى العمر اليرقى السادس خاصة فى معاملة تغذية اليرقات عند ١٥ يوم، وأن أعلى نسبة موت للعذارى كانت عند التغذية على المعاملـة الاولـى عند صـفر يوم تليها المعاملة الثانية عند التغذية بعد ٥ يوم ثم ١٠ يوم واخيرا بعد ١٥ يوم وذلك للمركبات تيبيوفينوزيد و ديفلوبنزيورون و كلوروبيريفوس علـى التوالى، وكان اقل تكشف لعدد الفراشات كان لمركب تيبيوفينوزيد عند معاملة التغذية بعد ٥ ايام وبدون فـارق جوهرى مـع مركب ديفلوبنزيورون وذلك للعمر الرابع، وأن التكشف للحشرة الكاملة كان يزداد تدريجيا للمركبات الثلاثـة من العمر اليرقـى الرابـع ليـزداد للعمر الخـامس واعلـى عدد كـان للعمر السادس