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Research Article

Chlorfenapyr and Methomyl Deterioration on Spinach Plants and Their Residual Effects *in Vitro* on Egyptian Cotton Leafworm (*Spodoptera littoralis*)

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Abstract

Field trials conducted to determine the degradation of chlorfenapyr and methomyl insecticides in/on spinach leaves. Spinach plants sprayed with chlorfenapyr (Challenger Super™ 24% SC) and methomyl (Neomyl™ 90% SP) at the rates of 50 cm^3/100 L water and 715 g/ ha, respectively. The QuEChERS method used for the extraction and clean-up of the samples. Residue amounts determined at 2 h, 2, 4, 6, 9, 13 and 16 days after application by UHPLC-UV. The mean of recovery percentages was 98.78 and 99.05 % for chlorfenapyr and methomyl, respectively. The initial deposits of chlorfenapyr and methomyl on/in spinach leaves, two hours after a single application of the insecticides were 23.17 and 235.37 mg/kg, respectively. The percentages of dissipation of chlorfenapyr were 37.68, 55.29, 69.45, 84.45 and 96.83% for 2, 4, 6, 9 and 16 days after application. The corresponding dissipation percentages of methomyl were 38.27, 56.01, 71.44, 84.34 and 97.81%. The rates of degradation (k values) were 0.212 and 0.223, while the corresponding half-life times ($t_{0.5}$) were 3.27 and 3.11 days with chlorfenapyr and methomyl, respectively. It could be recommended that single application of chlorfenapyr on Spinach plants at the early ages followed by single application of methomyl at least 17 days before harvest.

Keywords: chlorfenapyr; methomyl; dissipation; residues; QuEChERS; HPLC-UV; residual effect; Egyptian cotton leafworm; *Spodoptera littoralis*

Introduction

Spinach is a widely cultivated edible Asian vegetable plant, with dark green leaves that are consumed raw or cooked. Spinach is rich in fiber, vitamins e.g. A, C, K1, folic acid, and minerals e.g. iron, calcium. The spinach leaves represent the consumed edible part, the healthy leaf is the main characteristics of quality. Healthy leaves free of chewing damage caused by arthropods. The cotton leafworm host range covers at least 87 species of economic importance plants of 40 families [24]. Spinach (*Spinacia oleracea*) is the main host [9]. The young larvae larval instars feed on one side of the leaf (lower epidermis) then older instars chews small holes and increased in volume to become a large hole of irregular shape converted gradually to skeletonized leaf and desiccation of leaves. High populations can produce significant defoliation and yield losses [27]. So, the develop management strategies for armyworm should be based on farmers' needs and priorities [14].

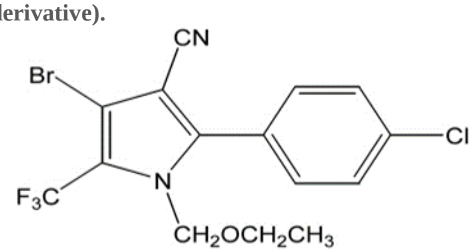
Involving pesticides in crop production is commonly used worldwide for plant protection, but potentially adverse effects resulted from the accumulation of considerable amounts of residues in the final products are a major global concern today. The excessive use/misuse results in widespread environmental contamination manifested as adverse health problems to consumers and local and global environmental impacts. Farmers often apply insecticides at regular intervals until harvest to protect spinach plants. Among the applied insecticides chlorfenapyr (arylpyrrole derivative) and methomyl (carbamate derivative) are commonly used. Both compounds are mainly stomach with some contact action. Chlorfenapyr exhibits good translaminar, but limited systemic activity in plants. While methomyl has a systemic activity [17].

The present study was conducted to determine dissipation kinetics for chlorfenapyr and methomyl insecticides on/in spinach under field conditions and monitoring their residual effect on the Egyptian cotton leafworm under laboratory conditions. From the generated data, the PHI will be established based upon dissipation patterns as well as the biological half-life.

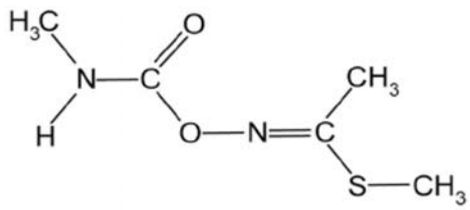
Materials and Methods

Insecticides used

A) **chlorfenapyr (Challenger Super™ 24% SC), (arylpyrrole derivative).**



B) **methomyl (Neomyl™ 90% SP), (carbamate derivative).**



Chemicals and reagents

The certified reference standard of chlorfenapyr and methomyl (purity>98%) were purchased from Sigma-Aldrich Co. Chlorfenapyr (Challenger Super™ 24% SC), was supplied by BASF Co. Ltd. Methomyl (Neomyl™ 90% SP), was supplied by kz pesticides and chemical Co. The other chemicals and reagents of the analytical grade used such as acetonitrile, HPLC grade (POUCH SA, Gliwice, Poland); acetic acid (El Nasr Pharmaceutical Chemicals Co., Abu-Zaabal, Cairo, Egypt); primary secondary amine (PSA), graphitized carbon black (GCB), C_{18}, anhydrous magnesium sulfate ($MgSO_4$) and anhydrous sodium acetate involved in QuEChRs kits purchased from Agilent Technologies Co., USA).

Field experiment

The field trial was carried out on forty days old spinach plants (*Spinaciaoleracea* L.) cultivar SakiatMekki (Maka Co.) planted in an open field located at AwladSaqr district, Sharkia governorate, Egypt. The plot dimensions were 5×5 m^2, designates by randomized blocks design with triplicates. The experimental area received routine horticultural practices.

The experiment was carried out in October 31st, 2019. The commercial formulations of chlorfenapyr (Challenger Super™ 24% SC) and methomyl (Neomyl™90% SP) were applied at the rats recommended by the manufacturer (50 cm^3/100 L and 715 g/ha respectively) using a knapsack sprayer motor. The spray volume was 1000 L ha^{-1}. The insecticide treatments were as follows: chlorfenapyr, methomyl and chlorfenapyr/ methomyl. The mixed treatment was implemented by spraying spinach plants with chlorfenapyr and after half-hour (ensure its dryness) followed by methomyl spraying to avoid tank-mix incompatibility.

Chlorfenapyr followed directly by methomyl. Untreated control plots designate in wind-blown and sprayed with water. During the experimental period, there was no rainfall at any time, and the average daily temperature was ranged from 15 to 24 °C. The spinach plots did not receive any further foliar applications until the end of the experiment.

Residue analysis of chlorfenapyr and methomyl in/on spinach leaves

Sample preparation

Three replicates of representative leaf samples were collected randomly from the experimental plots. The initial sample was collected after 2 h post-application. Subsequent samples were collected for recovery tests. The samples were collected in one kg paper bags and preserved in an icebox during transferring to the laboratory. A proportion of each sample (0.5 kg each) was blended using a food processor. From the homogenate of each sample, three replicates of 10 g each were transferred to a 50 ml polypropylene centrifuge tubes, labeled and stored at -18 °C.

Chlorfenapyr and methomyl residues were extracted and cleaned up by the QuEChERS modified method according to [16,21]. A 15 ml of acetonitrile, containing 1% (*v/v*) of acetic acid, was added to 10 g spinach leaf in a 50 ml polypropylene centrifuge tube. The sample was shaken manually for one minute hardly and vortexed for 15 sec. Then extract powder include: 6 g magnesium sulfate and 1.5 g anhydrous sodium acetate were added to the centrifuge tube contents, mixed manually for one minute and vortexed for 15 sec., then centrifuged for 5 min at 4000 r.p.m.

Of the resulted supernatant, eight milliliters volume was transferred to a15 ml centrifuge tube. This tube contains the required materials for purifying including 200 mg PSA, 200 mg $C_{18,}$ and 1200 mg magnesium sulfate, in addition to 40 mg GCB for samples. Then the sample was shaken immediately manually for one min, vortexed for 15 sec., and centrifuged for 5 min at 4000 r.p.m. Afterward, the upper layer was picked up by pipette and poured in a clean tube and evaporated to one milliliter with nitrogen flow. These prepared samples were stored at -18 °C until final quantitative determination.

HPLC-UV analysis

The HPLC analysis was performed according to [5,7] using a UHPLC-UV Agilent USA model 1100 infinity with a binary pump, vwd, auto sampler. The chromatographic column was Kinetex 2.6μm C_{18}100 Å (4.6mm × 100mm). The chromatographic apparatus was controlled by Chemstation software. The flow rate of the mobile phase was 1 ml/min, consisted of (methanol/water D 80/20, *v/v*) with chlorfenapyr (water D/ acetonitrile 75/25, *v/v*) with methomyl, and the injection volume was 20 μL. Detection wavelengths were set at 260 nm and 210 nm for chlorfenapyr and methomyl, respectively. The residues in the field-collected samples were tentatively identified by comparing retention times (RT) of the sample peaks with that of the injected standard.

Recovery Assay

Recovery assay was performed using untreated spinach leaves. The samples were homogenized before being spiked with 0.1, 1 and 10 mg/ kg concentration. The samples were processed for extraction, clean up and quantitative final determination according to the above-mentioned procedure. The obtained recovery percentage means were 98.775 and 99.048% for chlorfenapyr and methomyl, respectively. The residue results were corrected according to the recovery values.

Kinetic Study

The rate of degradation and half-life period of chlorfenapyr and methomyl follows first-order kinetics reaction were calculated according to(Stow *et al*., 1999). The degradation rate constant and half-life were calculated using the first-order rate equation: $C_t = C_0^{-kt}$, where C_t represents the concentration of the pesticide residue at time t, C_0 represents the initial concentration after application, and k is the dissipation degradation rate constant ($days^{-1}$). The half-life ($t_{1/2}$) was calculated from the k value for each experiment ($t_{1/2} = \ln 2/k$).

The residual effect of chlorfenapyr and methomyl on *Spodoptera littoralis* larvae.

Tested insect:

A laboratory colony of the Egyptian cotton leafworm, *Spodopteralittoralis* (Boisd.), (Lepidoptera: Noctuidae) was obtained from Plant Protection Institute, Agriculture Research Center, Dokki, Giza, Egypt. The colony was reared at 25 ± 2 °C, 65 ± 5% R.H. and photoperiod 12:12 L:D under constant conditions for successive generations according to [11].

Bioassay:

The first, second, and third instar larvae of the laboratory *S. littoralis* colony were fed for 24 h on spinach leaves obtained from chlorfenapyr and methomyl followed by chlorfenapyr/methomyl treatments. Whereas, control treatments fed on untreated spinach leaves. Each treatment contained 20 larvae with three replicates. After 24 h exposure, the dead larvae were recorded and the survival larvae were transferred to clean containers, fed on untreated leaves and kept under observation till the end of each larval stage under constant conditions as mentioned before. The dead larvae were recorded and the accumulative larval mortality was calculated. The corrected mortality percentage was calculated according to [1]. Regression toxicity lines were established for the residual effect of the tested insecticides at the indicated days post field application. The corrected mortality percentages were plotted against days post field application and the median protection time in days (LT_{50} values) were determined through probit analysis [12]. Data statistical analysis was performed using the Bio-stat 2009 software [version 5.8.4.3, 2010].

Results and Discussion

Dissipation of chlorfenapyr and methomyl in spinach

Results in (Table 1) reveal that the initial deposits two hours after a single application of chlorfenapyr and methomyl on/in spinach leaves were 23.17±0.64 and 235.37±2.93 mg a.i./kg respectively. The percentages of dissipation of chlorfenapyr were 37.68, 55.29, 69.45, 84.45 and 96.83% for 2,4,6,9 and 16 days after application. The corresponding dissipation percentages of methomyl were 38.27, 56.01, 71.44, 84.34 and 97.81%.

Table 1: *Residues of chlorfenapyr and methomylin spinach leaves.*

Time after treatment	Residues ($mg_{a.i.}$/kg spinach leaves)	
	Chlorfenapyr*	Methomyl*
2h	23.17±0.64 (0)	235.37±2.93 (0)
2days	14.44±0.05 (37.68)	143.29±3.31 (38.27)
4days	10.3583±0.11 (55.29)	103.542±3.11 (56.01)
6days	7.08±0.34 (69.45)	67.22±3.43 (71.44)
9days	3.61±0.19 (84.45)	36.87±3.32 (84.34)
13days	1.46±0.15 (93.71)	15.59±1.96 (93.38)
16days	0.74±0.08 (96.83)	5.16±1.52 (97.81)

-* chlorfenapyr (Challenger Super™ 24% SC) and methomyl (Neomyl™ 90% SP) were applied at 50 cm^3/100 L and 715 g/ ha, respectively.

- Numbers between parentheses refer to the % insecticide residue loss (%).

-Data expressed as mean ±SE (n=3).

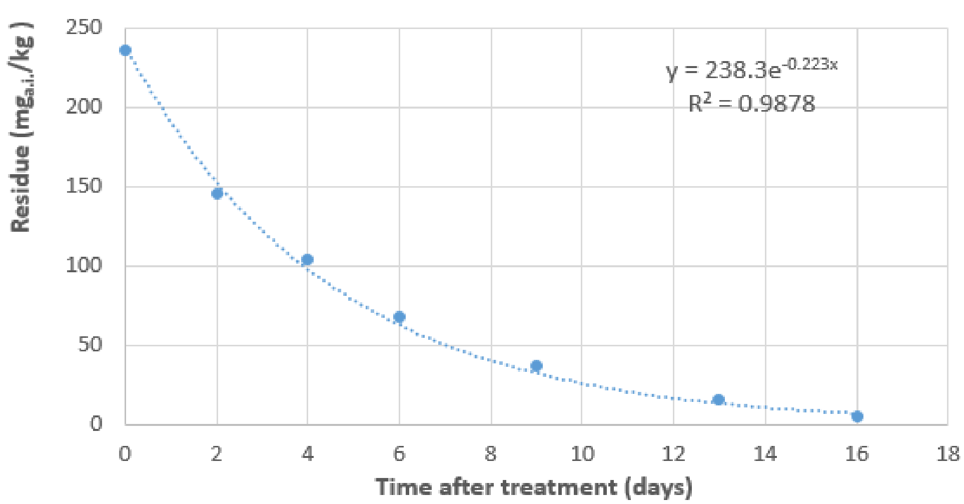


Figure 1: *Dissipation pattern of methomyl in spinach under open field condition.*

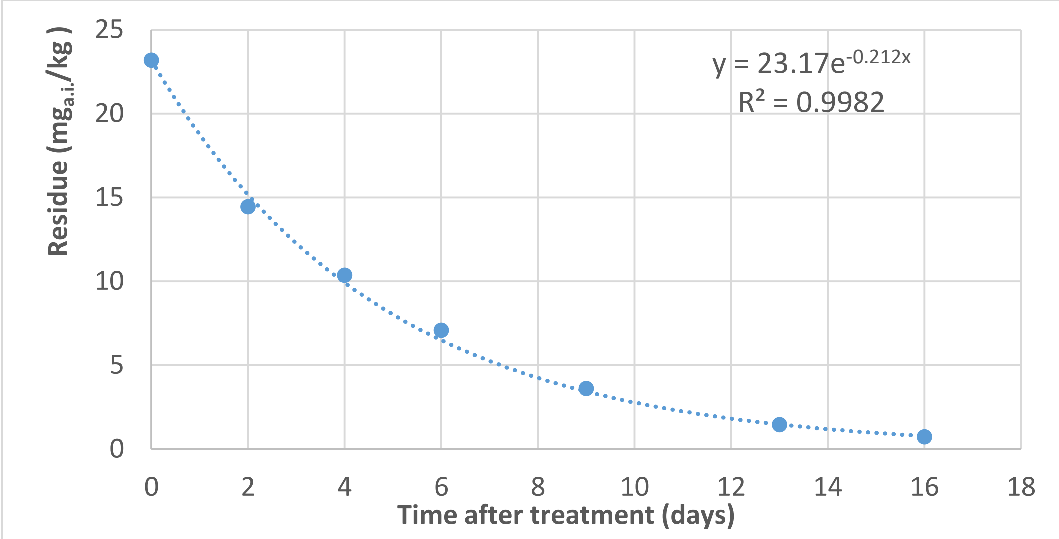


Figure 2: *Dissipation pattern of chlorfenapyr in spinach under open field condition.*

The obtained residue data fit the first-order model with R2> 0.9983 for chlorfenapyr and 0.9885 for methomyl (Figure 1 and Figure 2). The corresponding dissipation half-life ($T_{1/2}$) values were 3.27 and 3.11 days (Table 2).

Table 2: *Regression equation, correlation coefficient, and half-life of Chlorfenapyr and Methomyl in spinach.*

Insecticide	Dosage	Regression equation	Correlation coefficient (R^2)	Half-life (days)	MRL (mg kg^{-1})	PHI (days)
Chlorfenapyr	50cm³/100L	$C_t = 23.17e^{-0.212t}$	0.9983	3.27	0.01	36.55
Methomyl	715 g/ ha	$C_t = 238.30e^{-0.223t}$	0.9885	3.11	6	16.45

Regarding maximum residue limits (MRLs) of chlorfenapyr and methomyl, there is no available information in FAO [8]. The European Union (EU) Pesticides database showed MRLs as 0.01 mg kg^{-1} lower limit of analytical determination. Whilst, USA EPA's maximum residue limit in spinach was 6 mg $kg^{-1}$for methomyl residues and the absence of chlorfenapyr MRL value.
Based on our results, residues of chlorfenapyr 16 days post-treatment were above the prescribed MRL 0.01 mg kg^{-1} prescribed by the EU Pesticides Database. Meanwhile, methomyl residues were below the USA EPA prescribed MRL 6 mg kg^{-1} after 16 days following a single field application of the recommended rate. Therefore, it is recommended that growers should harvest spinach after 17 days of spraying of methomyl using good agricultural practices.

The observed DT_{50} of methomyl observed on spinach (3.11days) fits into the range of half-lives of methomyl on growing foliage (1-7 days) reported by [23,31]. Also, methomyl half-lives were 1.34 and 1.1867days on tomato fruits [3, 20].

The final amount of residue on the product is the result of a series of factors including volatilization after application, pesticide physicochemical properties, penetration and distribution inside the plant, rainfall, detoxification in plant, photo degradation and heat decomposition [19]. Also, the role of the plant growth dilution factor cannot be overlooked in reducing the amount of residue [9].

The higher deposits of methomyl on spinach leaves compared with chlorfenapyr may be due to the larger quantity of application rate of methomyl also, the higher percent of the active ingredient in formulation reach 90%. The high polarity of methomyl causing a high water-soluble pesticide [6,18]. The high solubility in water is 57.9 g/ L at 25°C [28] lead to commercialized as soluble powder formulation. The water solubility of methomyl leads to decrease penetration through the cuticle cover the spinach leaf leading to methomyl accumulation with high concentration after application accompanied by a low permeability rate. The accumulation on the outer surface of the plant makes methomyl residues vulnerable to washing by dew water to soil causing a potential for groundwater and surface water contamination [30]. So, methomyl residue after spraying was dissipated readily. In contrast, chlorfenapyr is low water solubility (0.12–0.14 mg/L) and is lipophilic based [22]. Also, chlorfenapyr has low volatility and binds strongly to soil particles [4]. Perhaps the systemic characteristic of chlorfenapyr, lipophilicity and low volatility is the main reason for the low field application rate.

The ratio of surface area/weight in spinach plants played a key role in the pesticide uptake amount regardless of the application date and frequency [15]. In contrast with other plants e.g. cabbage exposed the outer leaves while inner leaves away of pesticide uptake, the edible leaves of spinach are arranged in an open rosette from which a seed stalk emerges [10]. This arrangement provides the opportunity for the insecticide to reach the entire plant surface of the leaves, thereby increasing the amount of pesticide uptake and increasing the amount of the residue.

The residual effect of chlorfenapyr and methomyl on *Spodoptera littoralis* larvae.

The residual effect of chlorfenapyr and methomyl that applied to spinach plants under field conditions at the rates of 50 cm³/100 L water and 715 g/ ha, respectively, on the first, second and third instar larvae of the laboratory colony of *S. littoralis* is presented in Table (3). The larvae were feed for 24 h on the field treated spinach leaves and the accumulated mortality was recorded at the end of each larval stage.

Table 3: *Residual effect of chlorfenapyr, methomyl and chlorfenapyr/ methomyl against laboratory colony of Spodoptera littoralis larvae*

Time post treatment	Insecticide*	Exposed larval instars**			Mean	Interaction
		First	Second	Third		
2hr	**Chlorfenapyr**	18.67*** (93.33)	17.33 (86.67)	16.67 (83.33)	**17.56** ^{b} (87.80)	ns
	Methomyl	20.00 (100)	20.00 (100)	19.33 (96.67)	**19.78** ^{ab} (98.90)	
	Chlorfenapyr/ Methomyl	20.00 (100)	20.00 (100)	20.00 (100)	**20.00** ^{a} (100)	
	Mean	**19.56** ^{a} (97.80)	**19.11** ^{a} (95.55)	**18.67** ^{a} (93.35)		
2days	**Chlorfenapyr**	18.00 (90.00)	15.33 (76.67)	11.33 (56.67)	**14.89** ^{b} (74.45)	ns
	Methomyl	18.67 (93.33)	18.00 (90)	12.00 (60)	**16.22** ^{ab} (81.10)	
	Chlorfenapyr/ Methomyl	20.00 (100)	19.33 (96.67)	14.67 (73.33)	**18.00** ^{a} (90)	
	Mean	**18.89** ^{a} **(94.45)**	**17.56** ^{a} **(87.8)**	**12.67** ^{b} **(63.35)**		
4days	**Chlorfenapyr**	17.00 (85)	13.33 (66.67)	9.67 (48.33)	13.33^{b} (66.67)	ns
	Methomyl	18.00 (90)	15.33 (76.67)	11.33 (56.67)	14.89^{ab} (74.45)	
	Chlorfenapyr/ Methomyl	18.67 (93.33)	17.33 (86.67)	13.33 (66.67)	16.44^{a} (82.22)	
	Mean	17.89^{a} (89.44)	15.33^{a} (76.67)	11.44^{b} (57.23)		
6days	**Chlorfenapyr**	16.00 (80.00)	9.67 (48.33)	5.33 (26.67)	**10.33** ^{b} (51.65)	ns
	Methomyl	16.67 (83.33)	11.67 (58.33)	10.67 (53.33)	**13.00** ^{a} (65)	
	Chlorfenapyr/ Methomyl	17.00 (85)	13.33 (66.67)	11.33 (56.67)	**13.89** ^{a} (69.45)	
	Mean	**16.56** ^{a} (82.80)	**11.56** ^{ab} (57.80)	**9.11** ^{b} (45.55)		
9days	**Chlorfenapyr**	5.33 (26.67)	0.67 (3.33)	0.67 (3.33)	**2.22** ^{b} (11.10)	*
	Methomyl	7.33 (36.67)	6.67 (33.33)	1.33 (6.67)	**5.11** ^{a} (25.55)	
	Chlorfenapyr/ Methomyl	9.33 (46.67)	8.00 (40.00)	2.00 (10.00)	**6.44** ^{a} (32.20)	
	Mean	**7.33** ^{a} (36.65)	**5.11** ^{b} (25.55)	**1.33** ^{c} (6.65)		
13days	**Chlorfenapyr**	4.67 (23.33)	0 (0)	0 (0)	**1.56** ^{b} (7.80)	*
	Methomyl	6.00 (30.00)	5.33 (26.67)	0.67 (3.33)	**4.00** ^{a} (20)	
	Chlorfenapyr/ Methomyl	8.67 (43.33)	6.67 (33.33)	0.67 (3.33)	**5.33** ^{a} (26.65)	
	Mean	**6.44** ^{a} (32.20)	**4.00** ^{b} (20)	**0.44** ^{c} (2.20)		

*Chlorfenapyr and Methomyl were applied to spinach plants under field conditions at the rats of 50 cm3/100 L water and 715 g/ ha, respectively.

** Larvae were fed under laboratory conditions on field treated leaves at the indicated time post-application.

***Accumulative number of dead larvae.

-The figures in parenthesis are accumulative mortality percentages; Mean in each column or row followed by a different letter (s) significantly different from each other at $P < 0.05$ according to Duncan's multiple range tests.

The obtained data revealed that chlorfenapyr, methomyl and the dual chlorfenapyr/ methomyl treatments were effective against the three tested instar larvae of *S. littoralis* 2 hours post-treatment and the 1st instar was the most susceptible one followed by the 2nd and then the 3rd, The respective percentage mortalities were 93.33,100 and 100 % for the 1st instar; 86.67, 100 and 100 for the 2nd instar; and 83.33, 96.67 and 100 % for the 3rd instar. Nine days after treatment the percentages of mortality for chlorfenapyr were 26.67, 3.33 and 3.33 % for the 1st, the 2nd and the 3rd larval instars, respectively. The corresponding mortality percentages for methomyl were 36.67, 33.33 and 6.67%; whereas they were 46.67, 40.00 and 10.00 % for chlorfenapyr/ methomyl treatment.

Saleh *et al*. (2015) reported that the LC_{50} value for methomyl against the 4th instar larvae of the laboratory colony of *S* [25]. *littoralis* fed on treated leaves was 95.604 µg/ml. Moreover, the LC_{50} of chlorfenapyr on the 4th instar larvae of the susceptible strain of cotton leafworm *Spodoptera littoralis* was found to be10.12 µg/ml [13].

Data in (Table 4) and (Figure 3) showed the median protection time in days (LT_{50}) for the toxic residual effect of chlorfenapyr, methomyl and chlorfenapyr/ methomyl on the1st, 2nd and 3rd instar larvae of the laboratory colony of *S. littoralis*.

Table 4: *LT50 values for chlorfenapyr, methomyl and chlorfenapyr/ methomyl on the first, second, and third larval instar of the Egyptian cotton leafworm, Spodoptera littoralis.*

Insecticides	LT_{50} values in days* larval instar		
	1st	2nd	3rd
Chlorfenapyr	7.35	5.38	3.4
Methomyl	8.21	6.82	6.0
Chlorfenapyr/Methomyl	9.65	8.02	4.9

- Chlorfenapyr and methomyl were applied to spinach plants under field conditions at the rats of 50 cm3/100 L water and 715 g/ ha, respectively.
- Larvae were fed for 24 hours under laboratory conditions on field treated leaves at the indicated time post-application.

*Days post field application caused 50% accumulative mortality to the exposed larval instar.

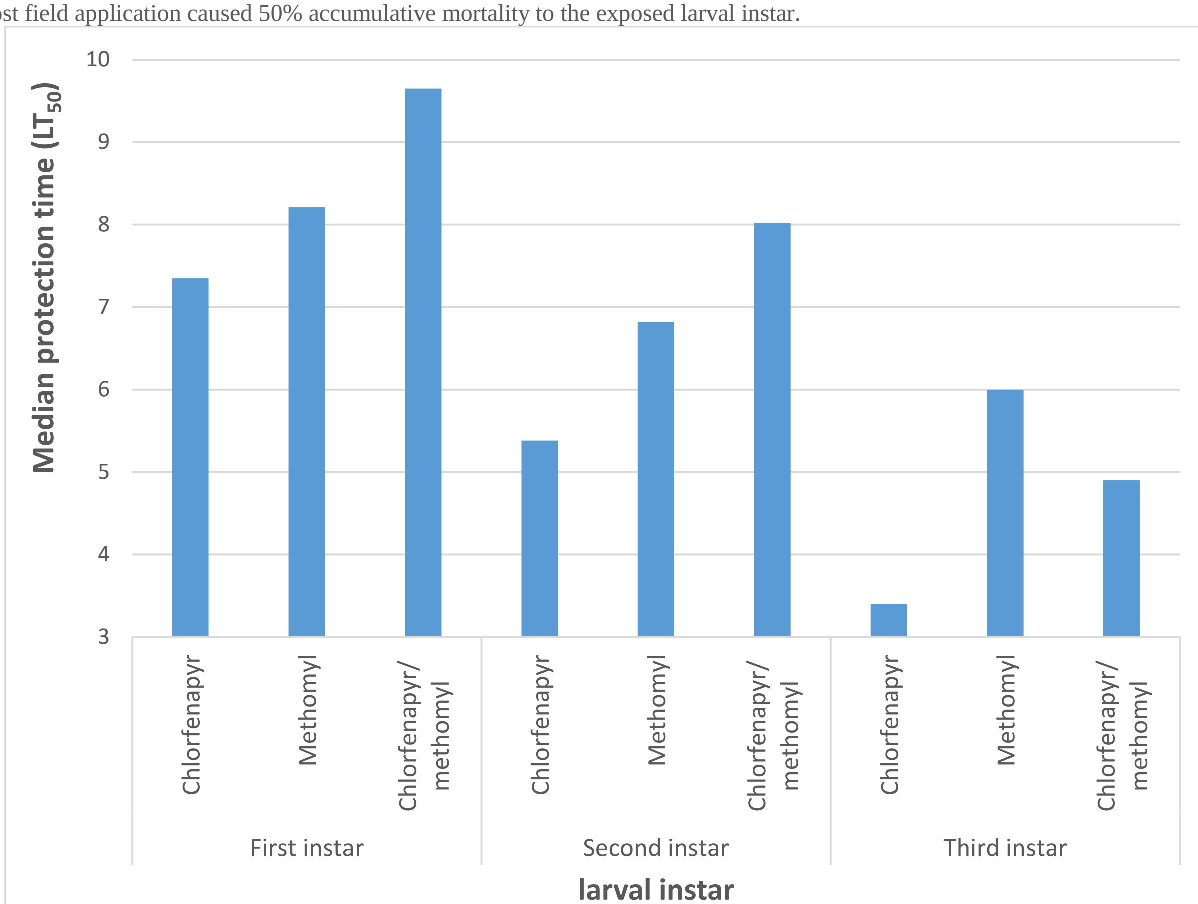


Figure 3: *median protection time in days (LT50) for the toxic residual effect of chlorfenapyr, methomyl and chlorfenapyr/ methomyl on the1st, 2nd and 3rd instar larvae of the laboratory colony of S. littoralis.*

Days post field application caused 50% accumulative mortality of the exposed larval instar for chlorfenapyr were 7.35, 5.38 and 3.4 for the 1st, the 2nd and the 3rd larval instars, respectively. The corresponding LT_{50} values for methomyl were 8.21, 6.82and 6.0 days; whereas they were 9.65, 8.02 and 4.9 days for Chlorfenapyr/ methomyl treatment. The toxic residual effect was more pronounced with the dual chlorfenapyr/ methomyl treatment followed by methomyl, whereas chlorfenapyr was the least. The results of the present work agree with those obtained by [2,3] who reported that methomyl controlled *S. littoralis*, infestations up to 12 days after treatment with LT_{50} value equal to 10.25 days.

Conclusion

In conclusion, residues of chlorfenapyr 16 days post-treatment were above the MRL 0.01 mg kg^{-1} prescribed by EU Pesticides database and methomyl residues were below the USA EPA prescribed MRL 6 mg kg^{-1} after 16 days following single field application of the recommended rate. The toxic residual effect was more pronounced with the dual chlorfenapyr/ methomyl treatment followed by methomyl, whereas chlorfenapyr was the least. However, it could be recommended that single application of chlorfenapyr on Spinach plants at the early ages followed by single application of methomyl at old ages at least 17 days before harvest are effective treatments to control the cotton leaf worms with no feasibility of the dual chlorfenapyr/ methomyl treatment.

These findings should be taken into consideration for harvest and post-harvest procedures as well as for sanitary, phytosanitary standards and health risk assessment.

Disclosure Statement

No conflict of interest exists.

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