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Investigation of diflovidazin and fenpropathrin on two-spotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae): population and interaction study

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Investigation of diflovidazin and fenpropathrin on two-spotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae): population and interaction study

Abstract: *Tetranychus urticae* is one of the most important pests of agricultural crops around the world. This research investigated the lethal effects of diflovidazin and fenpropathrin on different life stages of two-spotted spider mites, the interaction of binary mixture of these two compounds, and sublethal effects of diflovidazin on the deutonymphs under laboratory conditions. The Potter spray tower was used for the bioassay of acaricides on different life stages of *T. urticae*. The results showed that diflovidazin was effective on different developmental stages excluding female's adults. Also, fenpropathrin showed toxicity on all life stages except eggs. LC_{50} value and combination index (CI) of their mixture against deutonymph were 4.85 mg l^{-1} and 0.5 mg 1^{-1}, respectively, which revealed a synergistic effect on *T. urticae*. Sublethal effects of LC_{30} concentration of diflovidazin were evaluated on life table parameters of *T. urticae*. The value of the intrinsic rate of increase (r), the finite rate of increase (λ), and the net reproductive rate (R_0) significantly decreased in treated mites in comparison to control. These results suggested that diflovidazin could have significant roles in the control of *T. urticae* due to negative effect on population parameters as well as synergistic effect of binary mixtures of this acaricide with fenpropathrin.

Key words: two-spotted spider mite, life table, mixture of pesticides, interaction

Preučevanje učinkov diflovidazina in fenpropatrina na navadno pršico (*Tetranychus urticae* Koch, 1836) (Acari: Tetranychidae): populacijska in interaktivna raziskava

Izvleček: Navadna pršica (*Tetranychus urticae*) je eden izmed najpomembnejših škodljivcev gojenih rastlin širom po svetu. V raziskavi so bili preučevani letalni učinki diflovidazina in fenpropatrina na različne razvojne stopnje navadne pršice, učinek mešanic teh dveh snovi in subletalni učinki diflovidazina na deutonimfe v laboratorijskih razmerah. Za preučevanje učinkov teh akaricidov na različne razvojne stopnje navadne pršice je bil uporabljen Potterjev pršilni stolp. Rezultati so pokazali, da je bil diflovidazin učinkovit v vseh razvojnih stopnjah, z izjemo odraslih samic. Tudi fenpropatrin je bil strupen za vse razvojne stopnje pršice, z izjemo jajčec. LC_{50} vrednost in kombinacijski indeks (CI) mešanic akaricidov na deutonimfe sta bila 4,85 mg l^{-1} in 0,5 mg 1^{-1}, kar kaže na njun sinergijski učinek pri zatiranju navadne pršice. Vrednosti maksimalne rasti populacije (r), končne rasti populacije (λ) in neto reprodukcije (R_0) so se pri obravnavanih pršicah značilno zmanjšale v primerjavi s kontrolo. Rezultati nakazujejo, da bi diflovidazin lahko imel pomebno vlogo pri nadzoru navadne pršice zaradi njegovih negativnih učinkov na populacijske parametre kot tudi zaradi sinergističnih učinkov mešanic tega akaricida s fenpropatrinom.

Ključne besede: navadna pršica, preživetvena sposobnost, mešanica pesticidov, interakcija

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1 INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) is one of the most destructive polyphagous mites in the greenhouses and fields and causes significant damage to the yield of many horticultural, agricultural, and ornamental crops (Jeppson et al., 1975). Nearly 1,200 plant species have been reported as hosts of this mite, of which more than 150 species of these hosts such as cotton, corn, tomatoes, and ornamental trees have economic importance. This mite can produce more than 37 generations per year under favorable conditions (Riahi et al., 2013). The management of *T. urticae* populations has relied on application of different chemical acaricides (Cooper & Dobson, 2007) that have increased the public health concern (Owusu & Yeboah, 2007) and adverse effects on beneficial organisms (Bajc et al., 2017; Eziah et al., 2016). Due to high reproductive ability and short life cycle, two-spotted spider mite could develop resistance to many acaricides (Devine et al., 2001; Stumpf & Nauen, 2001). Despite the discovery and commercialization of acaricides belonging to different chemical groups, the number of acaricides on the market is much lower than insecticides that were estimated 7 % of the total insecticides market (Sparks & Nauen, 2015). Therefore, it is important to prevent the resistance of acari mites to acaricides and using a mixture of pesticides is one of the ways to prevent pest resistance to pesticides.

One of the resistance management programs is to use a mixture of pesticides for more effective control (Ahn et al., 1993). A mixture of two or more pesticides may improve pest control, enhance their pesticidal properties, decrease the costs and pesticide consumption and prevent pesticide resistance (Yuya et al., 2009). However, the influence of this way depends on the amount of application and the formulation of the pesticides. Widespread use of pesticide mixtures is usually common in greenhouses and seedling production centers where a collection of pests are present (Hosseininaveh & Ghadamyari 2013). In addition, multi-pesticide mixtures usually alter the uptake, transport, metabolism, and toxicity at the target site of pesticides, thus improving performance in many cases (Flint, 2002). On the other hand, sublethal insecticide exposure is expected to increase the competitiveness of resistant phenotypes (Wang et al., 2018), acting as a selection pressure for the evolution of insecticide resistance (Hamedi et al., 2010). The effects of sublethal concentrations of acaricides have been studied on spider mites (Landeros et al., 2002; Marčić, 2005, 2007). To better estimation of pesticides, the investigation on life table and population fitness could clarify the impacts of pesticide on pests and non-target species. Thus eco toxicological improvement could associate with altering the way of the evaluation of pesticide effects (Kammenga et al., 1996; Stark & Banks, 2003).

Different groups of pesticides are used for control of the tetranychid family in Iran (Saeidi & Arbabi, 2007). Fenpropathrin (Danitol®) is a pyrethroid insecticide used to control this pest (IRAC group 3A, sodium channel modulators) which was documented for reducing the rate of reproduction by feeding prevention and disruptive vital functions of adult mites (Nourbakhsh, 2019). Recently, diflovidazin (Flumite®) is a compound in the tetrazine chemical (IRAC group 10A, mite growth inhibitors affecting CHS1) (Merzendorfer, 2013) has been registered for management of *T. urticae* and *Panonychus ulmi* (Koch, 1836) (Acari: Tetranychidae) in Iran (Nourbakhsh, 2019).

Therefore, in this study, the lethal effects of fenpropathrin and diflovidazin were assayed on different stages of two-spotted spider mite. After determining the LC_{50}, the mixture of these compounds was examined for detection of synergistic, antagonistic, and potentiation effects on deutonymphs. Finally, the influence of LC_{30} concentration of diflovidazin was evaluated on life-table parameters as population growth parameters, female longevity, and life expectancy of *T. uticae*.

2 MATERIALS AND METHODS

2.1 MITE POPULATION GROWTH AND CHEMICAL COMPOUNDS

The mite population was collected from Siahkal (Gilan province, Iran) considered as susceptible to acaricides, in the summer of 2016 without a history of applying synthetic pesticides. The population was established on black-eyed pea (*Vigna unguiculata* (L.) Walp) under the laboratory conditions (25 ± 2 °C, 60 ± 10% relative humidity (RH), and a 16:8 h (L:D) photoperiod).

Commercial formulations of fenpropatrin 10 % EC (Danitol®) and difluvidazin 20 % SC (Flumite®) were obtained from Kimia Gohar Khak and Agro-Chemie Ltd, respectively.

2.2 BIOASSAYS ON DIFFERENT DEVELOPMENTAL STAGES

The toxicity status of pesticides was determined on each developmental stage of *T. urticae* (Vassiliou & Kitsis, 2013). Briefly, 10-15 same age larvae (0-24 hour old), deutonymph, and female adults were transferred

on the upside of each leaf discs of black-eyed pea, *Vigna unguiculata* (L.) Walp. (4 cm^2). After mite's settlement, different concentrations of fenpropathrin were sprayed on leaf discs containing deutonymph (4, 8, 16, 32 and 65 mg l^{-1}), larvae (2, 4, 6, 10 and 16 mg l^{-1}) and adult (10, 20, 40, 80 and 100 mg l^{-1}). Also, different concentrations of difluvidazin were applied on egg (1, 2, 3, 5, 7 and 10 mg l^{-1}), larvae (1, 2, 3, 5, 10 and 15 mg l^{-1}) and deutonymph (1, 2.5, 5, 10 and 20 mg l^{-1}) by a Potter spray tower (1 bar pressure, 1.5 ± 0.05 mg spray fluid deposit/cm^2). The distilled water was used as control. Mortality was assessed 48 h after treatment. Mites that did not walk normally after touching with a camel's hair brush were considered as dead.

For the egg developmental stage, 24 hours before bioassay, 10 female adult mites were put on a not-treated leaf disc for oviposition. Then, 20 eggs were used for each replication. For each treatment, 5 replications were prepared. All sample for each bioassay were transferred to the controlled conditions in an incubator at 25 ± 2 °C, 60 ± 10 % RH, and a 16:8 h (L:D) photoperiod.

2.3 THE MIXTURE BINARY ASSAY OF FENPROPATHRIN AND DIFLOVIDAZIN

Toxicity interaction studies were based on LC_{50} values for deutonymph stage of *T. urticae*. The mixture ratio was prepared based on LC_{50} values of fenpropathrin and difluvidazine using the potter tower bioassay method as discussed above. So, the binary mixture effects of these two pesticides were evaluated in 1 (diflovidazin): 2 (fenpropathrin) ratios. As above, the bean leaf discs containing deutonymph were sprayed with different doses (1, 2, 7, 14 and 20 mg l^{-1}) of a mixture of two pesticides, and the value of LC_{50} in the mixture was determined as described above.

2.4 DIFLUVIDAZIN SUBLETHAL EFFECT AND LIFE-TABLE ASSAY

For evaluation of the sublethal effects of diflovidazin, the LC_{30} concentration was investigated on deutonymph of two-spotted spider mite. In order for this assay, 100 deutonymphs were transferred on leaf discs and treated with LC_{30} concentration. Distilled water was used as control. After being adults, 15 pairs of *T. urticae* were transferred on new leaf discs, separately, and their oviposition as well as mortality were documented until the death of all individuals. Moreover, same aged-eggs laid on leaf discs (within a 24-h for both treatments) were used for life history experiments. During this test, developmental times and mortality of different immature stages were checked daily until adult emergence. Therefore, the raw life history data of treated and untreated *T. urticae* were obtained.

2.5 STATISTICAL ANALYSIS

LC_{30} and LC_{50} values, slopes, toxicity ratios between developmental stages in 95 % confidence intervals were determined by Probit analysis (POLO-PC, LeOra Software, Berkeley, USA). Furthermore, LC_{50} of two pesticides and LC_{50} of each pesticide on different life stages of two-spotted mite were compared by POLO-PC software and toxicity ratios were calculated to find the higher effects (Robertson et al., 2017).

Binary mixture of two acaricides were analyzed based on the method of Chou & Talalay, 1984. For this purpose, CompuSyn software was used which by certain ratios of each compound, the combination index, CI was calculated and the type of interaction effect was detected as synergistic, additive, and antagonistic. The combination index (CI) has been used for the quantitative determination of synergism (CI < 1), antagonism (CI > 1), and additive effect (CI = 1), and CI is calculated by Compusyn software.

The figures were designed by Microsoft Excel 16. The recorded raw data for life history and population growth parameters of *T. urticae* were analyzed based on the life table theory (Chi & Liu, 1985) and computer program TWOSEX-MS Chart (Chi, 2015). The age-specific survival rate (l_x), the age-stage-specific survival rate (s_{xj}), the age-specific fecundity (mx), the age-stage-specific reproductive values (v_{xj}), and population growth parameters such as intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0) and mean generation time (T) were calculated, accordingly. Moreover, the bootstrap method (100,000 replications) was used for the means and standard errors (Chi, 2015). The means were compared using the paired bootstrap test at 5 % significant level based on the confidence interval of difference (Efron and Tibshirani, 1993; Chi, 2015).

3 RESULTS AND DISCUSSION

3.1 EVALUATION OF THE LETHAL EFFECT OF DIFLOVIDAZIN AND FENPROPATHRIN

The LC_{50} and LC_{30} values of diflovidazin and fenpropathrin are presented in Table 1 and Table 2, respectively.

Table 1: Diflovidazin bioassay on different developmental stages of *Tetranychus urticae*

Developmental stage	N*	Slope ±SE	Chi-square	df	LC_{30} (mg l^{-1}) (95 % CI+)	LC_{50} (mg l^{-1}) (95 % CI+)
Egg	280	3.34 ± 0.57	2.29	4	2.21 (1.42-2.83)	3.18 (2.38-3.83)
Larvae	280	2.23 ± 0.44	4.50	4	3.29 (0.80-5.31)	5.65 (2.60-8.63)
Deutonymph	240	2.34 ± 0.49	1.01	3	3.93 (2.00-5.51)	6.59 (4.42-8.60)
Adult	240	-	-	-	NC	10000**

*The number of mites were used in bioassays
CI+: The upper and lower confidence interval at 95 % level
NC: Not calculated
**: It was not possible to calculate the LC_{50} value due to the phytotoxicity property

Table 2: Fenpropathrin bioassay on different developmental stages of *Tetranychus urticae*

Developmental stage	N*	Slope ± SE	Chi-square	df	LC_{30} (mg l^{-1}) (95 % CI+)	LC_{50} (mg l^{-1}) (95 % CI+)
Larvae	240	3.38 ± 0.60	1.11	3	3.95 (2.51-5.07)	5.65 (4.20-6.89)
Deutonymph	240	2.15 ± 0.32	1.02	3	7.94 (4.93-10.86)	13.92 (10.06-18.16)
Female adults	240	2.11 ± 0.35	1.78	3	20.04 (11.46-27.75)	35.51 (25.15-46.39)
Egg	-	-	-	-	NC	10000**

*The number of mites were used in bioassays
CI+: The upper and lower confidence interval at 95 % level
NC: Not calculated
**: It was not possible to calculate the LC_{50} value due to the phytotoxicity property

The LC_{50} values on the eggs, larvae, and deutonymphs indicated that the eggs had a higher susceptibility to diflovidazin. The greater slope of the logarithm-probit line in diflovidazin treatments was related to the egg stage that showed the lower increase in concentration caused more mortality in eggs in comparison to other developmental stages (Figure 1). Diflovidazin did not have any toxicity on females up to 10000 ppm (Table 1). Besides, fenpropathrin had no lethal effect on eggs up to 10000 mg l^{-1} (Table 2). The larval stage has the highest sensitivity to fenpropathrin rather than deutonymph and female adult (Table 2 and Figure 2). According to results (Table 3), the larval stage has the same sensitivity to fenpropathrin and difluvidazine (Figure 3). However, larvae showed most sensitivity to these pesticides.

The toxicity ratio comparison of LC_{50} of three developmental stages in diflovidazin treatment is shown in Table 3. Due to overlap of the diflovidazin LC_{50} values of deutonymph and larval

stage (1.16- fold), that involvement of number one between the high and low 95 % confidence limits. The toxicity ratio of fenpropathrin was found larva as the most sensitive stage. The various biological stages were as follows: larvae > deutonymph > female adults. The comparison of toxicity of diflovidazin and fenpropathrin on larval and deutonymph stages showed that there was not a significant difference in the larval stage (1- fold). However, the deutonymph stage had more sensitivity to diflovidazin with 2.11 folds more than fenpropathrin.

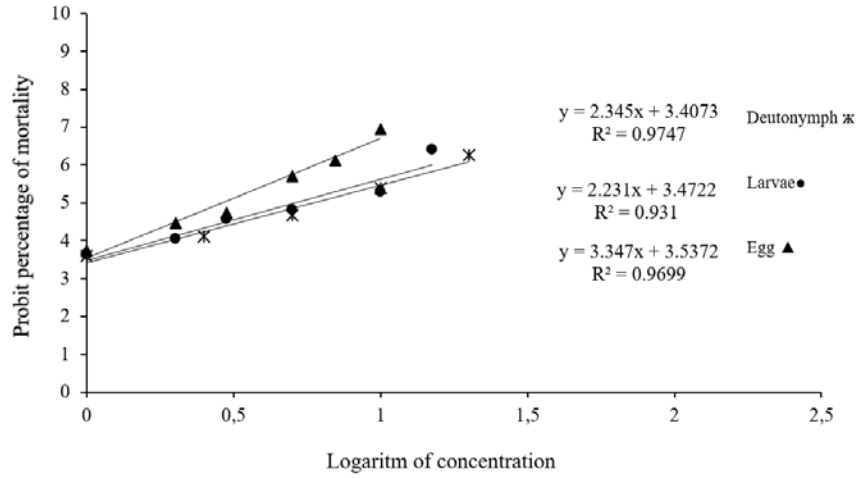

Figure 1: Logarithm of concentration-probit relationship percentage of mortality of different developmental stages of *Tetranychus urticae* in response to diflovidazin

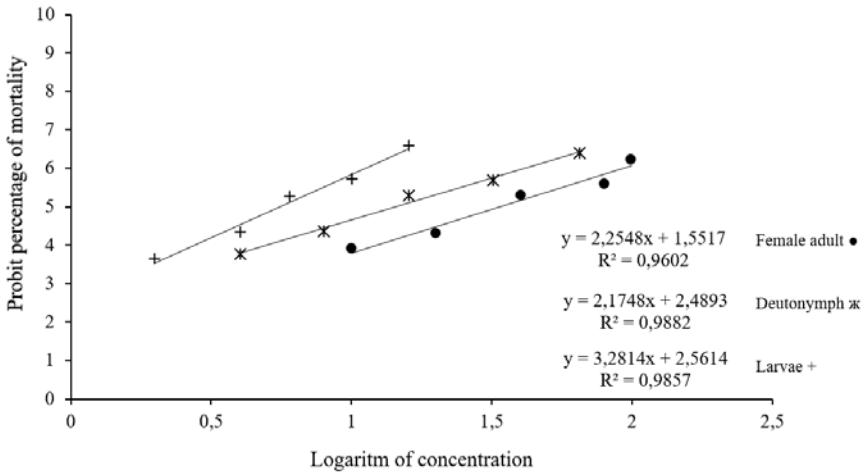

Figure 2: Logarithm of concentration-probit relationship percentage of mortality of different developmental stages of *Tetranychus urticae* in response to fenpropathrin

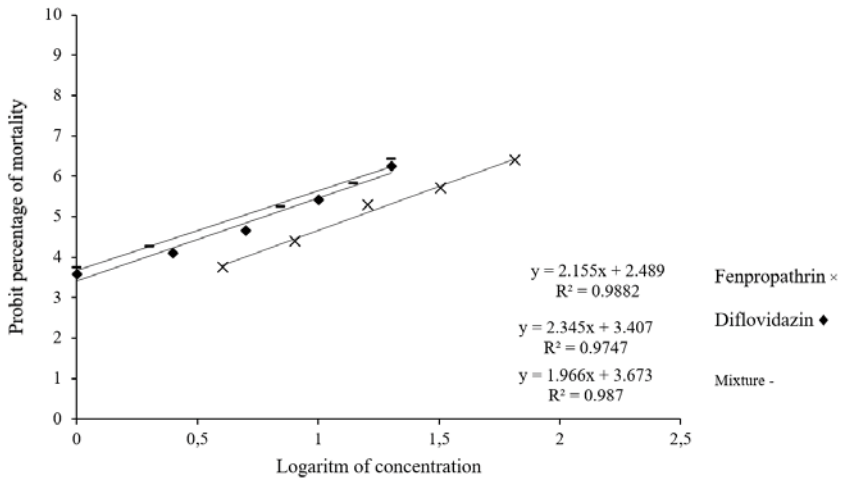

Figure 3: Logarithm of concentration-probit relationship percentage of mortality of different fenpropathrin, diflovidazin and their mixture on *Tetranychus urticae*

Table 3: The comparison of diflovidazin and fenpropathrin toxicity on different developmental stages of *Tetranychus urticae*

Treatment	Developmental stage	Toxicity ratio	(95 % Confidence Interval)*
Diflovidazin	LC_{50} of deutonymph/ LC_{50} of larvae	1.16	0.75-1.80
	LC_{50} of deutonymph/LC_{50} of egg	2.07*	1.42-3.01
	LC_{50} of larvae/LC_{50} of egg	1.77*	1.21-2.61
Fenpropathrin	LC_{50} of deutonymph/ LC_{50} of larvae	2.46*	1.70-3.55
	LC_{50} of adult/LC_{50} of deutonymph	2.55*	1.69-3.84
	LC_{50} of adult/LC_{50} of larvae	6.38*	4.32-9.11
$\frac{LC_{50}\ Fenpropathrin}{LC_{50}\ Diflovidazine}$	Larvae	1.00	0.67-1.47
	Deutonymph	2.11*	1.39-3.20

*The significant difference in Toxicity ratio: the upper and lower confidence at 95 % level

In the study of clofentzine (IRAC group 10A), propargite (IRAC group 12C), tetradifon (IRAC group 12D), etoxazole (IRAC group 10B), fenpyroximate (IRAC group 21A), amitraz (IRAC group 19), fenpropathrin, hexythiazox (IRAC group 10A), bromopropylate (unknown Mode of Action (MOA)), and fenazaquin (IRAC group 21A), the most effective compounds were reported hexythiazox and etoxazole, but fenpropathrin had the least effect on deutonymphs of Iranian population of *T. urticae* (Saeidi & Arbabi, 2007). The toxicity of difluvidazine on the larval stage of citrus red mite, *Panonychus citri* (McGregor, 1916) (Acari: Tetranychidae) (Gao et al., 2004) is 173.39- times higher than its adult stage which was consistent with the results obtained in this research. Since diflovidazin inhibits chitin synthesis (IRAC group 10A) less or no effective was observed on females.

Based on the results of Marčić (2000), eggs had more sensitivity to diflovidazin and clofentzin acaricides in comparison to larva and deutonymph of *T. urticae*. The LC_{50} value of diflovidazin on females of *T. urticae* was 2362.2 ppm (Havasi et al., 2018). Aveyard et al. (1986) reported the effectiveness of clofentzine acaricide on the egg, larval, and deutonymph of *T. urticae* without influence on adults. The egg stage had a lower LD_{50} (0.16 ppm) than other stages which were consistent with the results of this study. Fenpropathrin had no effect on the two-spotted mite egg stage. Similar to the results of this research, propargite did not show any toxicity effect on the egg stage of this pest (Ashley et al., 2006).

The acaricide activity of bifenazate and diazene was reported on all developmental stages of *T. urticae* and *P. citri*; while etoxazole and tebufenpyrad (IRAC group 21A) had no effect on adult comparison bifenazate (Ochiai et al., 2007). In the survey of clofentezine, deltamethrin (IRAC group 3A), fenpyroximate, and hexythiazox, hexythiazox had the lower LC_{50} on larvae of three laboratory strains of *T. urticae* (Nauen et al., 2001).

3.2 INTERACTION OF DIFLOVIDAZIN AND FENPROPATHRIN

The mortality of adult female *T. urticae* in response to different concentrations of a combination of diflovidazin and fenpropathrin (in a ratio of 1: 2 of LC_{50} values) were reported in Table 4. The CI of the binary mixture of diflovidazin and fenpropathrin was determined 0.5 which is in the range of $0.3 < CI < 0.7$, according to Table 4, the effect of the mixture was synergistic.

The combination toxicity of diflovidazin LC_{50} with fenpropathrin LC_{50} in a ratio of 1: 2 was tested on *T. urticae* deutonymph. The LC_{50} values obtained from the ef-

Table 4: The toxicity index values of diflovidazin and fenpropathrin mixture on *Tetranychus urticae*

Mortality percentage	Combination Ratio (2:1) diflovidazin + fenpropathrin	Combination Index (CI)
10	0.91	0.514
20	1.62	0.516
30	2.38	0.517
40	3.27	0.518
50	4.37	0.518
60	5.83	0.519
70	8.00	0.520
80	11.75	0.521
90	20.97	0.523
95	35.74	0.525

Table 5: Diflovidazin + fenpropathrin bioassay on deutonymph of *Tetranychus urticae*

Developmental stage	N*	Slope ± SE	Chi-square	df	LC_{50} (mg l^{-1})
Deutonymph	240	1.96 ± 0.26	0.89	3	4.85 (3.71-6.10)

*The number of mites were used in bioassays

fect of the mixture of them had been reported in Table 5. Based on the data in these table, the combined mixture of these two acaricides on the deutonymph stage is more toxic than either acaricide alone.

A comparison of the toxicity ratios of diflovidazin, fenpropathrin, and binary mixture of these compounds on deutonymph with 95 % confidence has been given in Table 6. If the confidence interval of LC_{50} ratios includes one, it indicates no significant difference between two treatments (Wheeler et al., 2006). Therefore, in this study, the LC_{50} of diflovidazin did not show a significant difference with LC_{50} of the pesticide mixture which was related to the overlap of their LC_{50} confidence limits and involvement of number one between the 95 % confidence limits of LC50 ratios (Table 6).

In the mixture of fenvalerate (IRAC group 3A) and some organophosphate pesticide, the most effective combination was fenvalerate and azinphos-methyl (IRAC group 1B) in a 1:1 ratio on *T. urticae* (Bruce Chapman & Penman, 1980). The result of this study showed the combination of diflovidazin and fenpropathrin, with different mode of action as sodium channel modulator and growth inhibitor, respectively, had a synergistic effect on *T. urticae* control. Similar to the results obtained for the mixture of difluvidazine with fenpropatrin (pyrethroid) in this research, a synergistic effect was observed in a mixture of chlordimeform with some pyrethroid compounds (El-Sayed & Knowles, 1984).

Table 6: Comparison of toxicity of fenpropathrin, diflovidazin and their mixture on deutonymph of *Tetranychus urticae*

Treatment	Toxicity ratio	Up - Down*
Fenpropathrin / Diflovidazin	2.11*	1.39 - 3.20
Fenpropathrin/ Mixture	2.86*	1.90 - 4.32
Diflovidazin/ Mixture	1.35	0.89 - 2.07

*The significant difference in confidence at 95 % level

3.3 THE EFFECT OF LC_{30} CONCENTRATION OF DIFLOVIDAZIN ON *T. urticae*

Developmental times (day) and longevity of the *T. urticae* are presented in Table 7. As shown, the length of the egg incubation period, as well as the protonymph duration, were significantly different in diflovidazin treatment and control (Table 7).

Analysis of variance showed that the adult preovipositional period (APOP) and total preovipositional period (TPOP) were not different in treatment and control (Table 8). The sub-lethal dose of diflovidazin significantly reduced the oviposition period of females, which was due to the reduction of female longevity in diflovidazin treatment (3.47 ± 0.25) in the comparison with the control (10.14 ± 3.5). Also, the number of eggs was significantly reduced by the treated females compared to the control.

In the present study, the effect of LC_{30} concentration of diflovidazin on fecundity, adult preoviposition period, total preoviposition period was calculated (Table 8). The intrinsic rate of population increase (*r*), the finite rate

Table 7: The effect of diflovidazin on different stage duration of *Tetranychus urticae* (mean ± SE)

Treatment	Egg	Larvae	Protonymph	Deutonymph	Pre-adult period	Female longevity	Life span
Control	3.75± 0.44	1.76 ± 0.43	2.11 ± 0.39	2.21 ± 0.52	9.83 ± 0.91	10.14 ± 3.5	19.97 ± 6.01
Diflovidazin	4.02 ± 0.02*	1.62 ± 0..05	1.83 ± 0.06*	2.29 ± 0.11	9.76 ± 0.12	3.47 ± 0.25*	13.22 ± 0.4*

*The significant difference in confidence at 95 % level

Table 8 . The sub-lethal concentration effect of diflovidazin on the reproduction capacity of *Tetranychus urticae* (mean ± SE)

Treatment	APOP** (day)	TPOP*** (day)	Oviposition period (day)	Fecundity (egg/female)
Control	0.0 ± 0.00	9.47± 0.16	10.97 ± 0.65	76.19 ± 5.64
Diflovidazin	0.0 ± 0.00	9.71 ± 0.14	3.64 ± 0.31*	16.39 ± 1.66*

*The significant difference at 95 % level
**APOP: Adult pre-oviposition period
***TPOP: Total pre-oviposition period

Table 9: The sub-lethal concentration effect of diflovidazin on the life table parameters of *Tetranychus urticae* (mean ± SE)

Treatment	r (day^{-1})	λ (day^{-1})	R_0 $(\frac{offspring}{individual})$	GRR $(\frac{offspring}{individual})$	T (day)
Control	0.266 ± 0.009	1.30 ± 0.012	47.24 ± 6.247	93.87 ± 5.764	14.455 ± 0.214
Diflovidazin	0.162 ± 0.012*	1.176 ± 0.014*	7.21 ± 1.090*	3.035 ± 3.72*	12.177 ± 0.158*

*The significant difference at 95 % level
r: Intrinsic Rate of Increase, *λ:* Finite Rate of Increase, R_0*:* Net Reproduction Rate, *GRR:* Gross Reproduction Rate, *T*: Mean Generation Time

of increase (λ), and the mean generation time (T) were significantly lower in diflovidazin. The net reproduction rate (R_0) was 7- folds lower than the control. Decrease in T value in diflovidazin treatment is probably due to shorter female longevity compared to the control treatment (Table 9).

The age-stage specific survival rate (S_{xj}) indicates the probability that a newborn will reach any age and stage of life. The egg incubation period, larval duration, and the preoviposition period in LC_{30} treatment of diflovidazin treatment were shorter than control (Figure 4).

The age-specific fecundity of female adults (m_x), the age-specific maternity ($l_x m_x$), and the age-specific survival rate (l_x) in different diflovidazin treatments and control are shown in Figure 5. In these curves, the age-specific fecundity indicates the rate of reproduction of females at different ages, as the onset time and reproduction termination in treatments. The age-specific survival rate (l_x) indicates a newborn egg will survive to x age. According to the results, m_x and l_x in diflovidazin treatment have a sharp decline and the fertility and survival of female adults in this treatment decreased rapidly (Figure 5).

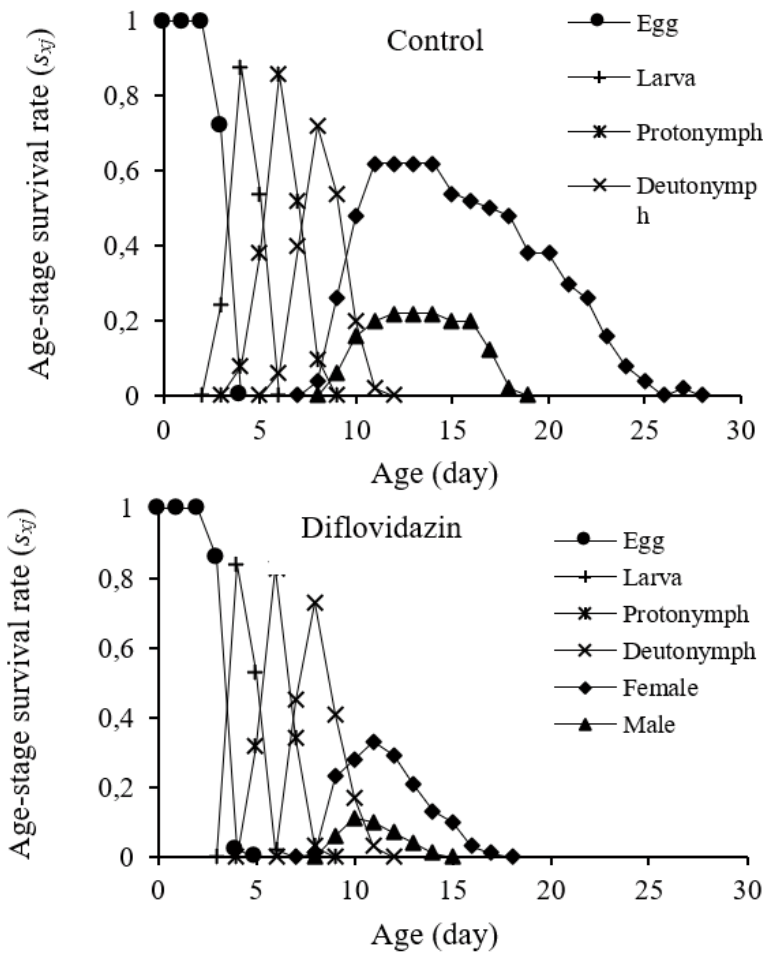

Figure 4: Age-stage survival rate (s_{xj}) of *Tetranychus urticae* at different stages in control and diflovidazin treatments

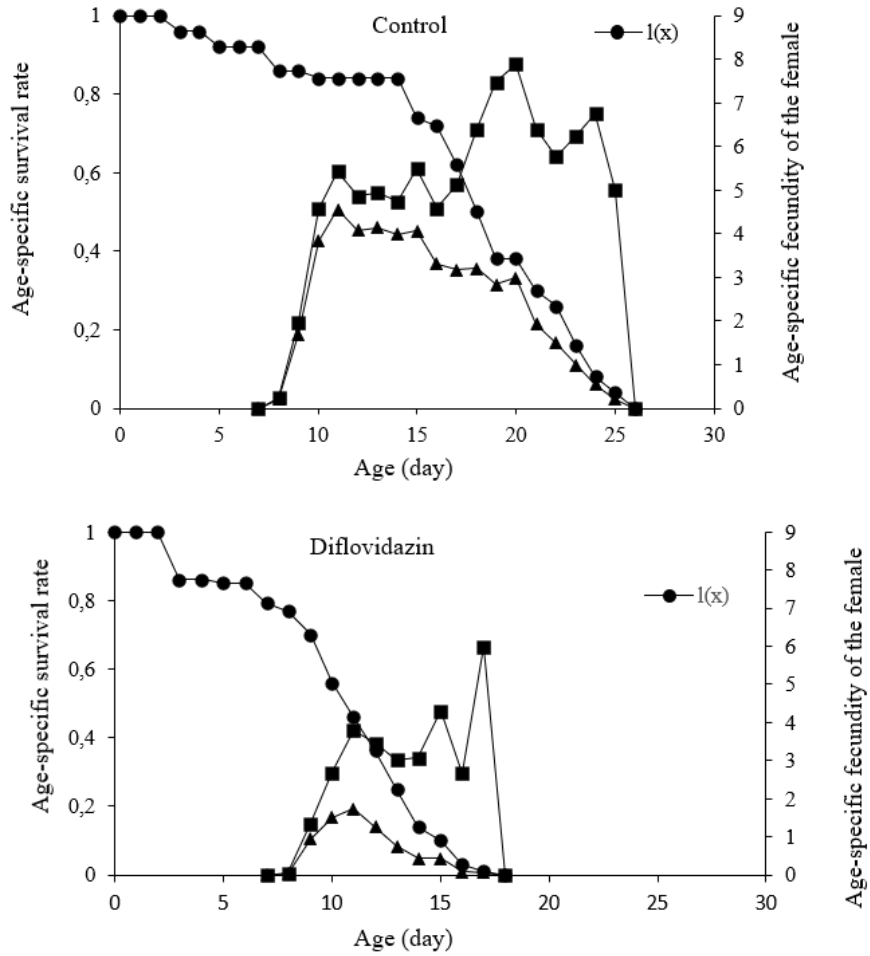

Figure 5: Age-specific survival rate (l_x), age-specific fecundity of the female (m_x) and age-specific maternity ($l_x m_x$) of *Tetranychus urticae* at different stages in control and diflovidazin treatments

Age-stage-specific reproductive value (v_{xj}) represents the number of offspring expected to be generated in the future by any person of x age and j growth stage (Carey, 1993; Fisher, 1958). There was a significant difference in comparison between the control reproductive value curves and the adult stage treatment (Figure 6) and the highest amount of reproductive value occurred in the control treatment on days 9-10. The age-specific life expectancy (e_{xj}) was summarized in Figure 7. Life expectancy changes had an inverse relation to mortality rate (q_x) and it was the lowest in diflovidazin treatment.

The acaricide sublethal effect measurements could clarify the different aspects of acaricides on mites. The sublethal effects of bifenazate (IRAC group 20D) affected the parental generation of *T. urticae* as survival rate, oviposition period, fecundity, and longevity (Li et al., 2017). The study of diflovidazin sublethal effects on *T. urticae* was associated with a significant reduction in biological parameters as female maturation duration, the oviposition period, the net reproductive rate (R_0), intrinsic rate of increase (r), and total fecundity (Havasi et al., 2018). Sublethal concentrations of tebufenpyrad significantly affected the offspring, longevity, fertility, and the intrinsic rate of increase (r) of *T. urticae* (Marčić, 2005).

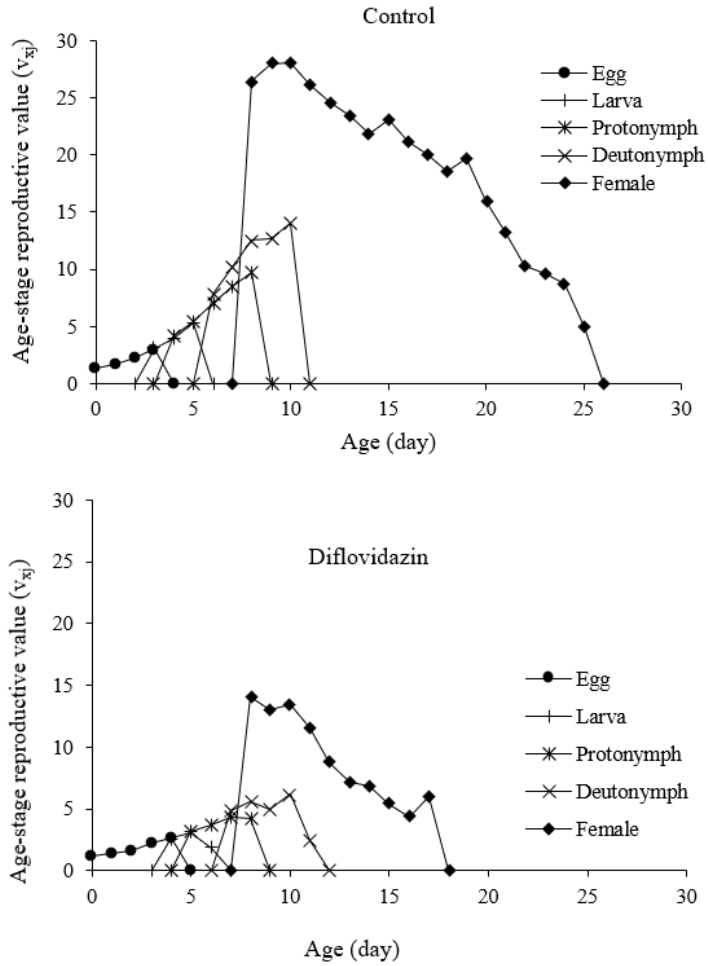

Figure 6: Age-stage reproductive value (*vxj*) of *Tetranychus urticae* at different stages in control and diflovidazin treatments

4 CONCLUSION

Although control of *T. urticae* has proven successful in many protected crops by some beneficial organisms, acaricides play a major role in control of this pest in field and greenhouse's crops (Van Leeuwen et al., 2010). Considering that the cost of chemical control is lower compared to biological control by releasing predatory mites in the field (Vidrih et al., 2020). This justifies the intensive use of acaricides in some areas. In addition to using biological control agents to control this pest, the mixture of pesticides should also be considered as a strategy to delay resistance. The results of diflovidazin studies on *T. urticae* showed that diflovidazin is an effective acaricide on immature stages especially eggs. However, it had no effect on the adult stage. Fenpropathrin had the most influence on the larval stage, without effect on the egg stage. The results of LC_{30} of diflovidazin acaricide were associated with the significant reduction of net reproduction rate (R_0), intrinsic population growth rate (r), and the finite increase in population (λ). Thus, the combination of these pesticides with complication effects on together and a synergistic effect on deutonymph, can be effective recommendation in *T. urticae* control and suitable for delay to acaricide resistance.

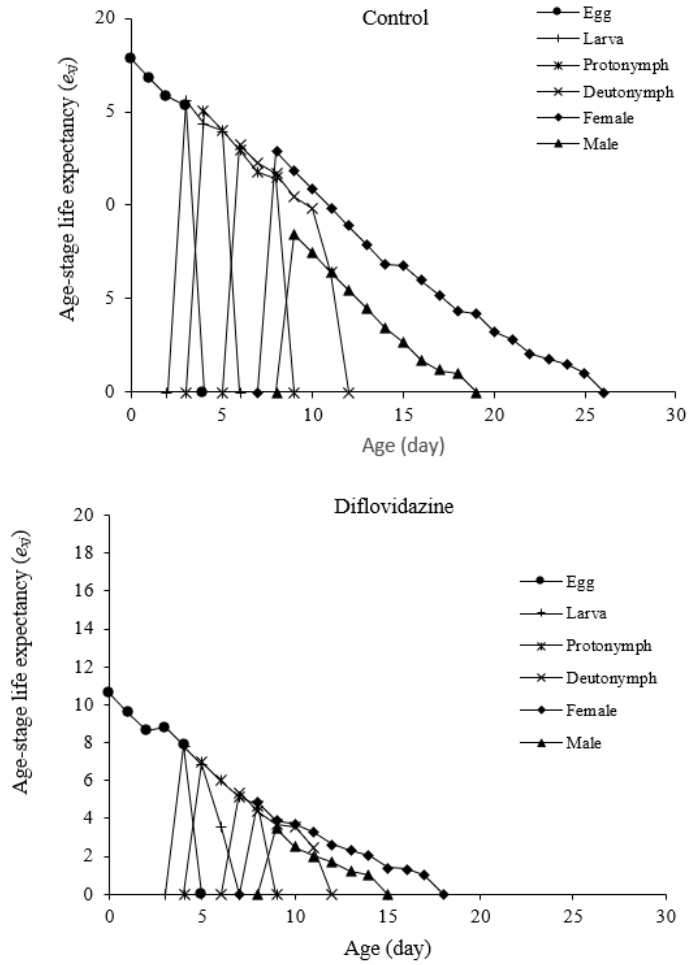

Figure 7: Age-stage life expectancy (e_{xj}) of *Tetranychus urticae* at different stages in control and diflovidazin treatments

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