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Lethal and sublethal effects of spirodiclofen on the biological performance of the predatory thrips *Scolothrips longicornis* (Thysanoptera: Thripidae)

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Abstract
Determination of negative non-target effects of insecticides on natural enemies by measuring only lethal effects is likely to undercalculate effects from sublethal doses. In this study, the sublethal effects of spirodiclofen on the predatory thrips *Scolothrips longicornis* Priesner fed on *Tetranychus urticae* Koch were estimated under laboratory conditions. Exposure to sublethal concentrations (LC_{10}, LC_{20} and LC_{30}) of Spirodiclofen significantly affected fecundity and longevity of treated females of *S. longicornis*. In addition, transgenerational effects on reproductive and life table parameters of the subsequent generation were established. The results from this study can be utilized to develop guidance for the use of spirodiclofen in order to reduce the impact on *S. longicornis*.

Keywords: fecundity, two-spotted spider mite, life table, non-target effects, biological control, Spirodiclofen

Introduction
The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a serious pest recognized from more than 200 economically important crops throughout the world [1]. Control of this pest is achieved almost exclusively with insecticides [2] with well-known drawbacks in terms of pest replacement, pest resurgence, negative non-target effects and development of resistance on the environment and non-target organisms, including natural enemies [3]. Spirodiclofen is a pesticide concerning to the chemical class of tetronic acids or ketoenols and acts as lipid biosynthesis inhibitor by acetylCoA-carboxylase inhibition [4]. It has registered uses in many countries to be effective against a variety of insect pests [5, 6].
Resistance problems together with heightened awareness of the environmental risks connected with the utilized of insecticides have excited development of integrated pest management (IPM), combining utilize of insecticides with biological control and with various protective methods. For *Tetranychus urticae* biological control could be based on a variety of natural enemies [7] including predatory thrips, such as *Scolothrips longicornis* Priesner (Thysanoptera: Thripidae) [8], which arise commonly in bean [9], cucumber and eggplant [10] in, among other places, the Middle East [11, 12]. Predatory thrips may be exploited in IPM strategies based on conservation through habitat manipulation incorporated with the utilized of selective insecticides [13] that are effective against spider mites but at the same time relatively nontoxic to predatory thrips [14].
The concentration of an insecticide usually redacts after insecticide application in the field, with concentrations depending on, among other things, the rate of degrading by abiotic factors such as rainfall, sunlight and temperature [14]. Predators surviving the first exposure to a full dose insecticide may thus become exposed to sublethal insecticide concentrations [15] leading to physiological or behavioral sublethal effects, e.g. effects on reproductive capabilities, rate of development, egg hatch, life span and feeding rate.
Sublethal effects of spirodiclofen a few studies have examined of natural enemies, such as *Orius niger* Wolff (Hemiptera: Anthocoridae) [16], *Hippodamia variegate* (Goeze) (coleopteran: Coccinellidae) [17] and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) [18]. But sublethal effects of insecticides in general on predatory thrips are scarcely documented [19, 20] and no information is currently available on sublethal effects of spirodiclofen on *S. longicornis*.

The aim of this study was to organize the compatibility of spirodiclofen and *S. longicornis* in the management of spider mite through evaluations of the sublethal effects of spirodiclofen on the biological performance and life table parameters of the predatory thrips.

Materials and Methods

All experiments were settled in a growth chamber (Binder KBWS 240, Germany) in Entomology laboratory of Islamic Azad University of Takestan (2015, June-September) at 25±1 °C, 60±10% RH, 16:8 h (L:D).

Mite and thrips rearing

A culture of the *Tetranychus urticae* was initiated utilizing individuals originally collected from fields of cucumber (*Cucumis sativa* cv. Sultan) of Shahriyar (Tehran Province). The spider mites were maintained on detached leaves of cucumber each placed upside down on a layer of wet cotton inside a Petri dish (150 mm in diameter). The lid of the dish had a 30-mm diameter hole covered with fine nylon mesh to allow ventilation. The Petri dishes were maintained in a growth chamber at 25±1 °C, 60±10% RH and a photoperiod of 16:8 (L:D) h. Similarly, a Petri dish-based culture of *S. longicornis* was initiated with adults accumulated from the same cucumber fields. The cultures were equipped by adding 40-50 female spider mites to a clean cucumber leaf (taken from plants in the 4^{th}-5^{th} leaf stage) placed in a Petri dish (180 mm in diameter) and allowing egg-laying (approx. 500-600 eggs) for 48 h at 25±1°C, 60±10% RH. After elimination of the female spider mites, five thrips were added to each Petri dish which were subsequently kept in another growth chamber at similar conditions as above. Adult thrips were transferred onto newly spider mite-infested cucumber leaves every 2 days. After a prescribed rearing period (2 and 3 months for thrips and spider mites, respectively), the colonies were used for the experiments.

Test arena

Seedlings of *Phaseolus calcaratus* Roxb. cv. Goli were grown to the 4^{th}-5^{th} leaf stage in a mixture of sand (33%), clay (33%) and peat moss (34%) in 20-cm in diameter pots. Excised bean leaf discs (30 mm in diameter) without major veins served as test arenas. Each disc was placed upside down on a layer of wet cotton inside a Petri dish (60 mm in diameter). The lid of the Petri dish had a hole (15 mm in diameter) covered with fine nylon mesh to allow ventilation.

Concentration–response bioassay for adults

A commercial formulation of spirodiclofen (Envidor® 24% SC, Bayer Crop Science), at a maximum recommended rate of 148 mg a.i. per litre, was utilize in the experiments. To evaluate the toxicity of the insecticide on the adult stage of *S. longicornis*, a modified leaf-dip technique was utilized [21]. The concentrations of the insecticide were chosen based on range-finding tests. The concentrations used in the final bioassay ranged from 2 µg a.i./ml to 15 µg a.i./ml. The fresh bean leaf discs (3.3 cm in diameter) were dipped in the spirodiclofen solution for 10 s and allowed to dry for about 3 h. The control leaf discs were dipped in distilled water and used to assess the natural mortality.

After dipping, the leaf discs were put in test arenas and spider mites eggs were added as food. Twenty newly emerged female or male predators were transferred to each individual experimental arena for each concentration (5 concentrations) and untreated control using a fine soft pointed brush. The test arenas were subsequently placed in the growth chamber. Each concentration and untreated control was replicated four times. Mortality was checked after 24 and 48 h. Dead treated thrips found in the wet cotton wool, as well as thrips that died because of improper handling or escaped from the leaf disc were excluded from the data analysis.

Effect of sublethal concentrations on treated females

Insecticide application was done with the modified leaf-dip technique, as above. Fresh bean leaf discs (3.3 cm in diameter) were treated with sublethal concentrations of spirodiclofen or distilled water (control) and allowed to dry for about 3 h. The sublethal concentrations were 1.23 µg a.i./ml (LC_{10}), 2.86 µg a.i./ml (LC_{20}) and 4.42 µg a.i./ml (LC_{30}), respectively. Each concentration was replicated four times. After dipping, the leaf discs were placed in test arenas with spider mite eggs added as food. One pair of newly emerged male and female was transferred to each leaf disc using a fine soft pointed brush; any males that died during the exposure period were replaced with new ones. A total of 30 test arenas for each concentration were set up. The test arenas were subsequently placed in the growth chamber. Forty-eight hours after treatment, each female was transferred to a fresh untreated leaf in a new test arena. The test arenas were subsequently changed in the growth chamber. Mortality, longevity, reproductive periods, egg hatch and oviposition were accounted daily until the death of the last female in both treatments and controls. Surplus eggs of *T. urticae* were provided as a food every day. Females that drowned in the wet cotton, died because of improper handling or escaped from the leaf disc were excluded from the data analysis. To prevent any contaminants such as fungi, prey cadavers and web accumulation, the predators were transferred onto new untreated leaf discs every other week.

Effect of sublethal concentrations on the offspring of treated females

To exanimate any possible carry-over activity of spirodiclofen on the offspring of treated females, eggs laid by treated and untreated females were collected daily and life table parameters of both groups estimated and compared. Eggs of the same age for each group of females were transferred collectively onto a fresh bean leaf disc (3.3 cm in diameter) using a fine needle. The leaf disc was then placed in a test arena and placed in the growth chamber. All the transferred eggs and subsequent stages were controlled daily and development time and survival recorded. After adult emergence, males and females were kept in individual pairs in test arenas. If there were not enough males to pair with females on a specific day, males from the stock colony were utilized. Daily controlling taken until the death of the last female scored data for mortality, oviposition period and number of eggs laid. These data were used together with data obtained with immature thrips for estimation of life-table data parameters (net reproductive rate, intrinsic and finite rate of increase, mean generation time and doubling time). Surplus eggs of spider mite were supplied as a food every day and the predators were transferred onto new leaf discs every other week.

Data analysis

Mortality was corrected utilizing Abbott's equation [22]. Estimation of LC values and the regression equation for the dose mortality line were obtained using the PROBIT procedure in SAS [23]. The 95% fiducial intervals of the LC_{50} values obtained for males and females from the 48 h acute concentration–response curves were utilized to compare the

susceptibility of male and female thrips.

Analysis of variance (one-way ANOVA, [23]) was utilized to analyze the effect of the sublethal concentrations of spirodiclofen on preoviposition, oviposition and post oviposition periods, total and daily fecundity, longevity and sex ratio of *S. longicornis*.

The life table parameters were calculated based on the age-stage, two-sex life table theory [24]. The standard errors of the life table parameters were estimated using the Jackknife method [25]. The computer program TWOSEX-MS Chart [26] was utilized for data analysis and Jackknife estimation. The LSD [23] procedure was utilized to calculate differences in population parameters, developmental times and fecundities among different treatments. In addition, we estimated the hazard quotient (HQ) value by dividing crop-specific application rates (g a.i. per hectare) with the obtained LC_{50}-value [27]. Hazard quotients give an indication of the risk posed by insecticide field rates.

Results

Concentration–response bioassay for adults

The estimated values of LC_{50} for predators were 9.86 µg a.i./ml (Table 1). The recommended field rate of spirodiclofen (500 g a.i. per hectare) was higher than the LC_{50} and HQ was equal to 50.71.

Table 1: Probit analysis for the concentration-mortality response of Spirodiclofen for *Scolothrips longicornis*

No.	LC_{50} (µg a.i./ml)	95% fiducial limits	Slope±SE	X^2	P
400	9.86	8.77-10.47	2.25±0.28	18.12	0.95

Effect of sublethal concentrations on treated females

Exposure of female predatory thrips to sublethal concentrations of spirodiclofen had a significant effect on all the measured parameters except the hatching percentage of eggs laid by treated females (Table 2). Thus the preoviposition period, oviposition period, post oviposition period, female longevity and fecundity were significantly affected (Table 2). The oviposition periods, fecundity and longevity were not significantly different between sublethal concentrations, whereas the post oviposition period was further decreased when the sublethal dose increased from LC_{20} to LC_{30} (Table 2).

Table 2: Effects of sublethal concentrations of Spirodiclofen on biological parameters of *Scolothrips longicornis*

Parameter	Treatment	Fenpropathrin					
	Control	LC_{10}	LC_{20}	LC_{30}	*df*	*F*	*P*
Pre-oviposition period (days ± SE)	1.83±0.18 [b]	2.08±0.28[a]	2.80±0.31[a]	3.20±0.20[a]	80	7.64	0.0001
Oviposition period (days ± SE)	14.30±1.53[a]	1.24±0.76[b]	1.67±0.71[b]	1.57±0.67[b]	80	423.23	0.0001
Post-oviposition period (days ± SE)	3.53±0.17[a]	1.36±0.08[b]	1.18±0.27[b]	0.66±0.37[c]	80	95.23	0.0001
Fecundity (egg ± SE)	56.17±1.71[a]	3.88±0.29[b]	2.66±0.37[b]	2.37±0.43[b]	80	658.13	0.0001
Egg hatch (% ± SE)	97.94±0.35[a]	91.40±3.94[a]	88.21±5.02[a]	87.89±5.22[a]	80	1.42	0.0001
Female longevity (days ± SE)	21.27±0.59[a]	5.28±0.25[b]	5.37±0.27[b]	5.12±0.19[b]	80	585.2	0.0001

*Values followed by different letters are significantly different (P<0.05, LSD).

Effect of sublethal concentrations on the offspring of treated females

No significant effects on the performance of offspring of *S. longicornis* treated with sublethal concentrations of spirodiclofen were found in terms of the duration of the 4 of the juvenile stages – eggs, first larvae, second larvae and pupa in comparison with the control (Table 3). However, prepupae, total immature female longevity, pre-oviposition period, oviposition period, post-oviposition period and fecundity of the offspring of female *S. longicornis* treated with sublethal concentrations were significantly decreased (Table 3), although not to the same extent as experienced by their mothers. The sex ratio (♀/(♀+♂)) among offspring of treated females remained unchanged compared with the control and was also unaffected by sublethal treatments of the mothers (F=0.21, *df* =(3, 123), P>0.023) with female-biased ratios (±SEM) of 0.62 (±0.067) for the control and 0.65 (±0.095), 0.61 (±0,086) and 0.39 (±0.152) for the doses of LC_{10}, LC_{20} and LC_{30}, respectively.

For the offspring life-table parameters of the females treated with sublethal concentrations (Table 4) significant declines were found for the net reproductive rates (R_0), intrinsic rate of increase (r_m) and finite rate of increase (λ), whereas the doubling time (*DT*) was found to be significantly raised for all sublethal concentrations. Although significant differences were also recognized for the mean generation time (*T*), the effect across the sublethal doses was not constant.

Table 3: Developmental durations of different life stages, oviposition periods, and longevity of offspring from female *Scolothrips longicornis* treated with sublethal concentrations of Spirodiclofen.

Parameter (days ± SE)	Treatment						
	Control	LC_{10}	LC_{20}	LC_{30}	*df*	*F*	*P*
Egg	6.26±0.18[a]	6.10±0.22[a]	6.18±0.24[a]	6.33±0.23[a]	85	0.42	0.512
Larva 1	1.52±0.12[a]	1.71±0.12[a]	1.66±0.13[a]	1.75±0.12[a]	85	0.14	0.845
Larva 2	2.56±0.10[a]	1.86±0.12[a]	2.14±0.11[a]	2.00±0.11[a]	85	0.32	0.785
Prepupa	1.46±0.09[ab]	1.2±0.18[b]	1.54±0.12[ab]	1.60±0.13[a]	85	1.64	0.112
Pupa	1.67±0.12[ab]	1.79±0.12[b]	2.06±0.13[ab]	2.25±0.27[a]	85	1.23	0.102
Total Immature	12.89±0.23[ab]	12.83±0.21[b]	13.42±0.78[ab]	13.88±0.42[a]	85	1.98	0.086

*Values followed by different letters are significantly different (P<0.05, LSD).

Table 4: Mean (±SE) life-table parameters of offspring from *Scolothrips longicornis* females treated with sublethal concentrations of Spirodiclofen

Parameter	Treatment						
	Control	LC_{10}	LC_{20}	LC_{30}	*df*	*F*	*P*
R_0 (females/female)	55.51±1.47[a]	22.30±1.38[b]	16.86±1.09[c]	15.15±0.84[c]	94	184.42	0.0001
r_m (females/female/day)	0.179±0.002[a]	0.154±0.030[b]	0.139±0.003[c]	0.130±0.003[d]	94	42.62	0.0001
λ (females/female/day)	1.254±0.004[a]	1.267±0.005[b]	1.162±0.004[c]	1.123±0.004[d]	94	51.58	0.0001
T (days)	21.47±0.30[a]	18.50±0.44[b]	19.34±0.33[ab]	22.04±0.20[a]	94	2.36	0.021
DT (days)	3.83±0.06[d]	4.56±0.10[c]	5.20±0.13[b]	5.86±0.12[a]	94	17.85	0.0001

*Values followed by different letters are significantly different (P<0.05, LSD).

Discussion

Effective integration of biological and chemical control which is necessary for the application of integrated pest management programs is only possible with the usage of selective insecticides. This research is the first to apply the population parameters and demographic data of offspring of treated *S. longicornis* with sublethal concentrations of spirodiclofen.

The low LC_{50} values of spirodiclofen to *S. longicornis* are similar with previous researches on the non-target effects of spirodiclofen on various natural enemies in which this insecticide has been classified as harmful to, for instance the *Orius niger* Wolff (Hemiptera: Anthocoridae) [16], *Hippodamia variegate* (Goeze) (coleopteran: Coccinellidae) [17] and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) [18]. The ratio between the LC_{50} and the field application rate, i.e. HQ, gives an illustration of the risk. For utilize of spirodiclofen, the HQ value is higher than the trigger value of 2 [27] which demonstrates a risk for *S. longicornis*. The recommended field rate for controlling *T. urticae* is 500 µg a.i./ml, in agreement to the instruction mentioned in the label (Bayer, Crop Science). In the present research, after conducting bioassay tests, the obtained sublethal concentrations were lower than the recommended rate.

Application of sublethal concentrations of spirodiclofen to newly emerged females had significant sublethal influences on almost all biological characteristics (Table 2). Very much alike results have been found for the sublethal effects of fenpropathrin and abamectin on *S. longicornis*, although abamectin affected fecundity more severely than did fenpropathrin and spirodiclofen [19, 20].

The conclusion that sublethal concentrations of spirodiclofen increased the pre-oviposition and decreased the oviposition period of treated females is in agreement with findings on sublethal effects of spirodiclofen on *O. niger* [16] and sublethal effects of fenpropathrin and abamectin on *S. longicornis*, [19, 20]. Prolonged pre-oviposition periods and decreased oviposition periods and longevity of treated females as a result of exposure to sublethal concentration of an insecticide may in part be explained as a result of reduced food [19, 20].

The effects of sublethal concentrations of spirodiclofen on *S. longicornis* were clearly followed in the life table parameters, capturing the overall effects on development, fecundity and survivorship [28]. So, the net reproductive rates (R_0), the intrinsic rates of increase (r_m) and the finite rates of increase (λ) of the treated females were obviously inferior to the rates for untreated females. This in turn was reflected in longer doubling times (*DT*) for treated females. Adverse influences manifested in life table parameters have been showed after treatment with sublethal concentrations of spirodiclofen for *O. niger* [16] and sublethal concentrations of fenpropathrin and abamectin on *S. longicornis*, [19, 20].

Our results have shown adverse influences on the biological performance of *S. longicornis* after exposure to sublethal concentrations. Insecticides in low concentrations may be utilized in combination with biological control agents within IPM systems [29] to decrease the selective pressure and development of resistance, but our demonstration of adverse influences of even the lowest concentration of spirodiclofen on *S. longicornis* suggests that this approach is not advisable for combined use with *S. longicornis* in IPM programs for controlling *T. urticae*. Hence, such laboratory-based information is only useful for selecting insecticides for further study under more natural conditions. Further studies should be carried out to using both laboratory and field populations as test insects.

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