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Sublethal effects of spirodiclofen on life table parameters of minute pirate bug *Orius niger* Wolff (Hemiptera: Anthocoridae)

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Abstract
Effects of spirodiclofen as a tetronic acid pesticide were studied on demographical characteristics of *Orius niger*, a voracious predator of *Tetranychus urticae*. Developmental time of the first, third and fourth nymphal stages of the predator was significantly influenced by the acaricide treatment, however, the total developmental time of pre-adult stages was not influenced significantly. There were also significant differences in adult preoviposition periods. The intrinsic rate of increase (r) was 0.0949 d^{-1} in spirodiclofen treatment, which is significantly lower than control with 0.1184 d^{-1}. Finite rates of population increase (λ) in control and treatment were 1.1257d^{-1} and 1.0995d^{-1}, respectively. Mean generation time (T) also varied between control (31.33 d) and treatment (34.30 d), significantly. Gross reproductive rate (*GRR*) and net reproductive rate (R_0) were not different after treatment, significantly. Although, spirodiclofen treatment revealed the species ability to survive, grow, develop, and reproduce successfully after the use of recommended doses.

Keywords: development time, fecundity, intrinsic rate of increase, mean generation time, Orius niger, spirodiclofen

1. Introduction
Flower bugs belonging to family Anthocoridae are small predators, variously attacking minute arthropods. Both adults and nymphs suck body fluids from their prey. The minute pirate bug *Orius niger* Wolff (Hemiptera: Anthocoridae) is a polyphagous species widespread in Western Palearctic, preying on aphids, psyllids, whiteflies, thrips, larvae of noctuids, and mites [1]. *O. niger* is a voracious generalist predator in all its active life stages, which means it can be released at various stages of its development. It also can feed on pollen and adapt to different habitats, which admits it to remain within a given cultivated area and lets small populations of the insect to be used successfully [2, 3, 4].

Spider mites are the most common mites attacking woody plants and the two spotted spider mite *Tetranychus urticae* Koch (1836) is considered to be one of the most economically important mites ever. This mite has been reported infesting over 200 species of plants [5]. Chemical control of spider mites generally involves pesticides that are specifically developed for mite control [5]. Spirodiclofen is an insecticide/acaricide belonging to the chemical class of ketoenols or tetronic acids and acts as inhibitor of lipid biosynthesis by inhibition of acetyl-CoA-carboxylase [6]. It has registered uses in many countries on fruits, fruiting vegetables, tree nuts and coffee. It is obviously not a neurotoxic compound, but its mode of action is similar to growth-regulating insecticides. It is effective against phytophagous mites and whiteflies [7, 8]. The recommended application rates for spirodiclofen range between 48-144 mg a.i./l [8].

Chemical insecticides, as a part of IPM program, are used in manner least disruptive to biological control agents. Researches so far showed spirodiclofen is a selective compound for predatory insects, such as green lacewing, *Chrysoperla-carnea*, the ladybirds *Coccinella-septempunctata* and *Stethorus punctillum* and the bug *Anthocoris nemoralis*, as well as for parasitoids *Aphidius rhopalosiphi* and *Trichogramma cacoeciae* [9].

Many studies have evaluated the selectivity of pesticides for natural enemies, but few take into account the sub-lethal effects. These effects can be studied through life tables, which can be used to assess the effects of chemical products across generations improve understanding of insect population dynamics in areas with pesticide use [10, 11, 12]. Studying the effects of chemical insecticides on demographical characteristics of natural enemies, including life table studies, could provide useful data for their integrated application in IPM programs.

Traditional age-specific life tables [13, 14, 15, 16] focused only on the survival and the fecundity of the female population, ignoring the male population. Chi and Liu (1985) [17] developed the age-stage, two-sex life table to take into account the male population and the variable growth rate occurring among individuals. This work focuses on the effect of spirodiclofen on the development of *O. niger* as a part of IPM program for two spotted spider mite in green houses.

2. Material and Methods

2.1 Collecting and rearing of species

A colony of *O. niger* was established from field collected specimens present on flowers of corn growing in field of the Agriculture Campus (2014, June-July). The colony was maintained at 25±2 ºC, 65±10 RH and a photoperiod of 16:8 h (L:D). The predatory bug was reared in groups of 50 individuals in plastic cylinder container (14 cm height and 8 cm diameter). A hole in wall of container fixed with a fine mesh net allowed ventilation. Perforated papers were placed in the container to prevent predator cannibalism. Eggs of flour moth *Ephestia kuehniella* and corn pollen were used as food. Green bean pods placed in the container served as the source of moisture and ovipositioning bed.

2.2 Bioassay

Spirodiclofen (Envidor® 240 SC, Bayer Crop Science, Germany) was used in recommended dose (120 mg a.i./l) for treatment. Since the preliminary tests revealed that the recommended dose leads to less than 40% mortality in the adult predator, sublethal dose was not applied.

Strawberry leaves cultivated in the greenhouse were used for treatment. The leaves were immersed in acaricide solution for 30 seconds, allowed to dry at room temperature for approximately 20 minutes and then transferred to small (5 cm diameter) ventilated plastic Petri dishes. Water was used for control treatment. A moistened cotton wick and some eggs of flour moth were placed in each Petri dish.

2.3 Life table studies

Approximately 100 pairs of *O. niger* were introduced to all Petri dishes for 24 h. Among the predators which survived, 20 pairs from each treatment (control and spirodiclofen treatment) were selected for oviposition. Each couple of predators was placed in a container described above and fed the same in the mentioned controlled laboratory conditions. Eighty eggs laid within a 24 h period were collected from each treatment and kept in growth chambers in the same condition for life table evaluation. Egg viability, duration of embryonic period, survival of nymphs, and duration of nymphal period were assessed daily. Adult longevity was recorded until their death. Adults were sexed by examination of genitalia.

2.4 Data analysis

The difference in developmental characteristics of *O. niger* was compared using one way analysis of variance. Student-Newman-Keuls tests were used to compare and separate means. In all cases alpha was 5% with respect to hypothesis testing. The life-history data of all individuals of *O. niger* were analyzed as directed by the age-stage, two-sex life table [17]. The means and standard errors of population parameters were calculated by using the bootstrap method [18, 19]. For life-table analysis a computer program, TWO-SEX MS-Chart, was used to estimate parameters [20].

The age-stage specific survival rate (s_{xj}) is the probability that a newly laid egg will survive to age x and stage j, and age-stage specific fecundity (f_{xj}), is the number of hatched eggs produced by female adult at age x. Age-specific survival rate (l_x), age-specific fecundity (m_x) were calculated as:

$$l_x = \sum_{j=1}^{m} s_{xj}$$

Where, m is the number of stages.

$$m_x = \frac{\sum_{j=1}^{m} s_{xj} f_{xj}}{\sum_{j=1}^{m} s_{xj}}$$

Population parameters (r, intrinsic rate of increase; λ finite rate of increase; R_0, net reproductive rate; and T, the mean generation time) were calculated as:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

R_0, the net reproductive rate is defined as the total number of offspring that an individual can produce during its lifetime. The intrinsic rate of increase was calculated using the Lotka–Euler equation with age indexed from zero, as:

$$\sum_{x=0}^{\infty} e^{-r(x+1)}\, l_x m_x = 1$$

The mean generation time represents the period that a population requires to increase to R_0-fold of its size as time approaches infinity and the population settles down to a stable age-stage distribution. Mean generation time was calculated as:

$$T = \frac{\ln R_0}{r}$$

Age-stage-specific life expectancy (e_{xy}), i.e. the time that an individual of age x and stage j is expected to live, was calculated according to the method described by Chi and Su (2006) [21] as:

$$e_{xy} = \sum_{i=x}^{n} \sum_{j=y}^{m} s'_{ij}$$

Where, s'_{ij} is the probability that an individual of age x and stage y will survive to age i and stage j.

3. Results

Minute pirate bug *O. niger*, embryonic development required 4.33±0.06 d after spirodiclofen treatment and no significant difference (F=0.02, P=0.8913) was detected between treatment (4.33±0.06 d) and control (4.35±0.09 d) (Table 1). Developmental time of the first nymphal stage was significantly (F=4.79, P=0.0304) enhanced by acaricide treatment (2.7±0.06 d) in comparison with control (2.48±0.07 d).

Likewise, significant differences in developmental times (F=5.30, P=0.0231) were observed between treatment and control in the third nymphal stage (1.60±0.07 vs. 1.37±0.08 d, respectively). Fourth nymphal stage was also increased after spirodiclofen treatment (2.73±0.10 d) in comparison with control (3.14±0.09 d), significantly (F=8.02, P=0.0056).

Table 1: Life-history statistics (mean ± SE) of *O. niger* in control and spirodiclofen treatment

Stage/objective	Control		Spirodiclofen				
	N	Mean±SE	N	Mean±SE	df	SNK	P
Egg	63	4.35±0.09 a*	66	4.33±0.06 a	1	0.02	0.8913
N1	63	2.48±0.07 b	66	2.7±0.06 a	1	4.79	0.0304
N2	60	2.58±0.07 a	66	2.59±0.07 a	1	0.01	0.9436
N3	56	1.37±0.08 b	60	1.60±0.07 a	1	5.30	0.0231
N4	50	3.14±0.09 a	56	2.73±0.10 b	1	8.02	0.0056
N5	49	4.90±0.09 a	50	4.82±0.07 a	1	0.29	0.5898
Male total pre-adult	20	18.55±0.38 a	26	19.19±0.34 a	1	0.18	0.2138
Female total pre-adult	27	19.37±5.20 a	21	19.42±0.31 a	1	0.02	0.9017
Male longevity	20	31.75±3.34 a	26	29.28±2.10 a	1	2.03	0.1764
Female longevity	27	24.18±1.74 a	21	27.75±2.64 a	1	0.60	0.4519
Adult pre-oviposition	27	4.27±0.45 b	19	7.21±1.35 a	1	5.39	0.0251
Total pre-oviposition	27	23.62±0.55 b	19	26.58±1.32 a	1	5.20	0.0282
Fecundity	27	93.93±10.04 a	19	80.00±13.86 a	1	0.88	0.3538

* Different letters at a row, indicate significant difference (using Student-Newman-Keuls tests at 95% of confidence interval) between control and spirodiclofen treatment on that stage/objective.

However, the total developmental time of pre-adult stage was not different between treatment (19.08±0.21d) and control (19.02±0.26 d), significantly (F=5.30, P=0.0231). The mean total preoviposition period of the predator in treatment and control were 26.28±1.32 and 23.62±0.55 d, respectively which were different, significantly (F=5.20, P=0.0281).

There were also significant differences (F=5.69, P=0.025) in adult preoviposition period of *O. niger* between treatment and control. Adult preoviposition period was longer in acaricide treatment (7.21±1.35 d) than control (4.27±0.45 d). *O. niger* treated with spirodiclofen did its oviposition during a period of 16.74 days, with an average fecundity of 80.00±13.86 eggs per female which did not differ significantly (F=0.88, P=0.3538) with that of control (93.93±10.04 eggs). The mean length of oviposition period in control was 16.27 days. The s_{xj} value of *O. niger* (Figure 1) gives the probability that a newly laid egg will survive to age x and stage j. These curves also show the survivorship and stage differentiation of the predatory bug.

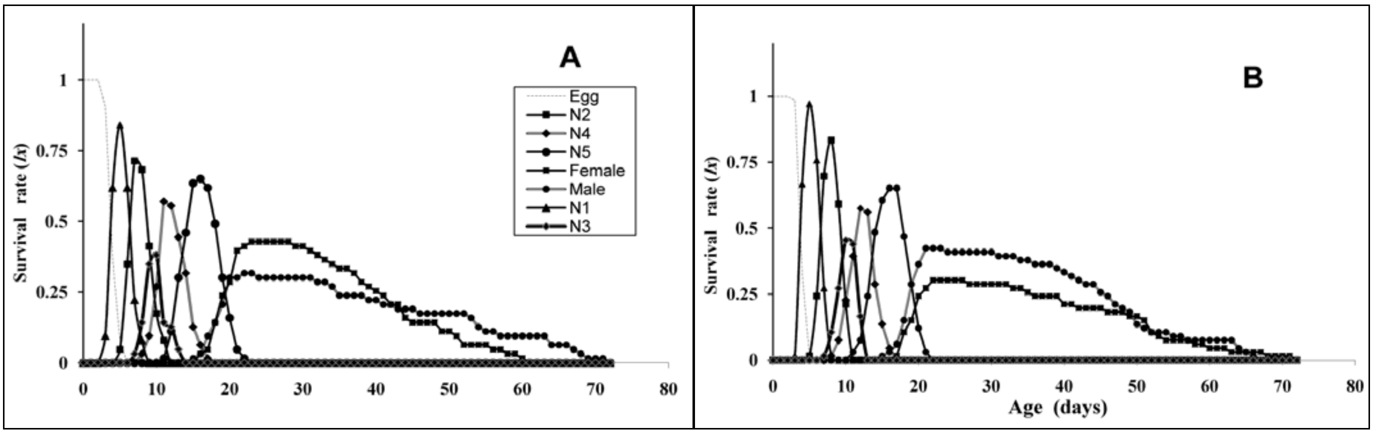

Fig 1: Age-stage specific survival rate (s_{xj}) of *O. niger* in control (A) and spirodiclofen (B) treat1ment.

l_x, f_x, m_x, and age specific maternity (l_xm_x) of *O. niger* illustrated in Figure 2. The *lx,* that describes the relationship between age specific survivorship and different treatments, is the probability that a new egg will survive to age x and can be calculated by pooling all surviving individuals of both sexes and those that died during the pre-adult stages. The curve of l_x is a simplified version of the curves in Figure1.

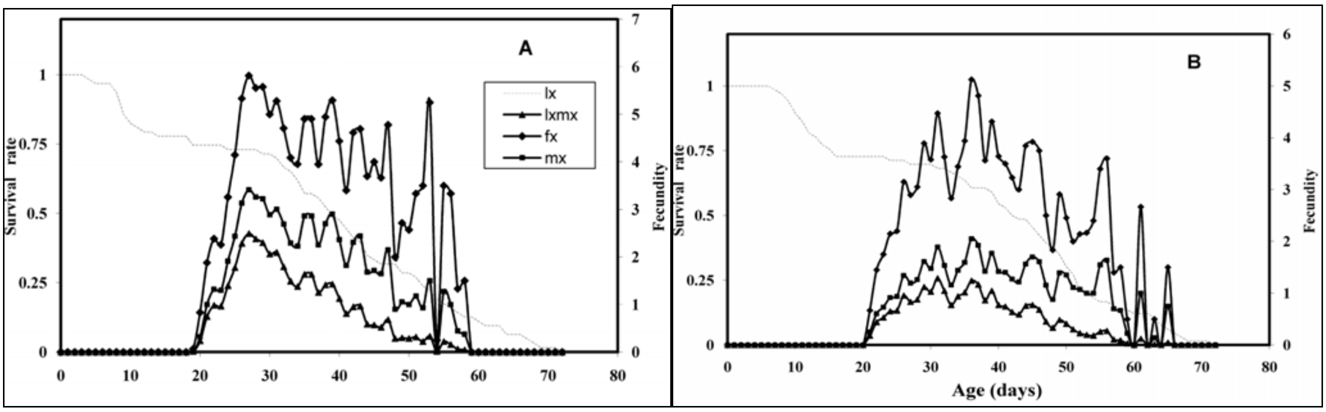

Fig 2: Age-specific survival rate (l_x), female age-stage specific fecundity (f_x), age-specific fecundity (m_x), and age specific maternity (l_xm_x) of *O. niger* in control (A) and spirodiclofen (B) treatment.

O. niger oviposition started with low value and increased gradually, in spirodiclofen treatment. Keeping them for 4 weeks, egg laying dropped to a lower level with small rises until female stopped laying eggs (Figure 2).

Figure 3 illustrates the life expectancy (e_{xy}) of each age-stage of *O. niger*. It gives the expected time that an individual of age x and stage j will live. The life expectancy of female, for example, was 32 and 27.5 d in control and spirodiclofen treatment, respectively.

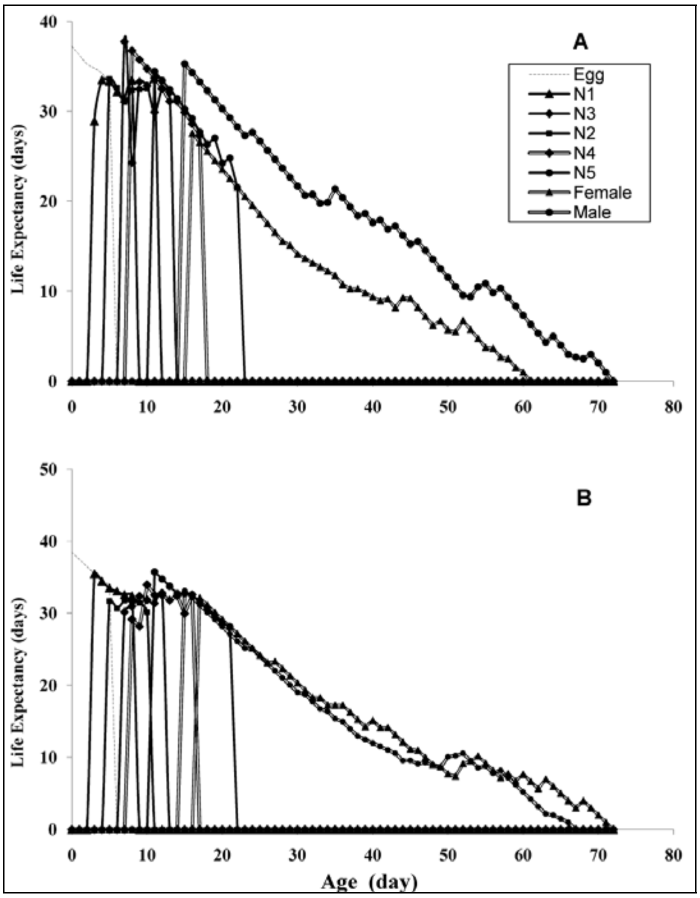

Fig 3: Age-stage-specific life expectancy (e_{xj}) of *O. niger* in control (A) and spirodiclofen (B) treatment.

The reproductive value (v_{xj}) gives the expected contribution of individuals of age x and stage j (Figure 4). It shows that a female at the peak oviposition can involve much more than a New born egg. For example, a newborn egg on spirodiclofen treatment has productive value of 1.09, but a female of age 29 has a much higher reproductive value of 32.57.

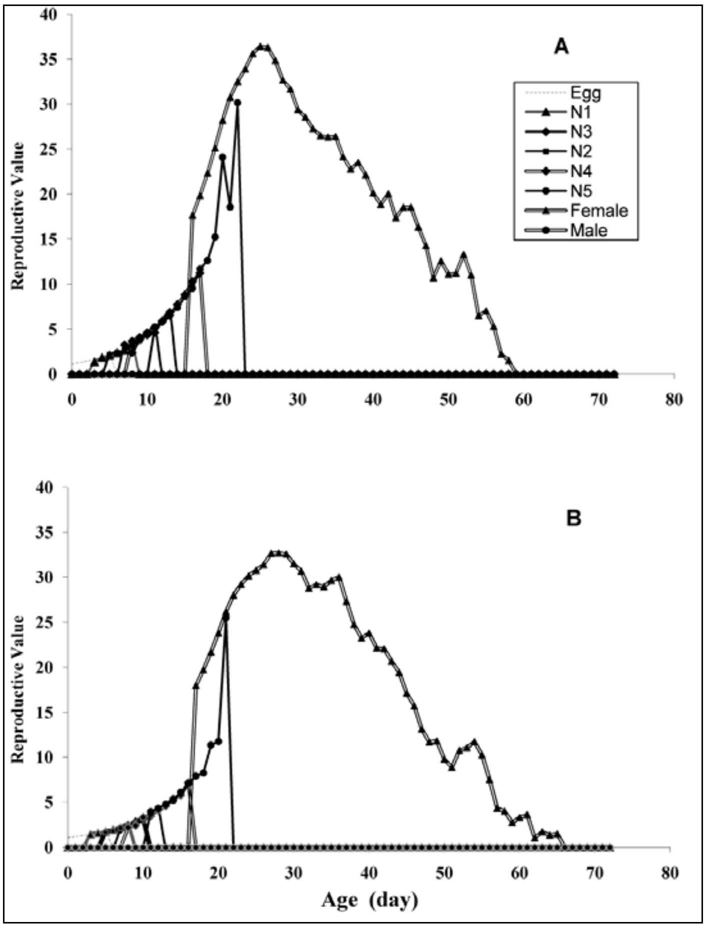

Fig 4: Age-stage specific reproductive value (v_{xj}) of *O. niger* in control (A) and spirodiclofen (B) treatment.

The means and SEs of the intrinsic rate of increase (r), net reproduction rate (R_0), gross reproductive rate (GRR), finite rate of increase (λ) and the mean generation time (T) were estimated using the bootstrap method are shown in Table 2. The intrinsic rate of increase was 0.0949±0.0078 d $^{-1}$ in spirodiclofen treatment, which is significantly lower than that of control (0.1184±0.0059 d^{-1}).

Table 2: Population parameters (mean ± SE) of *O. niger* in control and spirodiclofen treatment.

Parameter	N	Control	N	Spirodiclofen	df	T	p
r (d^{-1})	63	0.1184±0.0059	66	0.0949±0.0078	127	2.38	0.0187
GRR (offspring)	63	73.89±14.38	66	50.90±12.91	127	1.19	0.2356
R_0 (offspring/individual)	63	40.25±7.28	66	25.15±6.28	127	1.58	0.1175
λ (d^{-1})	63	1.1257±0.0066	66	1.0995±0.0086	127	2.39	0.0181
T (d)	63	31.33±0.75	66	34.30±1.03	127	2.30	0.0230

GRR, and R_0 were 50.90±12.91d^{-1} and 25.15±6.28 offspring in treatment and 73.89±14.38d^{-1} and 40.25±7.28 offspring in control, respectively, which were not differed significantly (F=1.19, P=23.56 and F=1.58, P=11.75). λ and *T* were 1.0995±0.0086 and 34.30±1.03 in treatment and 1.1257±0.0066 and 31.33±0.75 in control, which were differed significantly (F=2.39 , P=0.0181 and F=2.30 , P=0.0230).

4. Discussion

Effective integration of chemical and biological control which is important for the implementation of integrated pest management (IPM) programs is only possible with the use of selective pesticides. This study is the first to apply the age-stage, two-sex life table theory to evaluate the demographic characteristics of *O. niger*. The results provide useful insights for integrated pest management programs which incorporate chemical and biological control tactics. The research clearly shows the effects of spirodiclofen treatment on developmental characteristics of *O. niger*. Additionally it provides useful information on how the species demographic parameters are affected by acaricide treatment.

This investigation indicates that the acaricide treatment cannot influence the mean developmental duration of all stages of *O. niger*. It was also found the mean duration of embryonic development of *O. niger* was not affected by the treatment which can be related to selective properties of acaricide. Unfortunately little data available is for *O. niger* to compare with results of the current work. Spirodiclofen treatment had not influenced the total developmental duration of all nymphal stages of the predator. This result is in agreement with the finding reported by Azimzadeh *et al*. (2012)[22], which noted the residue of spirodiclofen was harmless for N1 and N5 instars of the predator *Deraeocoris lutescens* in the laboratory.

Adult exposure to recommended concentrations of spirodiclofen did not have considerable effect on the fecundity and longevity of female (Table 1). The same effect was reported by De Maeyer and Geerinck (2009) [23] who noted that spirodiclofen (Envidor®) is totally selective on predatory bugs *Anthocoris nemoralis.*

In this study, treatment by acaricide spirodiclofen showed significant effects on some life table parameters of *O. niger.* Among the life table parameters, the intrinsic rate of increase (*r*) has been recommended to be used for evaluating the sublethal effects of pesticides, because it reflects the overall effects on both survivorship and fecundity [24]. Lower intrinsic rate of increase (*r*) in acaricide treatments compared with the control indicated the adverse effects of spirodiclofen on this parameter. The mean generation time (*T*) of the predator was also influenced significantly by the acaricide.

As parts of IPM program, considering appropriate time interval between acaricide treatment and predator release can enhance this selective property. The result of this study is in agreement with Raudonis (2006) [25] that showed spirodiclofen was safe for *Amblyseius andersoni* and appears to be the promising candidate for use in integrated pest management programs where predatory mites are the major natural enemy. Also Bielza *et al*. (2009) [26] showed that spiromesifen, another tetronic acid derivative acaricide, had favorable selectivity to *Orius laevigatus* and *Eretmocerus mundus*, the biological control agents of whitefly *Bemisia tabaci*.

On the other hand, spirodiclofen unlike one of its relative, spirotetramat, is a contact acaricide and does not translocate throughout the plant [27]. Since *O. niger* feed on pollen and nectars of plants in the absence of prey, the lack of systemic action of the acaricide is a considerable advantage.

However, as Kramer and Nauen (2011) [28] proposed, in order to preserve spirodiclofen as an important tool in spider mite resistance management, the efficacy situation should be continuously monitored, and it is suggested that spirodiclofen be alternated with acaricides coming from different mode of action classes.

Nevertheless, because life table analysis takes into account age dependent mortality and reproduction in the estimation of demographic parameters, it is more suitable for the description pesticide treated population growth compared with simple reproductive summary statistics. Thus, randomized life table statistics and statistically derived parameters such as those calculated in this work, may provide useful values for summarizing the pesticide effect on the reproductive capacities of *O. niger*. Ultimately, more field and semi-field tests will have to be conducted to verify the result of this study.

5. Conclusion

Despite showing the negative effects, the life table parameters also showed that the predator can successfully survive, grow, develop and reproduce when treated with recommended dose of spirodiclofen. Therefore it could be concluded that spirodiclofen is selective acaricide for this predator.

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