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Action of some acaricides against *Tetranychus cucurbitacearum* Sayed and their side effects against the associated predator, *Stethorus gilvifrons* Mulsant

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Abstract
Three compounds are recommended as acaricides (Biofly, Abamectin and Ortus) for controlling a sucking- piercing mite *Tetranychus cucurbitacearum* (Sayed) by the determination of the median lethal concentration (LC_{50}). The newly emerged adults of the predator *Stethorus gilvifrons* Mulsant which associated with this mite exposed to bean leaves treated with such acaricides at LC_{50} that previously determined for *T. cucurbitacearum*. The estimated LC_{50} values for the mite were 9.2 ppm; 0.54 ppm and 9.53×10^7 conidia/ ml for Ortus, Abamectin and Biofly, respectively. While the slope values were 1.53; 2.11 and 1.97 for three acaricides. Results showed that, Abamectin, Biofly and Ortus have effect on the different biological aspects of the predator fed on the mite such as pre- oviposition, oviposition, post-oviposition, incubation periods and female fecundity. The longest period of oviposition and female longevity (22.9 and 32.8 days) accompanied with the highest fecundity (64.00 eggs) and high prey consumption (554.80 prey/couple) were recorded with Biofly treatment. These values reduced significantly in general with using Ortus and Abamectin, respectively. Also, the obtained results showed that the fourth larval instar was more highly voracious than the other three larval instars.

Keywords: Acaricides, *Tetranychus cucurbitacearum*, Toxicity, Biology, *Stethorus gilvifrons*

Introduction
Most of family tetranychidae are considered severe phytophagous pests; they attack some vegetables and other agricultural crops causing direct damage to the greenhouse and field crops [1]. Also, they are adapted to sucking out plant sap leading to death of the plants or affecting yield, quality and quantity of the crop [2-4]. These mites make indirect damages by transmitting some micro-organisms such as viral and fungal pathogens. The main difficulties in controlling of these mites are initial detection, economically damaging levels that are closely associated with acaricides applications [5, 6] or rapid development of resistance to acaricides than resistance in other pests and finally reduction of its natural enemies [7]. In Egypt, Italy, South Korea and other countries [8-12] reported that, most acaricides have been proved to be ineffective to control mite population in fields. Therefore, new approaches in pest management were applied, specially the use of natural enemies and bio- Aimee and Oscar 2007 pesticides that have received recently considerable attention. Many studies reported by [13, 14-21] succeeded through the use of bio-pesticides in management of mite pests in different fruit orchard, vegetable and field crops. However, several species of *Stethorus* Weise are used as biological control agent against mite pests because they are obligate predators of spider mites [22, 23]. So, the objectives of this study are to determine the toxicological and biological effects of three acaricides (Ortus, Abamectin and Biofly) under laboratory condition against the red spider mite (*Tetranychus cucurbitacearum*) and side effects of LC_{50} of these acaricides on the biological aspects of the predaceous *Stethorus gilvifrons*.

Materials and methods
Culture of the prey (*T. cucurbitacearum*)
The red spider mites *T. cucurbitacearum* were collected from infested beans leaves at Fayoum province (Egypt) during winter in 2016. Culture of mite was maintained on potted bean plants under laboratory conditions for different generations. The newly emerged adults were

collected and introduced into bean plants grown in plastic pots under wooden cages (60×60×60cm) covered with muslin to avoid cross contamination. Immature individuals were used for preparing small culture in Petri dishes provided with bean leaves, its ridges dipped in thin layer of agar 0.6% to prevent escape of the mites [24, 25].

Culture of the predator *(S. gilvifrons)*

Different stages of the predator, *S. givifrons* were collected from different plants especially the castor oil plant. Pupae of the predator were transferred into plastic Petri-dishes (9cm diameter and 1.5 high), the Petri-dishes bases provided with a layer of cotton wetted by water to preventing the dryness of leaf parts containing pupae. The dishes were introduced into the above mentioned a wooden cages containing *T. cucurbitacearum* for predator feeding. From this culture, progressive cultures were reared [26, 27].

Toxicity tests

Five concentrations were used from each chosen acaricides (5, 10, 20, 40 and 80 ppm for Ortus; 0.480, 0.960, 1.920 and 3.840 ppm for Abamectin and 1.8×10^6, 3.60×10^6, 7.25×10^6, 14.50×10^6 and 29.00×10^6 conidia/ ml for Biofly). Bean leaves were dipped in each of the tested concentrations for five seconds and left to dry in shade for two second. For each concentration, twenty adult of *T. cucurbitacearum* were introduced into Petri- dishes containing treated leaves on a thin layer of agar 0.6% to prevent escape of mites. All treatments were replicated five times and mortality percentages were calculated and corrected for natural mortality [28] after 24hrs for Ortus and Abamectin and after 72 hrs for Biofly.

Biological experiments

From the toxicity tests on *T. cucurbitacearum*, median lethal concentrations (LC_{50}) were calculated and used to evaluate the biological effects of these acaricides on the prey *(T. cucurbitacearum*) and the predator (*S. gilvifrons*). Leaves of bean plants were treated with the LC_{50} concentration (water used as control) by using dipping technique for five second, and then airy dried. In case of mite treatment, bean leaves were fixed on a thin layer of agar in Petri dishes. Twenty of newly emerged adults of the predator or the prey were transferred to the leaves and kept in a glass chimney cages. Five replicates and control were conducted for each treatment. The alive adults were confirmed for 24 hrs after treatment with Ortus or Abamectin and 72 hrs for Biofly, the survivors were transferred to clean cages and fed on *T. cucurbitacearum* nymphs. The cages were inspected daily to record the preying capacity, deposited eggs and longevity of adults. Some of the deposited eggs incubated to record the incubation period and hatchability. The successive immature were provided with *T. cucurbitacearum* in order to record preying capacity, duration of the different stages.

Statistical analysis

Biological data were statistically analyzed using LSD (least significant difference at $p = 0.05$) to separate means using SAS program according to the methods given by [29]. Mortality percentages were corrected according to Abbott's formula (1925).

Results

Toxicity of tested acaricides against *T. cucurbitacearum*

Toxicity effects of three acaricides on *T. cucurbitacearum* are given in Table 1. Depending on LC_{50} values results indicated that Abamectin was the most toxic acracides followed by Ortus and finally Biofly. These values were 9.2 ppm; 0.54 ppm and 9.53×10^7 conidia/ml Table 1. While the slope values were 1.53, 2.11 and 1.97 for Ortus, Abamectin and Biofly, respectively. In addition, Ortus, Abamectin and Biofly were recommended pesticides to control sucking piercing- pest, therefore the latent effect of these pesticides was evaluated to determine their side effects on *S. gilvifrons* under laboratory conditions(27±2 ºC and 70±5% RH) using the LC_{50} values for *T. cucurbitacearum*. The estimated LC_{50} values for *T. cucurbitacearum* were 9.2 ppm; 0.54 ppm and 9.53×10^7 conidia/ ml, while the slope values were 1.53; 2.11 and 1.97, respectively.

Table 1 Toxicities of three acaricides againsts *T. cucrbitacearum*

Acaricides	LC_{50}	Fiducial Limits for LC_{50} 95%		Slope value ±SE
		Lower	Upper	
Ortus	9.2(ppm)	8.75	9.95	1.53±0.45
Abamectin	0.54(ppm)	0.47	0.60	2.11±0.51
Biofly	9.53×10^7 (conidia/ml)	9.0×10^7 (conidia/ml)	10.20×10^7 (conidia/ml)	1.97±0.69

Preying capacity

For the preying capacity of adult couple on the different stages of *T. cucurbitacearum*, data indicated that, the highest total and daily consumed prey by were 701.5 and 25.00 nymphs of *T. cucurbitacearum* for the control treatment. Such numbers were reduced either slightly to 555 and 16.9 nymphs for Biofly or drastically to 200.5 and 10.2 for Ortus and 124 and 8.9 nymphs for Abamectin, respectively (Table 2). On the other hand, the preying capacity of the immature stages of the predator was increased with increasing the developmental larval instars Table 2. Where the larvae were characterized by highly movement and activity and sometimes take short time for resting. Also the preying capacity of the different larval instars ranged between them, where the highest capacity of total and daily consumed (24.3 and 9.5) was recorded with the fourth larval instar, while the lowest capacity (4.5 and 2.4) was recorded with the first larval instar, respectively. Finally, the second and third larval instars were intermediate in between the first and the fourth in the preying capacity where recorded 7.55 and 3.9 prey for the second larval instar and 13.4 and 7.3 prey for third instar, respectively.

Table 2 Effect of LC_{50} of three acaricides on the predaceous activity of different stages of *S. gilvifrons* reared on *T. cucurbitacearum*

Stage Acaricide	1st Larval instar		2nd Larval instar		3rd Larval instar		4th Larval instar		Adult couple	
	Daily prey Consumed	Total prey consumed	Daily prey Consumed	Total prey consumed	Daily prey Consumed	Total prey consumed	Daily prey Consumed	Total prey consumed	Daily prey Consumed	Total prey consumed
Ortus	2.4±0.17	4.5±0.18	3.9±0.43	7.55±0.21	7.3±0.11	13.4±o.19	9.5±0.54	24.3±0.16	10.2±0.54	200.5±0.67
Abamectin	1.8±0.75	4.1±0.23	3.75±0.78	6.5±0.24	4.65±0.12	8.5±0.20	5.9±0.36	11.4±0.65	8.9±0.58	124.±0.97
Biofly	4.2±0.86	10.3±0.94	8.4±0.61	17.9±0.55	12.9±0.21	28.7±0.50	14.9±0.86	28.7±0.90	16.9±1.7	555 ± 0.79
Control	2.65±0.66	5.22±0.17	3.75±0.69	7.90±0.41	5.10±0.68	10.5±0.56	5.55±0.33	11.3±0.48	25.0±0.96	701.5±0.60
LSD value	1.3	2.45	1.04	1.98	2.33	4.8	1.09	4.85	5.42	45.87

Effect of LC_{50} of three acaricides on biological parameters

a. Effect on *S. gilvifrons* adult

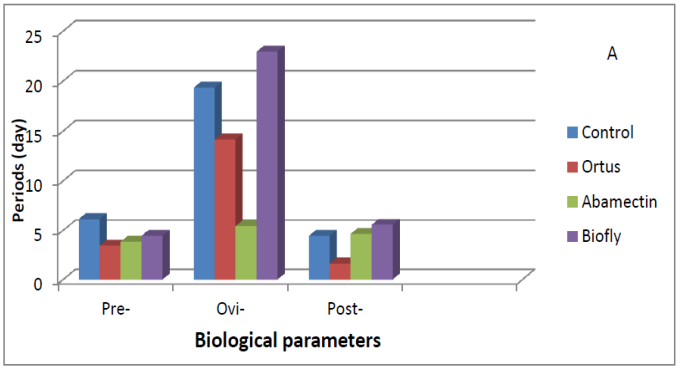
Treatments with Ortus and Abamectin significantly shortened pre-oviposition periods to 3.40 and 3.80 days in comparison with the control which recorded 6.10 days (Fig 1 a). When the female treated with Biofly the pre-oviposition period ranged from 2 to 6 day6 with an average 4.40 days.

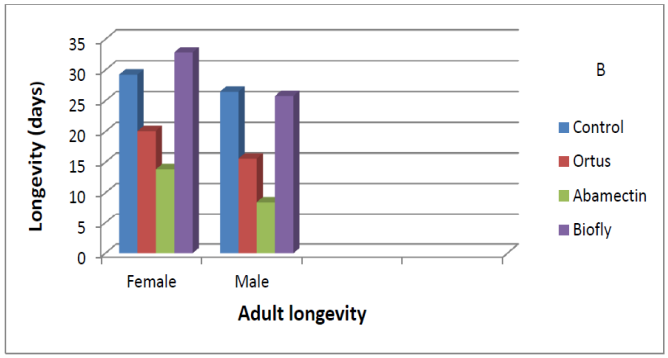
Data described in Fig(1 a) indicate that, The longest oviposition period was recorded for female treated with Biofly (22.9 days), while significant reduction was observed in the treatments of Abamectin, Ortus and control (5.40 14.0 and 19.30 days).

Treatment of female spider mite with Ortus shortened the post-oviposition period to1.60 days when compared with other treatments, on the other hand, no significant difference was observed among Abamectin, Biofly and control to record 4.60, 5.50 and 4.40 days, respectively.

The female longevities which recorded were 20.0, 13.80, 32.8 and 29.20 days for Ortus, Abamectin, Biofly and control, respectively. For male, the longevities were 15.00. 8.40, 25.20 and 26.40 days for the above treatment. These results suggested that Ortus and Abamectin could be effective on the longevity of adults which significantly reduced compared to Biofly and control (Fig 1b).

The weekly deposited eggs/ female and mean number of eggs/ female / day of oviposition period were significantly affected obviously by exposure to Ortus or Abamectin, while in case of Biofly treatment, the total deposited eggs (64.40/ female with a rate of 2.85eggs/ female/day) outnumbered those obtained at control (48.00 egg/females with the rate of 2.46eggs/ female/day).The lowest obtained egg number (13.80 eggs/female and 2.75 eggs/ female/day) was recorded using Abamectin. On the other hand, the Ortus treatment caused an increase to 22.00 eggs/female and 1.85 eggs/ female/day (Fig 1c).





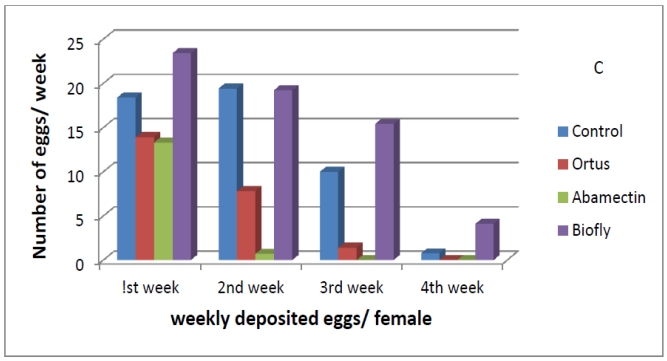


Fig 1: Effect of LC_{50} of three acacricides on the biological parameters of *S. gilvifrons.*

b. Effect on *S. gilvifrons* immature stages

Duration of embryonic development was not evidently influenced by the kind of treatment. Ortus and Abamectin prolonged insignificantly the incubation period to 2.19 and 2.13 days, respectively. For Biofly the incubation period was shorted to 1.94 days in comparison with the control treatment(1.85 days) (Fig 2a).On the other hand the immature larval instars which obtained from untreated females developed faster and required the shortest period to complete development. While treatments with Biofly, Ortus and Abamectin retarded the growth of the immature stages (Fig 2b).

According to results described in Fig (2c), different tested acaricides did not exhibit significant variations among pupal duration. However, pupae resulted from larvae treated with Abamectin required the shortest time (1.50 days) to complete development, while the longest period (2.28 days) was obtained for pupae resulted from larvae belonging to control. Pupae resulted from larvae treated with Ortus and Biofly had intermediate periods (1.91 and 1.77 days, respectively).

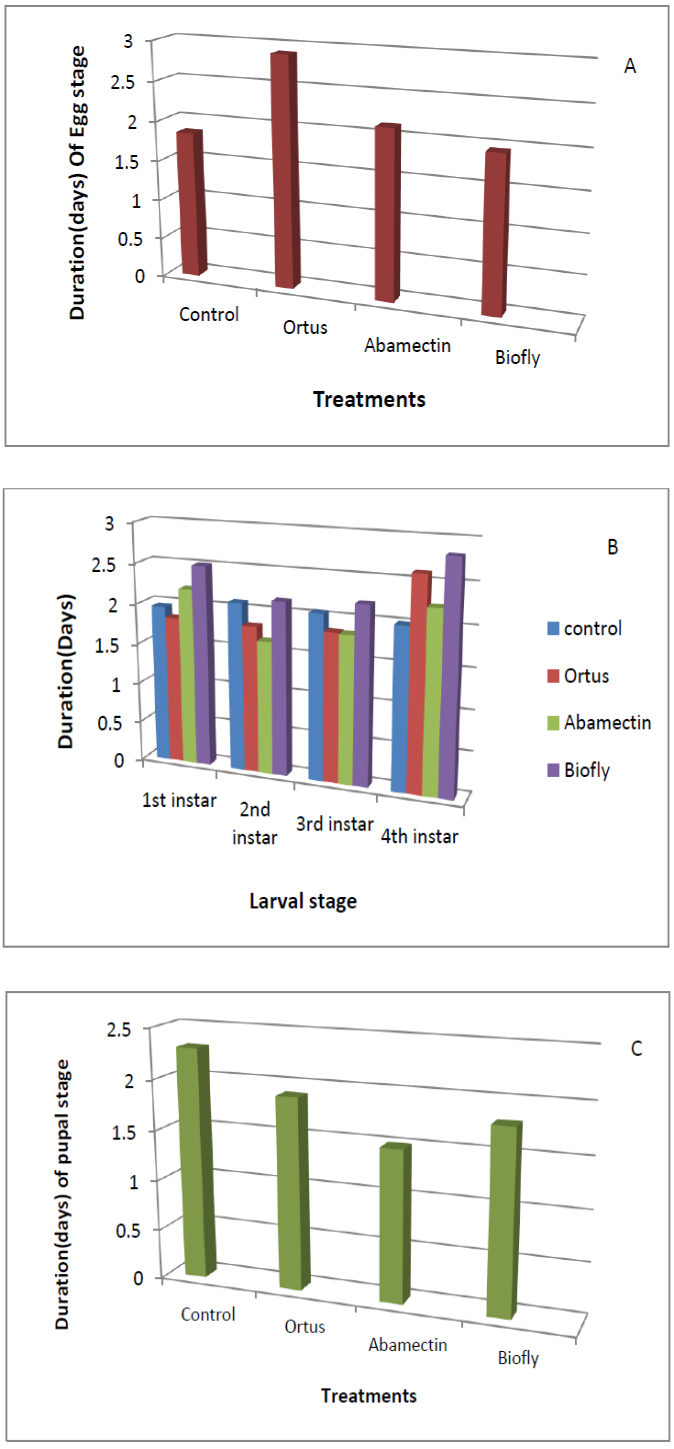


Fig 2: Effect of LC_{50} of three acaricides on the durations of immature stages of *S. gilvifrons*

Effect of LC_{50} of three acaricides on biological parameters

a. Effect on the *T. cucurbitacearum* adult

Fig (3 a) describe the differences between the three treatments and control as regard the pre- and post oviposition periods were showed a slight changes. The picture differed greatly with oviposition period, where the longest oviposition periods were observed in the control and Biofly treatments (17.80 and 17.55 days). On the other hand, this period was reduced significantly to 7.95 and 2.92 days by using Ortus and Abamectin, respectively.

The differences between fecundity of Biofly-treated females (48.20) and untreated ones (55.2) were insignificant (Fig 3c). While, these values were significantly reduced to 22.5 and 5.9 in case of Ortus and Abamectin treatments (Fig 3c).

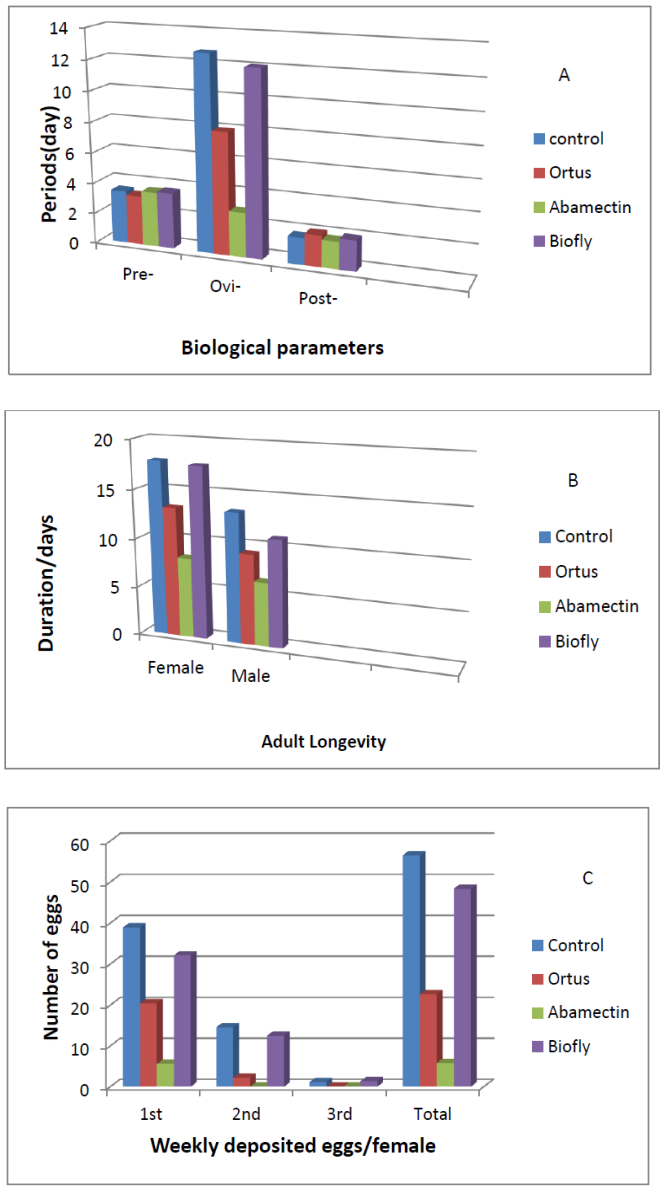
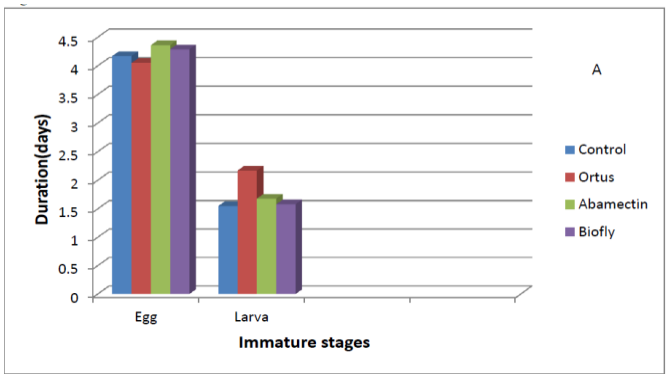


Fig 3: Effect of LC50 of three acaricides on the biological parameter of *T. cucurbitacearum*

b. Effect on *T. cucurbitacearum* immature stage

Incubation period of the deposited eggs obtained from treated females ranged between 4.0 – 4.35 days. So there is no significant effect for the three acaracides occurred (Fig 4 a). Duration of larval stage was significantly increased to 2.20 in the Ortus treatment, while differences were insignificant in the other treatments compared with control. Finally, no significant differences were recorded in the durations of the protonymph and deutonymph when compared with the control treatment (Fig 4b).



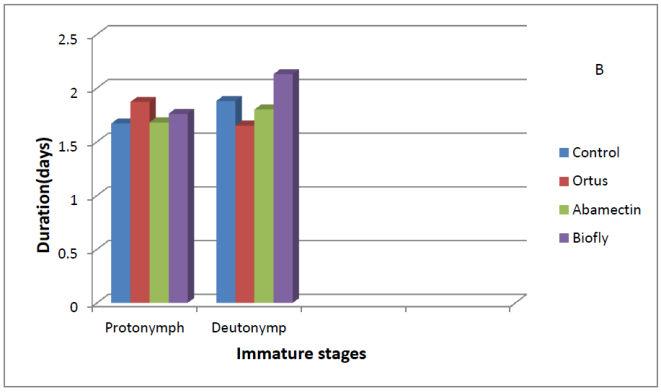


Fig 4: Effect of LC50 of three acaricides on the duration of immature stages of *T. cucurbitacearum*

Discussion

The present work confirmed the action of some acaricides on *T. cucurbitacearum* and its side effects on *S. gilviforns*. Ortus, Abamectin and Biofly are recommended as acaricides for controlling many species of family tetranychidae. The present work described the toxicity of three acaricides under laboratory conditions against the *T. cucurbitacearum*.

It was found that, Abamectin the most toxic acaricide is followed by Ortus and Biofly. Toxicity of these acaricides to some species of tetranychidae has been reported by various researchers. Ismail *et al*., 2006 calculated LC_{50} of Abamectin as 0.34 ppm against *T. urticae* which was higher than the LC_{50} (0.0135 ppm) reported by [30, 31] found that, Abamectin was more toxic acaricide than fenpyroximate against adult *T. urticae*. In addation, Ortus, Abamectin and Biofly were recommended as pesticides to control sucking piercing- pests.

S. gilviforns is a coleopterous predator collected from bean plants associated with spider mite *T. cucurbitacearum.* Our results concluded that, daily consumed prey larvae increased as the progressive larval instars. Our results a in reagreement with findings of [25, 32, 33] they reported that the fourth larval instar of *S. gilvifrons* is responsible for approximately 60% - 80% of total prey consumption at the larval stage. Being observed in our study, the fourth instar larvae were more voracious than first, second and third larval instars. The higher voracity of the fourth- instar larvae is possibly due to high requirements of energy intake for their growth and their critical weight attainment for the pupal stage [32]. However, Adult stage consumed more prey than any larval instar because it needed for egg deposition.

Treatment of female *S. gilvifrons* with the three acaricides made significant shorting in the pre- oviposition periods in comparison with the control treatment. On the other hand, treatment with Biofly longation in the oviposition period, while Ortus and Abamectin treatments caused significant reduction in the same period in comparison with control treatment. Similar results were obtained by [34] who found that, Actellic and Vertimec treatments caused significant reduction in the pre-oviposition period of *S. gilvifrons* when compared with control treatment. Also, the same author reported that the post-oviposition period decreased significantly in Actellic treatment. However significant reduction in the adult longevity was recorded in case of three acaricides treatments in comparison with the control. Also, these treatments caused significant reduction in the fecundity of predator female when compared with the control. The results were obtained agree with the results of [25] who observed that all organophosphorus compounds were highly toxic to Coccinellidae. Also, the same authors reported that Abamectin appeared to be the persistence under natural conditions and the most eradicative for *T. urticae* and *S. gilvifrons*, followed by Ortus, while Biofly appeared to be the lowest toxic one. Finally, [35, 36] indicated that the organophosphorus compounds killed all individuals of *S. bifidus* Biddinger. While, [37] observed that Abamectin was very toxic to *S. gilvifrons*.

These data lend credence to reports that augmentative biological control of *T. cucurbitacearum* mite with *S. gilvifrons* can be effective in different crops [15, 23].

The results of this study revealed that Ortus, Abamectin and Biofly acaricides showed high level of toxicity on the different stages of *T. cucurbitacearum*. Additionally, it was seen that the tested acaricides was more effective against the biological aspects of red mite. No significant differences were recorded in the durations of the nymphal stage or life span. Treatment with Biofly makes elongation in the oviposition period while treatment with Ortus and Abamectin make reduction in comparison with control. Also, the tested acaricides caused significant reduction in the fecundity of female when compared with the untreated females. This effect may be correlated with two factors; the range of oviposition period and the direct effect of the tested pesticides on the reproductive system. The previous results are in agreement with Salama *et al*. who found that Deltamethrin reduced the oviposition of *T. urticae* females on treated leaves compared with control. Also a sublethal concentration of Fenvalerate reduced the oviposition of *T. urticae* and its predator *Amblyesius fallacia* [38]. In contrary to these results, [39] found that Biopieren plus (pyrethrin) and Confidor increased the fecundity of the predator *Neoseiulus californicus* and unspecialized feeder *Tydeus califorincus*. But Confidor decreased *T. californicus* fecundity more than Biopieren plus.

Conclusion

A clear perspective that emerge from this investigation is that three acaricides (dOrtus, Abamectin and Biofly) can be used successfully in controlling the spider mite (*T. cucurbitacearum*) in presense of the natural enemy (*S. gilvifrons*) because of lower or negligible side effect on this natural enemy. In comparison between the toxicity of tested acaricides it was found that, Abamectin the most toxic acaricide is followed by Ortus and Biofly. So it can be recommended to use these products in the Integrated Pest Management Program of spider mites in different fields.

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