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Demographic Studies for Evaluating the Side Effects of Insecticides Proteus® and Pymetrozine on Variegated Lady beetle *Hippodamia variegata* (Goeze)

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Abstract
Variegated lady beetle *Hippodamia variegata* is considered as an important biological control agent. There is a need to use pesticides in combination with biological agents to get a better controlling result in some cases. In this study, various stages of the predator were exposed to the sublethal concentration (LC30) of two insecticides, Proteus® and pymetrozine. Results showed that the duration from adult emergence to the first oviposition (APOP), as well as the total preoviposition period (TPOP) from egg stage to the first oviposition day (TPOP) of the predator was also significantly increased after exposure to sublethal concentrations of both insecticides. The intrinsic rate of increase (r) was 0.195 ± 0.005 and 0.191 ± 0.007 day-1 in pymetrozine and Proteus® treatments, respectively, which are significantly lower than the control (0.225 ± 0.005 day-1). Conclusion, this study revealed that at sublethal concentration, Proteus® could adversely affect the predator at a demographic level.

Keywords: Hippodamia variegata, Biological control, Integrated Pest Management, Two-sex life table

1. Introduction
Butterflies the variegated lady beetle, *Hippodamia variegata,* is a widespread insect and is regarded as the most important naturally occurring predator in many countries [1, 2, 3, 4] and both adults and larvae consume large numbers of soft bodied insects (e.g. aphids, caterpillars, etc.) and insect eggs during their life time. In recent times, studies have been conducted on their predation capacity based on the life table theory [5]. One of the key components of integrated pest management is the use of biological control agents. However, the chemical control of pests is applied while various natural enemies are present Nevertheless, in some cases, there is the need to use pesticides in combination with these living agents to get a better controlling result The application of incompatible compounds with parasitoids and predators has led to pest resurgence or occurrence of secondary pests in many agro-ecosystems [6, 7]. Hence, choosing selective pesticides to protect biocontrol agents is highly recommended [8].
Most studies on the side-effects of pesticide on biocontrol agents are short term bioassays considering lethal or reproductive responses. The inadequacy of such studies has been discussed comprehensively and life table response experiments have been introduced as the best way to study the population level effects of pesticides [9, 10, 11].
Pymetrozine is a pyridine azomethine, systemic contact compound, used to control sucking pests on various crops. This insecticide disables the salivary pump of insects in order to stop their feeding. This compound has a low toxicity to mammals and is safe for birds and non-target arthropods, making it appropriate for IPM programs [12]. This selective pesticide targets plant-sucking homopterous insects such as aphids, whiteflies, plant hoppers, etc., [13], and has been shown to be harmless towards a large spectrum of beneficial arthropods [14, 15, 16,17,18].
Beside its selectivity, which is mainly due to the particular mode of action, insect parasitoids, especially endoparasitoids such as aphid parasitoids, can be affected indirectly while developing inside an insecticide-contaminated host [19]. Proteus®, a combination of thiacloprid and deltamethrin, is a contact and systemic insecticide, providing knockdown and residual control against insects [20]. Thiacloprid impacts by interfering with the transmission of nerve messages. It is active against various chewing and sucking pests [21]. Deltamethrin, on the other hand, is a pyrethroid insecticide used to control a wide range of pests. It causes paralysis by blocking the Na channels in nerve cells, thereby resulting in rapid death [22].

The purpose of this study was to investigate the total and ecologically relevant effects of pymetrozine (Chess®) and Proteus® sublethal concentrations on *H. variegata* by using the latest method, the age-stage, and two-sex life table theory.

2. Materials and Methods

2.1 Plant rearing

The broad bean *Vicia faba* L. cultivar Serakhsi was planted in plastic pots containing saw dust. The seeds were treated with the fungicides benomyl and mancozeb, before planting. Irrigation was done every day, with Horti® fertilizer (2% solution in water) added every 4 days. On reaching the 5 to 6 leaf stage, the plants were transferred to a growth chamber and kept under the environmental condition set at 25±1 °C, 65±10% relative humidity (RH), and photo period of 16:8 (L: D). Broad bean cultivation was continued for two year.

2.2 Insect rearing

The black bean aphid, *Aphis fabae* Scopoli, was collected from broad bean fields of the Agricultural Campus, Karaj. After the establishment of a colony, young plants of the bean cultivar Serakhsi were introduced every three days to keep the colony healthy. Adults of *H. variegata* were collected from an alfalfa field of the Agricultural Campus. They were transferred to plastic chambers (15×20×12 cm) and covered with 200 mesh gauze. Pieces of paper and leaves were used as oviposition bedding to protect the predators' eggs, and a piece of moist cotton was used to provide moisture in the chamber. After hatching eggs, each larva was introduced to a 9 cm plastic Petri dish containing aphids. Honeydew collected from infected plants was also used to feed the predator.

2.3 Insecticides

The insecticides used in this study were Proteus® OD 110, a combination of thiacloprid 100 g/L + deltamethrin 10 g/L (Bayer Agricultural Products, Germany) and pymetrozine (Chess®) WP 25 g/kg (Syngenta Crop Protection, Switzerland).

2.4 Life table response experiments

Sublethal concentrations of the two insecticide compounds were chosen based on a previous study [23]; the LC_{30} of Proteus® (227 ppm) and pymetrozine (2500 ppm). The one-day-old female adults (not mated) were treated by spraying 1.5 ml of each insecticide or water (as control) directly in the Potter tower (Standard model, Burkard Scientific, Rickmansworth, Herts, United Kingdom). The Potter tower was calibrated at 15 PSI according to the IOBC/WPRS Methodology and corresponded to an average deposition of 1.5 mg/cm^2 water droplets. Was used for treating the glass plates (10x10 cm) which formed the bottom of the exposure cages. Treated plates were allowed to dry for 1 h. Plastic glass rings (6 cm in diameter and 3 cm in height) containing ventilation windows, with another untreated glass plate, completed the cage. A piece of moist cotton was used as a water source. Fifteen individuals were introduced into each cage. Thereafter, the cages were transferred to the growth chamber. The growth chamber had the above mentioned conditions. After 72 h, the alive adults were selected and allowed to reproduce. Eggs of the same age (six-hours-old) from these females were used to construct the life tables.

Two cohorts of 106 eggs were used for Proteus® and control and a cohort of 114 was used for pymetrozine treatment. The developmental time of each instar for all individuals were recorded daily. In all treatments, the larvae were fed with aphids. After adult emergence, each female was paired with one male and their longevity and number of eggs produced by them were recorded, daily. All experiments were performed at 25 ± 1 °C, light period of 16:8 (L: D) and 65 ± 10% RH. All experiments are done in 2012 and 2013.

2.5 Data analysis

The raw data were analyzed based on the age-stage, two-sex life table theory, according to Chi and Liu [24] and Chi [25] using a user friendly computer program, TWOSEX-MS Chart [26]. The age-stage specific survival rate (s_{xj}) (where x is the age and j is the stage), age-stage specific fecundity (f_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), and population parameters (r, intrinsic rate of increase; λ finite rate of increase; R_0, net reproduction rate; and T, the mean generation time) were calculated. The means and standard errors of the life table parameters were estimated with paired bootstrap technique [27] included in the above mentioned program. Life history statistics were subjected to analysis of variance (ANOVA) using Tukey test. When the data were not distributed normally, they were analyzed by the two-tailed Kruskal-Wallis H tests; and a two-tailed Mann-Whitney U test was used as the post-hoc test.

3. Results

The developmental time for each stage, adult longevity, and fecundity of *H. variegata* at different treatments are shown in Table 1. The embryonic development of *H. variegata* required 3.18±0.04 days after pymetrozine and 3.05±0.02 days after Proteus® treatment. A significant difference (Kruskal-Wallis H test, H = 24.65, df = 2, P<0.001) was detected among treatments and control (2.98±0.01 days) (Table 1).

Table 1: Effects of thiacloprid+deltamethrin and Pymetrozine on the life history of *H.variegata.*

Life history statistics	Stage or Sex	Control		thiacloprid+deltamethrin		Pymetrozine		*P*
		n	Mean±SE	n	Mean±SE	n	Mean±SE	
Stage duration[1]	E g g	95	2.98±0.01a	84	3.05±0.02b	95	3.18±0.04c	<0.001
	1st larval instar	94	2.18±0.04a	81	2.32±0.05b	93	2.37±0.05b	0.015
	2nd larval instar	93	1.8±0.042	79	1.86±0.04	91	1.88±0.03	0.264
	3rd larval instar	91	1.93±0.03a	74	1.99±0.01a	90	2.24±0.05b	<0.001
	4rh larval instar	89	2.71±0.05a	73	2.68±0.05a	89	2.92±0.03b	<0.001
	Pupa	89	4.21±0.05	72	4.29±0.05	89	4.21±0.04	0.312
Adult longevity[2]								
	Female	44	47.8±1.36a	36	38.61±1.44b	43	45.84±1.80a	<0.001
	Male	45	70.87±1.71a	36	59.58±1.60c	46	66.13±1.82b	<0.001
APOP[1]	Female	44	2.73± 0.11a	36	3.56±0.52a	43	3.58±0.17b	<0.001
TPOP[1]	Female	44	18.36±0.18a	36	19.67±0.55b	43	20.12±0.18c	<0.001
Fecundity[2]	Female	44	1311±49.46a	36	686±48.28c	43	1120±60.54b	<0.001

Data in a row followed by different letters are significantly different (P<0.05) (Kruskal-Wallis and Mann-Whitney U tests) [2]Data in a row followed by different letters are significantly different (P<0.05) (Tukey).

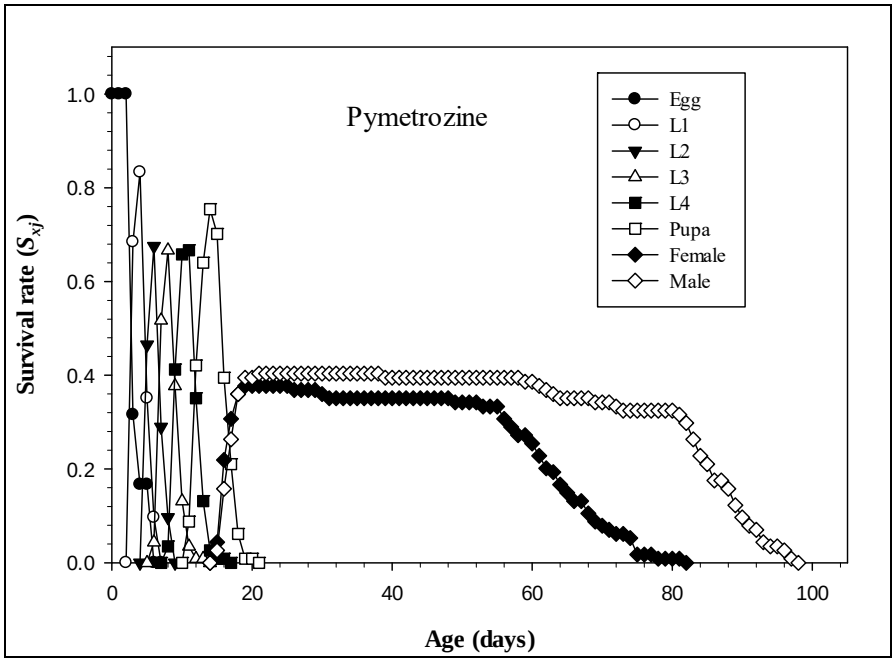
The developmental time of the first larval stage was significantly influenced (Kruskal-Wallis H test, $H = 8.40$, $df = 2$, $P = 0.015$) by insecticides treatment (2.32±0.05 for Proteus®, 2.37±0.05 for pymetrozine, and 2.18±0.04 days in control).

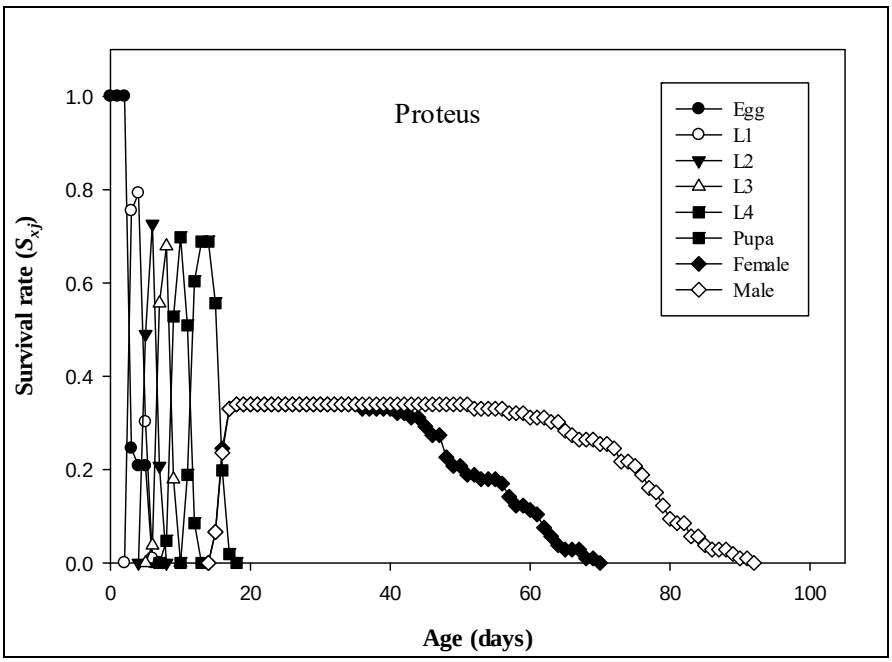
Likewise, significant differences in the developmental times were observed among treatments and control in the third larval stage (Kruskal-Wallis H test, $H = 40.80$, $df = 2$, $P<0.001$). The fourth larval stage was also influenced significantly (Kruskal-Wallis H test, $H = 15.74$, $df = 2$, $P<0.001$) by insecticides treatment (2.68±0.05 for Proteus®, 2.92±0.03 for pymetrozine, and 2.71±0.05 days in control). No significant differences were identified among treatments in the second larval stage (Kruskal-Wallis H test, $H = 2.66$, $df = 2$, $P = 0.264$) and pupal stage (Kruskal-Wallis H test, $H = 2.33$, $df = 2$, $P = 0.312$).

Besides, there were also significant differences (Kruskal-Wallis H test, $H = 15.39$, $df = 2$, $P<0.001$) in the adult preovipositional period (APOP) of *H. variegata* (3.56±0.52 days for Proteus®, 3.58±0.17 days for pymetrozine and 2.73± 0.11 days in control). The total preoviposition period (TPOP) of the predator in Proteus®, pymetrozine and control was 19.67±0.55, 20.12±0.18, and 18.36±0.18 days, respectively which differed significantly (Kruskal-Wallis H test, $H = 35.87$, $df = 2$, $P<0.001$).

The mean fecundity of control (1311 eggs/female) was the highest among the three treatments, but was significantly different from that of pymetrozine (1120 eggs/female) and Proteus® (686 eggs/female).

The age-stage specific survival rate (s_{xj}) value of *H. variegata* (Figure 1) gives the probability that a newly laid egg will survive to age x and stage j. These curves also show survivorship and stage differentiation.





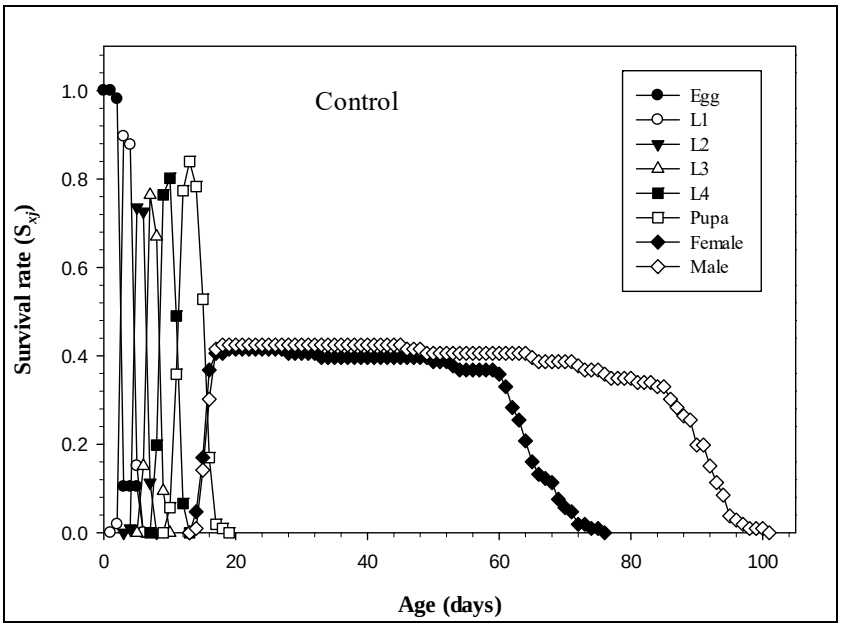
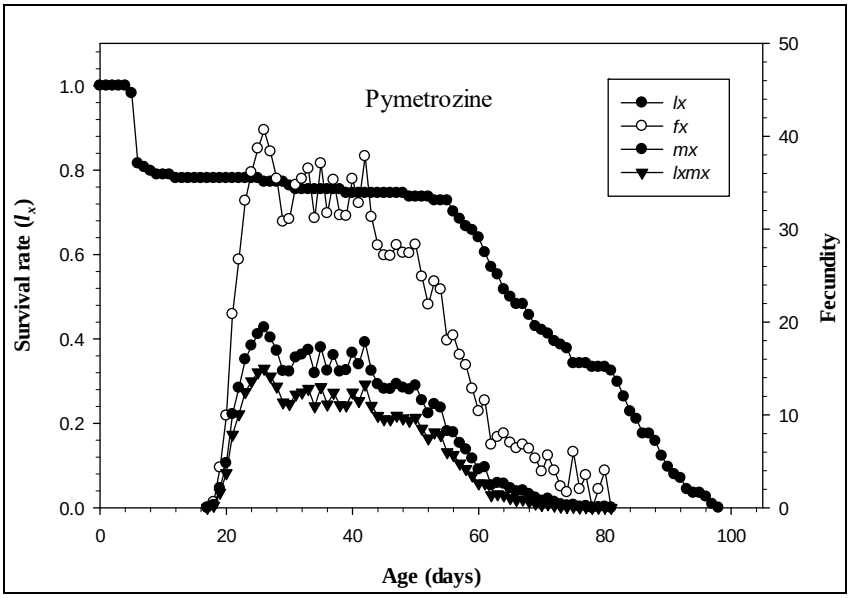
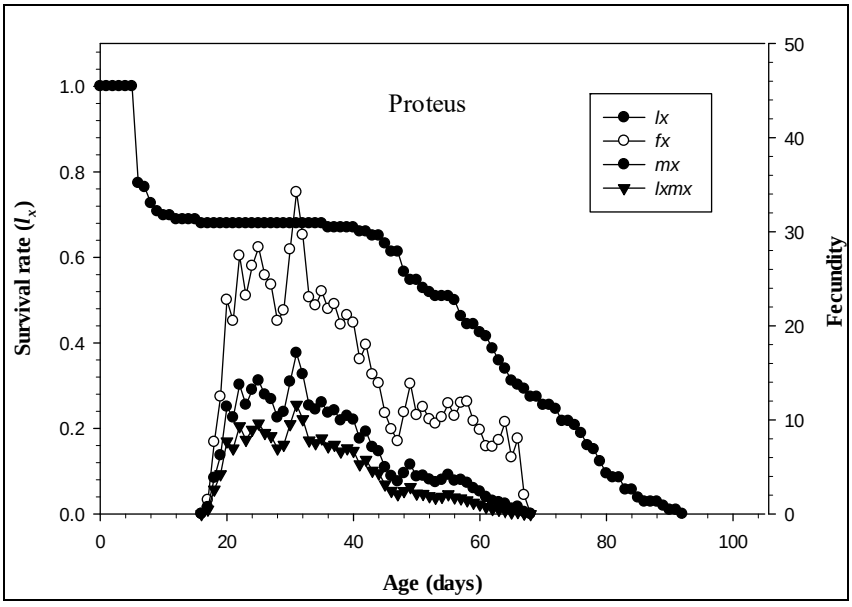


Fig 1: Age-stage specific survival rate (s_{xj}) of *H. variegata* in control and insecticides treatments.





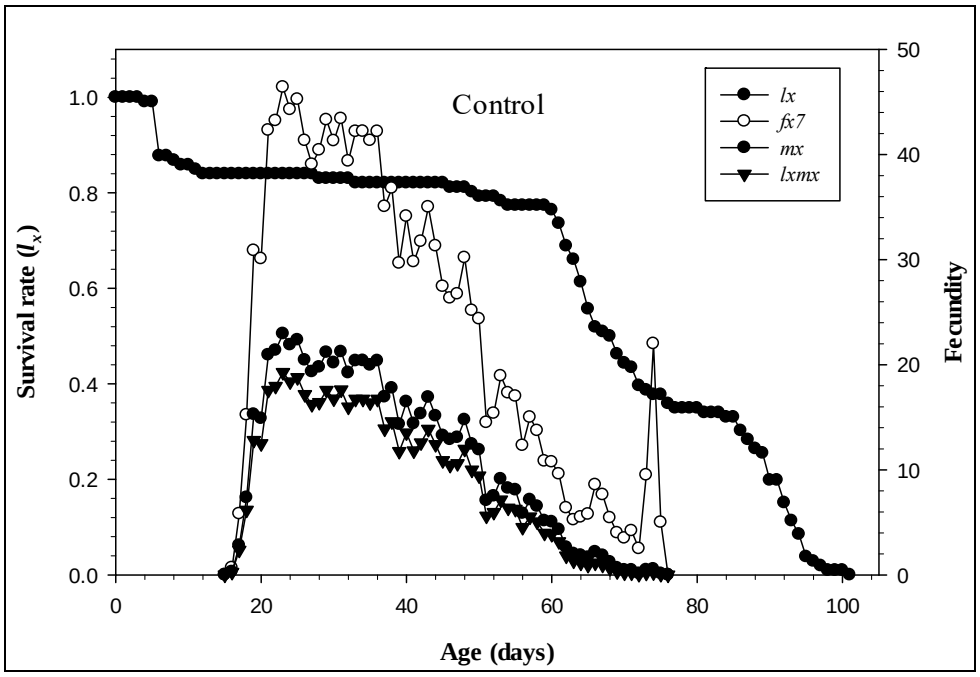
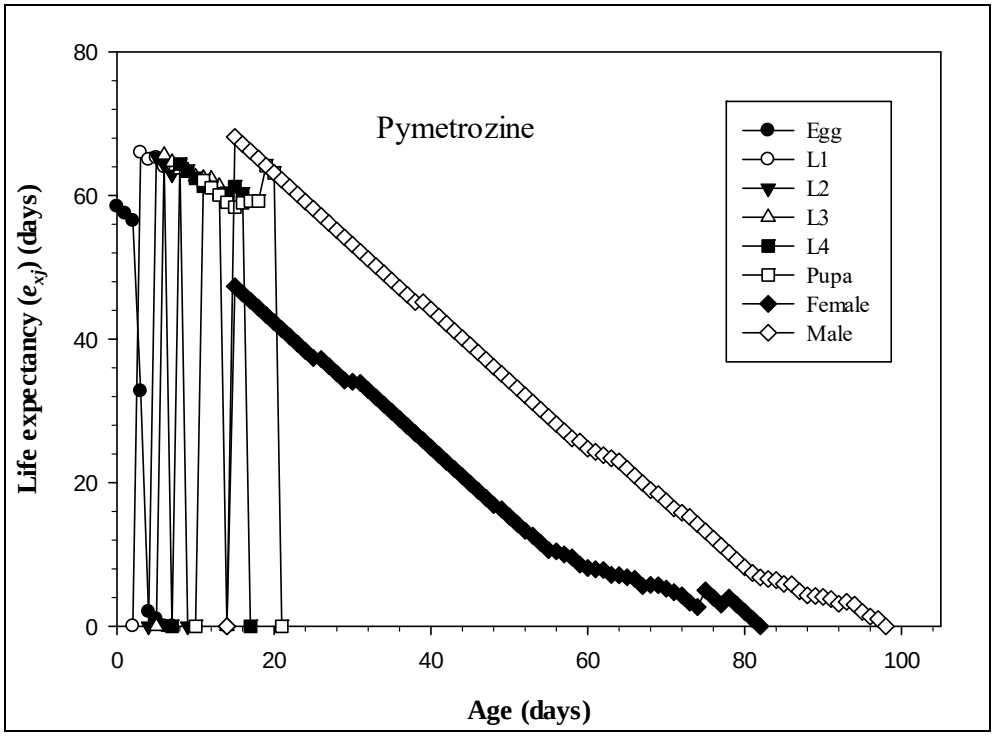


Fig 2: Age-specific survival rate (*lx*), female age-specific fecundity (fx7), age-specific fecundity of the total population (m_x), and age-specific maternity (l_xm_x) of *H. variegata* in control and insecticides treatments.

The curves of *lx, fx, mx,* and age-specific maternity (l_xm_x) of *H. variegata* are illustrated in Figure 2. The age-specific survival rate, l_x, 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 Figure 2.

The average age specific fertility (m_x), expressed as number of eggs per female per day, on Proteus® treatment oviposition started at age 17 with low value, increased gradually, and lasted for 7 weeks. Thereafter, it dropped to a lower level with small increments until the female stopped laying eggs some 3 weeks before the last adult died. On pymetrozine treatment oviposition started at age 18, lasted for 8 weeks, and egg laying stopped some 2 weeks before the death of the last female.

Figure 3 illustrates the life expectancy (e_{xj}) of each age-stage of *H. variegata*. It gives the expected time that an individual of age x and stage j will live. The life expectancy of the female, for example, was 49.4, 39.7, and 47.3 days in control, Proteus®, and pymetrozine treatment, respectively.



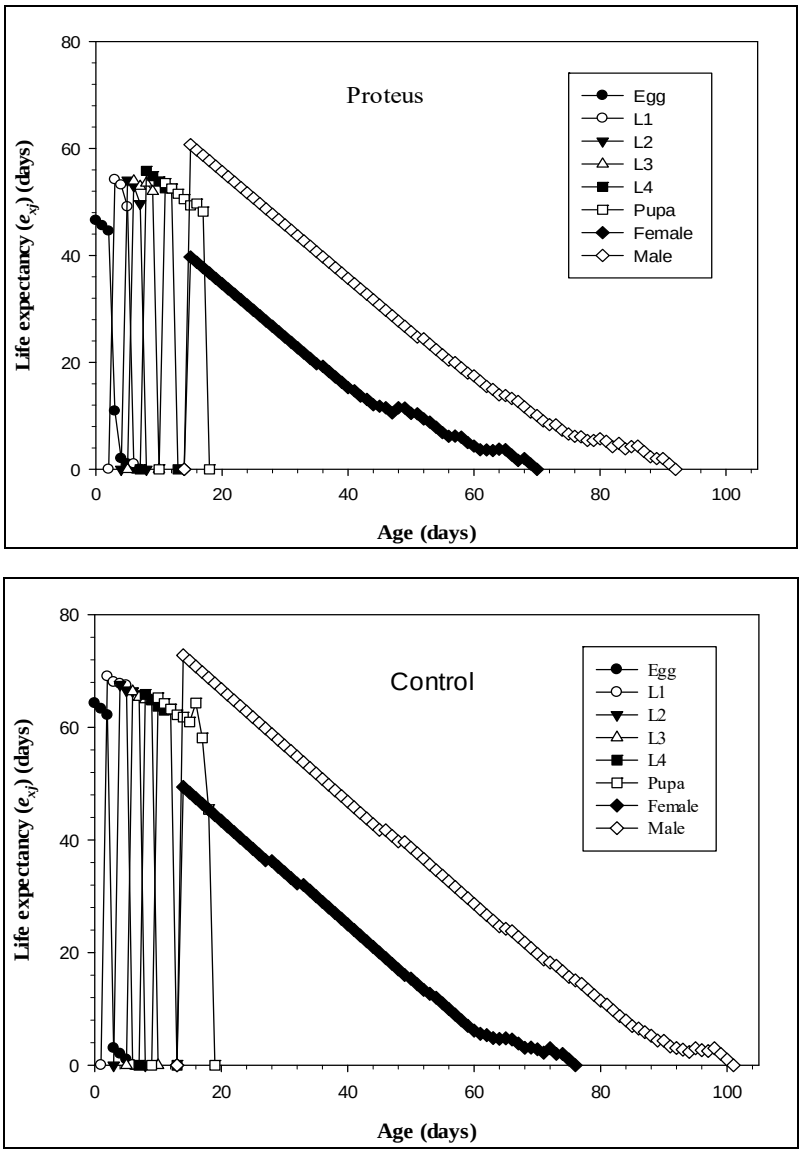
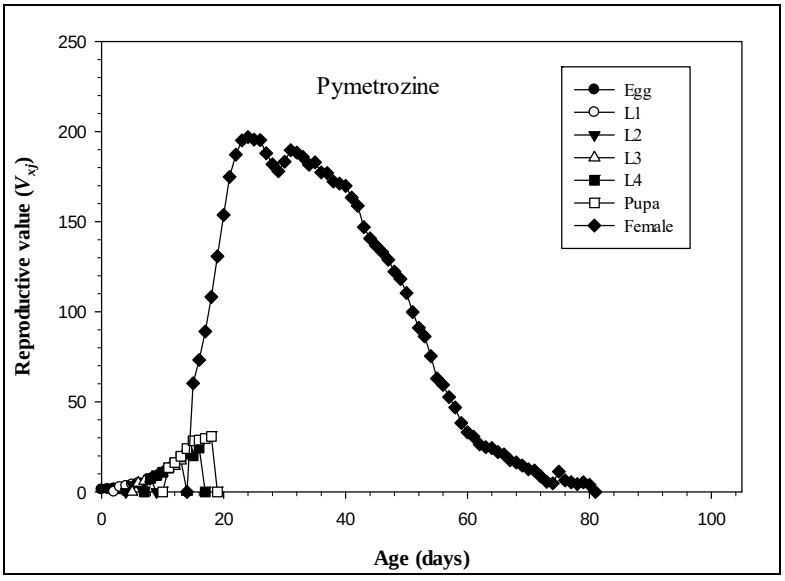


Fig 3: Life expectancy (e_{xj}) of *H. variegata* in control and insecticides treatments.

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 of oviposition can impart much more than a new born egg. For example, a newborn egg on Proteus® treatment has a productive value of 1.21, but a female of age 49 has a much higher reproductive value of 52.61.



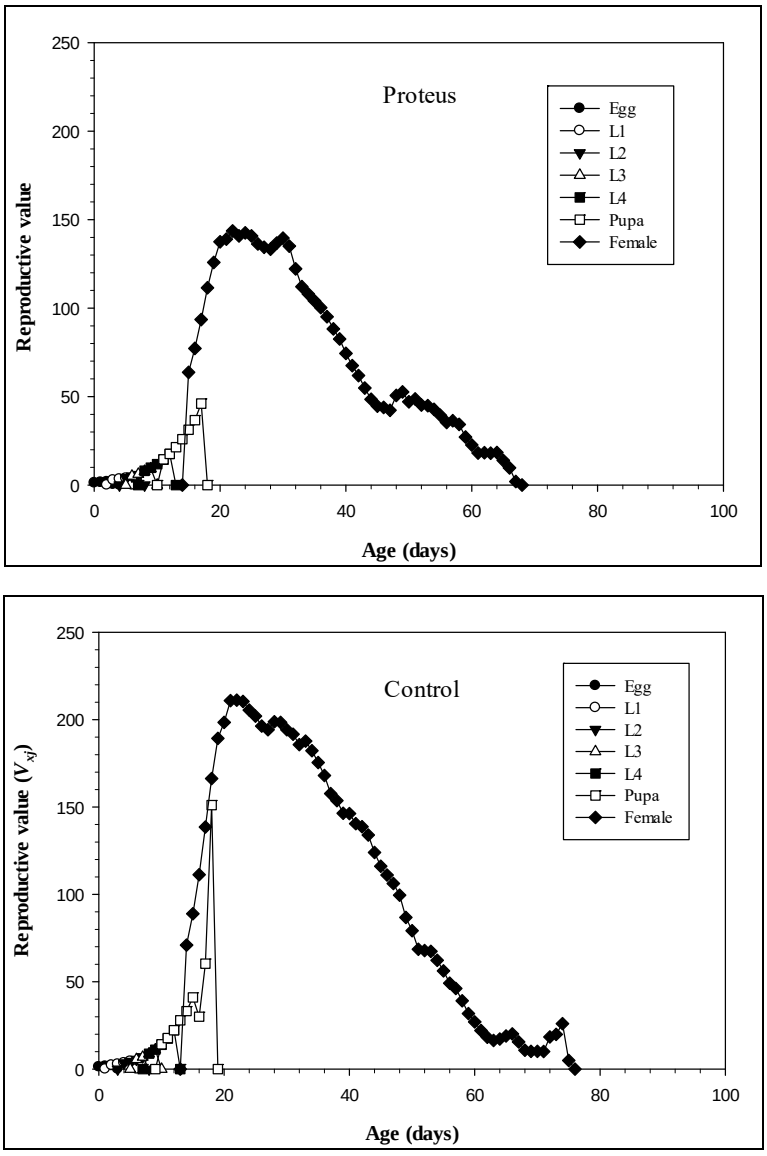


Fig 4: The reproductive value (v_{xj}) of *H. variegata* in control and insecticides treatments.

The means and SEs of *r, R0, GRR, λ,* and *T* were estimated using the Bootstrap method as shown in Table 2. The intrinsic rate of increase (*r*) was 0.195±0.005 and 0.191±0.007 day^{-1} in pymetrozine and Proteus® treatments, respectively, which is significantly lower than that of the control (0.225±0.005 day^{1}). The finite rate of increase (λ) was 1.215±0.006 and 1.211±0.008 day^{-1} in pymetrozine and Proteus® treatments, respectively and 1.252±0.006 day^{-1} in control, which differed significantly.

Table 2: Effects of thiacloprid+deltamethrin and Pymetrozine on life table parameters of *H. variegata*.

Population parameters	Control		Pymetrozine		thiacloprid+deltamethrin	
	n	Mean±SE	n	Mean±SE	n	Mean±SE
Intrinsic rate of increase (*r*) (d^{-1})	106	0.225±0.005a	114	0.195±0.005b	106	0.191±0.007b
Finite rate of increase (λ) (d^{-1})	106	1.252±0.006a	114	1.215±0.006b	106	1.211±0.008b
Net reproductive rate (R_0) (offspring per individual)	106	543±66a	114	422±56b	106	232±35c
Mean generation time (*T*) (d)	106	27.97±0.26b	114	30.98±0.30a	106	28.40±0.46b

Means in the same row followed by the same letter are not significantly different (P>0.05) using the paired bootstrap test.

The net reproductive rate (R_0) was 422±56 and 232±35 offspring per individual in pymetrozine and Proteus® treatments, respectively and 543±66 offspring in the control which also differed significantly. The mean generation time (*T*) was 30.98±0.30 and 28.40±0.46 days in pymetrozine and Proteus® treatments, respectively and 27.97±0.26 days in control, which differed significantly.

4. Discussion

This study provides useful applied information for an integrated approach to control aphids as an important pest of agricultural commodities. It revealed how life history traits and

consequently demographic parameters of *H. variegata* are affected by Proteus, an insecticide which is used to control sucking pests on various crops. Life table statistics and statistically derived parameters may provide useful values for summarizing the effects of pesticide on the reproductive capacities of *H. variegata*. Results from the study showed that both tested insecticides caused a decrease in survival of adults, extended the time for instars to become adults, and reduced the fecundity of *Hippodamia* females. To corroborate the result of this study, Kumar and Santharam [28] reported a significant adverse effect of imidacloprid on the longevity of *Chrysoperla carnea* (Stephens) larvae and adults. Other studies have reported the reproductive stimulation of pests or beneficials with imidacloprid [29, 30, 31].

In this study, a significant difference was also observed in the time between adult emergence and the first oviposition of *H. variegata* at various treatments; thus, tested insecticides increased during this period. Papachristos and Milonas [32] obtained a similar result showing that the application of imidacloprid reduced the preoviposition period of *Hippodamia undecimnotata.*

The preadult and preoviposition periods may be prolonged due to physiological factors. When insects are treated with insecticides, a considerable amount of energy is usually allocated to detoxification. Hence, the total energy was neither spent on growth nor development, thereby resulting in decreased growth rate and delayed development [33]. On the other hand, the insecticides may disrupt hormonal balance in the body of insects which may also lead to delayed growth.

The tested insecticides also had significant effects on the life table parameters of the predator. These parameters included net reproductive rate, intrinsic rate of increase, mean generation time, and finite rate of increase which is adversely affected by the insecticides.

The lowest value for intrinsic rate of natural increase occurred during Proteus® treatment, which was significantly different from the control; with the lowest net reproductive rate observed in Proteus®, this was significantly different from the control or pymetrozine. The maximum value for the mean generation time was observed in pymetrozine treatment which was significantly different from the control or Proteus® treatment. Sarmadi *et al* [34]. Also obtained the same results for some pesticides, such as thiacloprid and deltamethrin on parasitoid *Habrobracon hebetor* (Say) and reported that the insecticide treatments had negative effects on the stable population parameters including intrinsic rate of increase and net reproductive rate. Rezai *et al.* [35] showed that pymetrozine insecticide mean generation time (T) on *Chrysoperla carnea* (Stephens) increased more significantly than the control.

Forbes and Calow [36]. Stated that demographic toxicology had been considered as a better measure of response to toxicants than individual life history traits. The intrinsic rate of increase (rm) was recommended for evaluating the total effects of insecticides, because it is based on both survivorship and fecundity [37].

Furthermore, in this study, both insecticides caused reduction in this parameter of *H. variegata.* Rahmani and Bandani [38] also observed this result after the treatment of *H. variegata* with thiamethoxam.

The results obtained by Joseph *et al.* [19] on aphid parasitoid *Aphidius ervi* (Haliday) also showed that Plenum®, a trade name for pymetrozine, is not as selective as it had been reported previously. They concluded that this may be related to the methodology used, which allowed the parasitoid to develop inside contaminated hosts, whereas previous studies had evaluated the effect of pymetrozine or commercial formulations sprayed on to mummy or adult stages of the aphid parasitoid. They suggested that more care should be taken when these insecticides are used in IPM programs.

Results from this study showed that Proteus® even at reduced rates, had more negative impacts on the important life history parameters of *H. variegata* than pymetrozine. Hence, there is a little compatibility between *H. variegata* and the use of Proteus®. In another study, based on the acute lethal toxicity tests, pymetrozine was shown to be harmless to the predator [23].

Many evaluations have shown that ecotoxicological analysis based on population growth rate results in more accurate assessments of the impacts of insecticides and because measures of population growth rate combine lethal and sublethal effects [39].

Nonetheless, as these data were obtained under semi-field condition, the effects of pesticides on *H. variegata* should be confirmed by further tests under field conditions.

5. Conclusion

Overall, our results suggest that pymetrozine are compatible with the use of *H. variegata* for IPM. In contrast, thiacloprid+deltamethrin have deleterious effects on this predator and accordingly are not recommended to be applied during times of predator release. Semi-field and field studies that assess efficacy of the combined use of these insecticides and *H. variegata* are still needed to better understand the applicable conditions for the field.

6. Acknowledgements

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