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# Sub-Lethal Effects of Thiamethoxam and Pirimicarb on Life-Table Parameters of *Diaeretiella rapae* (Hymenoptera: Braconidae), Parasitoid of *Lipaphis erysimi* (Hemiptera: Aphididae)

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***Abstract*—**Integrated Pest Management (IPM) aims to combine biological and chemical strategies and measures, hence highlighting the study of acute toxicity and sub-lethal effects of pesticides comprehensively. The present research focused on the side effects of thiamethoxam and pirimicarb sub-lethal concentrations on demographic parameters of *Diaeretiella rapae* (McIntosh Laboratory) (Hymenoptera: Braconidae). Adult parasitoids were exposed to $LC_{25}$ of insecticides as well as distilled water as the control. The results showed that thiamethoxam adversely affected population parameters ($r$, $\lambda$, $R_0$, $T$), adults' longevity, females' oviposition period and mean fecundity, and a similar trend was obtained for pirimicarb with the exception of generation time ($T$), the latter did not significantly change compared to the control. The intrinsic rate of increase ($r$) in the control and those treated with pirimicarb and thiamethoxam were 0.2801, 0.2064, 0.1525 $days^{-1}$, respectively, and the sex ratio was biased toward females in all treatments. Furthermore, none of the insecticides influenced total pre-oviposition period (TPOP) and offspring emergence rate. In general, these results indicated that both insecticides potentially distort the demographic parameters of the parasitoid even at sub-lethal concentrations, and then they should not be considered for IPM program in the presence of *D. rapae*.

***Keywords*—***Diaeretiella rapae*, *Lipaphis erysimi,* life-table study, pirimicarb, thiamethoxam.

## I. Introduction

INSECT pests are the foremost destabilizes of canola production; aphids are, however, of more serious concerns [1]-[3], among which, mustard aphid *Lipaphis erysimi* (Kaltenbach) is a hugely destructive pest of Brassica crops with a worldwide distribution causing up to 90% yield loss in rape seed [4]-[6]. The aphid reportedly dominates canola-bound aphid fauna with a frequency of 67%, in the Khuzestan province in Iran [7].

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Certain natural enemies prey on mustard aphid; however, they emerge somehow late after the aphids would have already severely damaged the crops, and also, their populations are too small to successfully control the aphids [7], [8]. One of them is parasitoid *Diaeretiella rapae* (McIntosh Laboratory), a ubiquitous wasp with more than 30 host species [9]. It is ranked as the major parasitoid of Brassica feeding aphids with a great potential in preventing an aphid outbreak in Brassica crops [4], [10], [11]. According to [12], 82% of aphid parasitoids collected from cruciferous vegetable crops in Northwest U.S.A. belongs to the *D. rapae* species.

Existing cultivars lack sufficient plant resistance to give protection against aphids [13], [14]. It is, thus, recommended to use insecticides in order to relieve drastic losses caused by the mustard aphid [15]-[18].

Application of a given pesticide in IPM programs requires prior evaluation of its potential side effects on beneficial species. The susceptibility of species to toxicants cannot be solely assessed by simplistic models such as acute half lethal concentration estimations ($LC_{50}$) with regard to individual effects [19]-[22]. Exposure to chemicals may induce different sub-lethal effects on organisms which can appear simultaneously and are largely overlooked [19], [21]. The time period used in these studies are not long enough to determine the impacts in population levels [20], [23]. For instance, the surviving individuals may suffer from shortened life-span, reduction in the number of progeny, pre-reproduction time, changes in sex ratio, etc. [19], [24]-[26]. Furthermore, behavioral changes induced by toxicants will interrupt the matting process and cause a reduction in the natural enemy's ability to capture prey, mobility, orientation, learning, etc. [20], [26]. A demographic approach can obviously estimate most impacts of pesticides on a population through measuring the effects on survival and reproduction [20], [22]. In order to achieve the protection of beneficial and other non-target organisms under the IPM-based system, it is necessary to adopt demographic and modelling approaches to toxicological studies [22]. Several researchers analyzed the toxicity of different pesticides for *D. rapae* taking screening and demographic approaches, and revealed that the parasitoid species is tremendously susceptible to most of broad spectrum insecticides perceptibly deteriorating its performance as a biological control agent [27]-[31]. Numerous efforts also indicate that certain insecticides pose high risks to *D. rapae* by

reducing the longevity of adults [28], [32], parasitism rate [33]-[35] as well as specific behavioral outcomes [33]-[36].

The acute toxicity of pirimicarb on *D. rapae* and to a lesser extent its side effects in a demographic trend have been frequently investigated [31], [35], while only a few reports are available on thiamethoxam acute mortality effects [31], and its detrimental impacts on population level have been overlooked. The present research is the first demographic approach to the side-effects of thiamethoxam on *D. rapae*. Even though the research aimed at assessing the sub-lethal effects of studied insecticides on parasitoid of the mustard aphid, also, a new model of the Age-stage, two-sex life-table was employed in this life-table study reported to be of huge advantages over most common method [37]-[39] (Chi & Liu, 1985; Chi, 1988; Chi & Su, 2006).

## II. Material and Methods

### A. Insect Culture

The mustard aphid *L. erysimi* and its parasitoid *D. rapae* were collected in canola fields across the Khuzestan province (Southwest Iran) during February 2013. The insects were reared on canola seedlings *Brassica napus* L., cultivar Hyola 401 in netted hyaline cages (110×80×80 cm) at 25±2°C, 50-60% R.H and a photoperiod of 16:8 h (L: D).

### B. Chemical and Toxicity Bioassays

Formulated insecticides were used: pirimicarb (Pirimor® 50 W.P, Moshkfam Fars, Iran) and thiamethoxam (Actara® 25 W.G, Syngenta, Switzerland). These are two common insecticides in canola crops of Khuzestan province. A group of newly emerged female adults (<24 h old) was used to carry out the residual contact toxicity bioassay at five different concentrations of insecticides. The inner side of glass vials (diameter: 4.5 cm; length: 7.5 cm) were treated with 150 µl of each concentration and distilled water as the control. The vials were manually rotated to get a homogeneous layer of solutions and were left for two hours at room temperature to dry. Then the wasps were introduced to the tubes and fed on a honey solution (30% v/v). Five replications were used with 15 wasps each, with each test repeated three times at 23±2°C, 70±5% RH, and a photoperiod of 16:8 h (L: D). Mortality was assessed 24 h after treatments. Data was analyzed by Polo-Plus software (LeOra Software, Version 2, 2013) to estimate $LC_{25}$ values of each insecticide.

### C. Demographic Parameters

In order to study the life-table parameters, each five pairs of young adults (24-48 h old) of *D. rapae* were provided with the canola seedlings at 4-5 leaf- stage infested with approximately 100 third instar nymphs of *L. erysimi*. After a 24 h period, the adult parasitoids were removed and the aphids were checked daily for mummy formation. The mummies were then transferred into individual plastic petri dishes (diameter: 6 cm; height: 1 cm) and observed on a daily basis until emergence of adults occurred. About 50 parasitized aphids were used to develop a life-table for *D. rapae* in various treatments. The newly emerged adults were treated with $LC_{25}$ dosage of the studied insecticides, and distilled water (control) as mentioned above. $LC_{25}$ were chosen as the sub-lethal concentration because it is below 30% mortality threshold recommended for the use of insecticides in IPM [40], [41]. After 24 h, each pair of survived adults was transferred to an opaque cylindrical container (diameter: 7.5 cm; length: 18 cm) containing 4-5 leaf-stage canola seedlings pre-infested with 50 third instar nymphs of *L. erysimi*, the preferred nymphal instar of this host for *D. rapae* [42]. On a daily basis, the parasitoid pairs were re-transferred to a new container with 50 aphids; it continued until the death of the female parasitoids. Dead males were also steadily replaced with treated males of the same age. The date of mummification and the number of mummies produced in each day were recorded; newly formed mummies were separately kept until the emergence of an adult occurred. The sexes of all emerged off-springs were determined to estimate the offspring sex ratio.

### D. Age-Stage Two-Sex Life-Table Analysis

Obtained data was analyzed according to the age-stage, two-sex life-table theory [37], [38], [43]. The age-specific survival rates ($s_{xj}$) (where $x$ is the age in days and $j$ is the stage; the first, second, third and fourth stages are the pre-pupa, pupa, female and male, respectively), the age-specific survival rate ($l_x$), the age-stage specific fecundity ($f_{xj}$), the age-specific fecundity ($m_x$) and the population parameters (the intrinsic rate of increase ($r$), the net reproductive rate ($R_0$), the finite rate of increase ($\lambda$), $\lambda = e^r$ and the mean generation time ($T$)) were estimated accordingly. The intrinsic rate of increase was calculated by using the iterative bisection method from the Euler-Lotka equation with age indexed from zero [44]:

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

According to [37], the $l_x$ and $m_x$ was calculated from the daily records as:

$$l_x = \sum_{j=1}^{k} s_{xj} \quad (2)$$

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}} \quad (3)$$

Here, $k$ is the number of stages. The net reproductive rate ($R_0$) was calculated as the mean number of offspring that an individual can produce during its lifetime:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \quad (4)$$

The mean generation time ($T$) was defined as the length of time that a population needs to grow to $R_0$- fold of its size at the stable age-stage distribution and was estimated by $T = [\ln R_0/r]$. The age- stage life expectancy ($e_{xj}$) was calculated according to [39] as follows:

$$e_{xy} = \sum_{i=x}^{n} \sum_{j=y}^{m} s'_{ij} \quad (5)$$

Here, $n$ is the number of age groups, $m$ is the number of stages and $s_{ij}$ is the probability that an individual of age $x$ and stage $y$ will survive to age $i$ and stage $j$. Reference [45] defined the reproductive value as the contribution of individuals of age $x$ and stage $y$ to the future population. In the age-stage, two-sex life-table theory is calculated as:

$$v_{xy} = \frac{e^{-r(x+1)}}{s_{xy}} \sum_{i=x}^{n} e^{-r(i+1)} \sum_{j=y}^{m} s'_{ij} f_{ij} \quad (6)$$

The mean and standard errors of population parameters, fecundity, longevity, developmental time were calculated by using bootstrap method (B= 100000) [46]-[48]. Differences in life history traits and population parameters among different treatments were compared by paired bootstrap procedure (P< 0.05) [46].

## III. Results

According to the results of bioassay, pirimicarb and thiamethoxam caused 25% mortality in female adults at 39.95 µg (a.i) ml$^{-1}$ and 0.015 µg (a.i) ml$^{-1}$, respectively. As the pre-adult development of *D. rapae* occurs inside the aphid's body, its life history had been divided into three stages: pre-pupa, pupa and adult. The duration of pre-pupa was 6.3± 0.2, 6.6± 0.2 days (t= 1.02, d.f. = 115, P= 0.307) and pupa stages was 4.5± 0.1, 4.4± 0.154 days (t= 0.55, d.f. = 115, P= 0.579) in females and males, respectively. No significant differences were found in any of these pre-adult stages in female and male individuals using t- test at the 5% significance level. It is essential to note that these two developmental stages had not been exposed to pesticides. The number of emerging males and females showed that the sex ratio was female based (0.56: 0.44).

The age-stage specific survival rate ($s_{xj}$) (Fig. 1) gives the probability that a newborn will survive to age $x$ and develop to stage $j$. The negative effect of testing pesticides can be observed in the adult stage of both females and males. This difference is also evident from adult longevities (Table I). The overlap in stage survival rate curves is related to variation in the developmental rates among individuals. The age-stage specific fecundity ($f_{x3}$) gives the daily mean number of off-springs produced by adult females (the third stage defined in life history) of age $x$, where the age $x$ is counted from the egg stage (Fig. 2). Age-specific survival rate ($l_x$) is the probability that a newborn survives to age $x$, which is a deviation from the normal trend (Fig. 2). The rapid decrease of survival rate in the adult stage, shown in Fig. 2, is related to insecticide treatments.

The age-specific fecundity ($m_x$) is also plotted in Fig. 2. This curve depicts that reproduction started at age 8, 9, 10 days in control, pirimicarb and thiamethoxam treatments, respectively. The oviposition period indicates significant differences in all treatments (Fig. 2; Table I). The maximal daily oviposition rate in parasitoids treated with insecticides is lower than control treatment (Fig. 2). The parameter age-specific maternity ($l_x m_x$) is also plotted in Fig. 2, which shows periodic peaks in reproduction. The TPOP and the adult pre-oviposition period (APOP) did not differ significantly among treatments (Table I). The significant negative effects of both insecticides can also be observed in the longevity of male and female adults (Table I). The data revealed a dramatically significant decrease in the mean fecundity of parasitoid in both insecticide treatments as compared with control (Table I). The lowest and the highest value were observed in *D. rapae* females treated by thiamethoxam and control, respectively (Table I).

In the age-stage, two-sex life-table procedure, life expectancy ($e_{xj}$) represents the time that an individual of age $x$ and stage $j$ is expected to live. The age-stage life expectancy of female and male parasitoids in thiamethoxam treated cohort was shortest followed by pirimicarb treated cohort (Fig. 3). The reduction is more noticeable in the male individuals. As the exposure to insecticides occurred in adult stage, during pre-adult developmental time, the life expectancy does not differ among all three treatments. Age-stage reproductive value ($v_{xj}$) predicts the contribution of an individual of age $x$ and stage $j$ to the future population. As shown in Fig. 4, both chemicals reduced reproduction.

The means and standard errors of population parameters *D. rapae* in different treatments estimated by using bootstrap method are represented in Table II. The highest intrinsic rate of increase ($r$) (0.2801± 0.0151 days$^{-1}$) of *D. rapae* was related to control treatment and the lowest value related to the thiamethoxam treatment (0.1525± 0.0165 days$^{-1}$) (Table II). There are statistical differences among all three treatments. According to the equation $\lambda = e^r$, the finite rate of increase exhibited the same trend as $r$ (Table II). The mean generation time ($T$) in thiamethoxam was statistically longer than pirimicarb and control (Table II). The net reproductive rate ($R_0$ in thiamethoxam and pirimicarb treatments was significantly lower than control (Table II).

The stable-age distribution showed that the highest trend of population tended to pre-adult stages and the lowest rate of population was seen in the adult stage, in all three treatments. The sex ratio of off-springs in control, pirimicarb and thiamethoxam is female based (0.59: 0.41), (0.53: 0.47) and (0.55: 0.45), respectively. This parameter was not significantly affected by either insecticide, when compared with control (F= 0.65, d.f. = 2, P= 0.52). The values of offspring emergence in control, pirimicarb and thiamethoxam were 0.8± 0.05, 0.77± 0.042 and 0.73± 0.039, respectively. There were no significant differences among all three treatments (F= 0.59, d.f. = 2, P= 0.55).

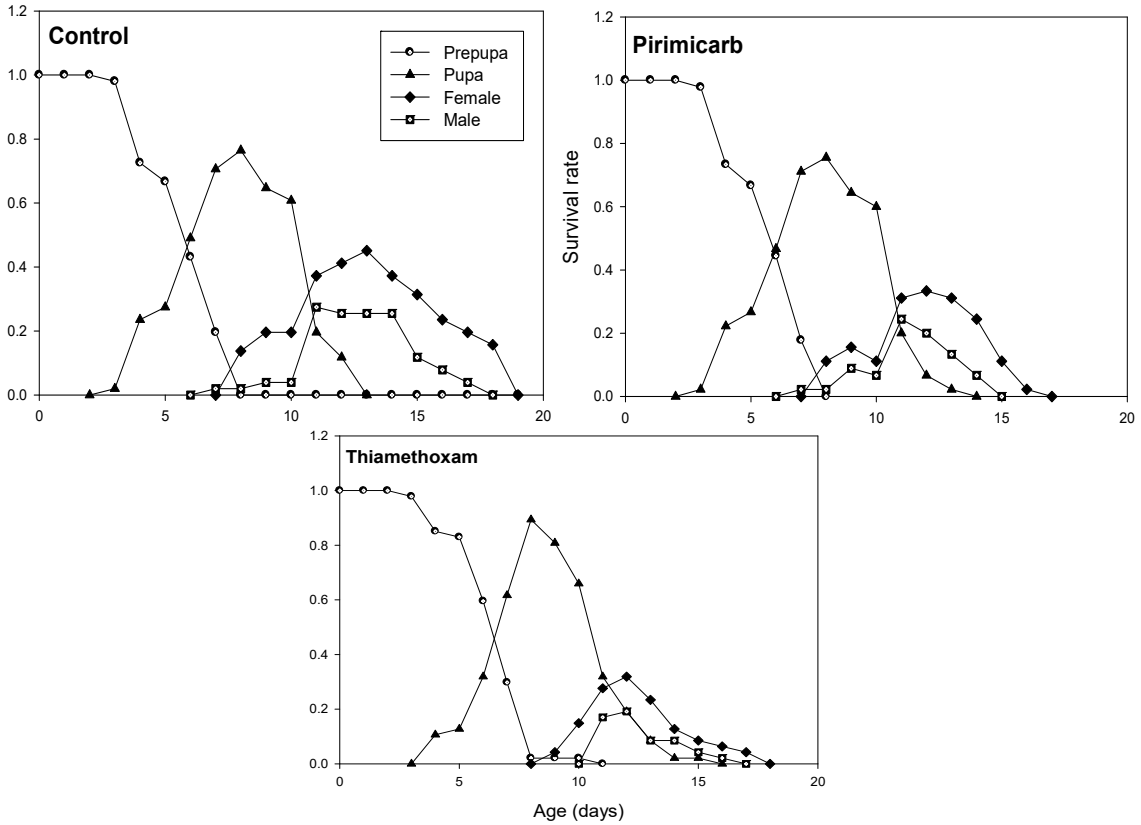


Fig. 1 Age-stage specific survival rate of *D. rapae* exposed to $LC_{25}$ of pirimicarb and thiamethoxam

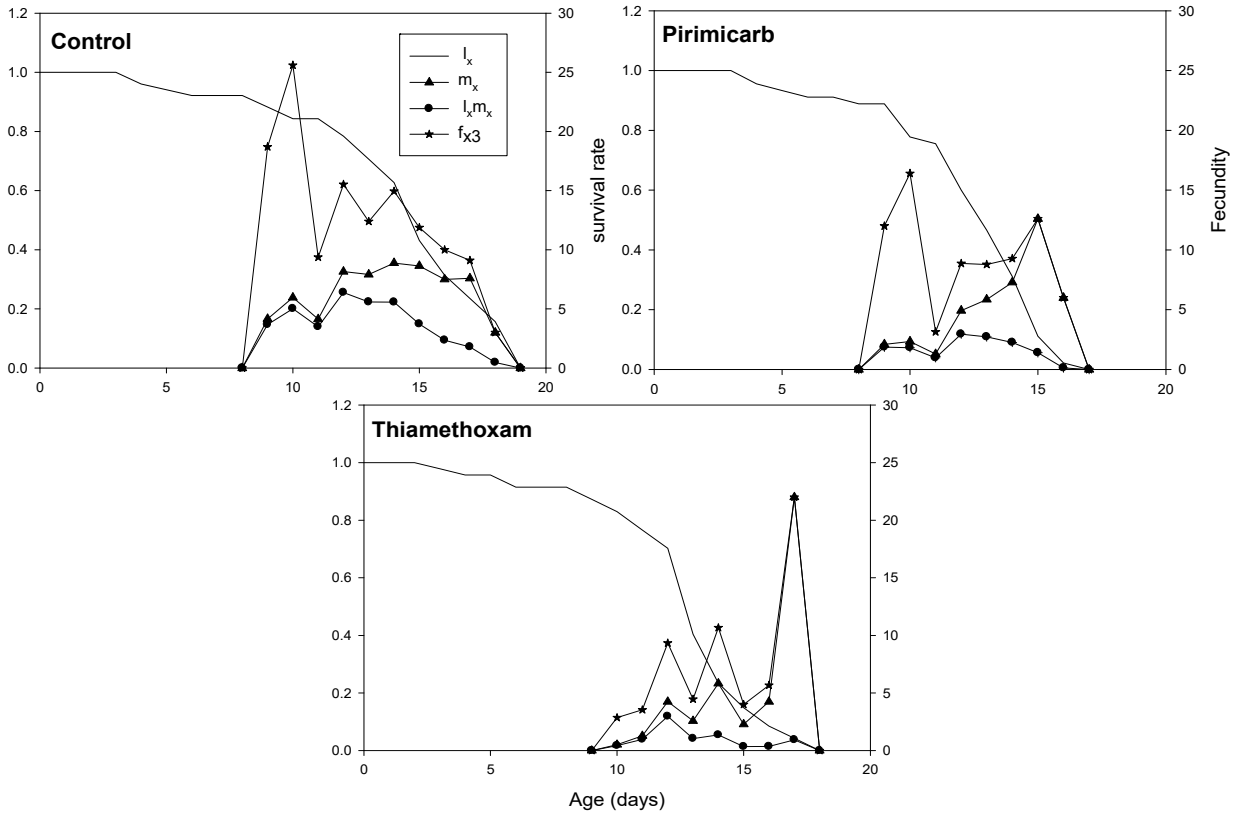


Fig. 2 Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$), maternities ($l_xm_x$) and age-stage specific fecundity ($f_{x3}$) of *D. rapae* exposed to $LC_{25}$ of pirimicarb and thiamethoxam

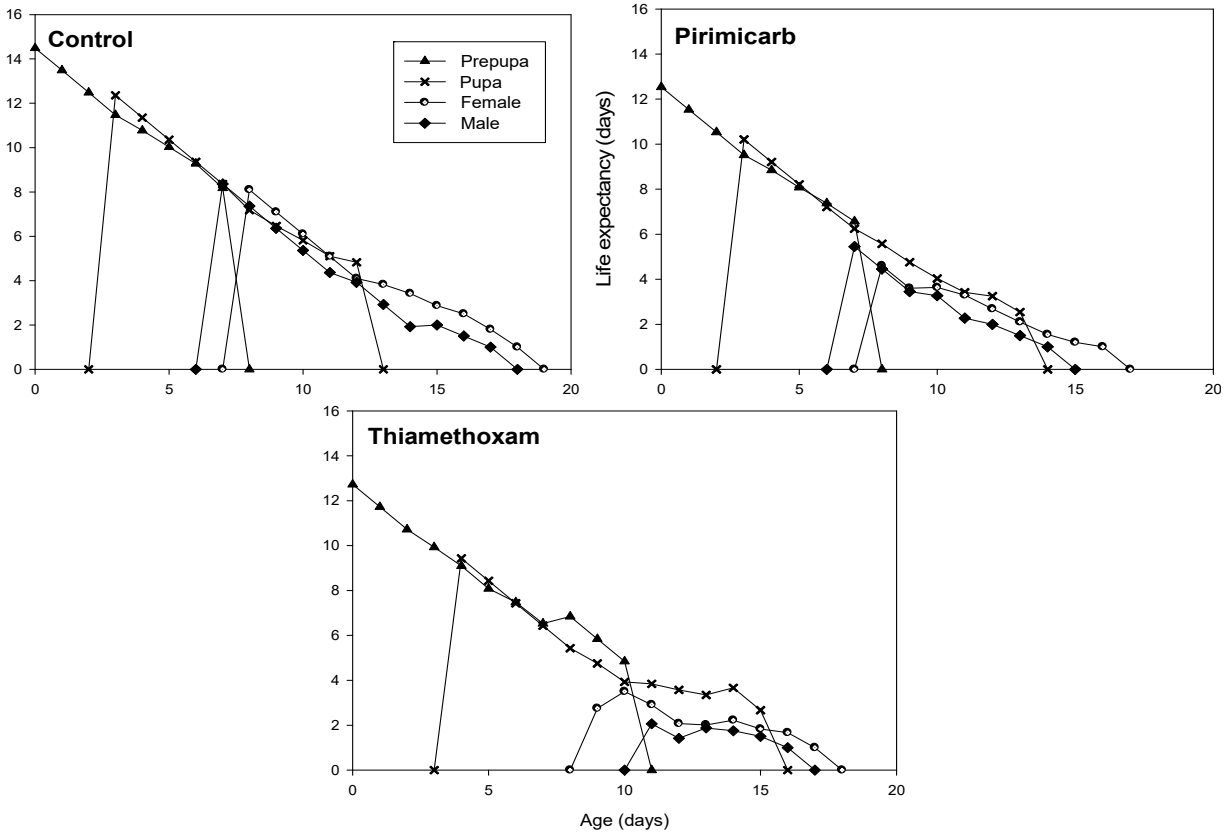


Fig. 3 Age-stage specific life expectancy ($e_{xj}$) of *D. rapae* exposed to $LC_{25}$ of pirimicarb and thiamethoxam

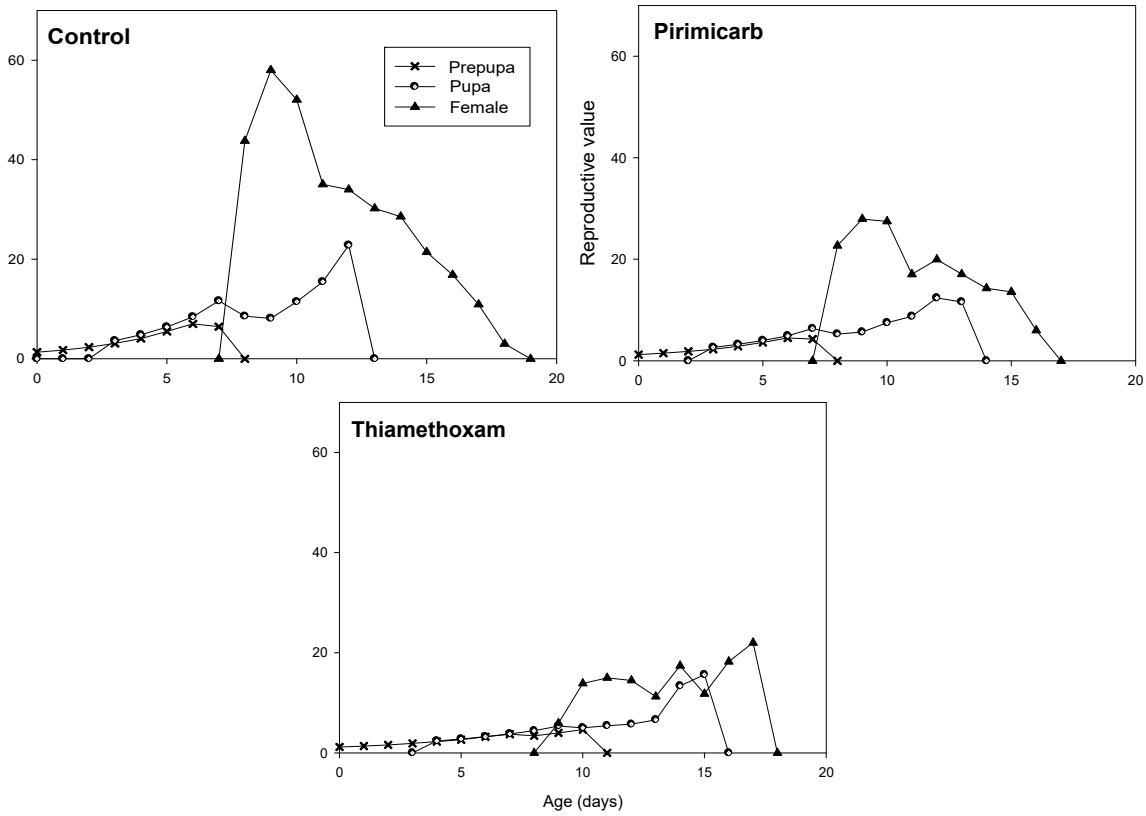


Fig. 4 Age-stage specific reproductive value (vxj) of *D. rapae* exposed to LC25 of pirimicarb and thiamethoxam

TABLE I
ADULT LONGEVITY AND FECUNDITY OF *D. RAPAE* EXPOSED TO $LC_{25}$ OF THIAMETHOXAM AND PIRIMICARB

| | | | Control | | Pirimicarb | | Thiamethoxam | | |
|---|---|---|---|---|---|---|---|---|---|
| Stages and Statistics | n | mean± SE | n | mean± SE | n | mean± SE | F | df | P |
| APOP (days) | 27 | 1.0±0.04a | 23 | 1.0±0.04a | 19 | 1.2±0.1a | 2.6 | 2 | 0.0815 |
| TPOP (days) | 27 | 11.56±0.4a | 23 | 11.6±0.4a | 19 | 12.6±0.4a | 2.2 | 2 | 0.1175 |
| Adult (male) (days) | 15 | 4.7±0.4a | 14 | 2.7±0.3b | 15 | 1.9±0.1b | 28 | 2 | <0.0001 |
| Adult (female) (days) | 27 | 5.5±0.2a | 23 | 3.4±0.2b | 23 | 2.7±0.3b | 43.6 | 2 | <0.0001 |
| Fecundity (egg/♀) | 27 | 71.9±3.3a | 23 | 27.7±2.8b | 19 | 17.2±2.6c | 101.4 | 2 | <0.0001 |
| Oviposition Period (day) | 27 | 4.11±0.0a | 23 | 2.3±0.1b | 19 | 1.7±0.0c | 40.8 | 2 | <0.0001 |

APOP, adult pre-oviposition period; TPOP, total pre-oviposition period (from egg to first oviposition). Means in the same row followed by different letters are significantly different ($p<0.05$) using Paired-bootstrap procedure

TABLE II
POPULATION PARAMETERS PF *D. RAPAE* EXPOSED TO LC25 OF THIAMETHOXAM AND PIRIMICARB

| Population parameters | Control | Pirimicarb | Thiamethoxam |
|---|---|---|---|
| Intrinsic rate of increase ($r$) ( $days^{-1}$) | 0.2801±0.0151a | 0.2064±0.0164b | 0.1525±0.0165c |
| Finite rate of increase ($\lambda$) ( $days^{-1}$) | 1.3234±0.0200a | 1.2294±0.0201b | 1.1645±0.0191c |
| Net reproductive rate ($R_0$) (offspring) | 38.0551±5.3537a | 14.1304±2.4483b | 8.4019±1.7655b |
| Mean generation time ($T$) (days) | 12.972±0.41a | 12.772±0.419a | 13.82±0.456b |

Means in the same row followed by different letters are significantly different ($p<0.05$) using Paired-bootstrap procedure

## IV. DISCUSSION

Direct contact with toxicant chemicals is corroborated to cause the greatest effects on natural enemies in short term sub-lethal impacts [49]. According to [50], the life-table assay provides more detailed information about the adverse effects of pesticides in comparison with the "Total Effect Index" or "E" method proposed by [51]. The present results show that the acute lethal concentration ($LC_{25}$) of pirimicarb and thiamethoxam caused different effects on life-table parameters of *D. rapae*. Previous studies have shown the susceptibility of *D. rapae* to pirimicarb and thiamethoxam [31]-[36], [52]. The obtained results clarify that thiamethoxam have negative effects on *D. rapae* population parameters, mean fecundity, oviposition period and adult longevity. The same results also were observed in pirimicarb treatment unless in $T$ (generation time) parameter which did not have statistical differences compared with control treatment. Moreover, the toxic effects of thiamethoxam on $r$, $\lambda$ and mean fecundity parameters are higher than pirimicarb.

There were no significant differences in TPOP, sex ratio and survivorship of offspring among all three treatments. Other authors such as [32] found the detrimental effects of these insecticides on natural enemies. They reported that thiamethoxam and pirimicarb scored the highest efficacy against *D. rapae* followed by imidacloprid and the natural oil of jojoba plant. According to [31], observations confirm these findings which indicated that acute toxicity of thiamethoxam is higher than pirimicarb. Additionally, in other experiments the high acute toxicity and adverse effects of thiamethoxam on behavior and life-table parameters of other non-target organisms have been reported [53]-[56].

According to [33], permethrin and malathion are more effective than pirimicarb to reverse the stereotypic upward foraging pattern of *D. rapae*. Furthermore, these results are matched with those obtained by [35] who reported the reduction of population parameters of *D. rapae* exposed to pirimicarb, imidacloprid, dimethoate and pymetrozine. Reference [52] found that dimethoate decreased the oviposition behavior of *D. rapae* by a repellent effect, whereas pirimicarb changed the sex ratio by affecting male sterility or mating behavior. However, in the case of sex ratio, their results contradict those obtained in the present study that can be related to the differences in laboratory conditions and experimental set-up such as adults' age at exposure time. Abnormality and effects on the viability of spermatozoa which interrupt the production of diploid females, maternal behavioral control of primary sex ratio and sex-specific mortality during progeny development could involve changing the sex ratio [57], [58].

The repellent effect of pirimicarb and other pesticides on *D. rapae* also have been recorded in other studies [33], [34]. Furthermore, the results of this study are very close to the findings of [59] who reported that residual contact of adult parasitoid *Habrobracon hebetor* Say with insecticides adversely affected the population parameters, but there were no effects on offspring sex ratio. Additionally, in different studies, it is obvious that high rates of mortality are achieved when adults are exposed to dry residue or direct spray of pirimicarb [33], [34], [36], [60]-[62]. However, indirect exposure in the mummy stage or parasitism of contaminated host caused less acute or sub-lethal effects [35], [52], [63], [64].

Based on these results, pirimicarb and thiamethoxam are not suitable choices for an IPM program and their application on canola fields should be restricted to periods when this parasitoid is not active or when they are mostly in the mummy stage. However, even the survived wasps may come in contact with insecticide residues after emergence from the sprayed mummies. This exposure can cause biological and behavioral impairments in adults. Thus, it is necessary to consider the feasibility of these pesticides' timing in future studies. Moreover, to assess the potential sub-lethal effects of these insecticides completely, it is necessary to conduct further studies in more realistic and semi-field techniques.

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