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Research Article

Sublethal effects of some biorational pesticides on population growth parameters of cabbage aphid, *Brevicoryne brassicae*

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Abstract: The cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae), is an important pest of canola that can considerably limit crop production through direct or indirect damage. In this study, the effects of Azadirachtin, Tondexir, Palizin, and Thiamethoxam, common insecticides used in managing the cabbage aphid, were investigated on the population growth parameters of this pest. Based on the results, the LC_{50} values for Thiamethoxam, Palizin, Azadirachtin, and Tondexir were 0.19, 0.63, 1.00, and 3.82 g/L, respectively. Moreover, LC_{30} concentrations of Thiamethoxam, Palizin, Azadirachtin, and Tondexir were 0.11, 0.10, 0.41, and 1.68 g/L, respectively, applied in the sublethal studies. The cabbage aphids reared on the plants treated with these insecticides had lower longevity, fecundity, and reproductive period than the control treatment. The intrinsic rate of increase (r_m), finite rate of increase (λ), net reproductive rate (R_0), and generation time (T) were lower on Thiamethoxam treatment. Tondexir and Palizin treatments had lower values than Azadirachtin. However, there was no significant difference between the Tondexir and Palizin treatments**.** The population treated by Azadirachtin had the highest values of growth parameters. According to the results and available information in the context of risk assessment of the studied insecticides, this research recommends the application of Thiamethoxam in the integrated pest management of cabbage aphid.

Keywords: Demographic toxicology, Life table, Botanical insecticides, Neonicotinoids, Integrated pest management

Introduction

The cabbage aphid, *Brevicoryne brassicae* L. (Hem: Aphididae), is native to Europe and has a worldwide distribution (Rivnai, 1962). The cabbage aphid is one of the most important pests of plants belonging to Brassicaceae, and is generally distributed in all regions of Iran (Farahbakhsh, 1961). It attacks leaves, stems, and flowers and in case of severe infestations can

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cause young plant death (Farahbakhsh, 1961). The cabbage aphid also interrupts photosynthesis through its honeydew secretion and creates an environment for the growth of black mold fungus (Anwar and Shafique, 1999; Ellis *et al*., 2000).

Despite having many natural enemies, the cabbage aphid is not completely controlled by them due to its rapid reproduction (Van Emden and Harrington, 2007). Currently, chemical pesticides are often used to control this pest. Unfortunately, natural insect enemies, e.g., parasitoids and predators, are most susceptible to insecticides and are severely affected. Moreover, pesticide application has adverse effects, such as toxic residues on agricultural products, aphid resistance, and environmental pollution (Van Lenteren, 2003). These concerns have led to increased restrictions on the use of pesticides (Wright and Welbourn, 2002).

Growing attention has been given to using environmentally friendly insecticides in the recent decade. The primary active ingredient in neem trees, Azadirachtin, is a potent antifeedant and insect development disruptor with deficient residual power and toxicity to biocontrol agents (Kilani-Morakchi *et al*., 2021). Thiamethoxam, marketed as Actara® for foliar and soil treatment, is the first commercially available second-generation neonicotinoid. Low use rates, flexible application methods, excellent efficacy, and a favorable safety profile make it well-suited for integrated pest management programs (Maienfisch *et al*., 2001).

In addition to investigating the direct or acute effect of insecticides, which is determined by median lethal dose (LD_{50}) and median lethal concentration (LC_{50}) indices, sublethal concentrations of insecticides can chronically affect the insect population. Although the impact of insecticides on insect populations is generally estimated in laboratory conditions, based on LD_{50} and LC_{50} indices, this method estimates only by the lethal effect indices. Therefore, demographic toxicology studies, or the effect of toxins on the life table parameters of insects, are recommended, which provide more accurate information (Robertson *et al*., 1992). Demographic toxicology is an ecotoxicological technique that incorporates life-table parameters in the context of toxicology (Stark and Wennergren, 1995). Life-table parameters of populations exposed to various pollutant concentrations are predicted under laboratory conditions. Since ecological and toxicological parameters are combined, the general assumption is that predictions of the effects of pollutants at the population level can be made (Stark and Wennergren, 1995).

We need to know more about the typical insecticides used for cabbage aphid. Some non-chemical insecticides, such as Azadirachtin, Palizin, and Tondexir, are commonly used, formally or informally, on cabbage aphid. Therefore, the objective of this study was to evaluate their sublethal effects and compare them with a well-known chemical insecticide, Thiamethoxam, on population growth rates parameters of cabbage aphid on rapeseed plants.

Materials and Methods

A cabbage aphid population was collected from Kerman Province, Iran canola fields. The collected population was reared on canola seedlings, var. Nepton in the laboratory at 20 ± 2 ºC, 60 ± 10% RH, and a 16:8 h L:D regimen photoperiod. This colony was used as a source for all laboratory experiments. The 4-5 leaf stage plants were used in acute and chronic toxicity tests (Lashkari *et al*., 2007).

The following formulations were used: Azadirachtin (Neem Azal®, EC 1%, formulated by Trifolio-M.), Thiamethoxam (Actara®, 25% WG, Syngenta Inc), Tondexir (Tondexir®, EC 80%, Kimia Sabzavar Co.) and Palizin (Palizin®, SC 65%, Kimia Sabzavar Co.).

To estimate LC_{50} and LC_{30} values, the bioassay tests were done with the leaf-deep method in the 4-5 leaves stage of the canola (Lashkari *et al*., 2007). Canola leaves containing 1st instar nymphs of aphids were dipped for 5 s in the insecticide dilutions of the formulated compounds or deionized water as control. Then, allow it to air dry for 30 min. Four replicates of at least 20 aphids in leaf clip-on cages were used for each insecticide concentration, and five serial concentrations were used for each insecticide. Mortality was assessed after 48 h. The experiments were conducted at 20 ± 2 ºC, 60% ± 5% RH, and a photoperiod of 16: 8 h L: D.

The life history parameters of cabbage aphids were predicted with LC_{30} concentrations of commercial formulations of the insecticides. The experiments also were carried out using clip cages. Adult apterous aphids individually were placed on each clip cage. A total of 30 clip cages were used in each treatment. After 24 h, adult aphids and all nymphs except one were removed. The treatments were done the same way as determining the lethal dose. Each cage was monitored daily until the maturity of the aphids to determine the nymphal developmental time and survival rate of *B. brassicae* for each treatment. After maturity, daily observations were followed until each female aphid died. The number of nymphs produced per female aphid was recorded daily, and then nymphs were removed entirely from the cages. The obtained data was used to determine the population growth parameters.

The LC_{50} and LC_{30} were determined by Probit analysis, POLO-PC. The raw life history data were analyzed using the TWOSEX-MSChart program (Chi, 2019). 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), age-specific maternity ($l_x m_x$), age-stage life expectancy (e_x) and reproductive value (v_{xj}) were evaluated from the daily records of the survival and fecundity of all individuals. Moreover, the fertility life table parameters, including the intrinsic rate of natural increase (r_m), net reproductive rate (R_0), finite rate of increase (λ), and mean generation time (T), were estimated. The means and standard errors of life table parameters were determined using the bootstrap technique (Huang and Chi, 2012) with 100,000 resamplings in the TWOSEX-MSChart program. The paired bootstrap test was used to evaluate the differences between treatments.

Results

The bioassay tests showed LC_{50} and LC_{30} values with 95% confidence limits for the first instar nymphs of cabbage aphids 48 h after treatment (Table 1). Based on the results (LC_{50}), more toxicity was observed in the treatments of Thiamethoxam, Palizin, Azadirachtin, and Tondexir, respectively. Thiamethoxam was 3.26, 5.15, and 19.73 times more toxic than Palizin, Azadirachtin, and Tondexir, respectively.

The results of biological parameters of cabbage aphid exposed to the sublethal concentration of insecticides showed that the pre-adult period, mean longevity, fecundity, and reproduction period were significantly different between the treated populations ($P < 0.01$) (Table 2). There was no significant difference in the pre-adult period among the three treatments: Azadirachtin, Palizin, and Tondexir. However, tested treatments significantly influenced this aphid's longevity, reproductive period, and fecundity (Table 2).

Table 1 Susceptibility of the first instar nymphs of cabbage aphids to treatments of Azadirachtin, Thiamethoxam, Tondexir, and Palizin.

Insecticide	n	Slope ± SE	LC_{50} (g/L) 95% C. L.	LC_{30} (g/L) 95% C. L.	df	Heterogeneity	x^2
Thiamethoxam	480	2.45 ± 0.335	0.194 (0.057-0.613)	0.119 (0.013-0.158)	3	0.995	0.995
Azadirachtin	480	1.369 ± 0.21	1.00056 (0.910-1.106)	0.414 (0.341-0.531)	3	0.00837	4.35
Tondexir	480	1.708 ± 0.466	3.829 (2.349-4.404)	1.6826 (1.594-3.256)	3	0.0724	7.24
Palizin	480	5.13 ± 0.173	0.634 (0.0289-8.724)	0.1068 (0.0123-0.1711)	3	1.2489	1.115

C. L., confidence limits.

Values of R_0, rm, λ, GRR, and T on Control were higher than those exposed to sublethal concentrations ($P < 0.01$). There was also a significant difference among the biological parameters of the treated populations, as the parameters were higher in Azadirachtin and lower in Thiamethoxam insecticides ($P < 0.01$) (Table 3). There was no significant difference between the remaining insecticides, Palizin and Tondexir.

The age-stage specific survival rate (s_{xj}) curve showed a relatively similar pattern in the different treatments, as the average adult emergence in control, Azadirachtin, Tondexir, Palizin, and Thiamethoxam treatments occurred on 6.25, 6.06, 6.04, 5.92 and 5.7 days, respectively. The probability that a nymph would survive to the adult stage was 90, 67.67, 66.33, 66.24, and 59.74% for control, Azadirachtin, Palizin, Tondexir, and Thiamethoxam, respectively (Fig. 1).

The l_x curve indicated survival declined in the pre-adult period after days 2-3 in all treatments except for Thiamethoxam, which declined after day 1. Then, the curve slowly fell in the adult period until the death of the last adult. Death of the last female under treatments of Control, Azadirachtin, Tondexir, Palizin, and Thiamethoxam occurred on days 25, 20, 20, 19, and 17, respectively (Fig. 2). The age-specific fecundity (m_x) and the age-specific maternity (l_xm_x) are also shown in Fig. 2. The highest peaks of mx and l_xm_x were 10.13 on control (day 14), 10.82 on Azadirachtin (day 12), 6.33 on Tondexir (day 11) and 6.19 on Palizin (day 11) and 5.11 on Thiamethoxam (day 11) (Fig. 2).

The reproductive value (v_{xj}) gives the expected contribution of individuals of age x and stage j (Fig. 3). At age 0, the reproductive values (v_{01}) were 41.94 (at day 11), 38.43 (at day 11), 25.12 (at day 10), 24.83 (at day 10) and 18.38 (at day 10) for control, Azadirachtin, Tondexir, Palizin and Thiamethoxam, respectively.

The life expectancies (e_x) curve at age 0 (e_{01}) were 13.98, 9.97, 9.33, 9.18, and 7.23 days in treatments of Control, Azadirachtin, Tondexir, Palizin, and Thiamethoxam insecticides, respectively (Fig. 4).

Table 2 Pre-adult period, longevity, fecundity, and reproduction period of cabbage aphid, *Brevicoryne brassicae*, populations exposed to Azadirachtin, Tondexir, Palizin, and Thiamethoxam.

Treatment	Pre-adult period (day)	Adult longevity (day)	Fecundity (off spring/female/day)	Reproduction period (day)
Control	6.25 ± 0.11^a	24.49 ± 0.94^a	107.20 ± 6.55^a	13.36 ± 0.12^a
Azadirachtin	6.06 ± 0.11^c	19.18 ± 0.68^b	63.80 ± 2.64^b	8.31 ± 0.12^b
Palizin	5.92 ± 0.11^c	18.02 ± 0.65^c	41.52 ± 2.15^c	8.00 ± 0.12^c
Tondexir	6.04 ± 0.12^c	18.58 ± 0.65^c	41.67 ± 2.01^c	7.91 ± 0.13^c
Thiamethoxam	5.70 ± 0.11^d	15.27 ± 0.5^d	25.12 ± 1.15^d	5.75 ± 0.15^d

Means in a column followed by the same letter are not statistically different (Paired bootstrap test, $P<0.01$).

Table 3 Estimating biological parameters (Mean ± SE) of cabbage aphid populations exposed to Azadirachtin, Tondexir, Palizin, and Thiamethoxam.

Treatment	Intrinsic rate of increase (rm) (day^{-1})	Net rate of reproduction (R_0) (off springs)	Finite rate of increase (λ) (day^{-1})	Gross reproductive rate (GRR) (off springs)	Generation time (T) (day)
Control	0.259 ± 0.01^a	43.97 ± 5.94^a	1.295 ± 0.013^a	100.29 ± 7.55^a	14.6 ± 0.18^a
Azadirachtin	0.236 ± 0.01^b	22.33 ± 3.3^b	1.266 ± 0.015^b	61.58 ± 4.09^b	13.25 ± 0.15^b
Palizin	0.197 ± 0.01^c	13.70 ± 2.02^c	1.218 ± 0.014^c	41.63 ± 2.91^c	13.16 ± 0.21^c
Tondexir	0.199 ± 0.01^c	13.75 ± 2.02^c	1.220 ± 0.015^c	41.15 ± 2.8^c	13.16 ± 0.2^c
Thiamethoxam	0.146 ± 0.03^d	6.03 ± 1.17^d	1.157 ± 0.018^d	25.89 ± 2.79^d	12.28 ± 0.17^d

Means in a column followed by the same letter are not statistically different (Paired bootstrap test, $P < 0.01$).

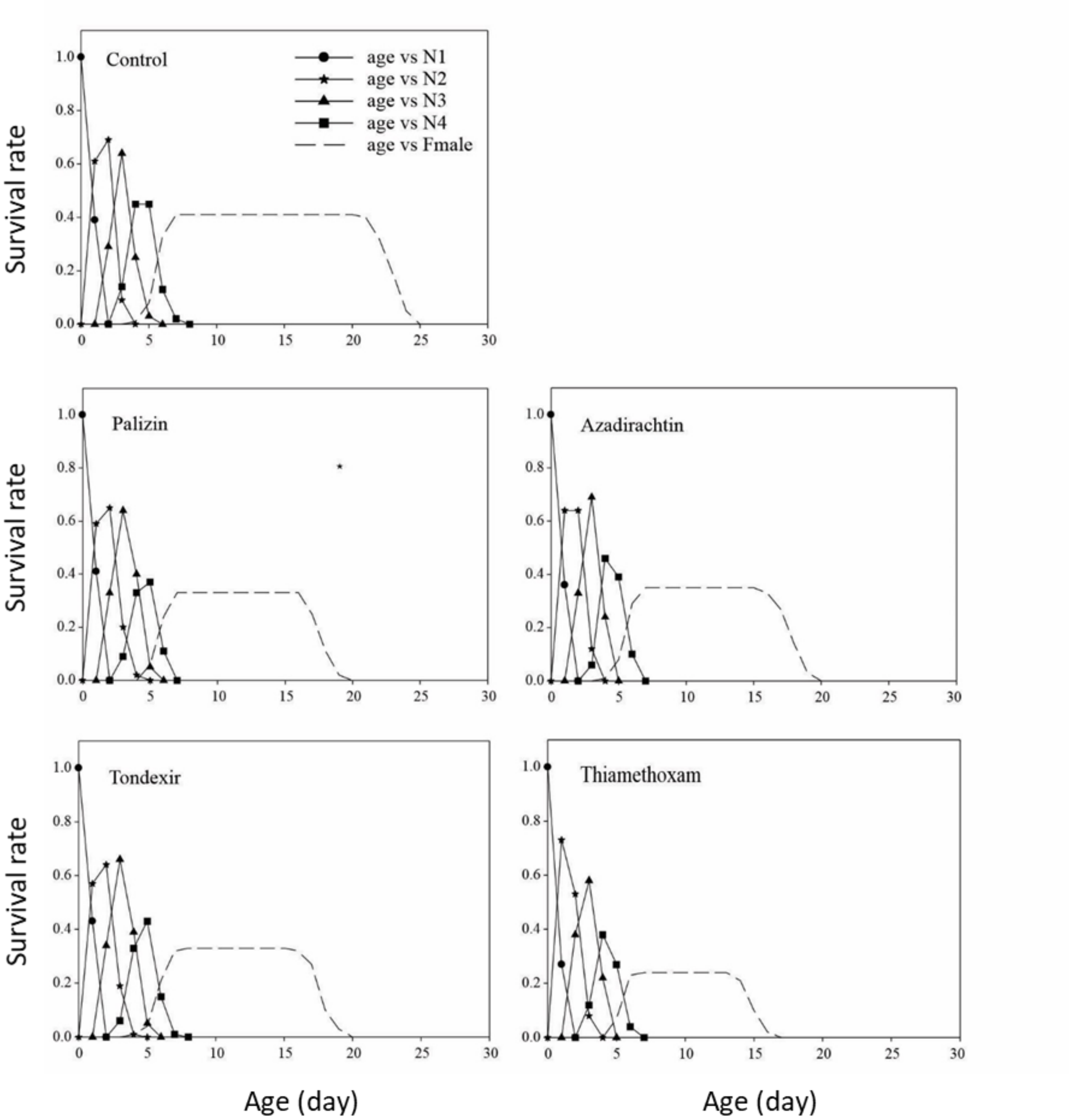

Figure 1 Age-stage specific survival rate (s_{xj}) of cabbage aphid, *Brevicoryne brassicae*, on canola plants treated with Azadirachtin, Tondexir, Palizin, and Thiamethoxam.

Discussion

Our results showed that the sublethal concentrations of applied insecticides in cabbage aphid control caused significant effects on survival, reproduction, and longevity. In a similar study, Romasi *et al*. (2021) investigated the impact of Tondexir and Palizin on the life table parameters of the cabbage aphid population from Khorasan Provinces on canola (Romasi *et al*., 2021). They showed that the life table parameter of treated aphids was negatively affected, as the intrinsic rate of population increase was 0.27, 0.21, and 0.2 per day for control, Palizin, and Tondexir, respectively (Romasi *et al*., 2021). Our results are near their findings. The sublethal concentration (LC_{25}) of Palizin on *Aphis gossypii* Glover significantly reduced the biological indicators, such as intrinsic rate of population increase, net rate of reproduction, and finite rate of population increase (Amini Jam, 2013). Moreover, using Palizin (in 2000 ppm) has been effective on a population of *A. gossypii*. Still, because of the possibility of plant toxicity, it should not be used at temperatures above 30 ºC (Baniameri, 2006).

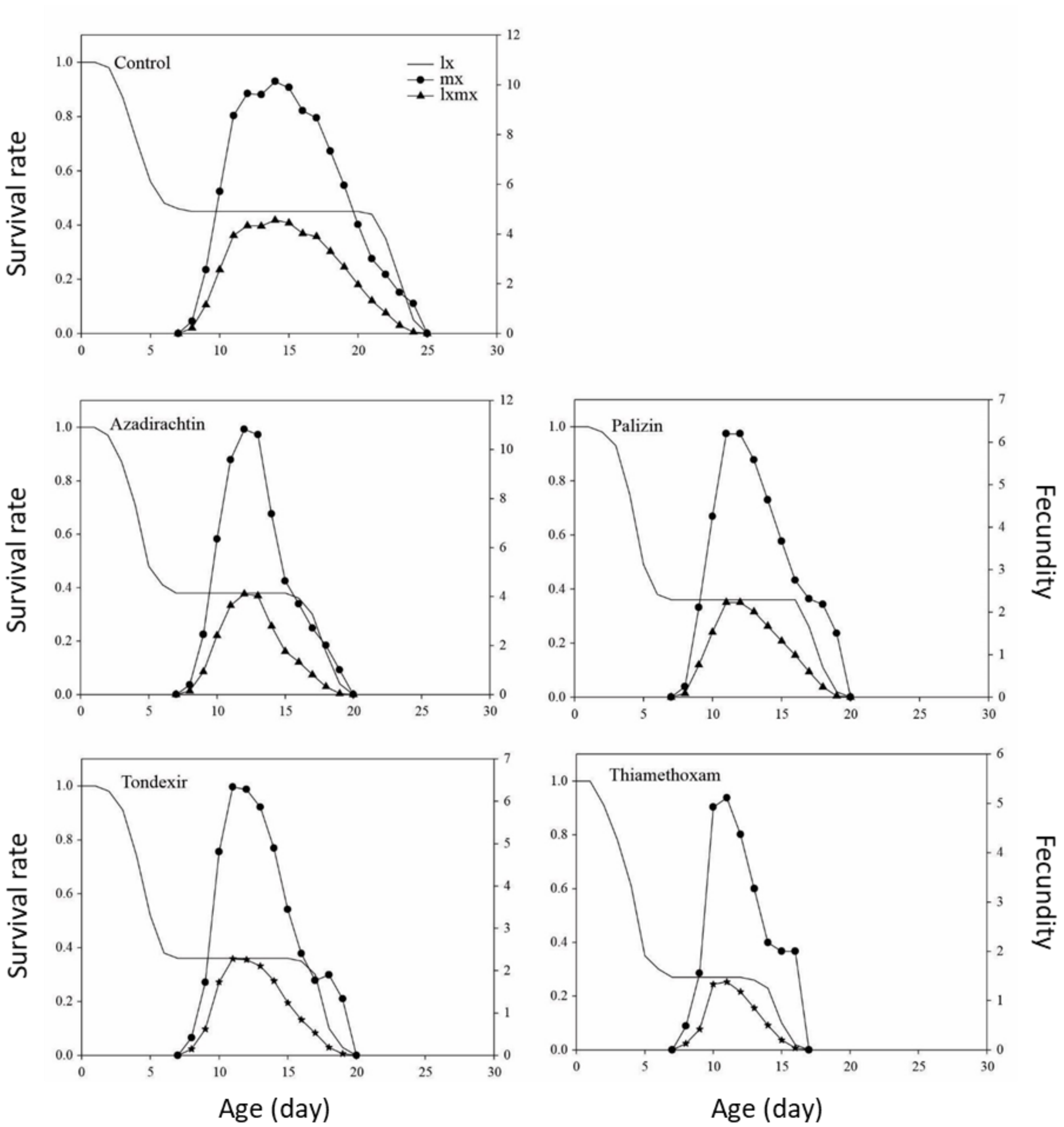

Figure 2 Age-specific survival rate (l_x), age-specific fecundity (m_x), and age-specific maternity (l_xm_x) of cabbage aphid, *Brevicoryne brassicae*, on canola plants treated with Azadirachtin, Tondexir, Palizin and Thiamethoxam.

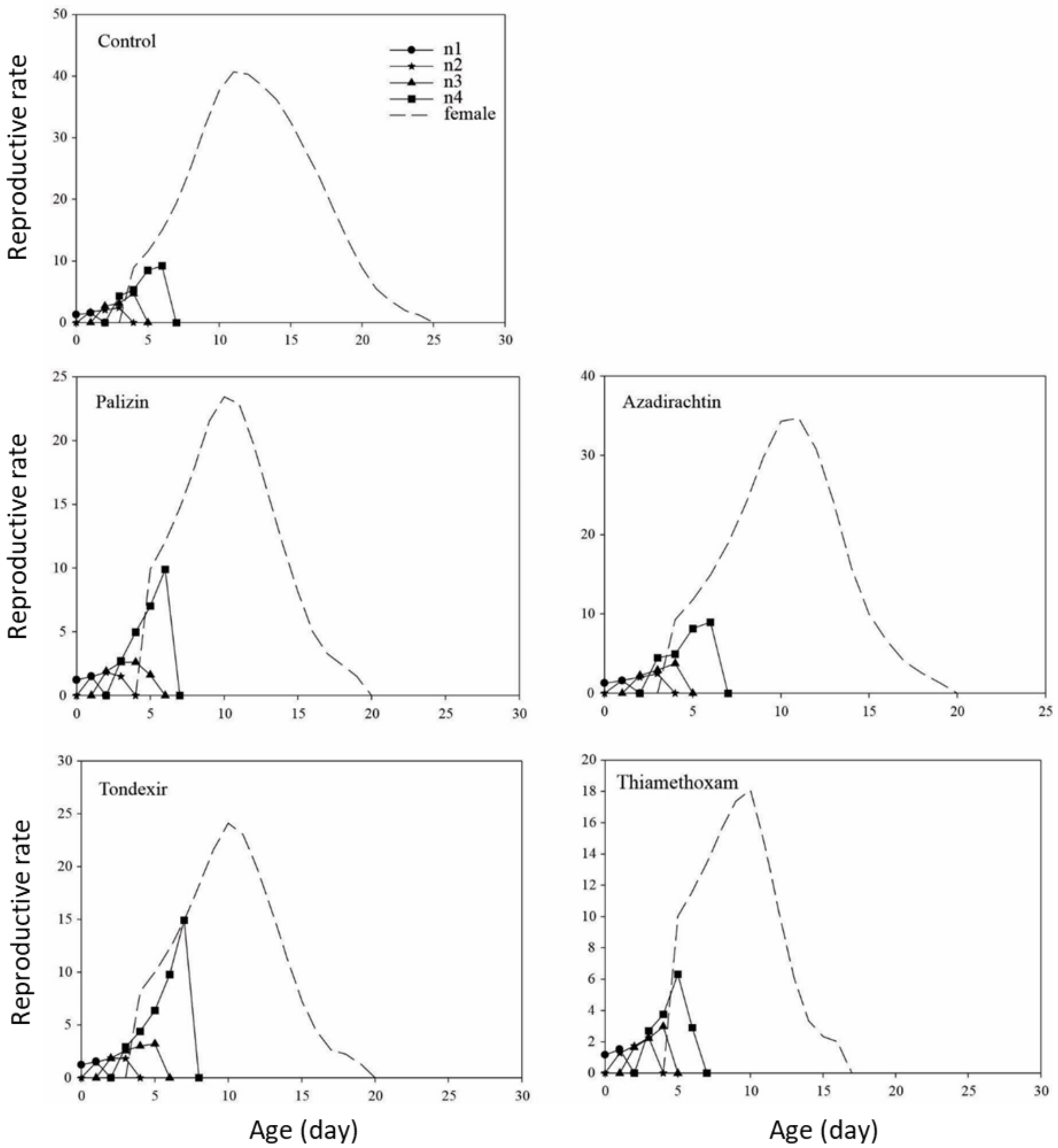

Figure 3 Age-stage reproductive value (V_{xj}) of cabbage aphid, *Brevicoryne brassicae*, on canola plants treated with Azadirachtin, Tondexir, Palizin and Thiamethoxam.

In other studies on non-chemical insecticides, the sublethal concentrations of bergamot, *Laurus nobilis*, and eucalyptus, *Eucalyptus camaldulensis*, extracts (Hosseini Amin *et al*. 2013), Galbanum, *Ferula gummosa*, and Cumin, *Cuminum cyminum*, extracts (Kiani, 2015) have significantly decreased the lifespan parameters of cabbage aphid populations.

In a study, the effect of Thiamethoxam, Thiacloprid, and the insect pathogenic fungus, *Lecanicillium longisporum*, was investigated on cabbage aphid in greenhouse conditions. The results showed that the simultaneous use of sublethal concentrations of the insecticides with the pathogenic fungus has greater effects in reducing the biological parameters of cabbage aphids (Taheri, 2013).

The effective blending of biological and chemical control in an IPM program also depends on understanding how pesticides affect natural enemies. Little research has been done on the sublethal effects of pesticides on natural enemies, although many studies have been done on the lethal effects of pesticides on natural enemies. Studies on the sublethal effects of insecticides on natural enemies can provide important information about the compatibility of chemical and biological control as well as the effect of insecticides on natural enemies. Amini Jam (2017) studied the sublethal effect of palizin on *Aphis fabae* Scopoli and the functional response of the parasitoid wasp, *Lysiphlebus fabarum* (Marshall).

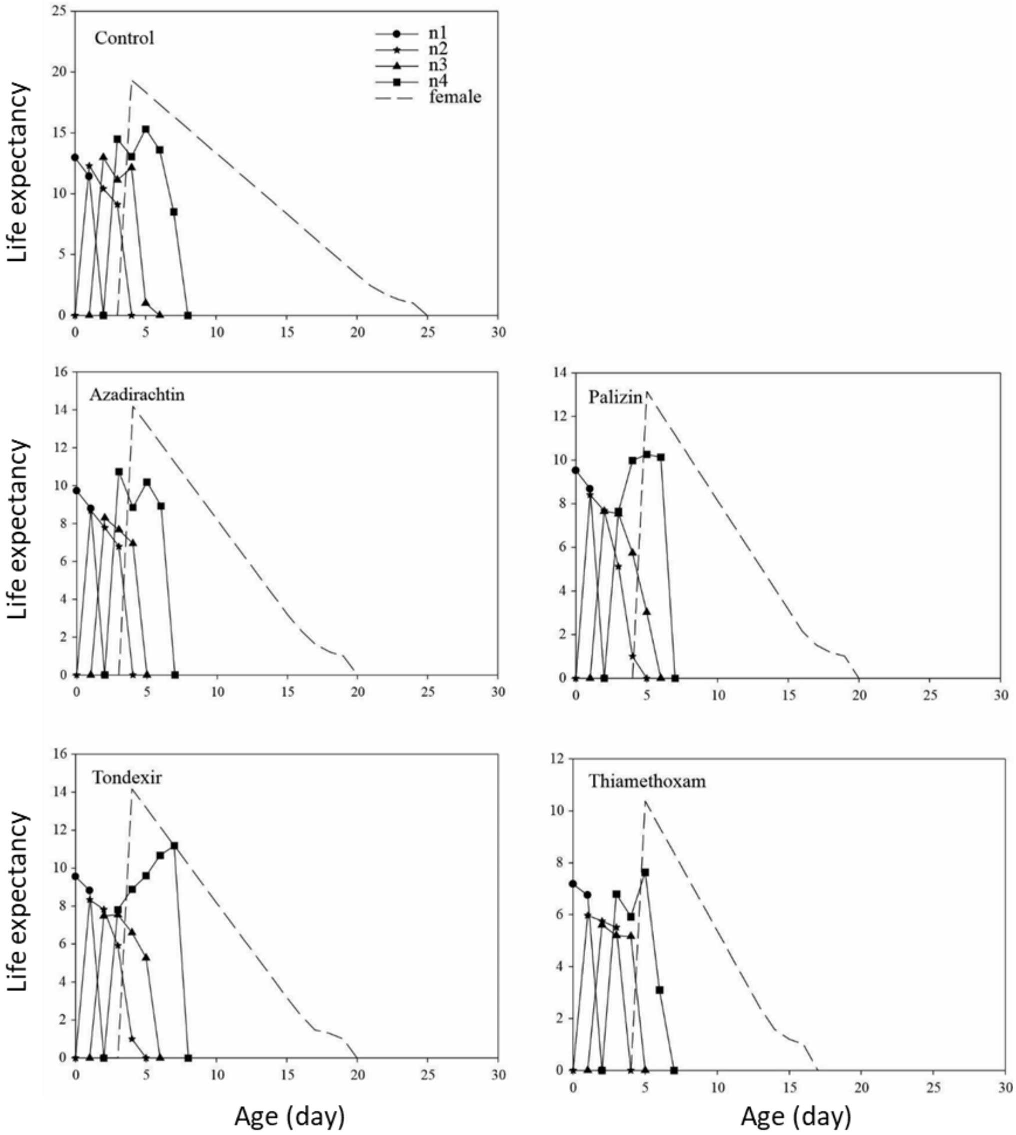

Figure 4 Age-stage specific life expectancy (e_{xj}) of cabbage aphid, *Brevicoryne brassicae*, on canola plants treated with Azadirachtin, Tondexir, Palizin, and Thiamethoxam.

Their results revealed no differences in the functional response parameters between the treated and untreated populations. They suggested that Palizin is a suitable candidate for controlling *A. fabae* in combination with *L. fabarum* (Amini Jam, 2017). Moreover, Abedi *et al.* (2012) investigated the lethal and sublethal effects of two commercial formulations of Azadirachtin on *Hebrobracon hebetor* (Say). They reported a reduction in the lifespan of the insects (Abedi *et al*., 2012).

Conclusion

It is well known that pesticides can affect not only the target insects but also non-target organisms, the environment, and humans by pathways including wind, rainfall, surface runoff, and treated crops. Thus, pesticide residue analysis and the risk assessment of pesticides are necessary for not only IPM but also for environment and food safety. According to the obtained results and the literature review on the risk assessment of the studied insecticides, this study recommends Thiamethoxam for use in IPM of the studied pest. The results of this study cannot recommend using Tondexir in the IPM of the studied pest because there is no available confirmed evidence on its risk assessment. Despite informal use by farmers, it is not registered by the responsible organization in Iran (i.e., Plant Protection Organization). Finally, it is suggested to 1) conduct risk assessment studies of Tondexir, such as its effect on non-target insects, environments, human health, and Phytotoxicity, and 2) combine sub-lethal concentrations of the recommended insecticides with entomopathogenic fungi.

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تأثیر زیرکشنده برخی آفت‌کش‌های کم‌خطر بر پارامترهای رشد جمعیت شته مومی کلم، *Brevicoryne brassicae*

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چکیده: شته مومی کلم، *Brevicoryne brassicae* L.، یکی از آفات مهم کلزا است که باعث کاهش قابل توجه عملکرد محصول از طریق خسارت مستقیم و غیرمستقیم می‌شود. در این تحقیق، تأثیر حشره‌کش‌های آزادیراختین، تنداکسیر، پالیزین و تیامتوکسام (به‌عنوان حشره‌کش‌های رایج در مدیریت کنترل این آفت) بر پارامترهای رشد جمعیت شته مومی کلم بررسی شد. براساس نتایج، غلظت زیرکشنده (LC_{50}) برای حشره‌کش‌های تیامتوکسام، پالیزین، آزادیراختین و تنداکسیر، به‌ترتیب ۰/۱۹، ۰/۶۳، ۱/۰۰ و ۳/۸۲ (گرم بر لیتر) برآورد شد. به‌علاوه، غلظت زیرکشنده (LC_{30}) برای حشره‌کش‌های تیامتوکسام، پالیزین، آزادیراختین و تنداکسیر، به‌ترتیب ۰/۱۱، ۰/۱۰، ۰/۴۱ و ۱/۶۸ (گرم بر لیتر) برآورد شد. براساس نتایج، شته‌های پرورش یافته روی گیاهان تیمار شده با حشره‌کش‌های ذکر شده دارای طول عمر، باروری و دوره پوره‌زایی کم‌تری در مقایسه با شاهد بودند. نرخ ذاتی افزایش جمعیت (r_m)، نرخ متناهی افزایش جمعیت (λ)، نرخ خالص تولیدمثل (R_0) و میانگین مدت زمان یک نسل (T) در تیمار تیامتوکسام کم‌تر بود. بعد از آن، تیمارهای تنداکسیر و پالیزین دارای کم‌ترین مقدار بودند و بین این دو تیمار تفاوت معنی‌داری مشاهده نشد. جمعیت تیمار شده با حشره‌کش آزادیراختین دارای بیش‌ترین مقدار پارامترهای رشدی بود. براساس نتایج و اطلاعات موجود در زمینه ارزیابی ریسک حشره‌کش‌های مورد مطالعه، این تحقیق حشره‌کش تیامتوکسام را جهت استفاده در برنامه مدیریت شته مومی کلم توصیه می‌کند.

واژگان کلیدی: سم‌شناسی دموگرافیک، جدول زندگی، حشره‌کش‌های گیاهی، نئونیکوتینوئیدها، مدیریت تلفیقی آفت