



Research Article

Efficacy of Dayabon[®], a botanical pesticide, on different life stages of *Myzus persicae* and its biological control agent, *Aphidius matricariae*

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Abstract: The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is one of the most important pests in greenhouses and its damages are economically important. The overuse of chemical pesticides has caused resistance in green peach aphid to many common insecticides. *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae) is an effective biological control agent against aphid populations especially in greenhouse crops. Currently, use of safe and biocompatible botanical pesticides in combination with biological control agents is being recommended. In this study, contact toxicity of Dayabon (SL 10%), a new botanical pesticide, was investigated on different life stages of *M. persicae* at 1000 to 7000ppm. Also, the toxicity was studied on mummies and adult parasitoids at 2000 to 14000ppm. The estimated LC₅₀ on first, second, third, fourth instar nymphs and adults were 3254, 3387, 4194, 3839 and 3508ppm, respectively. However, concentrations up to 5000ppm caused less than 50% mortality in parasitoid mummies. Moreover, the residues of Dayabon did not cause any mortality in *A. matricariae* adults. Based on the standard method of IOBC/WPRS Working Group for side-effects of pesticides on natural enemies of insects, the Dayabon is recognized as safe for *A. matricariae* while it has acceptable mortality on green peach aphid. Therefore, the integration of Dayabon with *A. matricariae* could be recommended in order to achieve better control of *M. persicae* in greenhouse products.

Keywords: green peach aphid, insecticide, parasitoid, Dayabon, LC₅₀

Introduction

Aphids are the most important pests that adversely affect crop yield and quality. There are plenty of agriculturally important species in the subfamily Aphidiinae such as green peach aphid, *Myzus persicae* (Sulzur) (Hemiptera: Aphididae). This species is a destructive pest both in greenhouse and field (Blackman and

Eastop, 2008; Van Emden and Harrington, 2017). The *M. persicae* infestation not only weakens the plant, but also transmits more than 100 viral or phytoplasma diseases that end up with the plant death, if efficient control methods are not applied (Van Emden *et al.*, 1969). At present, farmers use a great quantity of chemical insecticides against *M. persicae*. Overuse of insecticides has led to the development of aphid's resistance (Bass *et al.*, 2014) and decrement of the aphid's natural enemies specially parasitoids and predators. Biological control is a major component in the integrated pest management (IPM) program of

Handling Editor: Azam Mikani

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Received: 22 October 2018, Accepted: 18 December 2018
Published online: 7 January 2019

aphids (van Lenteren and Woets, 1988). There are a large number of reports on the efficacy of aphid natural enemies (Rakhshani *et al.*, 2007, 2012; Talebi *et al.*, 2009).

Aphidius matricariae (Haliday) (Hymenoptera: Braconidae) is an effective biological control agent against aphid populations especially in greenhouse crops that is produced by a number of commercial companies around the world (Zamani *et al.*, 2006; Tahriri *et al.*, 2007). This polyphagous parasitoid species probably originated from northern India or Pakistan but it is now found in various parts of the world such as, North and South America, Australia and Europe. Also, *A. matricariae* was reported from different parts of Iran such as Tehran, Khuzestan, Hamedan, Fars, Kurdistan and Mazandaran. It has been reported that *A. matricariae* has more than 50 aphid species as its hosts (Farahani *et al.*, 2016), however, *M. persicae* has been known as one of the preferred hosts (Tazerouni *et al.*, 2016). To achieve a successful IPM program with the *A. matricariae*, it is necessary to integrate the parasitoid with safe chemical control method in particular, compatible or botanical pesticides (Van Lenteren and Woets, 1988; Rezaei *et al.*, 2014).

Currently, use of safe and biocompatible or botanical pesticides in combination with biological control methods are very noticeable (Ndakidemi *et al.*, 2016). The pesticides and beneficial organisms working group (WPRS) of International Organization for Biological Control (IOBC) has published the standard methods for testing the side-effects of pesticides on natural enemies of insects and mites in laboratory, semi-field and field conditions (Hassan *et al.*, 1985). Also, they have described a laboratory method to evaluate the side effect of pesticides on *Diaeretiella rapae* McIntosh (Aphidiidae: Hymenoptera), a parasitoid of *Brevicoryne brassicae* (L.). Particularly, Polgar (1988) tested 82 pesticides on *A. matricariae*, using a unique glass plate method. *Aphidius matricariae* shows different levels of sensitivity to various kinds of pesticides. In addition, in the literature, some reports have been published about the side effects of chemical and botanical pesticides on different species of the genus

Aphidius (Longley, 1999; Bostanian *et al.*, 2005; D'Ávila *et al.*, 2018). However, little is known about the side effects of Dayabon on *A. matricariae*. Dayabon[®] is a botanical based pesticide consisting of castor oil and salts of fatty acids manufactured by Daya Nanotechnologists Company (Tehran, Iran). The effect of Dayabon on elm leaf beetles, *Xanthogaleruca luteola* (Muller) (Chrysomelidae: Coleoptera) was investigated by Vahabi Mashhour *et al.* (2016). They indicated the possibility of practical use of Dayabon in management of *X. luteola* in urban landscape. Dayabon has a suitable mortality when used in greenhouses to control two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) without any deleterious effects on the predatory mite, *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) (Mortazavi *et al.*, 2016). The toxicity of Dayabon has also been reported in a number of aphid species such as *Brevicoryne brassicae* L. (Rezaian *et al.*, 2015), *Aphis fabae* Scopoli (Amini Jam, 2017) and *Myzus nicotianae* Blackman (Rezaei *et al.*, 2017b).

Due to the deleterious effects of chemical pesticides on non-target organisms, beneficial insects and the environment, the use of botanical pesticides would be a safe and suitable method for pest control. In this study, the contact toxicity of Dayabon was evaluated on different life stages of *M. persicae*. Moreover, the side effects of Dayabon were investigated on potential parasitoid of green peach aphid, *A. matricariae*.

Materials and Methods

Plant and insect rearing protocols

Chinese cabbage, *Brassica pekinensis* cv. Hero, as a host plant, was grown in plastic pots (10 cm diameter) without application of any fertilizer or pesticides under greenhouse condition at 25 ± 5 °C, $65 \pm 5\%$ RH and L: D 16:8 h. Then, laboratory cultures of *M. persicae* were maintained on 5-leaf stage in ventilated cage (50 × 50 × 50cm) and in a constant environmental condition at 25 ± 1 °C, $70 \pm 5\%$

RH and L:D 16:8 h. Then cultures of *A. matricariae* were maintained on *M. persicae* in conditions as mentioned above. Also, cotton ball containing aqueous honey solution (40%) was placed in each rearing cage to feed the adult parasitoids and was renewed every 48 h. The rearing method was adopted from Zamani *et al.* (2006). Population of *A. matricariae* and *M. persicae* were first collected from greenhouses of Varamin County (35° 18' N, 51° 44' E, 969 m), located in Tehran province.

Experimental design

In this study, contact toxicity of Dayabon (SL, castor oil 10%) was investigated on different life stages (1st, 2nd, 3rd, 4th instar nymphs and adult) of *M. persicae*. In order to achieve the uniform aphids in age, apterous females from their stock cultures were transferred individually onto leaf discs of the host plant, placed in translucent ventilated plastic containers (11 × 10 × 4cm), to produce offsprings for 24h. Then, the newly produced nymphs were maintained at standard environmental conditions (25 ± 2 °C; 70 ± 5% RH; 16: 8, L: Dh) to achieve desired life stages. The bioassay was designed to determine both LC₅₀ and LC₉₀ values of 24h post treatments. Based on the preliminary experiments to cause 20 and 80 percent mortality, toxicity tests were conducted at 1000, 2000, 3000, 4000, 5000, 6000 and 7000ppm. The lethal effect of Dayabon was tested by dipping the established aphids on leaf discs (5 cm diameter) for 3 seconds in the pesticide (Rezaei *et al.*, 2017a).

Deleterious effects of Dayabon on the parasitoid wasp, *A. matricariae*, was studied based on the standard methods to test the side-effects of pesticides on natural enemies of insects developed by the IOBC/WPRS Working Group, Pesticides and Beneficial Organisms (Hassan *et al.*, 1985) with some modifications. The methods used in this study are much more rigorous than the IOBC methods. The methods developed by the IOBC/WPRS have been accepted by the EU for use in the registration of pesticides. The toxicity tests on parasitoid were conducted by two methods. In order to obtain

parasitoid mummies and adults, 400 individuals of aphids, established on the host plant, were exposed to 10 mated females of *A. matricariae* (< 1 day) for 24h. Then, the parasitized aphids were fed on the host plants until parasitoid mummies emerged. The mummies (< 1 day) were collected and used in the first part of experiment. Then, some mummies were maintained under standard environmental conditions until adult parasitoids were emerged. The newly emerged adults (< 1 day) were used in the second part of experiment. At first, parasitoid mummies (< 1 day) were dipped into aqueous solution of Dayabon at concentrations ranging from 2000 to 14000ppm for 3 seconds. Then, mummies were checked daily for adult emergence. The experiment was replicated five times per concentration and each replication contained 30 mummies of the parasitoid. In the second experiment, the tubes (1.5cm diameter and 10 cm high) were treated by Dayabon solution at 2000, 3000, 4000, 5000ppm. After drying the tubes for less than 2h, five adults (< 1 day) were introduced into each tube. A cotton ball of honey solution (40%) was placed in the tubes in order to feed the adult parasitoids. Then, the number of dead parasitoid wasps was recorded 24h after treatment. This experiment was replicated 10 times per concentration and each replication contained five adult parasitoids. All experiments were performed at 25 ± 2 °C; 70 ± 5% RH; LD 16: 8h. In addition, the control was treated with water only.

Statistical analysis

Data obtained from dose-response bioassays were subjected to probit analysis in order to estimate LC₅₀ and LC₉₀ values (lethal concentration for 50 and 90% mortality, respectively) (Finney, 1971). Relative median potency (RMP) test was used to compare the significant differences of LC₅₀s between treatments. Also, one-way ANOVA was used to test the effect of different concentrations of Dayabon on the mortality of *A. matricariae*. Then, post hoc pairwise comparisons between concentrations were performed using Tukey's honestly significant difference (HSD) tests at *P*

≤ 0.05 . All statistical analyses were conducted by SPSS version 22.

Results

The lethal concentration to cause 50% mortality (LC_{50}) and their 95% confidence limits on first, second, third and fourth instars, and adult aphids were 3254 (2835-3646), 3387 (2943-3791), 4194 (3980-4393), 3839 (3575-4080) and 3508 (3157-3840)ppm, respectively (Table 1). Moreover, LC_{50} value of Dayabon on parasitoid mummies was 7595.4 (6709.5-8843.4)ppm. Using relative median potency test, it was found that the 3rd instar aphid (4194ppm) was significantly more tolerant than

the other instars and adult aphids. Other instars and adult aphids were not significantly different to each other (Table 2). However, mummies was significantly more tolerant than the most tolerant aphid stage (3rd instar nymph) (Table 2). Probit lines also exhibited a distinct distance between aphid stages and mummies (Fig. 1). The toxicity values of Dayabon on 1-day-old parasitoid mummies are shown in Fig. 2. The minimum and maximum percent mortality of *A. matricariae* was observed at 2000 (14%) and 14000 (73.33%)ppm, respectively (Fig. 2). However, 24h after exposure to Dayabon residue, no adult mortality was observed at ranges from 2000 to 5000ppm. This means that all adult parasitoids stayed alive.

Table 1 The LC_{50} and LC_{90} values of Dayabon on different life stages of the green peach aphid, *Myzus persicae* and *Aphidius matricariae*.

Species	Stage	n ¹	χ^2 (df)	P-value	Slope \pm SE	LC_{50} (ppm) (95% confidence limits)	LC_{90} (ppm) (95% confidence limits)
<i>Myzus persicae</i>	1 st instar nymph	228	1.839 (5)	0.871	1.60 \pm 0.23	3254.1 (2834.7-3645.5)	5866.5 (5291.5-6719.2)
	2 nd instar nymph	250	0.650 (5)	0.986	1.52 \pm 0.22	3387.3 (2934.1-3791.0)	6242.7 (5667.3-7074.8)
	3 rd instar nymph	933	1.704 (5)	0.888	1.95 \pm 0.15	4194.1 (3979.8-4392.7)	6956.7 (6639.7-7348.6)
	4 th instar nymph	625	0.578 (5)	0.989	1.83 \pm 0.17	3838.7 (3574.5-4080.3)	6530.5 (6173.6-6982.2)
	Adult	322	0.909 (5)	0.970	1.62 \pm 0.21	3508.0 (3156.9-3840.1)	6279.3 (5722.7-7100.5)
<i>Aphidius matricariae</i>	Mummies	750	2.488 (3)	0.477	2.10 \pm 0.18	7595.4 (6709.5-8843.4)	30868.6 (23087.0-46143.4)

¹The number of tested insects.

Table 2 Comparison of LC_{50} values between different stages of green peach aphid, *Myzus persicae*, by relative median potency (RMP).

Stage (A / B)	RMP (LC_{50A} / LC_{50B})	95% confidence limits		Significance
		Lower	Upper	
Adult/1 st instar	1.078	0.888	1.449	ns
Adult/2 nd instar	1.036	0.918	1.292	ns
Adult/3 rd instar	0.836	0.658	1.022	ns
Adult/4 th instar	0.914	0.735	1.170	ns
4 th /1 st instar	1.180	0.926	1.611	ns
4 th /2 nd instar	1.133	0.907	1.494	ns
4 th /3 rd instar	0.915	0.738	1.074	ns
3 rd /1 st instar	1.289	1.055	1.783	*
3 rd /2 nd instar	1.238	1.032	1.650	*
2 nd /1 st instar	1.041	0.861	1.251	ns
Mummy/3 rd instar	1.811	1.368	2.980	*

*and ns indicate significant and non-significant differences between two groups based on lower and upper 95% confidence limits.

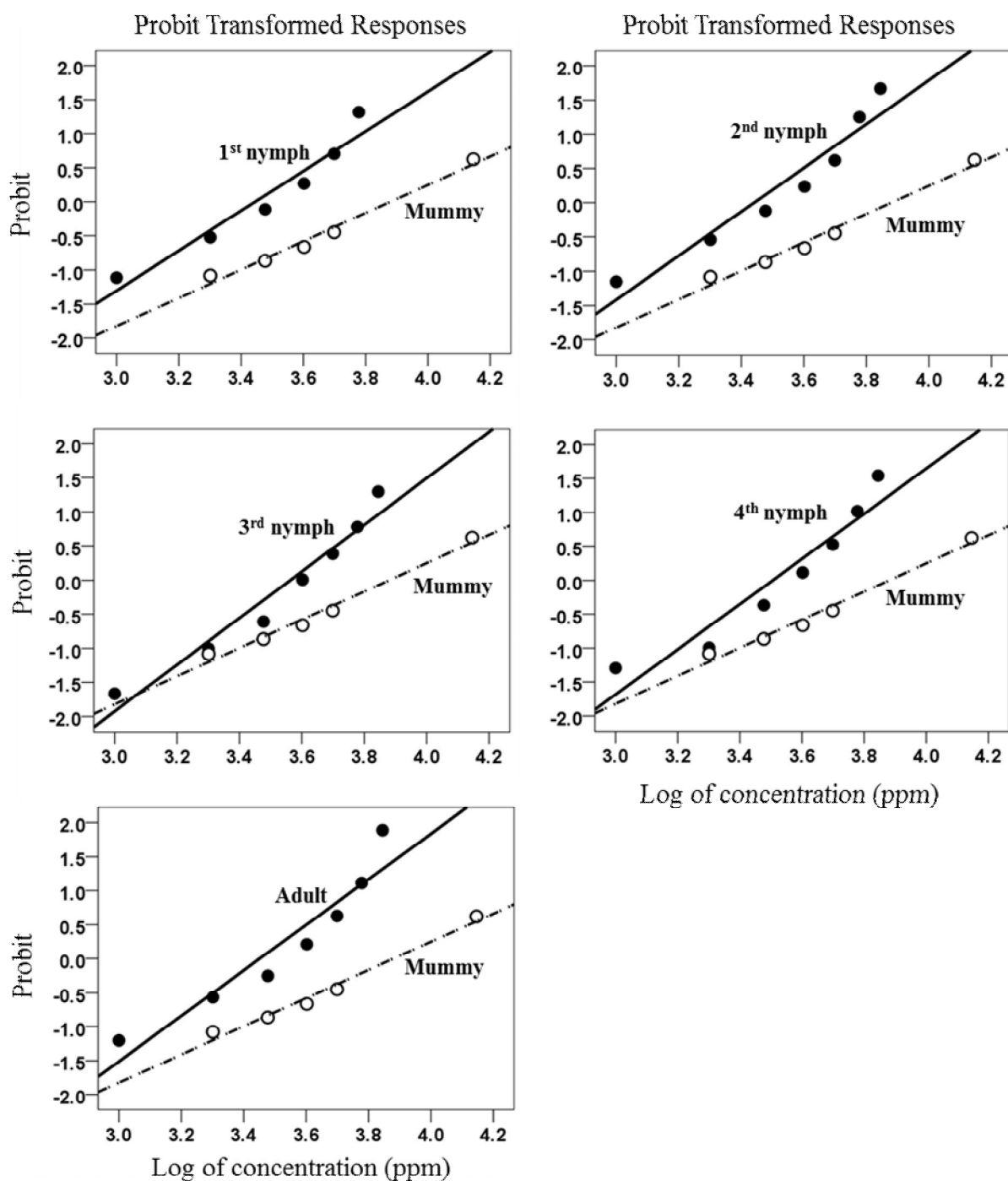


Figure 1 Lines of probit mortality of Dayabon on different stages of green peach aphid *Myzu persicae* and parasitoid wasp mummies *Aphidius matricariae*.

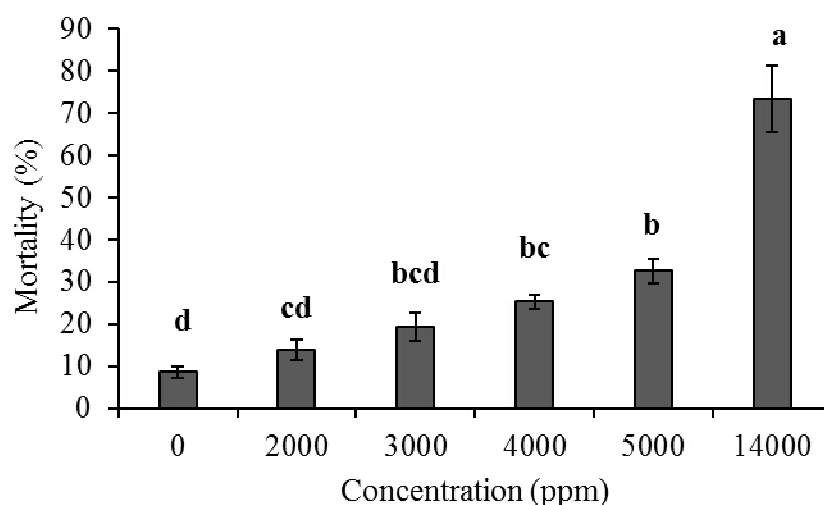


Figure 2 Impact of Dayabon on the mortality of *Aphidius matricariae* mummies calculated by number of emerged adult wasps. Vertical bars indicate standard error of mean. Means followed by the same letters are not significantly different (Tukey's test, $P \leq 0.05$).

Discussion

Our results obviously show that Dayabon can be considered as an effective pesticide against green peach aphid, *M. persicae* (Table 1). Dayabon is a new botanical pesticide which is safe for human and other mammals. Generally, the Dayabon is registered as an acaricide in particular, for the control of two-spotted spider mite, *T. urticae* in greenhouse crops (Mortazavi *et al.*, 2016). However, this is the first experiment reporting the toxicity of Dayabon on *M. persicae*. The LC_{50} of Dayabon varied for different aphid life stages. In this regard, the first and second instar as well as adults were more tolerant than the third instar nymphs. The high susceptibility of adult stage could be as a result of more coverage of insecticide on insect body surface. Additionally, susceptibility of the first and second instars could be attributed to small body size facilitating coverage of the body. This finding is in agreement with Vahabi Mashhour *et al.* (2016) who showed the adult stage of *X. luteola* is more susceptible than the third instar larvae to Dayabon.

The effects of a number of essential oils and botanical pesticides have been documented on *M. persicae* (Hori, 1998; Pavela, 2009; Nzanza and Mashela, 2012). For instance, the toxicity

and synergistic effect of fermented plant extracts of neem leaf and wild garlic has been reported on *M. persicae* (Nzanza and Mashela, 2012). Also, the seed extracts of *Melia azedarach* L. (Meliaceae) has insecticidal effects on *M. persicae* (Salari *et al.*, 2012). Compared to other botanical compounds, Dayabon has a suitable mortality effect on *M. persicae* at a reasonable concentration. Moreover, Dayabon is recognized as safe for some greenhouse natural enemies (Mortazavi *et al.*, 2016).

The side effects of insecticides have been studied on a number of natural enemies (Longley, 1999; Sterk *et al.*, 1999; Arnó and Gabarra, 2011; Rezaei *et al.*, 2014; D'Ávila *et al.*, 2018). The effect of pesticides on biological control agents should first be evaluated under laboratory condition. The pesticides found to be harmless to biological control agents in laboratory are most likely be harmless in greenhouse conditions (Hassan *et al.*, 1985). In this regard, the members of the IOBC/WPRS working group stated four evaluation categories to classify initial toxicity test on natural enemies in laboratory including, harmless (< 50%), slightly harmful (50-79%), moderately harmful (80-99%) and harmful (> 99%). According to results, the percent mortality of

Dayabon on aphid mummies was no more than 32.66% at 5000ppm. So, it could be concluded that Dayabon is a harmless botanical pesticide on *A. matricariae*. Besides, no mortality was observed for adult parasitoids exposed to dry residues of Dayabon.

An important issue threatening the successful augmentation of natural enemies is the use of pesticides (Elzen *et al.*, 2003). Thus, many studies have documented the detrimental effects of pesticides on natural enemies (Tedeschi *et al.*, 2001; Urbaneja *et al.*, 2008; Pavela, 2009) in particular the genus *Aphidius* parasitoids (Jansen, 1996; Joseph *et al.*, 2011; D'Ávila *et al.*, 2018). However, when the population of aphids is in outbreak, the parasitoids especially *A. matricariae* cannot succeed to acceptable control of aphids in greenhouse (Jacobson and Croft, 1998). In this case, Dayabon could be used as an ideal botanical pesticide for the control of aphid outbreaks. In addition, according to detrimental effects of pesticides on fertility and fecundity of natural enemies (Arnó and Gabarra, 2011), further research is needed to elucidate the sublethal effects of Dayabon on reproductive performance of *A. matricariae*.

In conclusion, according to the IOBC index, the Dayabon is recognized as safe for *A. matricariae* while it has acceptable mortality on green peach aphid. Therefore, integration of Dayabon with *A. matricariae* could be recommended in order to achieve suitable control of *M. persicae* in greenhouse products. However, further experiments are necessary to elucidate the efficacy of Dayabon in the natural field and greenhouse conditions.

Acknowledgments

We thank Mohammad Bashiri for the technical supports.

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تأثیر آفت‌کش گیاهی دایابون روی مراحل مختلف زیستی شته سبز هلو *Aphidius matricariae* و عامل کنترل بیولوژیک آن *Myzus persicae*

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دریافت: ۳۰ مهر ۱۳۹۷؛ پذیرش: ۲۷ آذر ۱۳۹۷

چکیده: شته سبز هلو، *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) یکی از مهم‌ترین آفات گلخانه‌ای محسوب می‌شود که خسارت آن از لحاظ اقتصادی بسیار مهم است. مصرف بیش از حد سموم شیمیایی سبب مقاومت شته سبز هلو به بسیاری از حشره‌کش‌ها شده است. زنبور پارازیتوئید *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae) یکی از عوامل مؤثر کنترل بیولوژیکی علیه جمعیت شته به‌ویژه در محصولات گلخانه‌ای به‌شمار می‌رود. در حال حاضر استفاده از آفت‌کش‌های گیاهی پایه سالم و زیست‌سازگار در ترکیب با عوامل کنترل بیولوژیکی توصیه می‌شود. در این مطالعه، سمیت تماسی با دایابون (SL 10%)، آفت‌کش گیاهی جدید در مراحل مختلف رشدی شته سبز هلو در غلظت ۱۰۰۰ تا ۷۰۰۰ پی‌پی‌ام بررسی شد. هم‌چنین اثرات دایابون روی مومیایی‌ها و حشرات کامل زنبور پارازیتوئید *A. matricariae* در غلظت ۲۰۰۰ تا ۱۴۰۰۰ پی‌پی‌ام مطالعه شد. مقادیر LC_{50} تخمین زده شده در سنین پورگی اول، دوم، سوم، چهارم و افراد بالغ به ترتیب ۳۲۵۴، ۳۳۸۷، ۴۱۹۴، ۳۸۳۹ و ۳۵۰۸ پی‌پی‌ام بود. با این حال، دایابون تا غلظت ۵۰۰۰ پی‌پی‌ام باعث مرگ‌ومیر مومیایی‌های زنبور کم‌تر از ۵۰ درصد بود. هم‌چنین باقی‌مانده دایابون هیچ‌گونه مرگ‌ومیر در حشرات کامل زنبور پارازیتوئید *A. matricariae* ایجاد نکرد. براساس روش استاندارد ارائه شده برای آزمایش اثرات جانبی آفت‌کش‌ها روی دشمنان طبیعی حشرات توسط گروه کاری IOBC/WPRS، آفت‌کش گیاهی دایابون برای زنبور پارازیتوئید *A. matricariae* به‌عنوان حشره‌کش بی‌خطر شناخته شد، این درحالی است که دایابون مرگ‌ومیر قابل‌قبولی در شته هلو سبز ایجاد نمود. بنابراین کاربرد تلفیقی دایابون و زنبور پارازیتوئید *A. matricariae* می‌تواند برای رسیدن به کنترل مناسب شته سبز هلو *M. persicae* در محصولات گلخانه‌ای توصیه شود.

واژگان کلیدی: شته سبز هلو، حشره‌کش، پارازیتوئید، دایابون، LC_{50}