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Effects of amitraz, buprofezin and propargite on some fitness parameters of the parasitoid *Encarsia formosa* (Hym.: Aphelinidae), using life table and IOBC methods

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Abstract

The side-effects of amitraz, buprofezin and propargite were studied on *Encarsia formosa* (Gahan) in the laboratory, using a life table response experiment and the IOBC system for their toxicity. Bioassays were conducted by dipping bean leaves containing third instar nymphs of the whitefly *Trialeurodes vaporariorum* (Westwood), parasitized by *E. formosa*, in insecticide solutions or caging adult parasitoids in treated petri dishes. The insecticide buprofezin caused 19.87% and 11.87% mortality in adults and pupae respectively. Amitraz showed the highest level of toxicity for the adults (100% mortality) and the pupae of parasitoids (83.3% mortality). Both buprofezin and propargite caused no adverse effect on the fecundity and longevity of parasitoids, but amitraz significantly reduced the fecundity and longevity of adults. Life table assay indicated that buprofezin and propargite have no major impact on the intrinsic rate of natural increase (r_m), while amitraz lowered the r_m value by 7.03%. According to the IOBC classification of toxicity, buprofezin was found to be harmless for the pupae and adults and amitraz proved to be harmful for adults. Based on both the life table and IOBC methodology, buprofezin and propargite are found to be relatively safe for *E. formosa* and can be used in integrated pest management programs where this parasitoid is involved.

Key words: amitraz, buprofezin, propargite, *Encarsia formosa*, *Trialeurodes vaporariorum*, life table experiments, IOBC method

چکیده

اثرات جانبی آفت‌کش‌های آمیتراز، بوپروفزین و پروپارژیت روی زنبور پارازیتوئید *Encarsia formosa* (Gahan) بر اساس استانداردهای سازمان بین‌المللی کنترل بیولوژیک (IOBC) و ارزیابی جدول زندگی در شرایط آزمایشگاهی مطالعه شد. زیست‌سنجی به روش‌های غوطه‌وری برگ‌های لوبیا حاوی شفیره‌های ۳ روزه‌ی پارازیتوئید در محلول‌های سمی در حداکثر غلظت توصیه‌شده از هر آفت‌کش و روش محصور کردن حشرات کامل در تشتک‌های پتری تیمارشده صورت گرفت. از بین حشره‌کش‌های مورد آزمون، بوپروفزین به ترتیب با ۱۹/۸۷ و ۱۱/۸۷ درصد مرگ و میر در حشرات کامل و شفیره‌ها، مرگ و میر چشمگیری ایجاد نکرد، اما آمیتراز بیشترین سمیت را روی حشرات کامل (۱۰۰٪ مرگ و میر) و شفیره‌ها (۸۳/۳٪ مرگ و میر) نشان داد. بوپروفزین و پروپارژیت روی تولید نتاج و طول عمر حشره‌ی بالغ اثر معنی‌داری نداشتند، درحالی‌که آمیتراز باعث کاهش تولید نتاج و طول عمر شد. آزمون جدول زندگی نشان داد که بوپروفزین و پروپارژیت نرخ ذاتی افزایش جمعیت (r_m) را کاهش ندادند، درحالی‌که آمیتراز ۷/۰۳ درصد کاهش در این پارامتر ایجاد کرد. بر اساس طبقه‌بندی IOBC بوپروفزین روی حشرات کامل و شفیره‌ها بی‌زیان می‌باشد. همچنین بر اساس روش IOBC و جدول زندگی مشخص شد که بوپروفزین و پروپارژیت روی پارازیتوئید *E. formosa* کم‌خطرند و به احتمال قریب به یقین می‌توانند در قالب برنامه‌های مبارزه‌ی تلفیقی مورد استفاده قرار گیرند.

واژگان کلیدی: آمیتراز، بوپروفزین، پروپارژیت، *Encarsia formosa*، *Trialeurodes vaporariorum*، پارامترهای جدول زندگی، روش IOBC

Introduction

A key principle of IPM is to maximize the efficiency of a pest control program by improving the roles of the natural mortality factors such as predators and parasitoids. Biological control can be supplemented with pesticide applications by necessity (De Cock *et al.*, 1996). However, pesticides have a variety of lethal and sublethal effects on natural enemies over a range of scales and levels (Jepson & Lewis, 2003). The side-effects of some pesticides have been studied on the parasitoid wasp *Encarsia formosa* (Gahan) (van de Veire & Vacante, 1988; Senn *et al.*, 1994; Castaner & Garrido, 1995; Liu & Stansly, 1996; Sterk *et al.*, 1999; Chiasson *et al.*, 2004; Richter, 2006), which is widely used against the whitefly pest *Trialeurodes vaporariorum* (Westwood) (Hem.: Aleyrodidae) in greenhouses (van Lenteren & Martin, 1999).

Many studies have been focused on the side-effects of pesticides on natural enemies and the majority of them commonly used a standardized method of the International Organization for Biological Control (IOBC) for assessing lethal and sublethal effects. In this method sequential pesticide screening is done in the laboratory, semi-field and field tests (Dohmen, 1998; Hassan, 1998). IOBC classification is derived from mortality and fecundity data, so the sublethal effects such as shortened life expectancy, mutations in offspring, weight loss, reduced fertility rates, behavioral changes, altered developmental rates and pre-oviposition period, and changes in sex ratio are not considered (Stark *et al.*, 2007). Demographic approach can integrate both lethal and sublethal effects using life table experiment to estimate toxicity (Stark & Banks, 2003). Life table response experiments (LTRE) are conducted by cohorts exposed to a dose of a pesticide over their life cycle, recording their daily mortality and reproduction individually to calculate the population parameters. The intrinsic rate of increase (r_m) can be estimated from data such as fertility, fecundity, developmental time, survival, and sex ratio (Forbes & Calow, 1999). LTRE provide more reliable estimate than the acute toxicity tests (Forbes & Calow, 1999) and being widely used to evaluate the side effects of pesticides on a number of beneficial insects (*e.g.* Stark *et al.*, 1997; Dutton *et al.*, 2003; Acheampong & Stark, 2004; Stark *et al.*, 2004; Rezaei *et al.*, 2006).

Propargite, buprofezine and amitraz have been recommended mainly as initial protection measures against greenhouse and cotton pests by the Iranian Plant Protection Organization (IPPO). The objective of this study was to improve our knowledge about the susceptibility of *E. formosa* to these pesticides, using life table response experiments and the IOBC approach. We report here the categorized degree of damage of the pesticides on *E.*

formosa and their impact on fertility life table parameters, including net rate of reproduction (R_0), intrinsic rate of natural increase (r_m), generation time (T), doubling time (DT), and finite rate of increase (λ).

Materials and methods

Collecting and rearing the insect specimens

Adults of *T. vaporariorum* were collected from tomato crop, *Lycopersicon esculentum* P. Mill., greenhouses in the northern Iranian city of Rasht and reared on tobacco (Kuker 347) (*Nicotiana tabacum* L.) to establish the whitefly population. A total of 10 pupae of *T. vaporariorum* were randomly selected for identification (Suh *et al*., 2008; Lee *et al*., 2005). The pupae parasitized by *E. formosa* were collected from tomato plants and the parasitoid identified using Polaszek's (1992) key. The wasps were reared separately later on whitefly nymphs in a growth chamber at 25 ± 1ºC, 70 ± 5% RH and a 14: 10 h (L: D) photoperiod.

Pesticides and application

Pesticides were evaluated at the maximum recommended field concentration: buprofezin 1.25 L / ha of a 40 SC formulation (Applaud®) (Nihon Nohyaku, http://www.nichino.co.jp /eng/) (500 L pesticide solution per hectare); propargite 1.5 L / ha of 57 EC (Agricultural EXIR Co., http://www.agroxir.com) and amitraz 2 L / ha of 20 EC (Bayer CropScience, http://www.bayercropscience.com) (600 L pesticide solution per hectare). These pesticides are recommended for pest control in the greenhouse and open fields (Mosallanejad *et al*., 2002). The control was treated with distilled water.

Side-effects of pesticides on pupae

Adults of *T. vaporariorum* were released on bean plants, *Vigna angularis* (Willd) Ohwi & Ohashi, with two insect-free fully developed leaves for 24 h. After the removal of whiteflies, these plants were maintained at 25 ± 1ºC, 70 ± 5% RH and a 14: 10 h (L: D) photoperiod. The new third instar whitefly nymphs were then exposed to adult parasitoids for 48h (40 third instar whitefly nymphs: 1 wasp). When parasitized whitefly scales turned black, the healthy pupae were eliminated and bean leaves with 3-day-old parasitoid pupae immersed in the pesticide solutions or distilled water (control) for 30s, with nine replicates per treatment. The treated bean leaves blotted dry on filter paper. The adult emergence in control and all treatments were monitored daily. The number of emerged progenies and mortality were recorded within 7 days. Mortality percentages (M) were corrected for untreated mortality according to Abbott formula (Abbott, 1925).

The fecundity of the surviving female progeny was evaluated. Fifteen newly emerged (0-1-day-old) females were randomly selected and placed individually in an 8.5 cm long × 3 cm high × 6.5 cm wide plastic cage, with a 4 × 3 cm window screen on the lid containing non-treated bean leaves with third instar *T. vaporarium* nymphs. The effect of pesticide on oviposition (E_r) was calculated from: $E_r = R_t / R_c$; where R_t and R_c are oviposition in the pesticide treatments and water controls respectively.

The total effect index of each pesticide (E) was the mortality of exposed wasps to pesticide, as well as the impact on the oviposition of surviving females, using the equation by Overmeer & van Zon (1982): $E = 100\% - [(100\% - M) \times Er]$. The pesticides were classified according to the categories for toxicity, developed by IOBC Working Group as follows (Stark *et al.*, 2007): $E < 30\%$ harmless (class 1), $30 < E < 79\%$ slightly harmful (class 2), $80 < E < 98\%$ moderately harmful (class 3) and $E > 99\%$ harmful (class 4).

The percentage data were transformed by ($\sqrt{x} + 0.5$) for normalization to ensure homogeneity of variance. One-way analysis of variance was applied to analyze percentage mortality and average egg production. Means were separated by Tukey´s test, using the SAS (SAS Institute, 1997).

Side-effects of pesticides on adults

The experiment was set to determine the effects of pesticides on adult *E. formosa* based on the Thamson *et al.* (1996) method. Petri dishes (diameter 50 × height 15 mm) with three 15 mm holes on their lids (two holes covered with very fine voile for gas exchange and the third for access) were dipped in pesticide solutions at the maximum recommended field concentration (tables 1-2) and allowed to air dry. The control was treated with distilled water. The two halves of the petri dishes were later bound together with parafilm.

Table 1. Effect of pesticides on mortality, longevity and fecundity of *E. formosa* pupae (Mean ± SEM).

Treatments	Pupae tested	Concentration (a.i. in µg/mL)	Mortality (%)	Longevity of adults (days)	Fecundity (total female / female)
Control	189	---	1.1 ± 1.1 c	27.73 ± 2.56 a	283 ± 26.17 a
Amitraz	384	500	83.3 ± 3.97 a	16.93 ± 2.79 b	158 ± 29.31 b
Buprofezin	261	1000	11.87 ± 3.36 bc	27.68 ± 2.91 a	282.6 ± 28.19 a
Propargite	229	1425	16.98 ± 5.7 b	24.9 ± 3.17 ab	243.4 ± 28.71 ab

Means in a column with the same letter are not significantly different ($P < 0.05$) according to Tukey´s tests.

Table 2. Effect of pesticides on mortality and fecundity of *E. formosa* adults (Mean ± SEM).

Treatments	Adults tested	Concentration (a.i. in µg/mL)	Mortality (%)	Fecundity (total female/female)
Control	100	---	7.1 ± 1.5 c	240 ± 22.16 a
Amitraz	250	500	100 ± 0 a	---
Buprofezin	220	1000	19.87 ± 4.35 bc	230.2 ± 22.18 a
Propargite	190	1425	27.1 ± 4.3 b	210.2 ± 21.62 ab

Means in a column with the same letter are not significantly different ($P < 0.05$) according to Tukey´s tests.

A total of ten newly-emerged adults of *E. formosa* were led into the petri dishes via the access hole before it was quickly sealed with cotton wool. The dishes were held for 24 h at 25 ± 1˚C and their mortality was assessed by lack of response to a prod with a fine pin. Ten dishes were used per treatment. The survived parasitoids from each petri dish were put in an untreated 8.5 cm long × 3 cm high × 6.5 cm wide plastic cage, with a 4 × 3 cm window screen on the lid containing non-treated bean leaves with third instar *T. vaporarium* nymphs (40 third instar whitefly nymphs: 1 wasp) to measure their parasitism viability. The effect of pesticides on oviposition (Er) and total effect of each pesticide (E) was determined, with the same approach outlined for the pupae.

Life table assay

Bean leaves with 3-days-old parasitoid pupae were immersed in the pesticide solutions (mentioned as above). To build a fertility life table for each treatment, an insect cohort (18 adults per treatment) was conducted until the death of last individual. Age-specific survival rates (l_x) and average number of female offspring (m_x) for each age interval (x) were used to build age-specific fertility life tables. The intrinsic rate of natural increase (r_m) was obtained by solving Euler's equation (Andrewartha & Birch, 1954):

$$\sum_{x=0}^{y} L_x m_x e^{-rx} = 1$$

Where y is the oldest age class, L_x is the survival of a newborn female to the midpoint of an age interval, and x is the age of each female at each age interval (Rezaei *et al.*, 2006). The other main fertility life table parameters; the net reproductive rate ($R_0 = \sum l_x m_x$), finite rate of increase ($\lambda = \exp r_m$), mean generation time ($T = (\ln R_0) / r_m$), and doubling time ($DT = (\ln 2) / r_m$) were also estimated (Meyer *et al.*, 1986; Maia *et al.*, 2000).

This algorithm was used to estimate the uncertainties associated with the other parameters. Jackknife pseudo values for r_m, T, DT, and λ for each treatment were subjected to analysis of variance (ANOVA) followed by Ryan´s Q test, using Life test in SAS (SAS

Institute, 1997). All parameters were calculated with Persian´s Rm software (Naveh *et al.*, 2004). The Log-Rank test (Rosner, 2000) was used for survival curve analysis, using the Proc Life test SAS (SAS Institute, 1997).

Results

Effect of pesticides on mortality, longevity and fecundity

Mortality and effects of pesticides on fecundity and longevity of *E. formosa* are presented in table 1 and 2. Total mortality caused by pesticides was significantly different from control (F = 90.75; df = 3; $P < 0.01$). Buprofezin and propargite had no significant effect on fecundity and longevity, but amitraz significantly reduced both the fecundity (F = 4.40; df = 3; $P < 0.01$) and longevity (F = 3.20; df = 3; $P < 0.05$).

Total effect (E)

Comparing the total effects, the pesticides (table 3) buprofezin and propargite were classified as harmless compounds for the pupae, but based on the IOBC classification, amitraz proved harmful for the adults and moderately harmful for pupae. Propargite was classified as slightly harmful compound for adults (table 3).

Fertility life table parameters

The course of the age-specific survival rate (l_x) and age-specific fecundity (m_x) of *E. formosa* for treatments are presented in fig. 1. The survival curve analysis was significantly different among the four treatments ($\chi^2 = 9.525$; df = 3; $P < 0.05$). The survival curve of amitraz was significantly different from the control ($\chi^2 = 4.4102$; df = 1; $P < 0.05$), propargite ($\chi^2 = 7.895$; df = 1; $P < 0.01$), and buprofezin ($\chi^2 = 4.4102$; df = 1; $P < 0.05$). No significant differences were observed among the survival curves of the control, propargite, and buprofezin. The mean rates of the life table parameters are presented in the table 4. There are significant differences among some fertility life table parameters. The lowest value for the intrinsic rate of natural increase (r_m) belongs to amitraz (F = 35.99; df = 3; $P < 0.01$). The R_0 values for buprofezin and propargite are not significantly different in comparison with the control, but amitraz significantly lowers R_0 in *E. formosa* (F = 4.39; df = 3; $P < 0.01$). There are no significant differences among the mean generation time (T) for the treatments (F = 1.73; df = 3; $P = 0.1703$). Doubling time (DT) of buprofezin and propargite shows no significant differences comparing to the control, while amitraz is significantly different (F =

3.66; df = 3; $P < 0.05$). Finite rate of increase (λ) is not significantly different in buprofezin and propargite, while amitraz is significantly different from it (F = 4.14; df = 3; $P < 0.05$) (table 4).

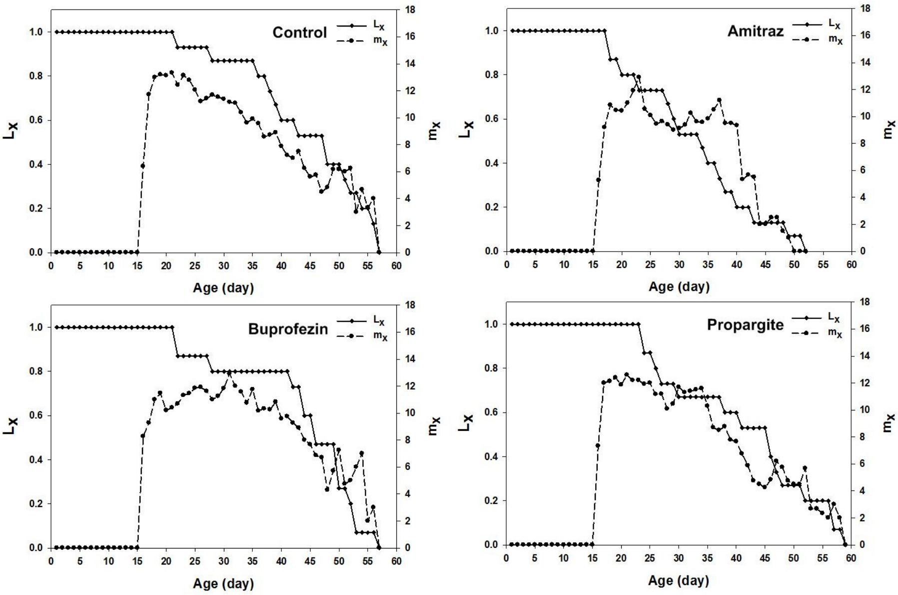

Figure 1. Effect of pesticides on age specific survival rate (L_x) and age specific fecundity (m_x) of *E. formosa*.

Table 3. Total effect and hazard classes of pesticides for *E. formosa* adults and pupae according to the IOBC evaluation categories.

Pesticides	Concentration (a.i. in µg/mL)	Total effect (%) (Classification of toxicity)	
		Pupae	**adult**
Amitraz	500	90.6 (3, Moderately harmful)	100 (4, Harmful)
Buprofezin	1000	10.9 (1, Harmless)	23.14 (1, Harmless)
Propargite	1425	27.8 (1, Harmless)	36.23 (2, Slightly harmful)

Discussion

Buprofezin and propargite had no significant effect on fecundity and longevity, when pupae used for assay, while amitraz caused a significant reduction in fecundity. Any change in the reproductive rate of *E. formosa* is likely as important as the direct toxicity to the parasitoid-host interaction. Reduction in reproduction is thought to be a result of either physiological interruption in the reproductive system of *E. formosa* and/or disorder of the searching and oviposition behavior of this beneficial insect as well as other parasitoids (Desneux *et al.*, 2007). Feldhege & Schmutterer (1993) believed that the parasitoid's longevity and emergence as well as the capacity of parasitism would be affected after indirect contact with Margosan–O. In addition, the application of 10 ppm azadirachtin appeared to be non-toxic, while the concentration of 20 ppm significantly damaged the fitness of *E. formosa*.

Table 4. Effects of pesticides on the fertility life table parameters of *E. formosa* (Mean ± SE).

Parameters	**Treatments**	**True calculations**	**Jackknife method**	
			Mean ± SE*	**95% Confidence interval**
R_0	Amitraz	153.78	153.5 ± 29.3 b	(90.5-216.6)
	Buprofezin	278.92	278.6 ± 28.3 a	(217.9-339.4)
	Propargite	239.92	239.6 ± 29.0 ab	(177.4-301.9)
	Control	280.15	279.9 ± 26.2 a	(223.5-336.3)
r_m	Amitraz	0.221	0.222 ± 0.007 d	(0.207-0.236)
	Buprofezin	0.232	0.232 ± 0.002 c	(0.229-0.236)
	Propargite	0.237	0.237 ± 0.002 b	(0.232-242)
	Control	0.238	0.238 ± 0.0018 a	(0.234-0.242)
T	Amitraz	22.72	22.76 ± 0.45 a	(21.7-23.7)
	Buprofezin	24.17	24.19 ± 0.44 a	(23.24-25.14)
	Propargite	23.108	23.12 ± 0.56 a	(21.9-24.3)
	Control	23.60	23.61 ± 0.4 a	(22.7-24.5)
DT	Amitraz	3.127	3.11 ± 0.09 c	(2.91-3.31)
	Buprofezin	2.976	2.975 ± 0.022 ab	(2.92-3.02)
	Propargite	2.922	2.92 ± 0.028 a	(2.86-2.98)
	Control	2.903	2.90 ± 0.021 a	(2.85-2.94)
λ	Amitraz	1.248	1.24 ± 0.008 b	(1.23-1.26)
	Buprofezin	1.26	1.26 ± 0.002 ab	(1.25-1.26)
	Propargite	1.267	1.26 ± 0.003 a	(1.26-1.27)
	Control	1.269	1.26 ± 0.002 a	(1.26-1.27)

* Means in a column with the same letter are not significantly different ($P < 0.05$), according to Ryan´s Q tests.

Reduced longevity due to exposure with sublethal doses of pesticides have been reported for many parasitoid species and some predators (Krespi *et al.*, 1991; Rumpf *et al.*, 1998; Stapel *et al.*, 2000; Alix *et al.*, 2001; Desneux *et al.*, 2004, 2006; Liu & Stansly, 2004; Schneider *et al.*, 2004). Sublethal effects of pesticides on longevity have been reported for many natural enemies and the reduced longevity in *E. formosa* exposing to amitraz may be resulted from a sublethal effect or delayed toxicity. Extrapolation of reduced fecundity and longevity of *E. formosa* treated with amitraz to the population level is difficult because they kill pests before their premature death (Jervis & Copland, 1996). Besides, another important factor is amount of feeding and reproduction between exposure to pesticide and death of natural enemies (Desneux *et al*., 2007). The consequences of reduced longevity on population dynamics can be considered in a study assessing pesticide impacts on life table parameters (Stark & Banks, 2000, 2003). Life table results indicate that buprofezin and propargite have the least negative effect on the intrinsic rate of natural increase (r_m) in comparison with amitraz, causing about 7.03% reduction in r_m. The intrinsic rate of natural increase is the most important parameter for describing the growth potential of a population, because r_m reflects an overall effect on development, reproduction and survival (Southwood & Handerson, 2000). The survival curve indicated that the survival rate decreased by amitraz. When the r_m is determined for risk assessment of pesticides, a reduction of survival (l_x) could lead to a strong reduction of the r_m, and consequently a negative effect at the population level (Stark & Banks, 2003). There were no significant differences among R_0, T, DT and λ values in the buprofezin and propargite treatments in comparison with the control. The former was evaluated as harmless based on the IOBC classification (class 1) (Stark *et al*., 2007). This compound and propargite, despite its significant mortality effects, could be used in IPM programs of crops in which *E. formosa* are released.

According to fertility life table parameters, amitraz showed a noticeable negative impact on the *E. formosa* population, causing higher mortality in comparison with the two other pesticides. Jones *et al*. (1995) reported that the adults of *E. formosa* were more susceptible to residues of amitraz than *Eretmocerus mundus* Mercet adults. Based on the IOBC, amitraz was found here to be harmful for adults and moderately harmful for pupae classification (class 3 and 4). We concluded that amitraz should not be used in integrated pest management programs where *E. formosa* exists. We found no different results between the IOBC and life table experiment methods. Rezaei *et al*. (2006) reported similar results for imidacloprid and propargite on *Chrysoperla carnea* (Stephens) (Neu.: Chrysopidae), but for pymetrozin, the life table assay showed more adverse effects compared to that of the IOBC method as considering only the total effect (E) had led to an underestimate of the pesticide effect. In comparison with r_m, the total effect included only fecundity and pre-imaginal mortality, excluding age-specific fecundity and survival.

According to the IOBC classification of toxicity, buprofezin is found to be harmless for the pupa and adult parasitiod, and amitraz is harmful only for the adult. Based on both the life table and IOBC methodology, buprofezin and propargite are relatively safe for *E. formosa* so can be used in the framework of an integrated pest management program where this parasitoid wasp is involved.

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