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Laboratory testing of the effects of diflovidazin on egg production and on transmaternal ovicidal activity in *Tetranychus urticae* Koch (Acari: Tetranychidae)

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ABSTRACT

The transmaternal properties of diflovidazin were examined under laboratory conditions on *Tetranychus urticae*. To assess its transmaternal ovicidal effect, leaf discs were dipped in a 100 ppm diflovidazin solution, after which adult females were allowed to feed on the treated surfaces for either 2 or 48 hours. The females then laid eggs on an untreated surface over five consecutive 7-hour periods. Egg mortality was evaluated based on exposure duration and time elapsed since exposure. After being removed from the treated surface, 100% of the eggs laid by females in the first 7-hour period were non-viable. However, mortality—and thus the effect—gradually decreased in subsequent periods, with eggs laid after 28 hours exhibiting mortality levels comparable to naturally occurring rates. Notably, the length of the exposure period did not influence the rate at which egg mortality declined. The effect on egg production was also examined. Females were allowed to lay eggs on treated leaf discs, but the treatment did not significantly alter the number of eggs produced per female. These findings indicate that while diflovidazin effectively induces high egg mortality, it does not reduce overall egg production. Our results suggest that diflovidazin temporarily sterilizes females, likely because developing eggs absorb the active substance from the female's system.

KEYWORDS

Acaricide, diflovidazin, egg production, transmaternal ovicidal activity, two-spotted spider mite

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INTRODUCTION

Diflovidazin (CAS: 162320-67-4) is a synthetic tetrazine introduced by the Hungarian company Chinoin (now Agro-Chemie Ltd.) in 1997 to prevent mite growth. It is effective against spider mites, gall mites, and rust mites and has favorable ecotoxicological properties (Pap *et al.* 1994, 1996; Szabó *et al.* 2023). Diflovidazin is sold and used in Eastern Europe and some Asian countries as a miticide for orchards, vineyards, vegetable crops, arable crops, and ornamental plants. In the EU, it is currently licensed only in Hungary for emergency use and is marketed under the brand name Flumite® 200 (200 g·l-1 diflovidazin suspension concentrate) (National Food Chain Safety Office 2024). The active substance is currently undergoing authorization in the European Union.

Similar to other active substances classified as mite growth inhibitors (MGIs) (IRAC Group 10), such as etoxazole, clofentezine, and hexythiazox, diflovidazin acts by inhibiting development. Its acaricidal effect targets eggs, larvae, and nymphs. The active substances allow the eggs to reach the embryonic stage where the prelarva (calyptostase) develops in the egg (Andre and Van Impe 2012). At this stage, two carmine eye spots become visible, but the eggs are unable to hatch into larvae (Bleicher

2003). In addition to their direct effects on eggs, these substances also impact progeny through the female organism, although they do not affect adult mites (Bretschneider and Nauen 2007). The transmaternal effect occurs through reduced egg viability and, in some cases, altered egg production (Bretschneider *et al.* 2003).

Previous studies have demonstrated that the expression of these sublethal effects differs among active substances. Hexythiazox causes temporary egg sterility, with egg viability gradually recovering after females are transferred to untreated surfaces (Marris 1988), whereas clofentezine does not induce transmaternal egg mortality and does not reduce oviposition activity (Aveyard *et al.* 1986; Neal *et al.* 1986; Brooker *et al.* 1987; Pap *et al.* 1996; Marčić 2002, 2003; Li *et al.* 2006). Other acaricides, including etoxazole, flufenoxuron, and spirodiclofen, have been reported to reduce egg production in addition to their ovicidal activity (Kim and Yoo 2002; Wachendorff *et al.* 2002; Marčić 2007; Askari Saryazdi *et al.* 2013; Saber *et al.* 2018).

Research on diflovidazin has primarily focused on its toxicity parameters at various developmental stages, its translocation properties (Pap *et al.* 1996), and its ecotoxicological effects on natural enemies (Naik *et al.* 2006). Some studies have explored the sublethal effects of the active substance in detail. When administered to adults at sublethal doses, diflovidazin reduced the expected lifespan of both males and females and decreased reproductive rates (Marčić 2000; Havasi *et al.* 2018); however, no data have been published on egg production per unit of time. Furthermore, the duration of the transmaternal ovicidal effect after females leave treated surfaces remains unclear, as does the extent to which this effect depends on the duration of exposure.

As a globally widespread and highly polyphagous pest, the two-spotted spider mite (*Tetranychus urticae* Koch) is a suitable target organism for laboratory testing of acaricides. Several other mite species also pose significant threats to field and greenhouse crops (Jeppson *et al.* 1975; Tomczyk and Kropczyńska 1985; Bolland *et al.* 1998; Zhang 2003). Even today, mite control relies heavily on chemical methods. Given the various challenges associated with acaricide use—such as optimal treatment timing, combining active substances, and resistance development (Van Leeuwen *et al.* 2010; Sparks and Nauen 2015)—it is essential to gather as much information as possible on available active substances.

Given the knowledge gaps, the aims of this study were threefold: (1) to quantify the duration of the transmaternal ovicidal effect of diflovidazin after removal of the females from treated surfaces; (2) to determine whether this effect is dependent on initial exposure duration; and (3) to assess the impact of the compound on egg production under the highest field-recommended concentrations. In addition, these practical aspects are particularly relevant for optimizing field applications, since diflovidazin is not a systemic active substance, and newly developed plant parts after treatment do not contain the active ingredient. Consequently, mites feeding on untreated plant parts may experience conditions similar to those modeled in the present experiment.

MATERIAL AND METHODS

The colony used and the conditions of the experiment

The studies were carried out on two-spotted spider mites (*T. urticae*). The colony was established from a pesticide-free population maintained on water traps at the Institute of Plant Protection (Hungarian University of Agriculture and Life Sciences) and was selected as a homogeneous strain susceptible to the active substance diflovidazin (LC_{50} = 0.05 ppm on eggs by dip method).

The colony was maintained and the experiments performed under laboratory conditions, with 16 hours of light, a temperature of 24 ± 2 °C, and relative humidity between 50–70%. The two-spotted spider mites were kept on the common bean (*Phaseolus vulgaris* L.), one of their preferred host plants that is easy to grow under laboratory conditions. Experiments were also conducted using bean leaf discs (diameter = 18 mm), which were cut from fully developed, primary bean leaves.

The acaricide used

A formulated product available on the market, Flumite® 200 (Agro-Chemie Ltd.), containing 200 $g \cdot l^{-1}$ of diflovidazin, was used to prepare the mixture for the experiments at the highest recommended field dose (100 $mg \cdot l^{-1}$ a.i.). The dose of 100 $mg \cdot l^{-1}$ was selected to mirror the manufacturer's

recommended label rate for commercial field application, as our objective was to determine practical post-exposure efficacy rather than baseline toxicity.

Experiment methods

In the experiments, mites were maintained on fresh leaf surfaces using Bleicher's wick method (Bleicher 2003). To create a damp surface, we placed 5 cm diameter PVC rings in Petri dishes filled with cotton, on top of which we placed two 5 cm diameter filter paper discs. A wick was made from the lower filter paper disc by making radial incisions and positioning it inside the PVC ring next to the cotton. The cotton channeled water to the filter paper, keeping the leaf discs continuously moist and ensuring their freshness for up to two weeks. The leaf discs were placed on the moist surfaces with their undersides facing up, and mites were transferred using fine brushes.

The leaf dip method was used to treat the leaf discs (Helle and Overmeer 1985), and excess solution was removed using filter paper. Adult female spider mites were placed on the leaf discs after the spray had completely dried. The control series was treated with deionized water.

The effect on egg production

To study the effect on egg production, a series of leaf discs treated with 100 ppm of diflovidazin and a control series were examined. Each leaf disc contained one adult female, and the number of eggs laid was recorded after 24 hours. The experiment was conducted with four replicates and 25 repetitions.

The examination of the transmaternal ovicidal action

The series treated with diflovidazin and deionized water was designated as Series 0. These leaf discs were used to feed adult mites, with five individuals per disc and 15 replicates. Two separate test series were created: in the first, mites were allowed to feed for two hours; in the second, they were fed for 48 hours on the treated surface. Afterward, they were transferred to a subsequent untreated series of leaf discs (Series 1), where they remained for an additional seven hours. This process was repeated five times to create Series 2, 3, 4, and 5, corresponding to the periods in Table 1.

Table 1. A schematic representation of the experimental protocol of transmaternal ovicidal activity (treated and untreated series).

	Series name					
	treated leaves	**untreated leaves**				
	0	1	2	3	4	5
time interval (h): number of hours after the removal of females from the treated surface	-	0–7	7–14	14–21	21–28	28–35
Time spent (h) on the surface	2	7	7	7	7	7
	48					

The seven hours were chosen due to the exceptionally fast metabolism of two-spotted spider mites. In previous experiments, we used 24-hour intervals, similar to a hexythiazox study (Marris 1988). However, literature data indicate that the active substance levels in female mites can drop significantly over 24 hours due to their rapid metabolism. While even shorter periods might be preferable, the minimum time required for arthropods to lay a sufficient number of eggs for evaluation must be considered. Based on the results, the seven hours were deemed appropriate, as no reduction in egg mortality was observed in Series 1.

In each series, the hatching of larvae was monitored until all the hatched individuals in the accompanying control series had passed the larval stage. At this time, the period for the hatching of larvae was considered closed, and we recorded the number of individuals that remained in egg form. The mortality data were adjusted with the use of Abbott's formula to determine the efficacy regarding the various periods. If the mortality rate in the control population exceeded 10%, the experiment was excluded from the evaluation.

Statistical analysis

The egg production of treated and control individuals was compared using a two-way Student's t-test. Normality was confirmed, as the absolute values of skewness and kurtosis for egg production

variables were both below 1 in both control and treated samples. The homogeneity of variances was tested with Fisher's F-test ($F(90,95) = 0.11$, $p = 0.74$).

The effect of treatment on egg mortality was evaluated using repeated-measures ANOVA, with treatment as the between-subjects factor and time as the within-subjects factor. Since mortality was zero after seven hours, the first series was excluded from the analysis. The unexplained variance ratio was assessed using Wilk's λ, while the within-subjects (time) effect and time × treatment interaction were tested using Greenhouse-Geisser's degrees of freedom correction ($\varepsilon = 0.71$). Normality of residuals was confirmed again via skewness and kurtosis.

However, homogeneity of variances was violated (Levene's test, $p < 0.05$). Therefore, significantly different treatment groups were identified using Games-Howell's post hoc test. We calculated the median of Abbott's efficacy values for all series and fitted a model (Abbott's Efficacy Median Model, AeMM) in the form:

$$Y(s) = 100 \times (1-\exp(-v \times (5-s)))$$

where Y represents the median Abbott's efficacy for the groups treated 2 h or treated 48 h, s is the series number, and v is the slope parameter. We then fitted the same model to the original Abbott's efficacy values (Abbott's Efficacy Model, AeM). Normality of the residuals was confirmed using Shapiro-Wilk's test ($p > 0.05$). The R^2 value and its significance, as well as the significance of model accuracy and the parameter v, were also calculated. Statistical analyses were performed using IBM SPSS v25 (Armonk, NY: IBM Corp.).

RESULTS

Egg production did not differ significantly between treated and control females (control: 5.92 ± 0.25, n = 91; treated: 5.74 ± 0.23, n = 96; $t(185) = 0.54$, $p = 0.59$). The multivariate effect of time and the time × treatment interaction were both significant (time: Wilk's $\lambda = 0.06$, $F(2.2, 116.5) = 111.5$, $p < 0.001$; interaction: Wilk's $\lambda = 0.06$, $F(6.4, 116.5) = 36.8$, $p < 0.001$). The treatment effect was also significant ($F(3, 55) = 174.7$, $p < 0.001$) (Table 2).

Table 2. Means and standard deviations of egg mortality (%) across time intervals 7–14, 14–21, 21–28, and 28–35 hours after treatment, adjusted using Abbott's formula. (In the first 7 hours, 100% mortality was observed.) Different letters indicate significantly different groups (Games-Howell test, $p < 0.05$).

	Series 2	Series 3	Series 4	Series 5
	7–14h	14–21h	21–28h	28–35h
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
control	3.95 ± 6.98a	2.69 ± 5.67a	3.56 ± 7.40a	3.24 ± 6.82a
treated 2 h	87.63 ± 12.98b	61.47 ± 28.72b	31.39 ± 23.37b	3.01 ± 5.48a
treated 48 h	93.94 ± 8.02b	57.62 ± 22.53b	31.53 ± 23.36b	3.22 ± 9.81a

Modeling Abbott's efficacy

When examining diflovidazin, we found that the active substance exhibited excellent efficacy during the first 7 hours after exposure ended (Series 1), with 100% egg mortality, similar to that observed on the treated surface. However, efficacy gradually declined over subsequent time periods (Series 2–4).

• Time Period 2 (7–14 hours): Efficacy remained high, with only a small percentage of eggs hatching.

• Time Period 3 (14–21 hours): Efficacy dropped to approximately 60–70%, which was no longer satisfactory.

• Time Period 4 (21–28 hours): Efficacy further declined to around 30%.

• Time Period 5 (28–35 hours): Mortality became negligible and was statistically indistinguishable from the untreated population (Fig. 1, Table 2).

When studying transmaternal ovicidal activity, we also examined the effect of exposure time. Results indicated that exposure time did not influence the outcome within the examined period (2–48 hours), as no significant difference was observed between individuals exposed for 2 hours or 48 hours

on the acaricide-treated leaf surface (Fig. 1, detailed performance metrics in Table 3).

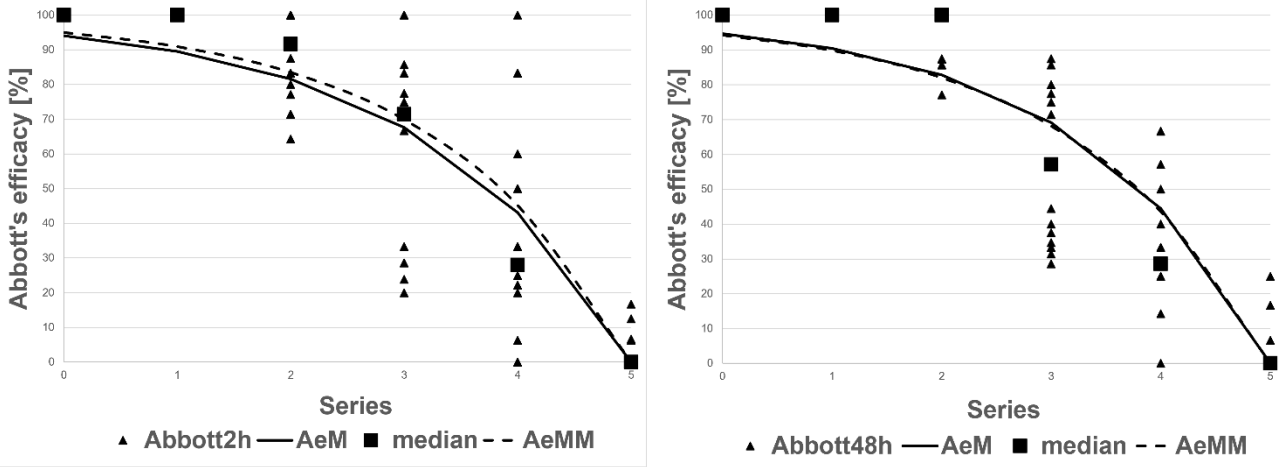

Figure 1. The Abbott's Efficacy Median Model (AeMM) and Abbott's Efficacy Model (AeM) for egg mortality were calculated for 2-hour (above/left) and 48-hour (below/right) treated samples. These models were fitted to the observed Abbott's efficacy values across five-time intervals: 0–7 h, 7–14 h, 14–21 h, 21–28 h, and 28–35 h.

Table 3. Estimated slope parameters (v) of the Abbott's Efficacy Median Model (AeMM) and Abbott's Efficacy Model (AeM) for egg mortality, calculated for 2-hour and 48-hour treated samples, along with model accuracy (F-test) and explained variance (R^2).

Model	Treatment	estimatedv	Model accuracy		R2
			F	df	
AeMM	2 h	0.60**	359.7***	1;6	0.95**
	48 h	0.57**	206.7***		0.91**
AeM	2 h	0.59***	1459.0***	1;89	0.79***
	48 h	0.56***	1932.0***		0.82***

** significant at $p < 0.01$; *** significant at $p < 0.001$

DISCUSSION

Impact on egg production

Several studies have reported sublethal effects of acaricides on reproduction. Havasi *et al.* (2018) reported a decline in reproduction rates caused by diflovidazin, although their experiments were conducted using a concentration of 941.2 $mg \cdot l^{-1}$ a.i.—nearly 10 times higher than the maximal recommended practical dose. In the present study, females feeding on diflovidazin-treated surfaces at the highest recommended field concentration (100 $mg \cdot l^{-1}$ a.i.) showed no reduction in egg production compared with untreated controls. However, all eggs laid during the exposure period were sterile. These findings indicate that diflovidazin exerts a strong transmaternal ovicidal effect without affecting oviposition under practical application rates.

Transmaternal ovicidal effect

The present study demonstrated that the transmaternal ovicidal effect was temporary and rapidly declined after females were transferred to untreated surfaces. Egg viability gradually recovered, while egg production remained unaffected throughout the experiment. Furthermore, the duration of initial exposure did not significantly influence the rate of recovery in egg viability.

These results suggest that diflovidazin –at the tested dose– acts directly on developing eggs rather than inducing persistent physiological changes in the female itself. The rapid decline in Abbott's efficacy after transfer to untreated surfaces indicates that the active substance is eliminated relatively quickly from the females.

Mechanism of action

Sterility occurs when reproductive organs are altered in a way that temporarily or permanently

impairs reproductive capacity (Bleicher 2003). Our results suggest that diflovidazin does not permanently impair female reproductive function, since egg fertility recovered after exposure ceased. Instead, the compound appears to affect developing eggs (prelarvae) during their maturation. Since oocytes develop on the surface of the ovary, which is in close contact with the midgut (Pijnacker 1985), they may have been exposed to diflovidazin through excreted bodily fluids during egg-laying. Thus, rather than sterilizing the female, diflovidazin exhibits a transmaternal ovicidal effect.

Excretion and metabolism

The rapid recovery of egg viability observed in this study is consistent with the high metabolic turnover reported for two-spotted spider mites. McEnroe (1961) observed that an adult female can consume 6×10^{-3} µL of water in 30 minutes, with an excretion rate of 5×10^{-3} µL in the same period. This means that 25–30% of the female's body weight passes through its alimentary canal every 30 minutes. Given this rapid metabolic turnover, it is unsurprising that diflovidazin is quickly eliminated from the organism.

Implications for pest management

Although diflovidazin showed excellent ovicidal efficacy through transmaternal exposure, the relatively short persistence of this effect should be considered in spider mite control strategies. The transmaternal efficacy of the compound may decrease under conditions of intensive shoot growth, when newly developed untreated plant tissues become available for feeding shortly after application. Therefore, the timing of application and coverage quality may strongly influence field performance, highlighting the importance of further field-based investigations.

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بررسی آزمایشگاهی اثر دیفلویدازین بر تولید تخم و فعالیت تخم‌کشی بین مادری در *Tetranychus urticae* (Acari: Tetranychidae)

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دریافت
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دبیر تخصصی
ا. ترابی

چکیده

خواص بین مادری دیفلویدازین در شرایط آزمایشگاهی روی *Tetranychus urticae* بررسی شد. برای ارزیابی اثر تخم‌کشی بین مادری آن، دیسک‌های برگی در محلول دیفلویدازین با غلظت ۱۰۰ پی‌پی‌ام غوطه‌ور شدند و پس از آن به ماده‌های بالغ اجازه داده شد تا به مدت ۲ یا ۴۸ ساعت از سطوح تیمار شده تغذیه کنند. سپس ماده‌ها طی پنج دوره ۷ ساعته پیاپی، تخم‌های خود را روی سطح تیمار نشده گذاشتند. مرگ تخم‌ها بر اساس مدت زمان قرار گرفتن در معرض و زمان سپری شده از زمان قرار گرفتن در معرض ارزیابی شد. پس از جدا شدن از سطح تیمار شده، ۱۰۰٪ تخم‌های گذاشته شده توسط حشرات ماده در دوره ۷ ساعته نخست، زنده نبودند. با این حال، مرگ و میر – و در نتیجه اثر آن – به تدریج در دوره‌های بعدی کاهش یافت، به طوری که تخم‌های گذاشته شده پس از ۲۸ ساعت، سطح مرگ و میر قابل مقایسه با میزان‌های طبیعی را نشان دادند. نکته قابل توجه این است که طول دوره در معرض قرار گرفتن تاثیری بر میزان کاهش مرگ و میر تخم‌ها نداشت. تأثیر آن بر تولید تخم نیز بررسی شد. به کنه‌های ماده اجازه داده شد تا روی دیسک‌های برگی تیمار شده تخم بگذارند، اما این تیمار تعداد تخم‌های تولید شده به ازای هر کنهٔ ماده را به میزان زیادی تغییر نداد. این یافته‌ها نشان می‌دهد که اگرچه دیفلویدازین به طور مؤثر باعث مرگ و میر زیاد تخم‌ها می‌شود، اما تولید کلی تخم‌ها را کاهش نمی‌دهد. این نتایج نشان می‌دهد که دیفلویدازین به طور موقت کنه‌های ماده را عقیم می‌کند، به احتمال به این دلیل که تخم‌های در حال رشد، ماده فعال را از سیستم کنهٔ ماده جذب می‌کنند.

واژگان کلیدی: کنه‌کش، دیفلویدازین، تولید تخم، فعالیت تخم‌کشی بین مادری، کنهٔ تارتن دو لکه‌ای

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