








Effects of solid and aqueous dietary diflubenzuron ingestion on some biological parameters in synthetic pyrethroid-resistant German cockroach, *Blattella germanica* L. (Blattodea: Ectobiidae)

Emre Oz¹  | Burak Polat²  | Aysegul Cengiz²  | Sevval Kahraman²  |
Zeynep Nur Gultekin²  | Cansu Caliskan²  | Huseyin Cetin² 

¹Department of Medical Services and Techniques, Vocational School of Health Services, Antalya Bilim University, Antalya, Türkiye

²Department of Biology, Faculty of Science, Akdeniz University, Antalya, Türkiye

Correspondence

Emre Oz, Department of Medical Services and Techniques, Vocational School of Health Services, Antalya Bilim University, Antalya 07190, Türkiye.

Email: emre.oz@antalya.edu.tr

Associate Editor: Emma Weeks

Abstract

Cockroaches, widespread pests found in metropolitan areas, are known as vectors of various disease agents, including viruses, fungi and antibiotic-resistant bacteria, as well as causing allergies in humans. Insect growth regulators have been used in pest management for several decades. These insecticides disrupt insect development and reproduction. Chitin synthesis inhibitors interfere with chitin biosynthesis in insects, causing abortive moulting and mortality, as well as inhibiting egg fertility, and larval hatching in insects. In this research, we evaluated the various effects of diflubenzuron, a chitin synthesis inhibitor, on synthetic pyrethroid-resistant German cockroach (*Blattella germanica* L. Blattodea: Ectobiidae), including ootheca production, oothecal viability, ootheca incubation time, the number of nymphs emerging from the ootheca and survivorship of nymphs. The cockroaches were fed diets that contained diflubenzuron, which was added to solid bait (impregnated fish food) and ingestible aqueous bait (impregnated cotton). Three concentrations (0.5%, 1% and 2%) were used in the experiments. As a result, diflubenzuron treatment led to ootheca production ranging from 60% to 100%; statistically, no difference was found between the treatment and the control groups. The number of nymphs emerging from the first and second ootheca was reduced by 40%–100% in the diflubenzuron-treated groups compared with the control. Nymphs exposed to diflubenzuron-impregnated solid bait and ingestible aqueous bait experienced mortality exceeding 92.1% and 66.27% within 15 days, respectively. In conclusion, diflubenzuron is a potential insecticide for use in cockroach baits to control *B. germanica*, as it caused high nymphal and embryonic mortality in the synthetic pyrethroid-resistant population and decreased the number of nymphs emerging from the ootheca.

KEYWORDS

benzoylphenyl ureas, chitin synthesis inhibitor, dietary toxicity, insect growth regulator, pest management, vector

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *Medical and Veterinary Entomology* published by John Wiley & Sons Ltd on behalf of Royal Entomological Society.

INTRODUCTION

One of the insect groups living in human dwellings together with human beings in the world is certain species of cockroaches. These insects are considered pests that pose a risk to public health due to their role as vectors of pathogens such as intestinal parasites, bacteria, fungi and viruses (Fotedar & Banerjee, 1992; Menasria et al., 2014). Over a 100 potentially pathogenic organisms have been identified in cockroaches collected in and around human and other animal environments (e.g., farms, zoos, aviaries and kennels) (Schal & DeVries, 2021). Although there are almost 4000 cockroach species, only 1% of them have been detrimental to human health according to the World Health Organisation (WHO, 2006). The German cockroach, *Blattella germanica* L. (Blattodea: Ectobiidae), is the most common species found in urban environments, living in homes and restaurants. In addition to facilitating the transmission of various pathogens, it causes allergic reactions, physiological stress and compromises food hygiene (Solomon et al., 2016; WHO, 2006).

Pyrethroids, oxadiazines, phenylpyrazoles, avermectins, neonicotinoids and sulfonamides are synthetic pesticides used worldwide for pest control (Gondhalekar et al., 2021; Scharf & Gondhalekar, 2021; WHO, 2006). However, the overuse of these insecticides for pest management has led to the development of resistance in cockroach populations (González-Morales et al., 2022; Jang et al., 2017). For example, Chai and Lee (2010) found low to extremely high resistance to deltamethrin (4.5- to 468-fold) in *B. germanica* populations collected from 22 districts in Singapore. In a previous study conducted in Türkiye, we found that five field populations of *B. germanica* had moderate-to-high resistance (resistance ratios between 7.7- and ≥ 1000 -fold) to synthetic pyrethroids; alpha-cypermethrin, deltamethrin, lambda-cyhalothrin and permethrin (Öz et al., 2021). Furthermore, the use of synthetic pesticides has been found to be harmful to non-target organisms and the environment (Tan et al., 2023; Vivekanandhan et al., 2021). Therefore, researchers have focused on identifying alternative products that are safer, less toxic and more selective toward target pests.

Insect growth regulators (IGRs) are highly selective insecticides primarily used to manage the immature stage of various pests in the Diptera, Hymenoptera and Hemiptera orders, including mosquitoes, houseflies, midges, fleas and bed bugs (Cetin et al., 2006; Joseph, 2017; Kawada & Hirano, 1996; Kimiaei et al., 2022; Mommaerts et al., 2006; Rubio et al., 2019; Sierras & Schal, 2020).

IGRs are classified based on their mode of action: JHAs (juvenile hormone analogs) and ecdysone agonists mimic insect hormones, whereas chitin synthesis inhibitors (CSIs) inhibit chitin biosynthesis in insects. Chitin is found in the cuticle of insects and other organisms such as crustaceans, protozoa, fungi and algae. CSIs are commonly utilised as larvicides in integrated pest management to control public health, veterinary and agricultural pests due to their minimal toxicity to mammals and their unique biosynthetic pathways that differ from traditional broad-spectrum insecticides (Merzendorfer, 2013). CSIs, which interfere with chitin biosynthesis in insects, inhibit the process of incorporation of N-acetylglucosamine into insect chitin, resulting in abortive moulting and mortality (Muthukrishnan et al., 2012), besides inhibiting egg fertility and larval hatching in insects (Mommaerts et al., 2006; Perez-Farinos et al., 1998).

Up to now, 15 benzoylurea CSIs (e.g., diflubenzuron, novaluron, hexaflumuron and triflumuron) have been commercialised (Sun et al., 2015). Although CSIs have been researched for their potential in cockroach control (Hamilton et al., 2021; King, 2005; Seccacini et al., 2018), the toxic effects of diflubenzuron on cockroaches have been investigated in a limited number of studies (Ross & Cochran, 1991; Wadleigh et al., 1991). In these studies, researchers have investigated the toxicity of insecticides, determining mortality and mating rates using various stages of cockroaches. Wadleigh et al. (1991) reported that various concentrations of diflubenzuron killed the first instar nymphs and adults (male and female) of *B. germanica* exposed as nymphs, and total cockroach numbers decreased by 67.3%, 93.0% and 98.2% after 12 weeks of exposure to 30, 60 and 120 mg/m², respectively. Ross and Cochran (1991) investigated the toxic effects of contact exposure to IGR including diflubenzuron on *B. germanica* nymphs and reported that small nymphs were more susceptible to diflubenzuron than large nymphs (100~ and 80–87~ kill at 600 ng/cm², respectively), and the lowest level of hatching was shown at the concentration of 600 ng/cm².

CSIs also have effects on reproduction in insects by inhibiting egg fertility and nymphal hatch. King (2005) studied the ovicidal activity of noviflumuron on adult German cockroaches through ingestion of treated bait and found that noviflumuron caused significant ovicidal effects after 5-day feeding exposure to bait concentrations ranging from 10 to 5000 ppm on virgin and fertilised females. The concentration of 5000 ppm caused 100% ovicidal activity over two ovarian cycles. Alamer and Hoffmann (2014) studied the toxicities of methoprene and pyriproxyfen against the Argentinian cockroach, *Blaptica dubia* Serville (Blattodea, Blaberidae) during the first vitellogenic cycle or during the period of gestation, and found that the treatment did not inhibit vitellogenesis or oocyte development during the first vitellogenic cycle (days 2–20 of adulthood), but it averted the ootheca formation. Besides, treatment of females in the gestation stage (days 30–70) resulted in full ootheca disintegration and resorption, as well as the induction of another vitellogenic cycle. Kaakeh et al. (1997) investigated the effect of lufenuron against *B. germanica* and reported that with increasing exposure time, the number of live oothecae decreased, the number of aborted oothecae increased, nymphal hatch decreased, nymphal survival at 7 days after hatch decreased, and nymphal survival of the first moult decreased. While diflubenzuron has been shown to be toxic to cockroaches, studies have not been done to assess its impact on the reproductive parameters of German cockroaches. This study aimed to investigate the effects of diflubenzuron on biological parameters (ootheca production, oothecal viability, ootheca incubation time, the number of nymphs emerging from the ootheca and survivorship of nymphs) of a synthetic pyrethroid-resistant isolate of the German cockroach *B. germanica*.

MATERIALS AND METHODS

Insects

A synthetic pyrethroid-resistant strain (≥ 1000 fold to alpha-cypermethrin, deltamethrin, lambda-cyhalothrin and permethrin) of

B. germanica originated from Dokuma region in Antalya, Türkiye, was used in the experiment (Öz et al., 2021). The insects were reared at the Vector Control and Ecology Laboratory of Akdeniz University, Antalya, Türkiye, in 5-L polyethylene cylindrical plastic jars since 2014. Cockroach colonies were fed a mixture of flour-honey and water ad libitum and were kept under laboratory conditions at a temperature of $25 \pm 2^\circ\text{C}$, 60% relative humidity, and a photoperiod of 12:12 h (L:D). Newly eclosed (1-day-old) adults (both males and females) were used for the experiments.

Insect growth regulator

The commercial insecticide Diflubenzuron 10 SC (suspension concentrate) used in the study was purchased from Bio-Sav Health Products Marketing Limited Company in Türkiye. The concentrations to be tested, which were 0.5%, 1% and 2% active ingredient, were prepared by diluting 10% active ingredient diflubenzuron solution. They were thoroughly agitated to ensure homogeneity before being used in the tests. The concentrations tested were determined by preliminary experiments. The concentrations selected did not result in sudden death or have any repellent or deterrent effect on *B. germanica*.

Experimental design

Bioassays were conducted in the laboratory (at a temperature of $25 \pm 2^\circ\text{C}$, 60% relative humidity and a photoperiod of 12:12 h L:D) using the methods of Koehler and Patterson (1989) and Hamilton et al. (2021) with minor modifications. Diflubenzuron-supplemented diets were prepared by impregnating pellet fish food bait (Sera Pond Granulat, Sera, France) and ingestible aqueous bait (water-impregnated cotton) with the appropriate diflubenzuron concentration. The cups with diflubenzuron-supplemented diets were then placed in each 5-L polyethylene cylindrical plastic jar separately. Control groups were fed untreated solid or aqueous diets. The experiments were conducted with a total of five repetitions, with one male and one female cockroach placed in each plastic jar. As sufficient

numbers of 1-day-old male and female cockroach pairs were not obtained at the same time, the repetitions were established at separate times (1–3 days intervals). The ootheca production rate, oothecal viability, incubation period and the number of nymphs emerging from the ootheca were recorded daily. In addition, all nymphs emerging from the ootheca in each repetition were exposed to the same diflubenzuron-supplemented diets for 15 days, after which the live nymph counts were recorded. The period of ootheca formation (days) was noted after the ootheca was fully formed (within 1 day after it appeared in the abdomen). The ootheca incubation time is the period of time between when the ootheca is entirely created and when it opens. These biological parameters were monitored for two ovarian cycles. Test solutions were re-prepared at 7–10 day intervals and stored in the refrigerator at 4°C for further processing. Diets were replenished as needed during the test duration. A cockroach was considered dead if it could not return to its normal position after its abdomen was touched.

Statistical analysis

The average percentage of ootheca production, ootheca formation period, ootheca incubation time, the number of nymphs emerging from the ootheca and survivorship was subjected to analysis of variance using SPSS 20.0. Differences in values were analysed using Duncan's Multiple Range Test. All statistical differences were evaluated at $p < 0.05$.

RESULTS

Ootheca production

When the results of feeding with diflubenzuron-supplemented diets (solid and aqueous baits) were analysed, it was observed that the ootheca production of German cockroach ranged between 60% and 100% (Table 1), and no statistical difference was observed between the treatment groups and the control group in either diet (Table 1).

TABLE 1 Ootheca production in *Blattella germanica* fed with diflubenzuron-impregnated baits (% mean \pm standard error) ($N = 5$).

Concentrations (%)	Diflubenzuron-supplemented ingestible solid bait		Diflubenzuron-supplemented ingestible aqueous bait	
	First ootheca production (%)	Second ootheca production (%)	First ootheca production (%)	Second ootheca production (%)
Control	80.0 \pm 20.0 a*	80.0 \pm 20.0 a	80.0 \pm 20.0 a	80.0 \pm 20.0 a
0.5	100.0 \pm 0.0 a	100.0 \pm 0.0 a	100.0 \pm 0.0 a	100.0 \pm 0.0 a
1	80.0 \pm 20.0 a	80.0 \pm 20.0 a	100.0 \pm 0.0 a	60.0 \pm 24.5 a
2	80.0 \pm 20.0 a	60.0 \pm 24.5 a	80.0 \pm 20.0 a	0*

Note: Means within a line followed by the same lowercase letter are not significantly different (Duncan's test, $p > 0.05$).

Abbreviation: N, number of repetitions.

*All tested females and males died after exposure to diflubenzuron.

Solid bait

No fatalities were observed in adult individuals that were provided solid food. The first ootheca formation period ranged from 9.75 to 13 days (df: 3.16; $p = 0.798$), but the second ootheca formation period was shorter ranging from 6.4 to 8.67 days (df: 3.15; $p = 0.181$). There was no statistical difference between the control and cockroaches fed CSI for either ootheca formation period (Table 2).

The ootheca incubation period for the first ootheca varied from 20.25 to 23.5 days (df: 3.16; $p = 0.002$), whereas the second ootheca incubation period ranged from 23.25 to 28.25 days (df: 3.15; $p = 0.000$). The groups fed with diflubenzuron-impregnated solid bait had a longer incubation period than the controls for both the first and second ootheca ($p \leq 0.05$, Table 2).

The number of nymphs emerging from the first ootheca decreased as the concentrations of diflubenzuron increased. At the

2% concentration, an average of 10 nymphs emerged, which is statistically fewer than the control group (38 nymphs). For the second ootheca, there were no live nymphs at the 1% concentration, and the number of nymphs reduced dramatically compared with the control group at the 0.5% and 2% concentrations (Table 2). Almost all the nymphs emerging from the first and second ootheca that continued to be fed diflubenzuron died by 15 days (Table 2).

Aqueous bait

All females fed ingestible aqueous bait supplemented with 1% diflubenzuron died during the second ootheca production or incubation period. At the 2% diflubenzuron-impregnated ingestible aqueous bait concentration, all adult cockroaches died without producing the second ootheca (Table 3). In addition, male cockroaches fed with a 2%

TABLE 2 First and second ovarian cycles in *Blattella germanica* fed with diflubenzuron-impregnated solid bait (% mean \pm standard error).

First and second ovarian cycles	Control	0.5%	1%	2%
First ootheca formation period (day)	13.00 \pm 3.67 a	12.60 \pm 3.11 a	9.75 \pm 0.25 a	11.00 \pm 0.41 a
First ootheca incubation period (day)	20.25 \pm 0.25 a	22.60 \pm 0.6 b	22.75 \pm 0.25 b	23.50 \pm 0.50 b
Number of the ootheca opened (first ootheca)	4	4	3	1
Number of nymphs emerging from the first ootheca	38.00 \pm 1.96 a	22.80 \pm 7.25ab	22.00 \pm 9.07 ab	10.00 \pm 10.00 b
Number of nymphs surviving from the first ootheca after 15 days of exposure to diflubenzuron	35.00 \pm 2.74 a	0.25 \pm 0.20 b	0.00 \pm 0.00 b	0.00 \pm 0.00 b
Second ootheca formation period (day)	7.00 \pm 0.82 a	6.40 \pm 0.40 a	7.75 \pm 0.75 a	8.67 \pm 0.88 a
Second ootheca incubation period (day)	23.25 \pm 0.48 a	27.00 \pm 0.55 b	28.25 \pm 0.48 b	27.33 \pm 0.88 b
Number of the ootheca opened (second ootheca)	4	3	0	2
Number of nymphs emerging from the second ootheca	36.00 \pm 3.34 a	16.00 \pm 8.00 ab	0.00 \pm 0.00 b	25.33 \pm 12.88 a
Number of nymphs surviving from the second ootheca after 15 days of exposure to diflubenzuron	34.25 \pm 3.20 a	0.00 \pm 0.00 b	0.00 \pm 0.00 b	2.00 \pm 1.00 b

Note: Means within a line followed by the same lowercase letter are not significantly different (Duncan's test, $p > 0.05$).

TABLE 3 First and second ovarian cycles in *Blattella germanica* fed with diflubenzuron-impregnated ingestible aqueous bait (% mean \pm standard error).

First and second ovarian cycles	Control	0.5%	1%	2%
First ootheca formation period (day)	13.00 \pm 3.67 a	9.80 \pm 0.37a	10.20 \pm 0.20 a	10.50 \pm 0.29 a
First ootheca incubation period (day)	20.25 \pm 0.25 a	22.20 \pm 0.80 ab	23.40 \pm 0.68 b	24.00*
Number of the ootheca opened (first ootheca)	4	3	3	1
Number of nymphs emerging from the first ootheca	38.00 \pm 1.96 a	20.00 \pm 8.50 ab	18.00 \pm 8.06 ab	6.50 \pm 6.50 b
Number of nymphs surviving from the first ootheca after 15 days of exposure to diflubenzuron	35.00 \pm 2.74 a	2.00 \pm 1.00 b	5.00 \pm 1.53 b	3.00*
Second ootheca formation period (day)	7.00 \pm 0.82 a	6.20 \pm 0.58 a	8.67 \pm 1.86 a	NC
Second ootheca incubation period (day)	23.25 \pm 0.48 a	27.20 \pm 1.07 b	NC	NC
Number of the ootheca opened (second ootheca)	4	3	NC	NC
Number of nymphs emerging from the second ootheca	36.00 \pm 3.34 a	15.80 \pm 6.62 b	NC	NC
Number of nymphs surviving from the second ootheca after 15 days of exposure to diflubenzuron	34.25 \pm 3.20 a	5.33 \pm 0.88 b	NC	NC

Note: Means within a line followed by the same lowercase letter are not significantly different (Duncan's test, $p > 0.05$).

*Since only one ootheca opened, statistics could not be conducted.

Abbreviations: NC, non-calculable because the females died before producing oothecae.

diflubenzuron-impregnated ingestible aqueous bait died on average 21.4 days, which is approximately 10 days earlier than females (31.6 days).

The first ootheca formation period ranged from 9.8 to 13 days (df: 3,17; $p = 0.544$), whereas the second ootheca formation period ranged between 6.2 and 8.67 days (df: 2,11; $p = 0.284$), with no significant difference between the control and treatment groups.

The ootheca incubation period for the first ootheca was 20.25, 22.2 and 23.4 days in the control group, 0.5%, and 1%, respectively. At the 2% concentration, only one of the four oothecae opened, and its incubation time was 24 days. At 1% concentration, the ootheca incubation period for the first ootheca was statistically longer compared with the control group (df: 2,13; $p = 0.024$). The second incubation times for the control and 0.5% concentration groups were 23.25 and 27.2 days, respectively.

As the concentration increased, the number of nymphs emerging from the first ootheca decreased, and there was a statistical difference between the cockroaches treated with 2% diflubenzuron and the control group (df: 3,17; $p = 0.073$). The number of nymphs emerging from the second ootheca in the control and 0.5% concentration groups was 36 and 15.8, respectively (Table 3).

The majority of newly hatched nymphs from the first and second ootheca that continued to be fed diflubenzuron died by 15 days (Table 3).

DISCUSSION

German cockroach populations have been shown to be resistant to a wide range of conventional insecticides around the world (Chai & Lee, 2010; González-Morales et al., 2022; Jang et al., 2017). Resistance to conventional insecticides has encouraged the development of other control methods and strategies for pest control, such as IGRs.

We conducted two different diet assays to determine the toxicity of diflubenzuron on *B. germanica*. Although we did not formally quantify how much bait was consumed between treatments, we observed that the cockroaches were actively consuming the baits and it suggests that the baits were not repellent. Our results indicated that exposing cockroaches to a diflubenzuron-supplemented impregnated solid food bait or ingestible aqueous bait had a significant impact on several biological parameters. Ootheca production varied between 60% and 100% when feeding with diflubenzuron-impregnated solid food bait or ingestible aqueous bait, and ootheca production in the control was 80%. Koehler and Patterson (1989) reported that newly emerged *B. germanica* adults fed 0.25% two IGRs produced between 73.6% and 95.8% ootheca, similar to our results.

We observed that female cockroaches fed a 2% ingestible aqueous bait produced darkened, nonviable oothecae in the first ootheca formation. This situation has been observed in several studies, including diflubenzuron and other CSIs. Weaver et al. (1984) examined adult sterility effects of Alsystin 25 WP (Bayer AG, Leverkusen, Germany), a commercial insecticide containing triflumuron, in *B. germanica* populations and reported that discoloration of oothecae from typical

chestnut brown to black occurred in 50% and 70% of oothecae generated by females on 0.25% and 0.5% (active ingredient) diets, respectively. Hamilton et al. (2021) reported that when adult females were fed novaluron at a 0.0001% concentration, they formed oothecae, but all of the embryos were inviable and turned black. The 2% ingestible aqueous bait diet was toxic to cockroaches and caused their death in the second ootheca formation.

Female German cockroaches developed oothecae by days 13 and 7 in the first and second ootheca. The incubation time for the control group lasted 20.25 and 23.25 days in the first and second ootheca and all nymphs hatched in 33.25 and 30.25 days, respectively. Moreover, the incubation period was significantly shorter by a few days in the control groups than in both treatment groups (the first and second ootheca incubation periods in the treatment groups ranged between 22.2 and 28.25 days). Hamilton et al. (2021) reported that in the control group, female German cockroaches developed oothecae by day 8, incubation time retained 21 days, and nymphs hatched between days 34 and 38.

An average of 36.5 first-stage nymphs emerged from the first and second ootheca cycles in the control group. Overall, the number of nymphs emerging from the ootheca decreased dramatically as diflubenzuron concentrations increased. No live nymphs hatched from the second ootheca in *B. germanica* fed with 1% concentration diflubenzuron-impregnated solid bait. In many studies, diflubenzuron and other CSI insecticides have been reported to reduce the number of hatching *B. germanica* nymphs. Koehler and Patterson (1989) found that control groups had an average of 30.1 first-stage nymphs per ootheca during the first oothecal cycle, whereas the benzoylphenyl ureas (i.e., CSIs: diflubenzuron, ChoCGA-112913 and UC-84572) entirely stopped nymph hatch, and also cyromazine and alsystin reduced significantly nymph hatch compared with the controls (8.8 and 0.3 nymphs per ootheca, respectively). Koehler and Patterson (1989) also examined whether the decrease in reproduction was permanent or reversible; only untreated bait was given to adult cockroaches after the first oothecal cycle and found that during the second oothecal cycle, similar results of reduced oothecal production and prevention of nymph hatch were achieved; but during the third oothecal period, the effect of the treatments reversed and normal oothecal production ranged from 75% to 100%, with 23.5–30.5 nymphs emerging per ootheca in the four of the five treatment groups, and 100% (oothecal production) with 30.3 (nymphs emerging per ootheca), respectively, for the controls.

Almost all first-instar nymphs died after being exposed to diflubenzuron for 15 days, and no nymphs were able to reach the adult stage. Nymphs either died within their exoskeletons or while trying to moult. These findings are consistent with previous research on the effectiveness of different IGRs and diflubenzuron in *B. germanica* (Hamilton et al., 2021; Wadleigh et al., 1991). Koehler and Patterson (1989) reported that bait formulations (0.25%) of CSI compounds ChoCGA-112913 and UC-84572 resulted in complete mortality occurring 1 week after treatment. Also, it took 4–6 weeks for the other baits (alsystin, diflubenzuron and cyromazine) to reach 100% mortality.

The effects of low concentrations of CSI compounds on adult insects are usually nonlethal. Hamilton et al. (2021) reported that novaluron did not cause significant mortality in adults. Koehler and Patterson (1989) reported that no significant mortality of newly emerged adult German cockroaches fed 0.25% baits of the various CSIs occurred during the first oothecal cycles. In our study, all of the females fed with a 2% diflubenzuron-impregnated ingestible aqueous bait died without forming the second ootheca. In addition, male cockroaches fed with a 2% diflubenzuron-impregnated ingestible aqueous bait died earlier than females. We think that the difference in mortality among adult cockroaches in the previous and current studies may be primarily attributed to the higher concentrations we tested in our research. In addition, the impact of testing different CSIs, or using exposure methods (i.e., uptake by contact or ingestion of baits) should be confirmed through further experiments in the future.

While our results were statistically significant, additional replicates would have resulted in more consistent findings. Furthermore, opting to test a specific formulation over an active ingredient in our study can be considered a risk. Despite the fact that the formulation we used did not exhibit a deterrent effect on cockroach feeding, it is recommended to exercise utmost care in formulation application and, if possible, use only the active ingredient. For researchers contemplating similar studies in the future, it is believed that conducting a larger number of replications with new generations of cockroaches and batches of pesticide and incorporating technical substances will contribute to obtaining more robust data.

German cockroaches have evolved resistance to many insecticides, so there is a need to produce new insecticides with a variety of modes of action and formulations to delay and minimise the emergence of insecticide resistance. To our knowledge, although there is limited availability of commercial products containing JHA (pyriproxyfen) and CSI (novaluron) for cockroach control, diflubenzuron has not yet received commercial authorization for cockroach control. The results of our study show that diflubenzuron causes high nymphal and embryonic mortality in the synthetic pyrethroid-resistant German cockroach population and could potentially be used in baits to control German cockroach populations. In the future, more research should be conducted on the toxicity of diflubenzuron and other IGRs on cockroaches.

AUTHOR CONTRIBUTIONS

Emre Oz: Conceptualization; data curation; project administration; investigation; methodology; formal analysis; writing – original draft; writing – review and editing. **Burak Polat:** Investigation; data curation. **Aysegul Cengiz:** Data curation; investigation. **Sevval Kahraman:** Investigation; data curation. **Zeynep Nur Gultekin:** Investigation; data curation. **Cansu Caliskan:** Investigation; data curation. **Huseyin Cetin:** Conceptualization; data curation; project administration; investigation; methodology; writing – review and editing; supervision; resources.

ACKNOWLEDGEMENTS

The authors are thankful to the staff of the Biology Department of Akdeniz University (Antalya, Türkiye) for their laboratory support.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Emre Oz  <https://orcid.org/0000-0001-5513-4960>

Burak Polat  <https://orcid.org/0000-0001-8975-4121>

Aysegul Cengiz  <https://orcid.org/0000-0001-9190-1821>

Sevval Kahraman  <https://orcid.org/0000-0003-2755-6677>

Zeynep Nur Gultekin  <https://orcid.org/0000-0001-5661-4931>

Cansu Caliskan  <https://orcid.org/0009-0002-5935-2732>

Huseyin Cetin  <https://orcid.org/0000-0002-9758-6356>

REFERENCES

- Alamer, A.H. & Hoffmann, K.H. (2014) Effects of juvenile hormone analogue treatment in adult females of the ovoviparous cockroach, *Blattella dubia* (Dictyoptera, Blaberidae). *Invertebrate Reproduction & Development*, 58, 53–59.
- Cetin, H., Yanikoglu, A. & Cilek, J.E. (2006) Efficacy of diflubenzuron, a chitin synthesis inhibitor, against *Culex pipiens* larvae in septic tank Water1. *Journal of the American Mosquito Control Association*, 22, 343–345.
- Chai, R.Y. & Lee, C.Y. (2010) Insecticide resistance profiles and synergism in field populations of the German cockroach (Dictyoptera: Blattellidae) from Singapore. *Journal of Economic Entomology*, 103, 460–471.
- Fotadar, R. & Banerjee, U. (1992) Nosocomial fungal infections—study of the possible role of cockroaches (*Blattella germanica*) as vectors. *Acta Tropica*, 50, 339–343.
- Gondhalekar, A.D., Appel, A.G., Thomas, G.M. & Romero, A. (2021) A review of alternative management tactics employed for the control of various cockroach species (Order: Blattodea) in the USA. *Insects*, 12, 550.
- González-Morales, M.A., DeVries, Z.C., Santangelo, R.G., Kakumanu, M.L. & Schal, C. (2022) Multiple mechanisms confer fipronil resistance in the German cockroach: enhanced detoxification and Rdl mutation. *Journal of Medical Entomology*, 59, 1721–1731.
- Hamilton, J.A., Wada-Katsumata, A., Ko, A. & Schal, C. (2021) Effects of novaluron ingestion and topical application on German cockroach (*Blattella germanica*) development and reproduction. *Pest Management Science*, 77, 877–885.
- Jang, C.W., Ju, Y.R. & Chang, K.S. (2017) Insecticide susceptibility of field-collected *Blattella germanica* (Blattaria: Blattellidae) in Busan, Republic of Korea during 2014. *Entomological Research*, 47, 243–247.
- Joseph, S.V. (2017) Effects of insect growth regulators on *Bagrada hilaris* (Hemiptera: Pentatomidae). *Journal of Economic Entomology*, 110, 2471–2477.
- Kaakeh, W., Reid, B.L., Kaakeh, N. & Bennett, G.W. (1997) Rate determination, indirect toxicity, contact activity, and residual persistence of lufenuron for the control of the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology*, 90, 510–522.
- Kawada, H. & Hirano, M. (1996) Insecticidal effects of the insect growth regulators methoprene and pyriproxyfen on the cat flea (Siphonaptera: Pulicidae). *Journal of Medical Entomology*, 33, 819–822.
- Kimiaei, M., Jalalizand, A. & Mahmoudi, E. (2022) Efficacy and horizontal transmission of *Beauveria bassiana* and its synergistic activity with diflubenzuron against the house fly, *Musca domestica* L. *Biocontrol Science and Technology*, 32, 551–563.

- King, J.E. (2005) Ovicidal activity of noviflumuron when fed to adult German cockroaches (Dictyoptera: Blattellidae). *Journal of Economic Entomology*, 98, 930–932.
- Koehler, P.G. & Patterson, R.S. (1989) Effects of chitin synthesis inhibitors on German cockroach (Orthoptera: Blattellidae) mortality and reproduction. *Journal of Economic Entomology*, 82, 143–148.
- Menasria, T., Moussa, F., El-Hamza, S., Tine, S., Megri, R. & Chenchouni, H. (2014) Bacterial load of German cockroach (*Blattella germanica*) found in hospital environment. *Pathogens and Global Health*, 108, 141–147.
- Merzendorfer, H. (2013) Chitin synthesis inhibitors: old molecules and new developments. *Insect Science*, 20, 121–138.
- Mommaerts, V., Sterk, G. & Smaghe, G. (2006) Hazards and uptake of chitin synthesis inhibitors in bumblebees *Bombus terrestris*. *Pest Management Science*, 62, 752–758.
- Muthukrishnan, S., Merzendorfer, H., Arakane, Y. & Kramer, K.J. (2012) Chitin metabolism in insects. In: Gilbert, L.I. (Ed.) *Insect molecular biology and biochemistry*. San Diego, USA: Academic Press, pp. 193–235.
- Öz, E., Çetin, H. & Yanikoğlu, A. (2021) Investigation of resistance to synthetic pyrethroids in *Blattella germanica* L., 1767 (Blattodea: Ectobiidae) and *Periplaneta americana* L., 1758 (Blattodea: Blattellidae) populations in Türkiye. *Turkish Journal of Entomology*, 45, 361–370.
- Perez-Farinos, G., Smaghe, G., Marco, V., Tirry, L. & Castañera, P. (1998) Effects of topical application of hexaflumuron on adult sugar beet weevil, *Aubeonemus mariaefrancisciae*, on embryonic development: pharmacokinetics in adults and embryos. *Pesticide Biochemistry and Physiology*, 61, 169–182.
- Ross, M.H. & Cochran, D.G. (1991) Effects on German cockroach nymphs of contact exposure to IGRs, singly and in combination. *Entomologia Experimentalis et Applicata*, 61, 117–122.
- Rubio, A., Cardo, M.V., Carbajo, A.E. & Vezzani, D. (2019) Assessment of combined tools and strategies for *Aedes aegypti* control with low environmental impact. *Parasitology Research*, 118, 411–420.
- Schal, C. & DeVries, Z.C. (2021) *Public health and veterinary importance*. Clayton South, Victoria, Australia: CSIRO Publishing, pp. 17–52.
- Scharf, M.E. & Gondhalekar, A.D. (2021) Insecticide resistance: perspectives on evolution, monitoring, mechanisms and management. In: Wang, C., Lee, C.Y. & Rust, M.K. (Eds.) *Biology and Management of the German Cockroach*, 1st edition. Wallingford, UK: CABI, pp. 1–304.
- Seccacini, E.A., Vassena, C.V., Zerba, E.N. & Alzogaray, R.A. (2018) Lufenuron kills deltamethrin-resistant *Blattella germanica* (Blattodea). *Revista de la Sociedad Entomológica Argentina*, 77, 32–35.
- Sierras, A. & Schal, C. (2020) Lethal and sublethal effects of ingested hydroprene and methoprene on development and fecundity of the common bed bug (Hemiptera: Cimicidae). *Journal of Medical Entomology*, 57, 1199–1206.
- Solomon, F., Belayneh, F., Kibru, G. & Ali, S. (2016) Vector potential of *Blattella germanica* (L.) (Dictyoptera: Blattellidae) for medically important bacteria at food handling establishments in Jimma town, Southwest Ethiopia. *BioMed Research International*, 2016, 1–6.
- Sun, R., Liu, C., Zhang, H. & Wang, Q. (2015) Benzoylurea chitin synthesis inhibitors. *Journal of Agricultural and Food Chemistry*, 63, 6847–6865.
- Tan, H., Wu, Q., Hao, R., Wang, C., Zhai, J., Li, Q. et al. (2023) Occurrence, distribution, and driving factors of current-use pesticides in commonly cultivated crops and their potential risks to non-target organisms: a case study in Hainan, China. *Science of the Total Environment*, 854, 158640.
- Vivekanandhan, P., Swathy, K., Thomas, A., Kweka, E.J., Rahman, A., Pittarate, S. et al. (2021) Insecticidal efficacy of microbial-mediated synthesized copper nano-pesticide against insect pests and non-target organisms. *International Journal of Environmental Research and Public Health*, 18, 10536.
- Wadleigh, R.W., Koehler, P.G. & Patterson, R.S. (1991) Age-specific reduction in German cockroach (Blattoidea: Blattellidae) populations exposed to diflubenzuron. *Journal of Entomological Science*, 26, 244–252.
- Weaver, J.E., Begley, J.W. & Kondo, V.A. (1984) Laboratory evaluation of alsystin against the German cockroach (Orthoptera: Blattellidae): effects on immature stages and adult sterility. *Journal of Economic Entomology*, 77, 313–317.
- WHO (World Health Organization). (2006) *Pesticides and their application for the control of vectors and pests of public health importance*, 6th edition. Geneva, Switzerland: World Health Organization, WHO/CDS/NTD/WHOPES/GCDPP/2006.1. Available from: <https://apps.who.int/iris/handle/10665/69223>. [Accessed 22nd May 2023].

How to cite this article: Oz, E., Polat, B., Cengiz, A., Kahraman, S., Gultekin, Z.N., Caliskan, C. et al. (2023) Effects of solid and aqueous dietary diflubenzuron ingestion on some biological parameters in synthetic pyrethroid-resistant German cockroach, *Blattella germanica* L. (Blattodea: Ectobiidae). *Medical and Veterinary Entomology*, 1–7. Available from: <https://doi.org/10.1111/mve.12704>