Eficiência de iscas tóxicas em gel para controle de *Blattella germanica* (LINNAEUS, 1767) (BLATTODEA)

Efficiency of toxics baits for the control of Blattella Germanica (LINNAEUS, 1767) (BLATTODEA)

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ABSTRACT: *Blattella germanica* is considered to be a pest insect that is difficult to control and which tends to be controlled by the use of chemical products. As such, the indiscriminate use of insecticides can result in cockroach resistance and environmental contamination. Aiming to promote a more conscientious and effective control method, formulations of toxic gel baits were developed. These baits result in less environmental contamination and can be applied in areas where the use of conventional insecticides is not possible. This study aimed to evaluate which toxic bait presented the most efficient control of *B. germanica*, taking into consideration characteristics such as attractiveness, feeding stimulation and mortality rate. For the bioassay, four compositions of toxic gel baits were used in a test arena, where, in the presence of the baits, (1) the time until consumption, (2) the bait consumed, (3) the time taken for feeding stimulation and (4) the time until death were evaluated. Toxic baits were compared using paired T-tests. We verified that the Fipronil toxic bait did not present attractiveness nor consumption and the Indoxacarb toxic bait presented the greatest attractiveness. There was no statistical difference between the baits when evaluating feeding stimulation and mortality, indicating, in this case, that both baits acted in a similar manner. The Imidacloprid and Indoxacarb toxic baits were recommended for the control of *B. germanica*, as they caused mortalities during a period of less than 24h. However, Indoxacarb presented a greater rate of attractiveness when compared to the Imidacloprid gel baits.

Keywords: Attractiveness. Bioassay. Feeding stimulation. German cockroach. Mortality.

RESUMO: A *Blattella germanica* é considerada um inseto praga de difícil controle e que tende a ser controlado com o uso de produtos químicos. Dessa forma, o uso indiscriminado de inseticidas pode resultar em resistência a baratas e contaminação ambiental. Com o objetivo de promover um método de controle mais consciente e eficaz, foram desenvolvidas formulações de iscas tóxicas em gel. Essas iscas resultam em menos contaminação ambiental e podem ser aplicadas em áreas onde o uso de inseticidas convencionais não é possível. Este estudo teve como objetivo avaliar qual isca tóxica apresentou o controle mais eficiente de *B. germanica*, levando em consideração características como atratividade, estímulo alimentar e taxa de mortalidade. Para o bioensaio, quatro composições de iscas de gel tóxico foram utilizadas em uma arena de teste, onde, na presença das iscas foram avaliados: (1) o tempo até o consumo, (2) a isca consumida, (3) o tempo gasto para estimulação da alimentação e (4) o tempo até a morte. Iscas tóxicas foram comparadas usando testes T emparelhados. Verificamos que a isca tóxica Fipronil não apresentou atratividade nem consumo e a isca tóxica Indoxacarbe apresentou a maior atratividade. Não houve diferença estatística entre as iscas na avaliação do estímulo alimentar e mortalidade, indicando, neste caso, que ambas as iscas agiram de forma semelhante. As iscas tóxicas Imidaclopride e Indoxacarbe foram recomendadas para o controle de *B. germanica*, pois causaram mortalidade em período inferior a 24h. Porém, o Indoxacarbe apresentou maior índice de atratividade quando comparado às iscas em gel de Imidaclopride.

Palavras-chave: Atratividade. Barata alemã. Bioensaio. Estimulação de alimentação. Mortalidade.

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INTRODUCTION

The majority of cockroaches, insects of the order Blattodea (BURMEISTER, 1829) or Blattodea (BRUNNER, 1882), are wild animals that inhabit natural habitats (ZORZENONZ, 2002). These insects present an ample geographic distribution, occurring in humid environments, however, they can withstand diverse thermal variations (7° a 47°C) (HARRISON *et al.*, 2016). In their natural environment, cockroaches inhabit different environments such as leaf litter, soil, tree hollows (DJERNAES, 2018), under stones, between dry flowers, on plants (CUI *et al.*, 2018), and can be found camouflaged under the barks of trees (EVANGELISTA *et al.*, 2015). It is estimated that cockroaches represent approximately 24% of arthropod biomass in tree tops, in fact many studies suggest that these insects can ecologically substitute termites (DJERNAES, 2018). However, 1% of cockroach species are synanthropic (POTENZA, 2005), presenting high adaptability to changes in the environment and large reproductive capacities, as well as being able to utilize diverse food resources and nesting environments (POTENZA, 2005; RAFAEL *et al.* 2008; ZORZENON, 2002).

Synanthropic cockroach species found in Brazil are: *Blattella germanica* (LINNAEUS, 1767), Periplaneta americana (LINNAEUS, 1758), Supella longipalpa (FABRICIUS, 1798) and Pycnoscelys surinamensis (LINNAEUS, 1758) (RAFAEL et al., 2008; ZORZENON, 2002). Within cockroach species, B. germanica and P. americana are considered to be pests as they are economically harmful and damaging to health (BRASIL, 2009; ZORZENON, 2002). In agriculture, these cockroaches have no relevant importance (RAFAEL et al., 2012), however, they cause significant impacts in food processing and storage environments (KINFU; ERKO, 2013; TACHBELE, 2006), in addition to spreading several diseases (RAFAEL et al., 2012; SCHAPHEER et al., 2018). In residences, they are mechanical vectors of diseases caused by bacteria and protozoans, contaminating food through direct contact or through their faeces (WANG et al., 2018). They also act as intermediate hosts for several helminth species, which habitually infest some mammals and eventually humans (NAZARI et al., 2016). Studies have demonstrated that cockroach pests are important disseminators of disease pathogens, through biological (through their faeces) or mechanical (through the bristles on their legs) transmission (REYS, 2003; SCHAPHEER et al., 2018; THYSSEN et al., 2004; ZORZENON, 2002). Blattella germanica, for example, can transmit bacteria, helminths, fungi, protozoans and viruses (MIRANDA et al., 2008; POTENZA, 2005; PRADO et al., 2002; REYS, 2003; RODRIGUES, 2014; THYSSEN et al., 2004).

Blattella germanica is a small cockroach (up to 1.5 cm in length) and has brown or yellow-brown coloration, preferring humid, warm and small areas for nesting (RAFAEL *et al.*,

2008; ZORZENON, 2002). As these species prefer warm, humid, urban environments, they are commonly found in areas of food processing and storing (TACHBELE, 2006; KINFU; ERKO, 2013), causing significant financial impacts (SCHAPHEER *et al.*, 2018). Faeces, empty oothecas, ecdysis and elevated rates of cockroach sightings during the day are indicators of an infested environment (POTENZA, 2005; RODRIGUES *et al.*, 2014).

In cases of high infestation, chemical control should be used, taking into consideration the risks, benefits and efficiency of each insecticide (POTENZA, 2005). This is important as, due to the lack of technical understanding, indiscriminate use of insecticides and the lack of rotation between chemical groups, *B. germanica* can become resistant to chemical compositions (LEE *et al.*, 1996; SALMERON; OMOTO, 2002; SOUZA *et al.*, 2011).

In the chemical control of pests, different application techniques and formulations of insecticides are used. When choosing the control method (technique and formulation), the biology, insecticide absorption routes and effects of chemical groups should be taken into consideration (FUNASA, 2001; FARIA, 2009). In terms of formulations, toxic gel baits stand out for (1) generating less environmental contamination, (2) being easy to apply, (3) having residual power, (4) only affecting the target species, (5) presenting attractiveness and (6) having a high mortality rate (APPEL, 1990; DURIER; RIVAULT, 2000a; OLIVEIRA, 2013). However, in order to be considered as an efficient method of control, the gel formulations must be toxic and palatable, as they compete with other food resources in an area (APPEL, 1990; DURIER; RIVAULT, 2000a).

With the aim of more efficient use and lower risks to the environment and to human health, insecticides in toxic gel bait formulation became the most adequate form of pest control in sensitive environments such as homes, hospitals and restaurants (JESUS, 2015; OLIVEIRA, 2013). Toxic baits with different chemical compositions can have different levels of toxicity and attractiveness, which can influence the control of *B. germanica*. As such, it is important to compare toxic baits with different chemical compositions, with the aim of verifying which baits present the most effective results against infestations. Therefore, the aim of this study was to evaluate which toxic bait is more efficient in the control of *B. germanica*, taking into consideration characteristics such as, attractiveness, feeding stimulation and mortality rate.

2 MATERIALS AND METHODS

2.1 COLLECTION AND COCKROACH BREEDING

In order to carry out the experiment, samples of *B. germanica* were collected from different establishments in the city of Salvador, Bahia (Brazil). The cockroaches that were collected were placed in plastic containers and went through a period of 30 days in observation. This procedure was used to verify the condition of the individuals and to avoid mortalities

within the system. After this period, 22 adult cockroaches were placed into a glass terrarium (45 cm x 22 cm x 25 cm) with water, food and shelter under room temperature and a natural photoperiod of 12 hours.

2.2 TOXIC GEL BAITS

Four compositions of toxic baits in gel formulation were tested, presenting different active ingredients and inert components (Table 1).

Table 1. Information about the composition of the toxic baits used in the efficiency bioassay. w/w = weight/weight and m/m = mass/mass.

Toxic bait	Concentration of active ingredient	Aggregates	Gross formula	Appearance
A	Imidacloroprid 2.15% w/w	Attractants, stabilizers, thickeners, dispersants, preservatives, denaturant, defoamer, solvents, humectant 97.85% w/w	$C_9H_{10}ClN_5O_2$	Pasty, light brown to dark brown colouring
В	Imidacloroprid 2.15% w/w	Denatonium benzoate 0.0001%, solvents, emulsifiers, attractive vehicles.	$C_9H_{10}ClN_5O_3$	Pasty, cream to beige colouring
С	Fipronil 0.05% w/w	Attractants, vehicles, and aggregates 100%	$C_{12}H_4Cl_2F_6N_4OS$	Pasty, yellow to cream colouring
D	Indoxacarb 0.6% m/m	Attractants, thickeners, humectants, preservatives, pH adjustment, biocide, and diluent 99.4% m/m	C ₂₂ H ₁₇ ClF ₃ N ₃ O ₇	Pasty to liquid, yellow to gold colouring

2.3 TEST ARENA AND EFFICIENCY BIOASSAY OF TOXIC BAITS

For the bioassays, 30 adult *B. germanica* specimens were used, without considering sex. The cockroaches were placed in a plastic Petri dish, with dimensions 150x20mm with the provision of water and shelter (Figure 1). Before the tests began, the insects were kept from feeding (fast) for 48 hours, following the procedure suggested by the ANVISA (2009) protocol. Using a Shimadzu AUY220 precision balance, with a maximum capacity of 220g and a minimum of 10mg, the different toxic baits were weighed at 0.02g each and were placed side by side. The weight of 0.02g was chosen to be the standard weight of the baits, as it fell within the minimum weight/area required by the manufacturers of the Petri dish.

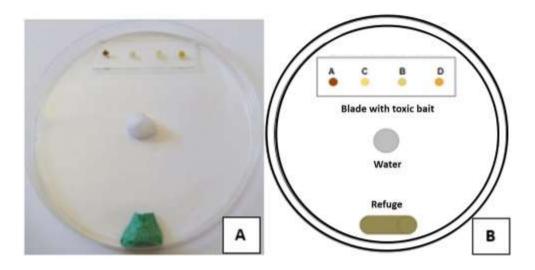


Figure 1. Image of the test arena and bioassay of real (A) and illustrative (B) attractiveness, containing the principal elements (slide with the availability of the toxic baits, moist cotton wool with water and a piece of an egg box to simulate the shelter) for the test of attractivity with the toxic gel baits. Adapted from ANVISA (2009).

For the experiment, the cockroaches were kept in the arena for a maximum period of 30 minutes or until they consumed one of the toxic baits. Following the protocol of Durier and Rivault (2000a), the most attractive bait was considered as the cockroaches' initial choice and its uninterrupted consumption for more than 30s. During the test, the (1) start and end time of the bioassay, (2) time until consumption, (3) bait consumed, (4) time consumed and finally (5) time until death after first contact with the bait, were recorded. Following the protocols by ANVISA (2009), mortalities were recorded in intervals of 12 hours up to a period of 72 hours, and death was defined as when the insect no longer presented any evident movement after a period of 60 seconds. The choice of the most efficient toxic bait was determined by (1) a greater attractive potential, taking into consideration the time of the insects' first choice, (2) a greater feeding stimulation, considering the longest time consuming the bait and (3) mortality in a shorter time, in accordance with the data collected.

2.4 DATA ANALYSIS

With the aim of comparing the efficiency of baits, we considered the attractiveness, feeding stimulation and the mortality rate of the baits consumed. Initially we used a Kruskal-Wallis test (KW), since although we did not identify dependency between the samples, the correlation was not significant p<0.05 and the data did not pass the test of normality using the Kolmogorov and Smirnov (KS) test, p<0.01.

To compare the efficiency of the baits pair by pair we also considered attractiveness, feeding stimulation and time until mortality. As such, we performed a paired T-test. All the statistical analyses were performed using the program Past version 3.24 - 2019.

3 RESULTS AND DISCUSSION

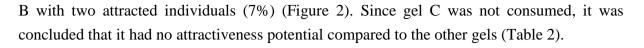
Based on the bioassay performed, for a quantity of 0.02g of toxic baits, the results demonstrated that gel C was not consumed and as such it was discarded from the statistical analyses.

Table 2. Cockroach exposure - *Blattella germanica* exposed to four types of gel (A, B, C e D) demonstrating attractivity (consumption time in minutes), feeding stimulation (time in minutes) and mortality (within 12 hours)

Cockroach	Toxic bait	Attractiveness	Feeding stimulation	Mortality
1	D	10:41	01:52	Х
2	D	02:00	02:03	Х
3	D	06:33	01:11	Х
4	А	07:06	01:02	Х
5	А	15:08	01:28	Х
6	D	05:22	01:19	Х
7	D	02:53	02:47	Х
8	D	02:06	01:31	Х
9	D	03:27	01:48	Х
10	D	01:35	00:40	Х
11	D	02:04	02:50	Х
12	D	02:30	00:50	Х
13	А	04:30	04:00	Х
14	D	14:35	01:08	Х
15	D	23:14	02:07	Х
16	А	20:38	03:57	Х
17	D	04:00	03:25	Х
18	D	10:49	01:26	Х
19	А	04:28	03:33	Х
20	D	08:55	02:31	Х
21	D	04:10	01:17	Х
22	В	20:26	00:35	Х
23	D	10:32	00:51	Х
24	D	07:03	01:24	Х
25	А	08:42	02:11	Х
26	D	15:21	01:16	Х
27	D	06:55	01:24	Х
28	В	18:14	01:32	Х
29	А	10:12	03:23	Х
30	D	07:57	02:18	Х

3.1 ATTRACTIVENESS

For the attractiveness tests, it was found that gel D was the most attractive with 21 attracted individuals (70%), followed by gel A with seven attracted individuals (23%) and gel



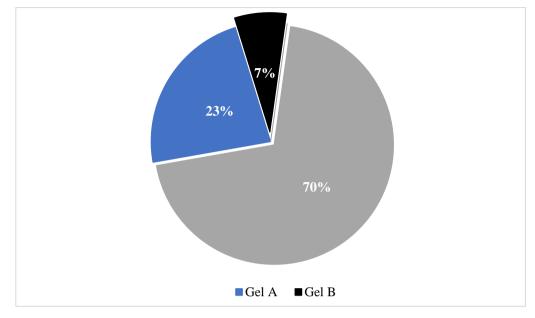


Figure 2. Graph of the percentage of attracted individuals in the test of attractiveness with *B. germanica*. *Gel C was not represented.

The potential attractiveness of a substance is measured by the first choice between the offered options (DURIER; RIVAULT, 2000a). Our results corroborated those found by Davari *et al* (2018), where the gels with Indoxcarb as the main active ingredient presented a greater attractiveness when compared to Fipronil. During the bioassay, gel C did not present attractiveness, which differed from other studies (DURIER; RIVAULT, 1999, 2000a). In terms of comparing Imidacloprid and Indoxacarb, no studies were found in reference to attractiveness and feeding stimulation bioassays. However, their effectiveness in causing mortality due to their active ingredients was found in several experiments (APPEL, 2003; DAVARI *et al.*, 2018; MILLER; MCCOY, 2005; NASIRIAN, 2008).

When we evaluated the gels individually, the average attractiveness time for gel A was 09:53min (DP=9.95), for gel B 19:20min (DP=1.5) and for gel D 07:16min (DP=5.49). The toxic gel baits A and B, which presented the same active ingredient but different aggregating components, differed in their average attractiveness times, 04:28min and 18:14min respectively. Thus, it is possible that toxic bait attractiveness may be related to the attractive substances present in the composition of the aggregating ingredients, making them more or less interesting to *B. germanica*.

The best attractiveness times were 04:28min for gel A, 18:14mins for gel B and 01:35mins for gel D (Table 2). Regardless of the choice of gel, a greater activity rate was found during the period of 01:01min-11:00min, with an average of 08:44min of attraction (Figure 3).

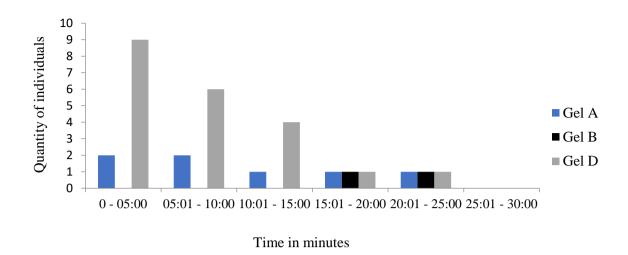


Figure 3. Graph of the temporal distribution, in minutes, of the most attractive toxic bait for *B. germanica*.

Several studies have demonstrated that the attractive components present in toxic gel baits are of great importance in terms of their attractiveness to species (TSUJI, 1965, 1996; KARIMIFAR *et al.*, 2011). Based on the results found in this study, the bait with Indoxacarb as its principal active component had a greater potential attractiveness when compared to the other baits with Imidacloprid. This may be related to the attractive components present in its composition.

Our results indicate that the attractants in gel D were more 'interesting', as they were more attractive and less repellent for *B. germanica*, reaching a potential attractiveness of 70%. Whereas for the Imidacloprid baits, gel A (23%) was found to be more attractive than gel B (7%), demonstrating that the principle active component was not an attractiveness factor.

The use of synergetic attractants (substances that when together, present a better result than when separated) proposed by Karimifar *et al.* (2011), in the formulations of toxic baits can be the differential factor in enhancing their attractiveness to target organisms, which may be another factor influencing the attractiveness of gel baits.

When comparing the attraction times of the three consumed baits, we observed that there was a significant difference (KW = 10.757, p=0.0046). When comparing the baits consumed pair by pair, we did not observe a significant difference (p>0.05, paired T-test; figure 4) between the gel A and gel B pair (T=1.7202; p=0.09604). Whereas, between the gel A and gel D pair (T=2.4538, p=0.02038) and the gel B and gel D pair (T=-3.0725, p=0.00458), significant differences were found (p<0.05, paired T-test). Gel D was found to be significantly more attractive when compared to the others.

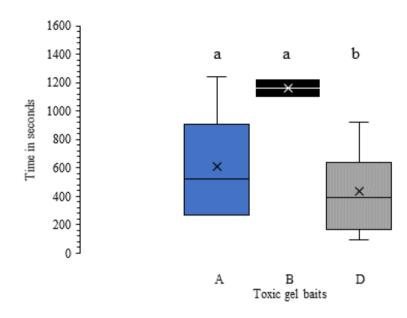


Figure 4. Average attractiveness times of the different toxic gel baits presented to B. germanica.

Another factor which influences attractiveness, is the distance of the toxic bait in relation to the individual, shelter and other food substances. Due to the reduced space and the presence of necessary elements (water and shelter) in the test arena, it is possible that the individuals were more easily attracted to the attractive components present in the baits. This would allow for a faster induction of individuals to where the bait was located.

In studies on the foraging of *B. germanica* in artificially designed environments, Durier and Rivault (2002a) found that cockroaches have an understanding of the space they are in, as well as the resources present and associated feeding and shelter areas. Other studies have shown that cockroaches tend to be guided more by their olfactory senses than their visual perception (DURIER; RIVAULT, 2000b, 2003), highlighting the importance of the attractiveness potential of insecticide formulations. Based on these studies, in order for attractiveness to be effective, the positioning of the bait in relation to possible shelters must be taken into consideration.

Therefore, the positioning of baits is a major factor in the control of infestations. This fact can be verified through the reduced attraction time demonstrated by the cockroaches in this study (Figure 3). The proximity of toxic baits to nesting areas reduces foraging time, competition with other food, as well as maximising the effects of the attractive components in baits, ultimately leading cockroaches to where baits are positioned (DURIER; RIVAULT, 2002b).

3.2 FEEDING STIMULATIONS

The maximum time for feeding stimulation was 04:00 mins and the minimum time was 00:35s (Figure 5). The minimum consumption time of gel A was 01:02mins and the maximum

time was 4:00mins (DP=1.18), for gel B the minimum time was 00:35s and the maximum time was 01:32min (DP=0.69) and for gel D the minimum time was 00:40s and the maximum time was 03:25mins (DP =0.73) (Table 2). When we evaluated the consumed toxic baits, regardless of the active ingredient, we observed that the average feeding stimulation time was 01:55minutes. When we evaluated the average feeding stimulation times of individual baits, gel A was consumed for 02:47mins, gel B for 01:03mins and gel D for 01:42mins.

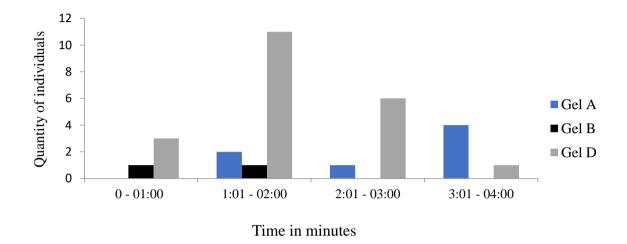
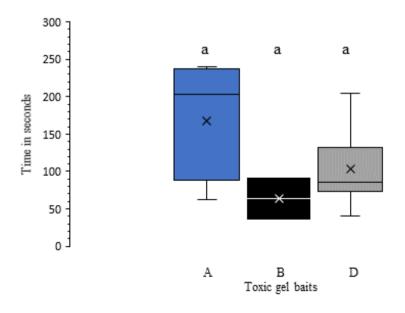
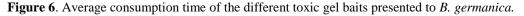


Figure 5. Graph of the temporal distribution, in minutes, of toxic bait feeding stimulation for B. germanica.

When comparing the time of consumption between the baits, gel A – Imidacloprid and gel B – Imidacloprid (T=1.2127, p=0.31204; figure 6), gel A and gel D (T=-1.4, p=0.25601) and gel b and gel D (T=-2.3098, p=0.10405), we observed no significant difference (p>0.05), demonstrating that statistically all three gels stimulated consumption in a similar way.





According to Durier and Revault (2000a), feeding stimulation is nothing more than the ability, in this case of the toxic bait, to instigate and increase consumption, which is evaluated using the time that the insects continue to consume the product provided. As such, the greater the ability of the toxic bait to stimulate feeding, the greater the guarantee that the active ingredient is being consumed.

The feeding stimulation time found in this study corroborates the results found by Durier and Rivault (2000a). With the maximum time being 04:00mins and the minimum 00:35s, which is expected for the consumption of toxic baits. When we evaluated the average consumption time, no significant differences were found between the baits, demonstrating that they stimulate consumption in a similar manner. However, the aspect and the texture of baits may or may not influence bait consumption.

The texture of the formulations is influenced by temperature, environmental humidity and by the rate of exposure to drafts, which are all factors that contribute to their dissection (APPEL, 2003). During the bioassays, the test arenas and toxic baits were kept under the same conditions. As such, it is possible to conclude that the environmental alterations influenced the texture of the baits equally.

According to Appel and Benson (1995), when abamectin B1 baits dried completely, they became hard and inedible. In other studies, Appel and Tanley (2000), demonstrated that Imidacloprid baits continued to be soft and edible even after exposure to the surrounding environment for three days. Whereas Appel (2003) showed that even seven days after its application, although dry, the Indoxacarb bait remained fresh and palatable. These studies demonstrate that the texture of toxic baits is not necessarily a major factor and that other factors, such as attractiveness, may be influencing the consumption of baits.

3.3 MORTALITY

All the consumed toxic baits presented 100% mortality within 24hours. A greater mortality rate was observed in the first 12hours, with a total of 69% of cockroaches dying in this period, followed by the period of 24hours with a total of 31% (Figure 7). This study demonstrated that all the consumed toxic baits showed mortality indices of 100% of the target organisms and therefore, corroborates the results from other studies of bait performance and efficiency (APPEL, 2003; APPEL; TANLEY, 2000; DAVARI *et al.*, 2018; MILLER; MCCOY, 2005; NASIRIAN, 2008).

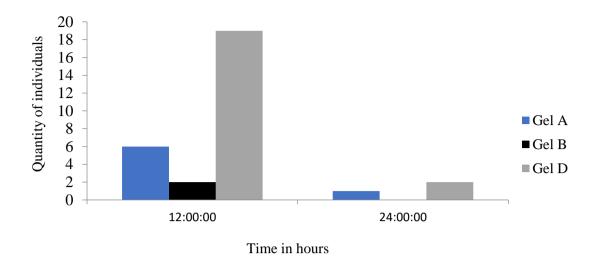


Figure 7. Graph of the temporal distribution, in hours, of the mortality effect of the toxic baits on B. germanica.

When comparing the time until mortality between the baits, gel A and gel B (T=1.3207, p=0.27832), gel A and gel D (T=-1.1022, p=0.35086) and gel B and gel D (T=-1.1556, p=0.33153), no significant differences were found (p>0.05) between the baits.

The active ingredient Imidacloprid, from the neonicotinoid class, takes effect after oral consumption and does not present environmental contamination as it does not vaporise (NASIRIAN, 2008). Previous studies have demonstrated that toxic baits of 2.15% Imidacloprid, presented good performances in the control of *B. germanica* in the absence of food competition (APPEL; TANLEY, 2000). Indoxacarb, belongs to the oxadiazine class, and was developed due to the necessity of extinguishing the resistance of organisms to older active ingredients (DIAS *et al.*, 2006). According to Appel (2003), toxic baits containing Indoxacarb are toxic, poorly repellent and act faster in attractiveness experiments, demonstrating their excellence i the control of *B. germanica* infestations.

The active ingredient of toxic baits is the factor that affects the mortality of the target organisms. Based on the literature and on the experiments performed in this bioassay, we can conclude that the active ingredients Indoxacarb and Imidacloprid, were effective against *B. germanica* and are recommended in the control of infestations.

4 FINAL CONSIDERATIONS

Based on our results, the active ingredients Imidacloprid and Indoxacarb are recommended for the control of *B. germanica*, as they caused the mortality of individuals in a period of less than 24 hours. However, when taking into consideration the statistical results of attractiveness, feeding stimulation and mortality, the active ingredient which stands out from the others was Indoxacarb, with an attractiveness rate of 70%.

Attractiveness is correlated with feeding stimulation and mortality, with both factors depending on each other. The absence, or low efficiency, of attractive compounds results in cockroaches using their poor visual perception to guide them, making consumption and consequently mortality, unfeasible.

As such, we noted that attractiveness is the factor which increases the effect of toxic baits. Therefore, we propose that gel D – Indoxacarb was the most efficient, although it presented feeding stimulation and mortality values that were similar to the other baits.

We recommend further studies on the effects of toxic baits, with a focus on bioassays to evaluate potential attractiveness, food stimulation ability and mortality.

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