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Artikel Asli/*Original Articles*

Toxicity of Imidacloprid Gel Bait against Laboratory Strain of *Periplaneta americana* (L.) (Dictyoptera : Blattidae) and *Blattella germanica* (L.) (Dictyoptera : Blattellidae)

(Ketoksikan Umpan Gel Imidakloprid terhadap *Periplaneta americana* (L.) (Dictyoptera : Blattidae) dan *Blattella germanica* (L.) (Dictyoptera : Blattellidae) Strain Makmal)

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ABSTRACT

The efficacy of a 2.15% imidacloprid gel bait against laboratory strain Periplaneta americana *and* Blattella germanica *was evaluated under laboratory conditions. The susceptibility trend of both species towards imidacloprid was: adult male < adult female < nymphs. All stages of both species were dead within 10 days in primary poisoning testing.* Periplaneta americana *adult male (LT_{50} = 0.47 h; LT_{95} = 5.24 h) died fastest, while nymphs of* B. germanica *took the longest time to reach 95% mortality (LT_{95} = 43.84 h). In indirect exposure via secondary poisoning, only adult males of* P. americana *(LT_{50} = 100.63 h) and* B. germanica *(LT_{50} = 54.66 h) obtained 50% mortality before the testing ended. No complete mortalities were achieved in any stages of both species within 10 days of secondary poisoning testing. Therefore, imidacloprid gel bait used in this study was able to cause complete mortalities within less than 2 days of 10-day primary poisoning testing but less effective in the 10-day secondary poisoning testing.*

Keywords: Imidacloprid; Periplaneta americana; Blattella germanica; primary poisoning; secondary poisoning

ABSTRAK

Keberkesanan umpan gel imidakloprid 2.15% terhadap Periplaneta americana *dan* Blattella germanica *strain makmal telah dinilai di dalam makmal. Corak kerentanan kedua-dua spesies terhadap imidakloprid adalah: jantan dewasa < betina dewasa < nimfa. Kesemua peringkat bagi kedua-dua spesies mati dalam masa 10 hari dalam ujian peracunan pertama. Jantan dewasa* P. americana *(LT_{50} = 0.47 jam; LT_{95} = 5.24 jam) mati paling cepat, sementara nimfa* B. germanica *mengambil masa paling lama untuk mencapai 95% mortaliti (LT_{95} = 43.84 jam). Dalam pendedahan tak langsung melalui peracunan kedua, hanya jantan dewasa* P. americana *(LT_{50} = 100.63 jam) dan* B. germanica *(LT_{50} = 54.66 jam) mencapai 50% mortaliti sebelum ujian tamat. Tiada mortaliti penuh dicapai dalam mana-mana peringkat bagi kedua-dua spesies dalam tempoh 10 hari ujian peracunan kedua. Justeru itu, umpan gel imidakloprid yang digunakan dalam kajian ini mampu menyebabkan mortaliti sepenuhnya dalam masa kurang daripada 2 hari dalam ujian 10 hari peracunan pertama tetapi kurang berkesan dalam ujian 10 hari peracunan kedua.*

Kata kunci: Imidakloprid; Periplaneta americana; Blattella germanica; peracunan pertama; peracunan kedua

INTRODUCTION

Cockroaches remain as public health and medically important pests (Ree et al. 2006) with cosmopolitan distribution (Kinfu & Erko 2008; Yoon et al. 2009). The German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) is the main pest in households, hospitals and residential areas (Limoee et al. 2012), whereas the American cockroach, *Periplaneta americana* (L.) (Dictyoptera: Blattidae) is a predominant pest cockroach in housing premises especially in Southeast Asia (Tee et al. 2011a). Both species serve as potential mechanical vectors of pathogenic agents (Nasirian et al. 2011; Tee et al. 2011b; Xiao et al. 2012).

Chemical applications including insecticide spraying, aerosol, dusting and bait inclusion are extensively used in the control of cockroach populations worldwide. Toxic gel baits are more frequently employed in controlling cockroaches nowadays (Ree et al. 2006; Nasirian 2010). Gel baits possess a long term residual activity, safe, rapid action and lesser odour (Agrawal et al. 2010). Moreover, gel baits could also be incorporated into integrated pest management (IPM) strategies. Gel baits could be easily discarded when control activities are accomplished (Oz et al. 2010).

Imidacloprid {1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine} is an important neonicotinoid insecticide (Buckingham et al. 1997; Kagabu 2011). Neonicotinoid insecticides including imidacloprid act on

multiple insect nicotinic acetylcholine receptors (nAChRs) (Buckingham et al. 1997; Ohno et al. 2007; Bodereau-Dubois et al. 2012). Phosphorylation / dephosphorylation of nAChR1 have been found to play an important role in the mode of action of imidacloprid (Courjaret & Lapied 2001). The use of imidacloprid gel bait is easily handled, effective and stable under various conditions (Agrawal & Tilak 2006). The main objective of this study was to determine the toxicity of gel bait containing 2.15% imidacloprid against the local laboratory strain of *P. americana* and *B. germanica*.

MATERIALS AND METHODS

TEST COCKROACHES

Laboratory strain of *P. americana* (L.) and *B. germanica* (L.) were obtained from the Medical Entomology Unit, Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. These strains were collected originally from Selangor over 30 years ago and have been maintained in the insectarium since then without any exposure to insecticide. The insectarium condition was conserved at temperature of 26 ± 2ºC, 70 ± 5% relative humidity and 12 hours dark:12 hours light of photo period.

Three weeks old adult male and female cockroaches and third instar nymphs of both species were utilized in this study. These cockroaches were separated from their parental colonies and acclimatized for three days for *P. americana* and one day for *B. germanica* prior to the testing. Only water was supplied during the acclimation period. The acclimation period was held in the same insectarium with similar conditions maintained for their parental colonies. These cockroaches were transferred to the laboratory with similar environmental conditions as the insectarium after the completion of acclimation period for primary poisoning and secondary poisoning, respectively.

INSECTICIDES

A gel formulation of imidacloprid (2.15%) was used in this study which was provided by the product supplier; Hextar Chemical Sdn. Bhd.

PRIMARY POISONING

Both primary and secondary poisoning procedures were conducted according to the evaluation method by Standards and Industrial Research Institute of Malaysia (SIRIM) (SIRIM 1993). For primary poisoning, *P. americana* and *B. germanica* were starved for 3 days and 1 day, respectively. Upon the completion of acclimation period, ten (10) male adult cockroaches were placed in each plastic aquarium (18.7 × 13.3 × 9.5 cm). As recommended by the manufacturer, 3 g of 2.15% imidacloprid and water were introduced into the same aquarium. Three replicates and one control were set up. Only mice chow (PicoLab® Rodent Diet 20, LabDiet®, St. Louis, Missouri, USA) as a food and water were supplied for the control. Time-dependent mortality rate was recorded hourly on the first day and every twelve hours throughout the next nine days for all replicates and control. Similar procedures were repeated using female cockroaches and nymphs.

SECONDARY POISONING

For secondary poisoning, ten male adult cockroaches placed in the same size of plastic aquarium were allowed to feed on water containing three carcasses of adult cockroaches killed by similar gel bait as in the primary poisoning. All samples of *P. americana* and *B. germanica* were starved for 3 days and 1 day, respectively, prior to the secondary poisoning testing. Three replicates and one control were prepared. Only water and clean cockroach carcasses as food were supplied for the control. Time-dependent mortality rate was recorded at every hour on the first day and every twelve hours throughout the next nine days for all replicates and control. Similar procedures were repeated using female cockroaches and nymphs.

ANALYSIS OF DATA

Mortality of cockroaches was recorded. A cockroach was considered to be dead if it was unable to return itself to its normal posture within 2 minutes after being touched on its abdomen using a pair of forceps (Chai & Lee 2010). If the control mortality was between 5% and 20%, the percentage of mortality was corrected by Abbott's formula (1925). Data obtained from all tests were subjected to a probit analysis computer programme to obtain the 50% lethal time (LT_{50}) and the 95% lethal time (LT_{95}) values (Raymond 1985).

RESULTS AND DISCUSSION

The toxicity of imidacloprid on all stages of both *P. americana* and *B. germanica* in primary poisoning was shown in Table 1. The gel bait was readily consumed by all cockroaches tested. Male cockroaches were the most susceptible against imidacloprid, followed by female cockroaches and nymphs for both species. At adult stage, half of the populations of both male (< 1 hour) and female (< 2 hours) *P. americana* died faster than male (< 2 hours) and female (< 3 hours) populations of *B. germanica*. In contrast, nymphs of *P. americana* (< 6 hours) were more tolerant towards imidacloprid compared to nymphs of *B. germanica* (< 2 hours). Similar results were obtained at 95% mortality for adult stage where *P. americana* was more susceptible than *B. germanica* towards imidacloprid. However, for 95% mortality of nymph stage, *B. germanica* (< 2 days) took longer time to be killed than *P. americana* (< 1 day). Complete mortalities for all stages of both species were achieved by the end of tenth day of testing.

TABLE 1. Toxicity of imidacloprid against *P. americana* and *B. germanica* in primary poisoning

Species	Developmental Stages	*n*	LT_{50} (h) 95% C.L.	LT_{95} (h) 95% C.L.	Slope	X^2 (df)
Periplaneta americana	Nymph	30	5.27 (4.59-5.94)	22.91 (18.35-31.29)	2.58	10.98 (13)
	Adult male	30	0.47 (0.05-0.91)	5.24 (3.44-17.35)	1.57	0.18 (4)
	Adult female	30	1.71 (1.12-2.30)	19.76 (15.62-27.20)	1.54	9.39 (23)
Blattella germanica	Nymph	30	1.55 (0.89-2.26)	43.84 (31.40-71.14)	1.13	13.93 (27)
	Adult male	30	1.58 (0.97-2.18)	20.93 (15.70-31.98)	1.46	2.39 (18)
	Adult female	30	2.99 (2.35-3.63)	22.58 (18.65-28.82)	1.88	21.09 (26)

C. L. = Confidence Limit (95%)

Meanwhile, the toxicity of imidacloprid against *P. americana* and *B. germanica* in secondary poisoning was shown in Table 2. Similar findings were obtained in secondary poisoning for both species where male cockroaches were the most susceptible towards imidacloprid, followed by female cockroaches and nymphs. However, more than 2 days were needed by all stages of both species to achieve 50% mortality after consuming carcasses from the primary poisoning treatment. Male and nymphs of *B. germanica* died faster than the same stages of *P. americana* at both 50% and 95% mortalities. On the other hand, for female cockroaches, 50% populations of *P. americana* (LT_{50} = 249.69 h) died earlier than *B. germanica* (LT_{50} = 306.33 h). However, populations of female *P. americana* (LT_{95} = 5611.22 h) were expected to reach 95% mortality few days later than female *B. germanica* populations (LT_{95} = 4769.14 h). Fifty percent (50%) mortality was reached only in male cockroaches of both species but no complete mortalities were obtained in any stages of both species towards the end of the tenth day of testing.

TABLE 2. Toxicity of imidacloprid against *P. americana* and *B. germanica* in secondary poisoning

Species	Developmental Stages	*n*	LT_{50} (h) 95% C.L.	LT_{95} (h) 95% C.L.	Slope	X^2 (df)
Periplaneta americana	Nymph	30	547.33 (367.08-1264.25)	5191.53 (1926.71-44841.48)	1.68	13.40 (40)
	Adult male	30	100.63 (84.32-123.32)	2580.16 (1601.49-4733.05)	1.17	8.89 (27)
	Adult female	30	249.69 (195.31-345.65)	5611.22 (2834.09-15011.96)	1.22	6.03 (17)
Blattella germanica	Nymph	30	461.55 (305.52-575804.50)	1348.52 (542.01-13581740000.00)	3.53	0.68 (7)
	Adult male	30	54.66 (47.65-62.78)	267.77 (207.07-351.01)	2.38	78.33 (38)
	Adult female	30	306.33 (240.47-417.88)	4769.14 (2682.44-10389.55)	1.38	40.79 (38)

C. L. = Confidence Limit (95%)

In comparison with another local study by Low et al. (2012), imidacloprid gel bait utilized in this study was more effective against male cockroaches, female cockroaches and nymphs of *B. germanica* in primary poisoning. However, in secondary poisoning, imidacloprid 2.15% gel bait used by Low and his co-researchers showed faster killing effects against all stages of *B. germanica* compared to the imidacloprid gel bait employed in this study. Even though imidacloprid 2.15% was used in both studies, different results were obtained which could be due to dissimilarity of the acclimation period, supply of mice chow concurrently with bait and water in the treated aquarium as well as the nature and contents of the containerized bait used in their study which consists of the mixture of food attractant and killing agent. Both baits could also be prepared using different techniques as they were provided by different product suppliers.

Each stage of both cockroach species demonstrated different level of toxicity against imidacloprid gel baits. Abd-Elghafar et al. (1990) indicated that adult females and large nymphs have larger proportions of body mass compared to gravid females and adult males, respectively. The present findings in this study were supported by Wang et al. (2006) who found that behavioural resistance to gel baits in *B. germanica* has weak sex-linkage, with a higher resistance level being inherited by female cockroaches compared to male cockroaches.

Moreover, according to Nalyanya et al. (2001), female cockroaches were usually the least responsive to gel baits as these cockroaches in normal populations would be expected to be carrying oothecae which lead to little feeding, but this scenario was not observed in this study. Besides that, it was also suggested that other factors such as metabolic rate contributed to the differential of toxicity effects among the stages of cockroach (Phillips & Appel 2010; Phillips et al. 2010).

Studies by Appel and Tanley (2000) showed that LT_{50} values for *B. germanica* consuming 2.15% imidacloprid gel bait without any other competitive food provided ranged from about 1.7 h for adult females to approximately 31

h for adult males. Meanwhile, Wei et al. (2001) reported that Apyr-R strain of *B. germanica* which was collected from Opelika, Alabama demonstrated cross-resistance to imidacloprid with a resistance ratio of 10 and failed to be suppressed significantly with the use of a synergist; piperonyl butoxide (PBO).

Later in 2004, Appel demonstrated that the LT_{50} value for imidacloprid gel bait consumed by *B. germanica* was 0.4 days, whereas the use of imidacloprid gel bait (2.15%) in cookhouses at Pune reduced the *B. germanica* density by eighth week of post-treatment between 96.3% and 98.8% compared to untreated areas (Agrawal & Tilak 2006). In Iran, Nasirian (2007) showed that LT_{50} for male *B. germanica* susceptible strain exposed to imidacloprid gel baits was 11.3 hours.

Few studies on the field effectiveness of imidacloprid gel bait in controlling the cockroach populations have been reported worldwide especially on *B. germanica*. Nasirian (2008) reported on the density reduction of *B. germanica* in residential units in Tehran which was increased from first to ninth week post-treatment of both fipronil 0.05% and imidacloprid 2.15% gel baits. *B. germanica* populations were fully eliminated from these areas after 60 days of treatment (Nasirian 2008).

In other studies by Agrawal et al. (2010), a significant reduction in *B. germanica* infestation by 94% at the end of eighth week post-treatment in catering establishments and houses at Uttar Pradesh was recorded. Furthermore, Chai and Lee (2010) demonstrated that there was an absence or low resistance level for the imidacloprid (0.8 – 3.8×) amongst 22 field strains of *B. germanica* collected from different localities in Singapore.

Other than that, the toxicity of imidacloprid could also be transmitted through the copraphagy (consumption of faeces), necrophagy (ingestion of dead cockroaches) and emetophagy (ingestion of excretions from dying cockroaches) behaviors of cockroaches (Khadri & Lee 1995; Wang et al. 2008). By consuming the lethal dose of imidacloprid gel bait, cockroaches will then infect their colonies within the same harborage when they return to their nests. These colonies are diminishing throughout time by contact with excreted faeces and fluids as well as carcasses of cockroaches exposed to primary poisoning treatment which holds sufficient level of toxicant activity to kill cockroaches. This phenomenon will help in the long term control approach as whole exposed colonies of cockroaches will be wiped out over the time.

Gel baits usually consist of various attractive ingredients as carriers with high moisture content (Oz et al. 2010). Imidacloprid gel form is suitable to be used as a bait as it has no contact effects and its active ingredients do not evaporate into the environment. Hence, the cockroaches have an optimum access time to the imidacloprid gel bait to be consumed which will increase the mortality rate of these cockroach populations. Moreover, imidacloprid also possesses low toxicity to non-target organisms like mammals, birds and fishes (Agrawal & Tilak 2006). Nevertheless, placement of toxic baits including the imidacloprid gel bait is only effective against the adult and nymphal stages but not in destroying eggs inside oothecae (Tee et al. 2011a).

CONCLUSION

Male cockroaches of both *P. americana* and *B. germanica* were the most susceptible towards imidacloprid compared to female cockroaches and nymphs. Imidacloprid gel bait used in this study was effective in direct poisoning for cockroaches control. Furthermore, imidacloprid toxicity was able to be transmitted via copraphagy behaviour of cockroaches. Nevertheless, the use of imidacloprid gel bait took much longer time for complete mortality of cockroaches when it was used in this indirect poisoning.

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