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Gil G Padonou
[1] Centre de Recherche entomologique de Cotonou (CREC), Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Casimir Dossou Kpanou
[1] Centre de Recherche entomologique de Cotonou (CREC), Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Razaki Osse
[1] Centre de Recherche entomologique de Cotonou (CREC), Benin
[2] École de gestion et d'exploitation des systèmes d'élevage, Université Nationale d'Agriculture de Porto-Novo, Bénin

Albert Salako
Centre de Recherche entomologique de Cotonou (CREC), Benin

Boulais Yovogan
[1] Centre de Recherche entomologique de Cotonou (CREC), Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Hermann W Sagbohan
[1] Centre de Recherche entomologique de Cotonou (CREC), Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Alphonse Konkon
[1] Centre de Recherche entomologique de Cotonou (CREC), Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

David Zoungbedji
[1] Centre de Recherche entomologique de Cotonou (CREC), Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Bruno Akinro
Centre de Recherche entomologique de Cotonou (CREC), Benin

Haziz Sina
Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Lamine Baba-Moussa
Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Martin Akogbéto
[1] Centre de Recherche entomologique de Cotonou (CREC), Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Corresponding Author:
Casimir Dossou Kpanou
[1] Centre de Recherche entomologique de Cotonou (CREC), Benin
[2] Faculté des Sciences et Techniques de l'Université d'Abomey-Calavi, Benin

Evaluation of the efficacy of 2.15% imidacloprid (ROCO GEL) gel against *Periplaneta americana* under laboratory and field conditions in Benin

Gil G Padonou, Casimir Dossou Kpanou, Razaki Osse, Albert Salako, Boulais Yovogan, Hermann W Sagbohan, Alphonse Konkon, David Zoungbedji, Bruno Akinro, Haziz Sina, Lamine Baba-Moussa and Martin Akogbéto

Abstract
In Benin, cockroaches are infesting urban environments and developing resistance to commonly used insecticides. Therefore, developing alternative insecticides is important for resistance management. The present study aims to test the efficacy of a gel bait formulation-2.15% imidacloprid against the common cockroach *Periplaneta americana* under laboratory and field conditions in the commune of Abomey-Calavi. Dead and alive cockroaches were compared before and after exposure to 2.15% imidacloprid. In laboratory as well as in the community, the mortality rates of *Periplaneta americana* were more than 50% in 24 hours after consumption of 2.15% imidacloprid bait. Under laboratory conditions, the mortality rate was 100% after eleven (11) days while in the community, an overall mortality of 86.97% [84.3-89.3] was observed in the restrooms and 84% [76.3-89.9] in the kitchens 8 weeks after treatment with 2.15% imidacloprid. The mortality rate was similar in latrines and kitchens. Overall, mortality among adult cockroaches was 94.85% [92.8-96.5]) versus 65% [58.6-71.0] for nymphal cockroaches. The 2.15% imidacloprid did not prevent egg hatch from ootheca. These results showed that the use of 2.15% imidacloprid can be used to mitigate cockroach resistance to pyrethroid, carbamate and organophosphate insecticides in Benin.

Keywords: *Periplaneta americana*, imidacloprid, resistance, Benin

Introduction
Background
Cockroaches are the largest of common peridomestic insects that infest houses and apartments. There are about 3500 species worldwide (Kopanic *et al*., 1994) [7]. They can mechanically spread microbes by contaminating food with germs collected from sewers, latrines, and garbage piles. Most of these cockroaches live in tropical and subtropical regions where they are not recognized as pests (Vazirianzadeh B. *et al*., 2009) [17]. The common disease vectors species are *Periplaneta americana* and *Blattella germanica* (Zurek L. *et al*., 2004) [19]. In Southeast Asia, the German cockroach, *Blattella germanica* is an important pest in hotels and food processing companies. In Benin, sensitization to cockroach among atopic asthmatic people in Cotonou is common and is associated with increased sensitization and high frequency of rhinitis (Agodokpessi *et al.* 2015) [1]. Cockroaches are infesting in urban environments where they are unpleasant to populations simply by their presence. They are present in homes, including kitchens, toilets, and sewers. In this context, chemical or mechanical vector control is often applied to reduce their nuisance. For mechanical control, people use shoes or straps to kill cockroaches. Chemical control of cockroaches includes insecticide Aerosol spray, camphor (used as a repellent), and insecticidal chalks. Unfortunately, the results from these limited control solutions were generally not satisfactory. In addition, cockroaches are the second most resistant pests to insecticides after houseflies (*Musca domestica*) (Lee C. Y *et al*., 1996 and Lee L. C. *et al*., 2004) [9, 10]. This problem represents an important threat to vector control efforts suggesting that alternative control methods are necessary.
Baiting offers an alternative opportunity to manage cockroaches' insecticide resistance. This German cockroach control method became a popular method in Thailand, and no longer plays a complementary role to insecticide residual spraying (Sitthicharoenchai *et al*., 2006) [14].

The use of baits can help manage cockroach resistance by reducing the frequency of insecticide use.
The present study aims to test the efficacy of a gel bait formulation-2.15% imidacloprid against the common cockroach *Periplaneta americana* under laboratory and field conditions in the commune of Abomey-Calavi, Benin.

Materials and Methods

Study area

The Commune of Abomey-Calavi is in the south of Benin in the Atlantic Department, at 20 km from Cotonou, the economic capital of Benin. Abomey-Calavi is bordered in the north by the Commune of ZE, in the south by the Atlantic Ocean, in the east by the Commune of Cotonou (Littoral) and the Commune of So-Ava and in the west by the Commune of Ouidah and the Commune of Tori-Bossito (Figure 1). It is one of the most populous communes in Benin with 700,000 inhabitants. It is subdivided into nine (09) districts (Godomey, Calavi, Togba, Akassato, Hêvié, Ouèdo, Glo-Djigbé, Zinvié, Kpanroun), and includes 149 villages and sub-districts. Abomey-Calavi is a cosmopolitan city because it is a residential city for executives, students, and businessmen. The relief is mainly characterized by a sandy strip with coastal strips, a plateau of bar land and depressions and swamps. The climate is sub-equatorial with two rainy seasons and two dry seasons. The hydrographic network essentially includes a Lake (Lake Nokoue) and a coastal lagoon. In addition, the commune has a maritime façade close to the coastal lagoon, marshes, streams, and swamps. The health infrastructures include one (1) University Hospital Center which serves as a zone hospital also serving the sister Commune of Sô-Ava, one (1) Communal Health Center, eight (8) District Health Centers and a no less important number of private clinics.

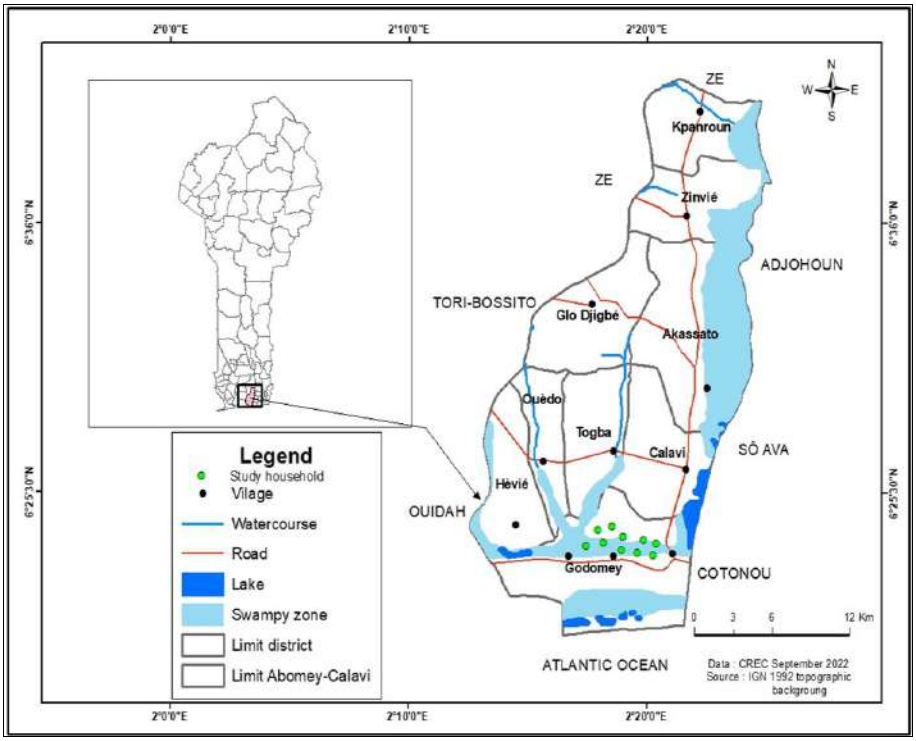

Fig 1: Map of the study site

Laboratory activities

We conducted efficacy tests of the 2.15% imidacloprid (ROCO GEL) on the cockroach *Periplaneta americana* samples (Figure 2). These cockroaches were collected in the field from septic tanks, cesspools and latrines in the commune of Abomey Calavi and carried out to the laboratory at the "Centre de Recherche Entomologique de Cotonou" to be morphologically identified according to the morphological identification keys for cockroaches (Choate, 2009; Harry, 2012) [5, 6]. And tested. After 24 hours of observation, cockroaches were isolated (5 cockroaches per cage) in 15 cm cubic cages (Figure 3) designed for this purpose and fed with corn paste. Fifty (50) cockroaches were used for the tests and 25 for the controls, representing 10 cages for the tests and 5 for the controls respectively. In each of the test cages, three (03) drops of the 2.15% imidacloprid of 0.004 g each (equivalent to an approximate diameter of 4 mm) were placed. Mortalities were recorded every 24 hours (1 day) in both the control and treated cages. Cockroaches in control cages were fed with cassava fufu, a common local food.

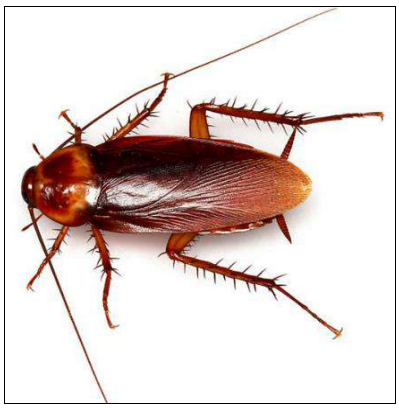

Fig 2: *Periplaneta americana*

Fig 3: Cages containing *Periplaneta americana* cockroaches

Field activities

In the commune of Abomey-Calavi, the kitchens and latrines of ten houses were randomly selected for a Phase III (community-based) evaluation of the 2.15% imidacloprid. The selected houses were mouse-free, each with a moderate level of cockroach infestation. Ten (10) supervisors (01 per house) were recruited and trained to monitor and count cockroaches in the treatment sites. The numbers of *Periplaneta americana* cockroach oothecae, nymphs, and adults found in the kitchens and latrines of the 10 houses before treatment were recorded (Figure 4). In each house, the treatment consisted of a deposit (Figure 5) of three (03) drops of 2.15% imidacloprid 0.004 g each per square meter in the latrine and in the kitchen.

Every 24 hours and for 7 days after treatment, dead cockroaches on the premises were collected and counted according to their developmental stages (Figure 6). Similarly, every 7 days after the treatment, mortalities were recorded for 8 weeks. The number of cockroaches collected, and their developmental stage (oothecae, nymphs and adults) were recorded each week on a record card.

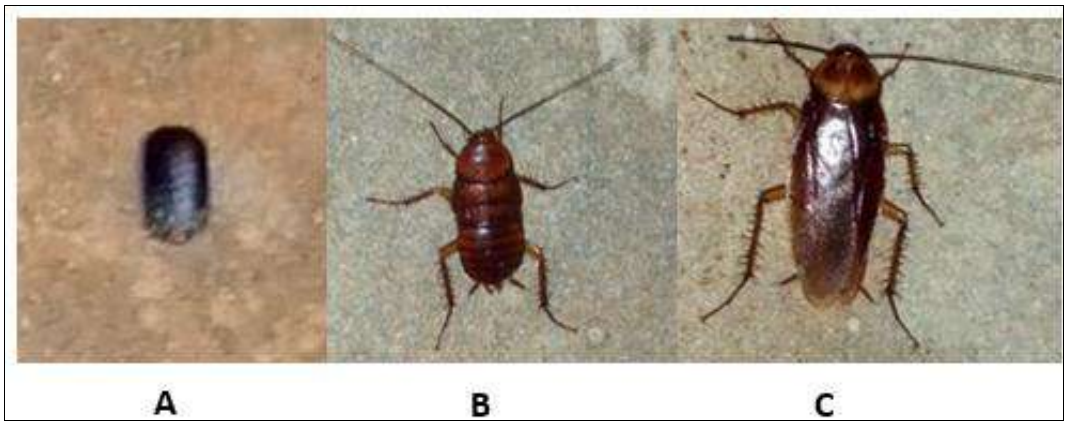

Fig 4: A (Oothecae); B (nymph); C (Adult

Fig 5: Deposit of ROCO GEL in a latrine

Fig 6: Dead *Periplaneta americana* cockroaches at the treatment site

Data analysis

Histograms were made in Excel and the Kaplan-Meier method was used for the survival curve. Stata and R software were used to calculate the mortality rate by log-probit regression and the confidence intervals were calculated by the binominal test.

Results

Laboratory test (phase 1 test)

2.15% imidacloprid induced mortality

The Kaplan Meier survival curve showed a continued decrease of the survival rate from 44.9% after 24 hours to 0% after eleven (11) days (Figure 7). From day 1 to day 2, more than 50% of the individuals exposed to 2.15%

imidacloprid were dead (Table 1). Figure 8 showed 100% mortality rates induced by imidacloprid 2.15% in cage 6 after the 2nd day of treatment. The same results were observed on day 3 for cages 9 and 10, and on day 4 for cage 4. For cages 1, 2, 3, 5, 7, and 8, the mortality rates were 100% on days 8, 10 and 11. However, no dead cockroaches were recorded in the control cages (Figure 7).

Table 1: Cockroach survival rate

Time (in days)	N. risk	N.event	Survival	Std.err lower	95% CI upper	95% CI
1	50.00	2.76e+01	0.4490	0.11628	0.1450	1.000
2	22.45	8.16e+00	0.2857	0.05128	0.0834	0.979
4	14.29	2.04e+00	0.2449	0.03923	0.0680	0.883
5	12.24	3.06e+00	0.1837	0.02365	0.0465	0.726
6	9.18	8.00e-07	0.1837	0.02365	0.0465	0.726
7	9.18	1.02e+00	0.1633	0.01961	0.0386	0.691
8	8.16	1.02e+00	0.1429	0.01598	0.0308	0.663
9	7.14	1.02e+00	0.1224	0.01274	0.0232	0.647

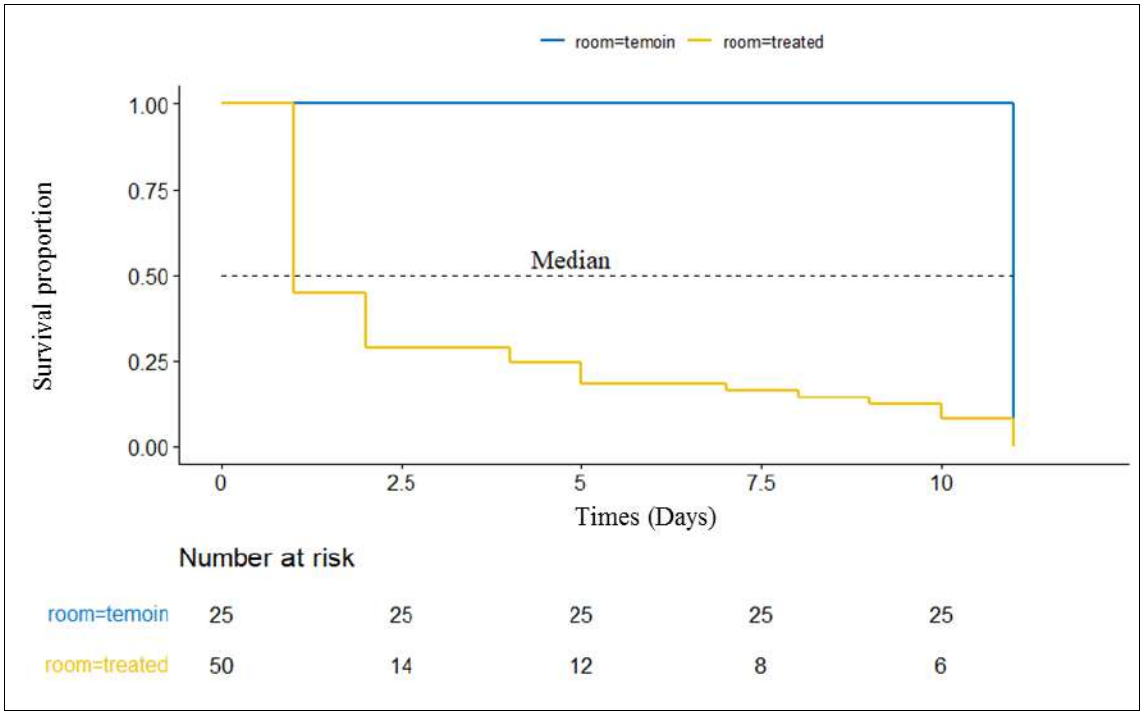

Fig 7: Cockroach survival curve

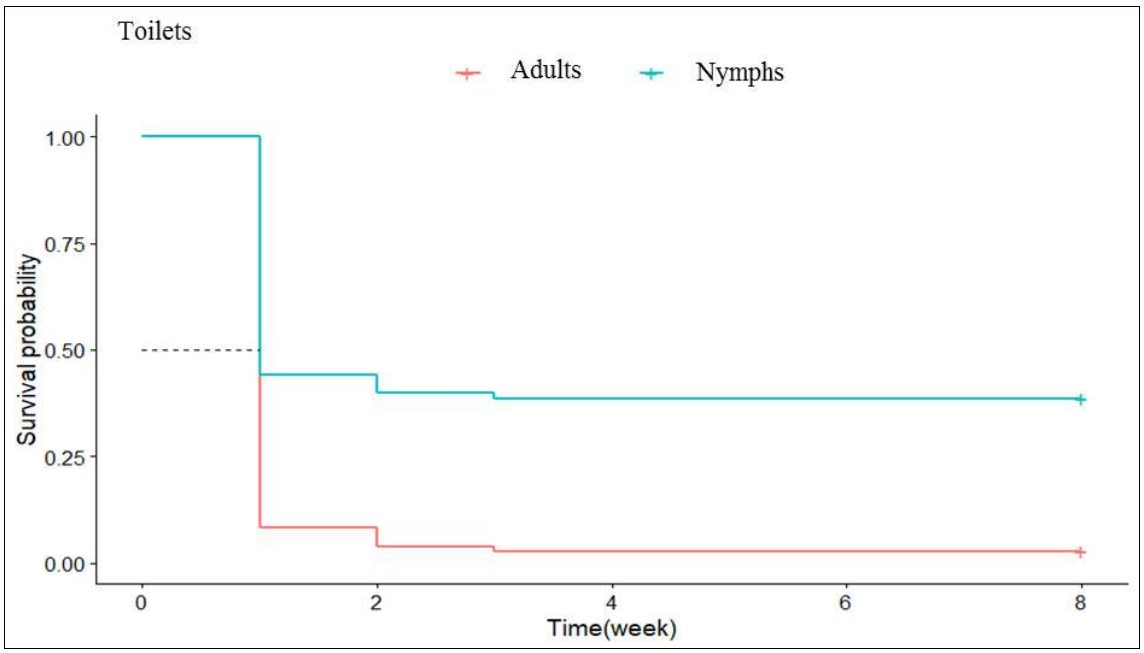

Fig 8: Survival curve of cockroaches 8 weeks after treatment with 2.15% Imidacloprid (ROCO GEL) in latrines

Field activities

Cockroach count before treatment with Imidacloprid 2.15% (ROCO GEL)

A total of 862 cockroaches were recorded in the ten (10) houses selected in the study site before treatment of the premises. According to Table 2, the proportion of cockroaches collected in latrines (85.5% [82.9-87.8]) were higher than in kitchens (14.5% [12.2-17]). Most of the cockroaches (72.15%, 622/862) were adults versus 27.85% (240/862) nymphs (Table 2).

Table 2: Number of cockroaches collected in latrines and kitchens before treatment with 2.15% Imidacloprid (ROCO GEL)

Location	Stage	Total	%	95%IC
	Ootheca	30	3.9	(2.7-5.6)
Latrine	nymphs	212	27.6	(24.5-31)
	Adults	525	68.4	(65-71.7)
	Ootheca	5	3.8	(1.3-8.7)
Kitchen	Nymphs	28	21.5	(14.8-29.6)
	Adults	97	74.6	(66.2-81.8)
Latrine Adults and nymphs		737	85.5	(82.9-87.8)
Kitchen Adults and nymphs		125	14.5	(12.2-17)

Mortality induced by Imidacloprid 2.15% (ROCO GEL) seven (7) days after treatment of latrines and kitchens

During seven days of post-intervention collections, 692 dead cockroaches out of a total of 862 (80.27%) were recorded after exposure to Imidacloprid 2.15% in both latrines and kitchens. In the latrines, most dead cockroaches were adults (80.30% [76.8-83.4]) (Table 3). In the kitchen, adults' mortality rate was 76.34% [66.4-84.5] (Table 3). For both adults and nymphs, the mortality rate was 81.27% [78.2-84.0] in the latrines versus 74.4% [65.8-81.8] in the kitchens (Table 3). No significant difference in mortality rate was observed between nymphs (78.6%; 95%CI: [58.5-90.9]) and adults (73.2%; 95%CI: [63.1-81.4]) in the kitchens. However, for latrines, adult mortalities (91.6%; 95%CI: [88.8-93.8]) were significantly higher than nymph mortalities (55.7; 95%CI: [48.7-62.4]) for nymphs (Table 3).

Table 3: Mortality rate of cockroaches in latrines and kitchens 7 days after treatment

Location	Stage	N (Before treatment)	N (7 days after treatment)	Mortality rate	95% IC
Latrine	Nymphs	212	118	55.7	(48.7-62.4)
	Adults	525	481	91.6	(88.8-93.8)
	Total	737	599	81.27	(78.2-84.0)
Kitchen	Nymphs	28	22	78.6	(58.5-90.9)
	Adults	97	71	73.2	(63.1-81.4)
	Total	125	93	74.4	(65.8-81.8)

N: number; CI: Confidence interval

Overall mortality rate of cockroaches 8 weeks after treatment of latrines and kitchens with Imidacloprid 2.15%

The observed mortality in latrines was 86.97% [84.3-89.3] and 84% [76.3-89.9] in kitchens 8 weeks after treatment with 2.15% Imidacloprid (Table 6). This suggests that the overall mortality rates were similar in latrines and kitchens. The overall adult cockroach's mortality was 94.85% [92.8-96.5]) versus 65% [58.6-71.0] for nymphal cockroaches.

Table 4: Survival rate of cockroaches 8 weeks after treatment with 2.15% Imidacloprid (ROCO GEL) in latrines

	Latrine					
	Time (week)	N. Risk	N. Event	Survival	95% CI upper	95% CI
Adults	1	526.0	4.82e+02	0.0838	0.00991	0.708
	2	44.1	2.40e+01	0.0380	0.00382	0.379
	3	20.0	6.01e+00	0.0266	0.00194	0.365
	4	14.0	4.77e-07	0.0266	0.00194	0.365
	5	14.0	4.77e-07	0.0266	0.00194	0.365
	6	14.0	4.77e-07	0.0266	0.00194	0.365
	7	14.0	4.77e-07	0.0266	0.00194	0.365
	8	14.0	4.77e-07	0.0266	0.00194	0.365
Nymphs	1	213.0	1.19e+02	0.442	0.1037	1
	2	94.1	9.07e+00	0.399	0.0827	1
	3	85.0	3.02e+00	0.385	0.0758	1
	4	82.0	7.28e-06	0.385	0.0758	1
	5	82.0	7.28e-06	0.385	0.0758	1
	6	82.0	7.28e-06	0.385	0.0758	1
	7	82.0	7.28e-06	0.385	0.0758	1
	8	82.0	7.28e-06	0.385	0.0758	1

Table 5: Survival rate of cockroaches 8 weeks after treatment with 2.15%Imidacloprid (ROCO GEL) in kitchens

	Kitchens					
	Time	**N. risk**	**N. event**	**Survival**	**lower 95% CI**	**upper 95% CI**
Adults	1	98.0	7.19e+01	0.266	0.0405	1
	2	26.1	4.05e+00	0.224	0.0280	1
	3	22.0	2.85e-07	0.224	0.0280	1
	4	22.0	2.85e-07	0.224	0.0280	1
	5	22.0	2.85e-07	0.224	0.0280	1
	6	22.0	2.85e-07	0.224	0.0280	1
	7	22.0	2.85e-07	0.224	0.0280	1
	8	22.0	2.85e-07	0.224	0.0280	1
Nymphs	1	29.00	2.28e+01	0.212	0.03290	1.000
	2	6.15	4.15e+00	0.069	0.00645	0.738
	3	2.00	6.35e-06	0.069	0.00645	0.738
	4	2.00	6.35e-06	0.069	0.00645	0.738
	5	2.00	6.35e-06	0.069	0.00645	0.738
	6	2.00	6.35e-06	0.069	0.00645	0.738
	7	2.00	6.35e-06	0.069	0.00645	0.738
	8	2.00	6.35e-06	0.069	0.00645	0.738

Table 6: General mortality rate of cockroaches 8 weeks after treatment of latrines and kitchens with 2.15% Imidacloprid (ROCO GEL) insecticide

Location	**Stages**	**Alive**	**Dead**	**Mortality (%)**	**% IC**
Latrines	Nymphs	212	130	61.3	(54.4-67.8)
	Adults	525	511	97.3	(95.4-98.5)
	Total	737	641	86.97	(84.3-89.3)
kitchens	Nymphs	28	26	92.9	(76.4-99)
	Adults	97	79	81.4	(72.2-88.6)
	Total	125	105	84	(76.3-89.9)
Latrines and kitchens	Nymphs	240	156	65.0	(58.6-71.0)
	Adults	622	590	94.85	(92.8-96.5)
	Total	862	746	86.54	(84.0-88.8)

Effect of 2.15%-Imidacloprid (ROCO GEL) on cockroach fecundity

According to table 7, the number of ootheca laid before treatment was 30 and 5 respectively in the latrines and in the kitchens. Four weeks after treatment 56.66% of the ootheca hatched in the latrines and 80% hatched in the kitchens (Table 7). 8 weeks after treatment, the number of ootheca laid was zero in the latrines and kitchens.

Table 7: Effect of 2.15% Imidacloprid (ROCO GEL) on cockroach fertility

Location	**Stages**	**Number before treatment**	**Number and rate (%) hatched after 4 weeks of treatment**		**Number and rate (%) not hatched after 8 weeks of treatment**		**Number of eggs laid after 8 weeks of treatment**
			Number	**Rate (%) (95% CI)**	**Number**	**Rate (%) (95% CI)**	
Latrines	Oothèques	30	17	56,66 (37.4-74.5)	13	43,34 (25.4-62.6)	00
Cuisines	Oothèques	5	4	80 (28.3-99.5)	1	20 (0.5-71.6)	00
Total	Oothèques	35	21	60 (42.1-76.1)	14	40 (23.8-57.9)	00

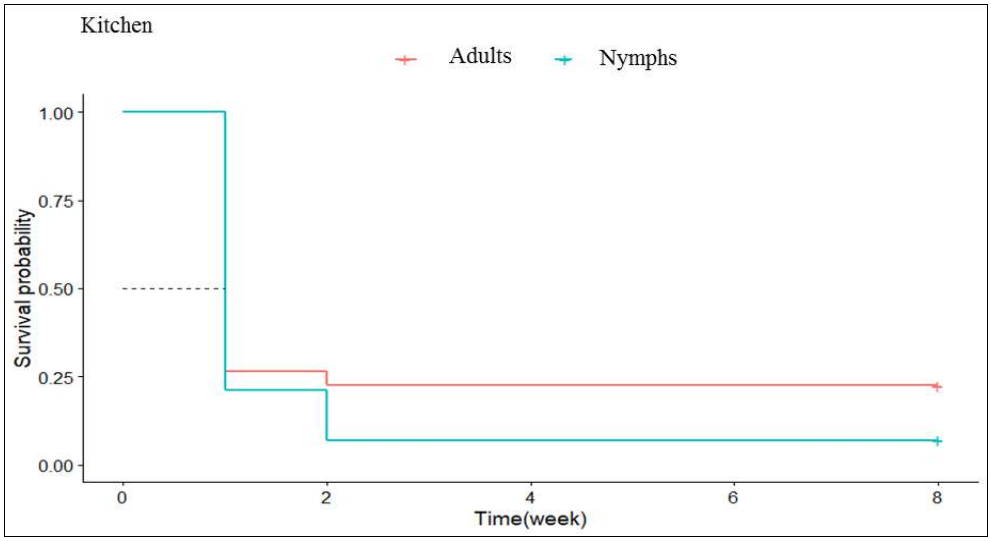

Fig 9: Survival curve of cockroaches 8 weeks after treatment with Imidacloprid 2.15% (ROCO GEL) in kitchens

Discussion

Periplaneta americana Cockroaches are disease vectors (Zurek L. *et al*, 2004) [19] that became resistant to insecticides and then reducing the impact of insecticide-based control interventions. It was necessary to develop alternative control products to manage this resistance. The present study was therefore carried out to evaluate the efficacy of a gel formulation of 2.15% imidacloprid (an alternative cockroach's control product) under laboratory and field conditions in Abomey-Calavi. In the laboratory, the results showed a mortality rate of 100% of cockroach's populations 11 days after exposure to the 2.15% imidacloprid. However, this mortality was only 44.9% after 24 hours suggesting a slow effect of the product. This slow effect could be explained by the variation in bait consumption by cockroaches and confirmed the results of Wan-Norafikah *et al*., in 2017 [18]. This could also be due to a slow effect of the imidacloprid at a 2.15% concentration which did not cause an immediate mortality effect on cockroaches. This slow effect is one of the characteristics of neonicotinoid insecticides including the imidacloprid as reported by Agossa *et al.* (2018) [2] in *Anopheles gambiae* s.l. during an experimental hut evaluation. In community, before the exposure to the 2.15% imidacloprid, more cockroaches were found in latrines than in kitchens. This finding could be explained by the low maintenance of latrines in comparison to the kitchen, since cockroaches prefer dirty places where they have an easy access to decaying wastes for feeding and breeding. Even if latrines are maintained in the same way as kitchens, the presence of on human feces was a bait that attracts more cockroaches. This observation was confirmed by Uckay I. *et al*., 2009, who stated that cockroaches frequently feed on human feces, garbage, and sewage, and are endowed with a strong capacity to spread pathogens to humans. Furthermore, we found in the households that no significant difference in mortality rate was observed among nymphs and adults in the kitchens in contrast to the latrines. This could be explained by the variation in feeding behaviors across developmental stages of *Periplaneta americana* and was confirmed by Wan-Norafikah (2017) [18]. But our results did not fully confirm the findings of Nasirian (2008) [11]. who reported that *Blatella germanica* was eliminated 9 weeks after treatment with 0.05% fipronil and 2.15% imidacloprid. The additional effect of Fipronil which is a chloride channel blocking phenylpyrazole and causing an additional toxic effect could explained the total efficacy observed in Nasirian's (2008) [11]. Study in contrast to our study where we had 86.54% [84.0-88.8] mortality rate for both latrines and kitchens. Our results also showed that imidacloprid gel bait was more effective against the adults and nymphs, but does not prevent egg hatching from the oothecae, because 4 weeks after treatment, 56.66% of the oothecae hatched in the latrines and 80% hatched in the kitchens. These results confirmed the findings of Tee *et al*., (2011) [15]. However, the imidacloprid at the concentration of 2.15%, is detrimental to the fecundity of *Periplaneta americana* which did no longer lay eggs in both kitchens and latrines 8 weeks after treatment.

The present study was limited to a single commune in Benin and did not demonstrate the effectiveness of the product in other localities. It would also be interesting to consider a study in localities where *Periplaneta americana* would have shown strong resistance to pyrethroids an can be used as an alternative insecticide for insecticide resistance management.

Conclusion

The present study showed that the use of 2.15% imidacloprid (ROCO GEL) baits might contribute to the management of cockroach resistance by reducing the frequent use of pyrethroid, carbamate and organophosphate insecticides. It is a product that acts by contact and ingestion and has systemic properties. Since no cases of food poisoning have been identified or reported in treated households, the product can be used in latrines as well as in cockroach-infested kitchens or restaurants. Despite its lethal efficacy, 2.15% imidacloprid has a delayed lethal action like most neonicotinoids.

Author Contributions

Conceptualization, G.G.P., R.O., C.D.K. and A.L.S.; methodology, G.G.P., B.Y., H.W.S., K.K.A. and D.Z.; software, A.B.; validation, M.C.A. and L.S.B; data curation, A.L.S. and C.D.K.; writing-original draft preparation, G.G.P.; writing-review and editing, C.D.K., H.S., R.O. and H.W.S.; visualization, A.L.S.;

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Data Availability Statement

The dataset is not available.

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