

Assessing the Susceptibility Status of Cypermethrin resistance in German Cockroaches (*Blattella germanica*: Blattellidae) to Hydramethylnon Gel Bait

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Abstract

Background: The German cockroach (*Blattella germanica*) is one of the most important vectors of public health interest due to its involvement in the transmission of infectious diseases. Different insecticides are used to control cockroaches; however, resistance developed against most of insecticides has made them ineffective. Attempts should always be made to find proper alternatives to control this insect.

Methods: In the present study, *B. germanica* was collected from a hospital in Tehran. Insecticide susceptibility of the cockroaches to cypermethrin was determined using the WHO glass jar method and discriminating doses. Mortality was assessed at 14 h intervals after exposure to bait.

Results: The results showed a resistance ratio of about 3.4 to cypermethrin and a complete susceptibility of the field strain to Hydramethylnon gel bait insecticide when they were compared with the susceptible strain. Therefore, there was no cross resistance pattern between these insecticides. This report is the first study on the susceptibility status of resistant *B. germanica* to Hydramethylnon bait in Iran.

Conclusions: We suggest that the use of Hydramethylnon gel bait could be used as an effective control method especially when other insecticides fail to control the cockroach populations.

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Introduction

Cockroaches are one of the most successful groups of insects that have been living on earth since the Pennsylvania period; perhaps one of the most important factors in this

success is their quick adaptation to the environment(1). A number of cockroach species are adapted to mechanical transmission of different pathogens to humans (2). Among vectors, four species: *Blattella germanica*, *Blatta orientalis*,

Polyphaga aegyptiaca and *Periplaneta americana* are important carriers of bacteria and other pathogenic agents (2-4). *Blattella germanica* has been reported as a significant health problem especially in urban areas due to its role in the transmission of infectious diseases as well as inducing asthma (5, 6). This species is known to carry microorganisms causing cholera, leprosy, diarrhea, typhoid fever, dysentery (7) and viral diseases such as poliomyelitis (8). Moreover, they carry the eggs of parasitic worms and cause allergic reactions such as itching, dermatitis, swelling of the eyelids and serious respiratory conditions (9-11).

Chemical control is one of the most frequently used interventions in the control of the German cockroaches. This has caused widespread insecticide resistance in this pest (12). The progression of insecticide resistance in German cockroach is an important problem in its management. Resistance to different classes of insecticides including organochlorines, organophosphates, carbamates and pyrethroids have been reported in the German cockroach (12, 13). Amongst the different groups of insecticides, pyrethroids have been immensely used for the German cockroach control in recent years due to their low mammalian toxicity. However, the frequent use of these compounds has led to the development of resistance (14). Control failures have been reported in field populations of the German cockroach due to the development of permethrin resistance in Iran (15), deltamethrin resistance in Singapore (16), and permethrin and deltamethrin resistance in Alabama (17). Consequently, attempts should always be made to find proper alternatives to control this insect.

Poison bait formulations such as Hydramethylnon are recent control strategies for the German cockroach, because

this method of control is cost effective and reduces insecticide exposure to the non-target organisms (18). Hydramethylnon which belongs to the amidinohydrazone group was approved by USEPA in 1980 (19, 20). The toxicity mechanism of the insecticide is a disruption of the electron transfer chain and the cellular respiration (18, 20). It was used in many countries including the USA against the German cockroach for many years (20). However, resistance to this insecticide was reported in 2016 in Puerto Rico (21). Therefore, evaluation of resistance to this insecticide in other areas in order to achieve a satisfactory control is helpful. There is no information about the susceptibility status of the insect to Hydramethylnon gel bait in Iran and its probable cross resistance pattern is unknown. Therefore, this study was undertaken to evaluate the susceptibility status of cypermethrin resistance in German cockroach to the Hydramethylnon gel bait in Iran.

Materials and methods

Study area and duration

Sina Hospital is located in the Hassan Abad district, center of Tehran in the catchment area of about 25, 000 m². The study was performed in this hospital from June 2011 to April 2012.

Cockroach strains

Two German cockroach strains were used in this study: 1) Standard Susceptible Strain (SUS) which has been rearing since 1975 in the insectary at the School of Public Health, Tehran University of Medical Sciences without exposure to insecticides; 2) field strain, S1, was collected in 2011 from Sina hospital in Tehran. Spraying with different insecticides including: organophosphates, carbamates and pyrethroids, we

were unable to control the German cockroaches in this hospital. All cockroaches were kept in an insectary at $27\pm 2^\circ\text{C}$, $60\pm 10\%$ RH, with a photoperiod of 12:12 h (L: D). Each strain was reared in the same size labeled glass jars. Cockroaches were provided with rodent food and water.

Chemicals

Chemicals were used including: cypermethrin, 97.5% (technical grade), (Zeneca, Haslemere, UK), Hydramethylnon (2%) gel bait which was provided by the American Cyanamid Company (Princeton, NJ), and CO₂ was used as an anesthetic and acetone as a solvent.

Susceptibility Test

Baseline insecticide susceptibility of the cockroaches to commonly used insecticide (cypermethrin) was determined using WHO glass jar method and discriminating doses, as described by Lee (22). The bait was provided to the insects and every day at a certain time they were checked for mortality until 100% of them die. According to Limoe et al, discriminating doses of 15 mg/kg and 30 mg/kg were used for susceptible and field strains of the German cockroaches respectively (15). Adult males of the first generation (F₁) of the strains were used for testing. The insects were anesthetized with CO₂ for 15 seconds and were placed inside the glass jar. Inside the jar was treated with 5 different concentrations of cypermethrin (technical grade diluted in acetone) 24 hours in advance. After a 25-minute contact time, they were transferred into a disposable cup and supplied with rodent food and water. The mortality was scored 24 hours later. Data were pooled and subjected to Probit analysis. The 95% confidence interval overlap of LD₅₀ was compared for statistical significance.

Resistance ratios were calculated by dividing the LD₅₀ values of the field strain to that of the susceptible strain (23).

Mortality test using Hydramethylnon 2% gel bait

Only adult males (F₁, 6-day old) were used in the bait tests because their weight and physiological conditions were more uniform than those of other strains (24). In addition, female cockroaches were needed for producing more generations. Ten adults of both laboratory and field strains of the German cockroach were anesthetized with CO₂ and transferred into a 2-liter glass beaker. Poisonous bait was made available to the insects that were to be tested only with the bait, and to a second group, rodent food plus poisonous bait were made available. The amount of bait consumed daily and cumulatively was determined by weighing the cup containing the bait, then adjusting for the weight gained or lost by the control.

Bait included 0.05g of Hydramethylnon 2% gel, which was placed in a bottle cover in the center of the beaker. The mouth of each beaker was smeared with Vaseline to prevent the cockroaches from escaping. Control groups (10 cockroaches for each replicate) ingested rodent food only. Three replicates were conducted for each gel bait test. The same number of insects from the SUS was tested with the same method and used for comparison. Mortality of the cockroaches was assessed daily at a certain time up to 14 days (23). Data were subjected to probit analysis according to the procedure described by Robertson & Preisler (25).

Results

Probit analysis of the results of the susceptibility tests performed on the strains in Sinai hospital and the control

susceptible strain using jar contact method revealed an LD₅₀ of 13.28 mg/kg and 45.12 mg/kg respectively. This indicates a resistance ratio of about 3.4 to cypermethrin (Figure 1).

The regression lines of the susceptibility data from the SUS and S1 strains of the German cockroach to cypermethrin

are depicted in Fig 1. As it can be deduced from the probit line equations of the strains (Figure 1), the S1 strain has a steeper line showing a more homogenous population towards resistance.

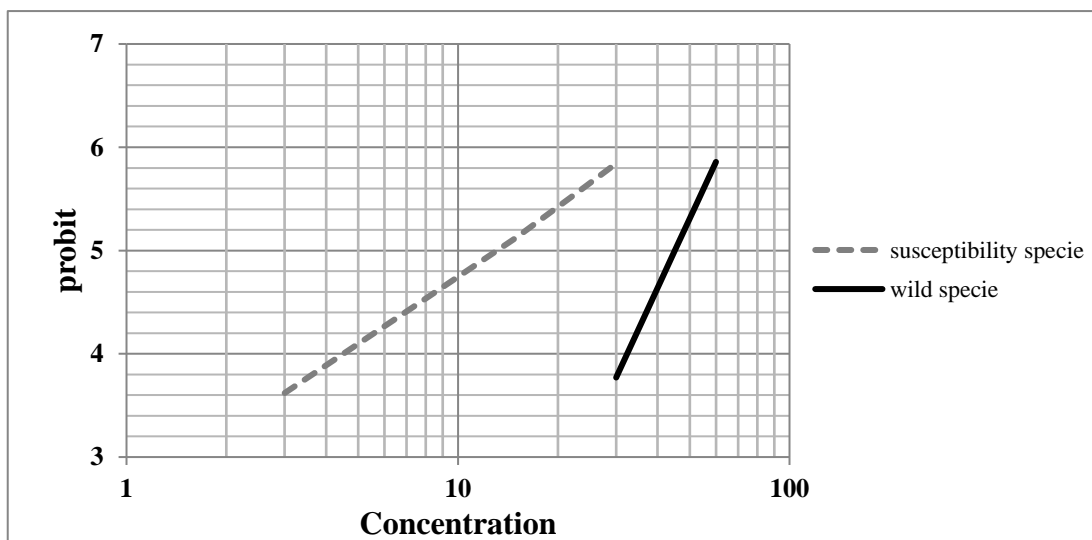


Figure 1. Probit regression lines of susceptibility test for SUS and S1 strains of adult male German cockroaches to cypermethrin

Hydramethylnon gel bait was found highly toxic to the field collected strain of the German cockroach from Sinai hospital in Tehran. The mortality of the SUS strain to

Hydramethylnon bait started from day 3 and 100% mortality occurred in day 8. However, the mortality of the S1 strain in this experiment reached 100% in day 11 (Figure 2).

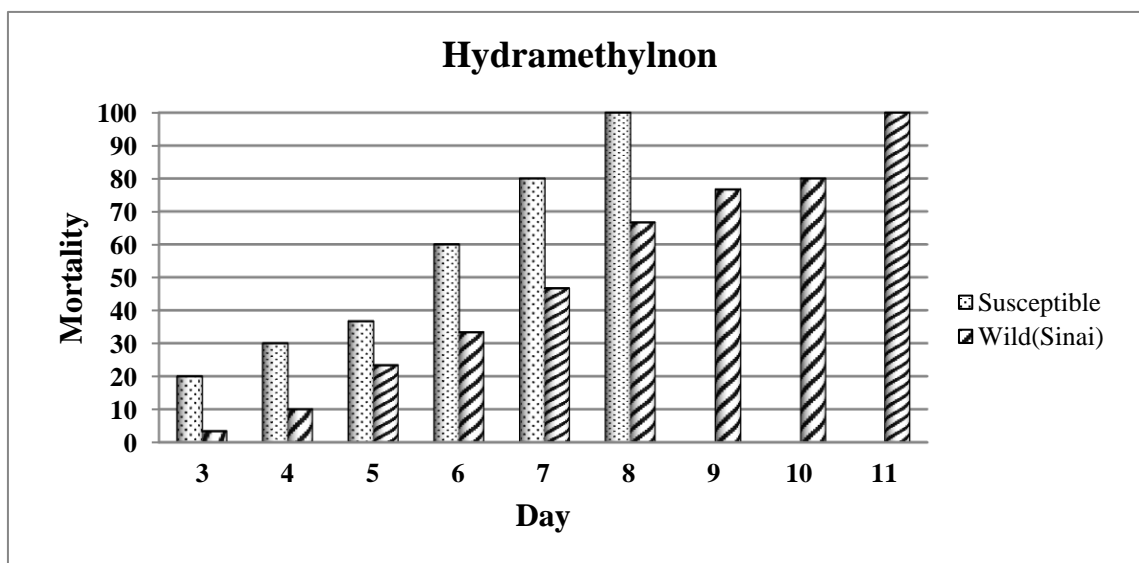


Figure 2. Comparison of the mortality of SUS and S1 strains of German cockroaches to Hydramethylnon bait in 2011.

The mortality of the SUS strain to Hydramethylnon bait (food started from day 3) was 100% and it occurred in day 9.

However, the mortality of the S1 strain in this experiment started in day 4 and reached 100% in day 13 (Figure 3).

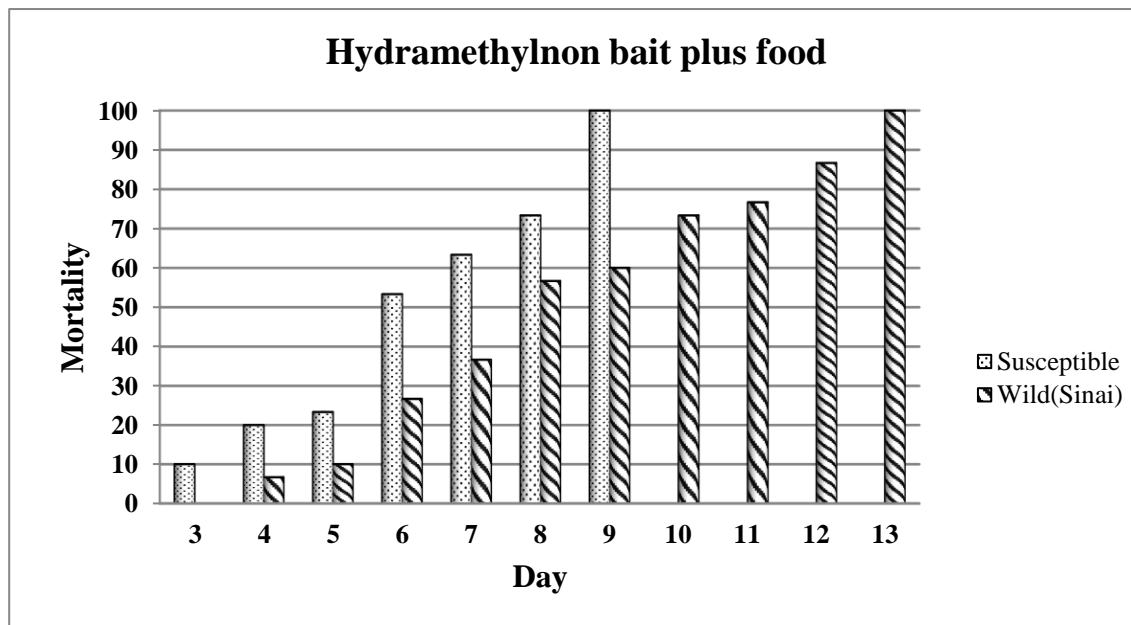


Figure 3. Comparison of the mortality of SUS and S1 strains of German cockroaches to Hydramethylnon bait plus food in 2011.

The LT50 values of the SUS and S1 strains were 4.92 and 6.47 days respectively for Hydramethylnon. The LT50 values of the SUS and S1 strains to Hydramethylnon plus food were 5.78 and 7.86 days respectively, not showing significant differences between the strains and treatments. The toxicity of Hydramethylnon to susceptible and field cockroach strains is shown in figure 2.

Discussion

The results of the susceptibility tests on SUS and S1 strains of the German cockroach to cypermethrin showed a resistance ratio of about 3.4. The regression line of the field population (S1) was steeper than the SUS strain. This phenomenon can be attributable to the fact that the field population has been under the selection pressure of

insecticides and more susceptible individuals were removed from the population leaving the remaining individuals proceeding towards homogeneity and tolerance/resistance.

The study on susceptibility levels of five strains of the German cockroaches collected from hospitals in Tehran using different insecticides including permethrin and cypermethrin showed some degree of resistance to those pyrethroids (26).

The nymphal stage of the German cockroaches collected from dormitories of Tehran University were resistant to permethrin from 3- to 20-fold in the field collected strains compared with the SUS (27). In a study on the German cockroaches collected from dormitories of Tehran University, Farzinnia in 1998 reported a resistance ratio of 8.67 and 12.8 (28).

According to a study on German cockroaches that were collected from hospitals in Sari, north of Iran, findings showed a resistance ratio of 2.27 and 2.15 to cypermethrin (29). This highlights a trend of tolerance to the insecticide, when compared with the results of another study undertaken on the same populations when they were susceptible (28).

Cypermethrin resistance in the German cockroach has been reported previously in Tehran. This is similar to the current study (30). Another study conducted by Limoe in 2006 showed a resistance ratio of 5.78 and 5.45 to cypermethrin. This result is in line with the present study (15). In another study in Kermanshah, some degrees of resistance to cypermethrin were reported (31). The results of the above-mentioned researches reveal the fact that the relatively long time of using cypermethrin as the insecticide of choice in managing the insect, has put it under a constant selection pressure, hence, resistance has been built against cypermethrin. Thus, it necessitates the need for an effective alternative to pyrethroids for controlling the German cockroaches in Iran.

The German cockroaches tested with Hydramethylnon bait showed a resistance ratio of 1.3. This indicates that the field strain is completely susceptible to the insecticide based on the criteria set by Ladonni 1993 (26). In the present study, the LT50 value of the SUS strains was 4.92 days. This finding is similar to the study by Shahraki and Farashiani in which Hydramethylnon gel bait produced 100% mortality in cockroaches within 1 to 5 days (LT50 = 5 D) (32). In another study, LT50 of the susceptible strain to Hydramethylnon bait was 3.1 days (33).

According to Scott's study, no cross-resistance was detected for Hydramethylnon in seven insecticide-resistant

strains of German cockroaches. This suggests that this insecticide (Hydramethylnon) can be useful for the continued control of this pest (34).

According to a study, 93% of the species died after 3 days and complete mortality was achieved after 14 days and laboratory susceptible strain of the German cockroach showed complete susceptibility to Hydramethylnon bait as all tested insects died after 48 hours (35).

In a study conducted by Scott, all strains were susceptible to Hydramethylnon bait and resistance ratio was 0.9-1, killing all the tested insects after 6 days of treatment (34). The results of this study are in accord with those of our study. It also confirms that there is no cross resistance pattern between different groups of insecticides and Hydramethylnon. Appel reported a mean lethal time LT50 of 57.6 h for the German cockroaches exposed to 25.4% hydramethylnon bait (35).

In another study on the German cockroaches collected from West London, 502 out of 504 insects died after 100 days of the study treating them with Hydramethylnon bait (36).

An earlier study by Milio et al. also reported >85% reduction in German cockroach populations at 4 weeks post-treatment with bait Hydramethylnon (19). Appel, however, found a lower reduction rate of about 50% in his study in Alabama (37).

According to a study by Ogg and Gold, the suppression of cockroach population was sustained with a reduction of >80% (38).

The first evidence for hydramethylnon resistance is recorded in Puerto Rico in 2016, where the performance of Maxforce Pro Roach Killer® (AI – hydramethylnon) gel baits was reduced (21).

These results indicate that Hydramethylnon-based bait stations can perform well against cypermethrin-resistant German cockroaches. Hydramethylnon is an inhibitor of mitochondrial respiration (20). This different mode of action compared to cypermethrin may explain the better efficacy when tested against resistant insects.

Laboratory assays of bait stations against S1 strain of the German cockroach showed comparable LT50 values to that of the susceptible strain. This shows that field strains tested were susceptible to Hydramethylnon-based baits. Lee after four weeks post-treatment revealed a reduction of >90 % in all strains of German cockroach and they did not differ significantly from each other (23).

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Conclusion

Continuous monitoring of insecticide resistance and evaluation of new active ingredient or formulation against the German cockroaches is necessary to find the best choice of insecticide for controlling this household pest. The results of the current study demonstrated a good performance of the bait formulation of Hydramethylnon against insecticide-resistant German cockroaches, both in the laboratory and the field, so this formulation is recommended for this purpose in Iran. However, more studies have to be conducted to further substantiate the current findings.

Conflict of interest statement

We declare that we have no conflict of interest.

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