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Evaluation of a newly modified eight-chamber-olfactometer for attracting German cockroaches *Blattella germanica* (Dictyoptera: Blattellidae)

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Abstract

Attractive Toxic Bait (ATB) is a low risk practical method to cockroach control. This study aimed to evaluate efficacy of a newly modified eight-chamber-olfactometer for attractiveness experiments. Different stages of German cockroaches were exposed to carbohydrate-rich and protein-rich foods and their attractions were tested both at ambient conditions and in the modified olfactometer. In the latter method, cockroaches were released into the main chamber of olfactometer and their attraction to food compounds placed in test chambers were recorded as the number of captured cockroaches in test chambers. The direct attractiveness test was performed in plastic pans. Repellency of fipronil insecticide was performed with prepared bait powder using olfactometer. Data were subjected to analysis of variance with Tukey's Test, $P \leq 0.05$. Different notes should be considered when using introduced olfactometer like precision when opening the test chambers valves, the fan speed, placement of cardboard shelter in both test and main chambers, and initial starvation of cockroaches. Roasted peanut, banana and biscuits powder were the most attractive foods for cockroaches. The females significantly preferred roasted peanut and the males significantly preferred banana and roasted peanut. These results were similar to the results of direct attracted cockroaches. The cockroaches have repellency to fipronil but attractiveness of bait with 0.01% concentration was significantly higher than cockroach diet. Generally, three factors should be considered in formulation of ATB like un-repellency of the a.i, attractiveness of bait compositions, and palatability of bait texture. The olfactometer introduced in this study could be used by researchers to study cockroach behaviors.

Keywords German cockroach · Olfactometer · Attractiveness · Repellency · Food compounds · Fipronil

Introduction

Cockroaches are among the most important health pests due to their role in transmission of pathogens to humans. German

cockroach, *Blattella germanica* (L.), is a common health pest worldwide. It causes health problems by transmitting pathogens and allergens as well as inducing psychological anxiety (Brenner et al. 2003; Lauprasert et al. 2006; William et al.

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2007; El-Sharabasy et al. 2014; Shahraki et al. 2013; Akbari et al. 2014; Hashemi-Aghdam and Oshaghi 2015; Memona et al. 2017; Hashemi-Aghdam et al. 2017; DeVries et al. 2019).

Intensified application of organophosphate and pyrethroid insecticides for German cockroach control has led to the development of resistance and also to food and environment contaminations. In this regard, Attractive Toxic Baits (ATB) are considered to be an alternative low risk method for cockroach control and as a common part in integrated pest management (IPM) strategies (Wang and Bennett 2009; Zha et al. 2018; Gondhalekar 2019; Rahimian et al. 2019; Wang et al. 2019). In general, the efficiency of ATB depends on its attractiveness and palatability as well as on toxicity of its active ingredients (Nalyanya et al. 2000, 2001; Nalyanya and Schal 2001; Memona et al. 2017).

The bait attractiveness is as a result of the odor of its nutritional composition which may be enhanced by addition of insect allelochemicals like sexual pheromones. In general, three factors are important in the formulation of ATB for cockroach control. These are: inclusion of non-repellent active ingredients, ensuring bait attractiveness, and using preferred bait composition and texture. Accordingly, evaluation of attractiveness of carbohydrate- or protein-rich foods combined with non-repellent insecticides is a prerequisite for ATB formulation (Appel 1990; Karimifar et al. 2011; Bayer et al. 2012; Pol et al. 2017a, 2017b).

There are many studies about food preference and feeding behavior of cockroaches (Silverman and Selbach 1998; Lauprasert et al. 2006). The researchers showed sex-biased preference for food in German cockroach due to reproductive needs. They also indicated that harborage-to-resource distance was the main factor influencing the frequency of feeding activity of German cockroach (Silverman and Selbach 1998; Lauprasert et al. 2006). Generally toxic baits must be attractive enough to be efficient in controlling German cockroach because this pest usually selects the first food when it is active during the night period. A highly ATB could assemble the active cockroaches toward the bait and dissuade them from consuming other foods in infested places. To estimate bait or food attractiveness, the olfactometer is a good instrument (Lauprasert et al. 2006). The attractiveness is measured on the basis of which food type is eaten first by the cockroaches. This method entails checking their movements over a period of time by highly sensitive video camera to count the attracted cockroaches to exposed baits/foods. However, the newly designed olfactometer allows assessment of attractiveness of foods, repellency of insecticides and efficiency of commercial baits without using camera and video check system.

The objectives of this study were to: (1) set up a Newly Modified eight-chamber-Olfactometer (NMO), (2) and using it both to determine the attractiveness of carbohydrate-rich and Protein-rich foods for different life stages of German cockroaches; and (3) to assess the repellency of different concentration of fipronil insecticides to the insect.

Materials and methods

Insect mass rearing

The colony of German cockroach was established from field-collected specimens and reared in the entomology laboratory of Yazd Science and Technology Park, Iran. All the specimens were confined in transparent plastic jars (300 height, 175 mm diameter) and maintained at 28 ± 5 °C and $50 \pm 5\%$ RH under 12:12 h light: dark photoperiod. To prevent escape, the top 5 cm of the inner surfaces of the rearing jars were coated with thin layer of petroleum jelly-mineral oil mixture (2:3). Cardboard rolls were placed in the rearing jars to serve as shelter and resting sites. The bottoms of the rearing jars were covered by wet tissue papers and cottons. A small plate containing two-layer wet cotton was used as the water source. The cockroaches were fed with diets including bread (baguette) powder (70%) (Sahar Pokht Comp, Iran), peanut butter (20%) (Kompass Comp, Iran) and milk powder (10%) (Pegah Comp, Iran).

The new modified eight-chamber-Olfactometer (NMO)

A new modified eight-chamber apparatus was redesigned based on Lauprasert et al. (2006). The NMO was extensively modified to suit experimentation without using camera or any video check system. This apparatus was constructed from polystyrene and Plexiglas materials to contain a main release chamber and eight small compartments as test chambers (Fig. 1). The outside of the main chamber was designed in an octagonal shape giving a spherical volume of 400 mm in diameter and 300 mm in height. The entrance of the main chamber was covered by a transparent plastic door (thickness: 2 mm, diameter: 450 mm) with a small opening in its middle (diameter: 25 mm). A small fan (V: 12, mA: 0.2) was installed on the opening (Fig. 1a) to create air flow from the test chamber to the main chamber (sucking fan). The test chambers were connected to the main chamber by tubes (length = 170 mm, diameter = 40 mm) (Fig. 1b). The inside volume of test chambers were designed to be $200 \times 140 \times 160$ mm (length \times width \times height). Each test chamber was covered with a glass door (170×230 mm) equipped with a net-sealed opening (20 mm diameter) (Fig. 1c). These openings allow air flow from the test chambers to the main chamber. The entrance of

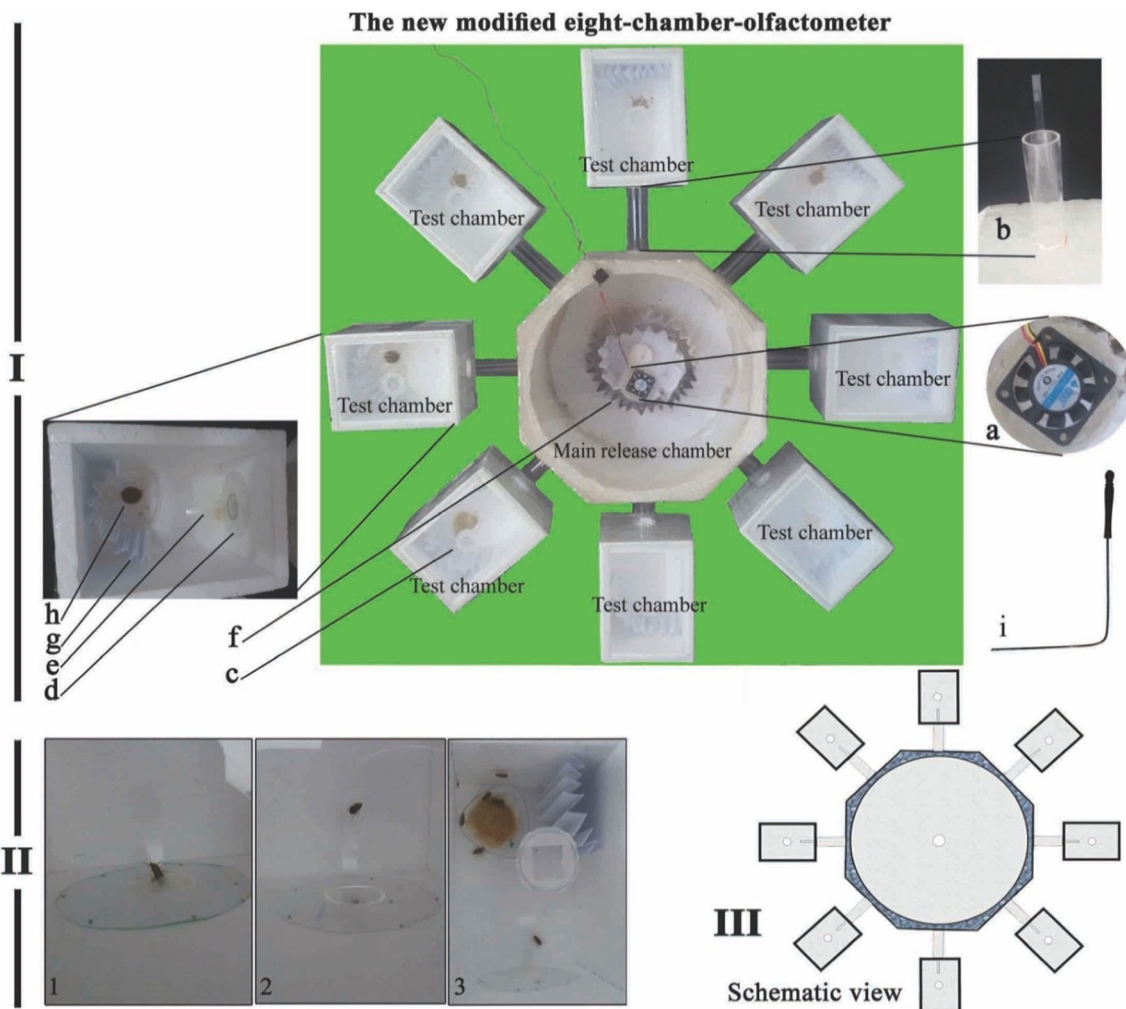


Fig. 1 The new modified olfactometer. I; (a) small fan (V12, mA 0.2), (b) tube (length 170 mm and diameter 40 mm) and thin plastic tape separated from the test chamber, (c) glass door (170 × 230 mm) equipped with a net-sealed opening (20 mm diameter), (d) coated circle plexiglas (100 mm diameter) with small hole (12 × 8 mm), (e) thin plastic tape (55 × 10 mm), (f) cylindrical cardboard in the main chamber, (g)

cardboard roll in test chamber, (h) plate in test chamber containing food, (i) L-shaped bar. II; cockroaches entering the test chamber by a thin plastic tape (1) cockroach walking on the tape, (2) The tape bent down due to the cockroach weight and connected to the bottom of the chamber, (3) The tape sprang back afterwards to close the opening. III. Schematic view of *eight-chamber-olfactometer*

the test chamber was designed in a way that allowed insects to enter but prevent them from escaping (Fig. 1d). This was achieved through three ways: circle coated plexiglas, flexible thin plastic tape, and the cylindrical cardboards in each chamber. A circle plexiglas (100 mm diameter) was adjusted on the output tube in the test chamber and coated with petroleum jelly: mineral oil mixture (2:3) (Fig. 1d). A small hole (12 × 8 mm) was created on the circle plexiglas and installed on a thin plastic tape (55 × 10 mm) at the bottom of the connecting tube (Fig. 1e). The tape was designed to suit different instars of cockroach from nymph to adult stage (Fig. 1e and b). When a cockroach walked on the tape, it bent down due to its weight which allowed its entrance and sprang back afterwards to close the opening (Fig. 1 II). Shelters were provided by placing perforated cylindrical cardboards in the chambers (Fig. 1f and g).

Cockroach foods

Seven different foods including four carbohydrate-rich (baguette, potato, banana, and biscuit powders) and three protein-rich (peanut butter, roasted peanut, and chicken liver powders) were prepared. The baguette powder was made by putting some baguette breads in a room temperature for 48 h and then subsequently grinded to create the powder. Potato powder was produced by putting some potatoes in a microwave oven for 15 min, then sliced and put in fruit dryer at 60 °C for 48 h. Finally, the dried potato slices were grinded to create the potato powder. Banana powder was produced by putting some banana slices (3 mm thickness) in fruit dryer at 55 °C for 48 h and finally grinded to create banana powder. Biscuit powder was prepared by grinding some biscuits in a mill. The peanut used (variety of North Carolina 2 (NC2) was

produced in Iran. some raw peanuts were pulverized to produce raw peanut butter by heating the raw peanuts at 150 °C for 25 min and then pulverized with a special apparatus to produce the roasted peanut butter. Chicken liver powder was made by crushing fresh chicken liver and spread on a nylon sheet at room temperature and then air flown by a fan for 72 h. Finally, the dried liver was grinded.

Cockroach diet

The cockroach diet including bread powder (70%), peanut butter (20%) and milk powder (10%) was prepared by mixing and sifting the powders and finally remixing them completely in a small mixer for 60 min.

Cockroach ATB

Based on the results achieved in the food tested, the bait was prepared by mixing 30% banana powder, 50% biscuit powder and 20% roasted peanut butter and then sifted. The bait was then contaminated with fipronil powder (technical grade, Sigma-Aldrich) at 4 different concentrations 0.01, 0.02, 0.04 and 0.08% (w/w). The contaminated bait was then mixed thoroughly in a small mixer for 60 min.

Attractiveness assay using a newly modified Olfactometer (NMO)

We evaluated the attractiveness of seven different foods by recording and counting the number of attracted cockroaches to each food in the test chambers of olfactometer. The attractiveness tests were done using fresh foods and cockroach specimens for each replication. The experimental layout was conducted in factorial arrangement in randomized complete design with four replications. In each replication, a combined number of ninety cockroaches including 30 adult males, 30 adult non-gravid females and 30 last nymphal stages were released into the main chamber of olfactometer and starved for 24 h. Then, plates containing 1.5 g of each of the seven foods were randomly placed inside each test chamber. After that, the entrance of the test chambers were opened using L-shaped bar (Fig. 1i). The bar was entered from the end through a small hole of circle plexiglas in the test chamber which opened the main chamber valves by pushing it. The sucking fan was turned on after the valves were opened. After 24 h, the number of attracted cockroaches in each test chamber was recorded based on their sex and nymphal stage. One of the test chambers was considered as control group without any material. After each experiment, the olfactometer chambers were cleaned and washed with distilled water.

Attractiveness test based on direct observation

We evaluate the attractiveness of foods by direct observation of attracted cockroaches to each food plate in plastic pans. This test was performed in 4 plastic pans (500 mm in diameter, 270 mm in height) covered with glass lids each having a 20 mm diameter hole in the center. The top 5 cm of the pans were coated with thin layer of petroleum jelly: mineral oil mixture (2:3), (Henan Daken Chemical Comp). Sheltering was provided by placing perforated cylindrical cardboards in the pans. A small plate containing two layers of wet cotton was used as water source in the pans. Hundred cockroach specimens of each stage including non-gravid females, gravid females, males, and nymph were released into the pans separately and starved for 24 h. Finally, the plates containing 1.3 g of each of the seven food stuff were provided randomly to the pans. The cockroaches attracted to each food were counted during one hour after the start of the test. The experimental layout was conducted in factorial arrangement in randomized complete design with three replications. The direct attractiveness tests were performed using fresh group of insects in each replication. After each experiment, the pans were cleaned and washed with distilled water.

Fipronil repellency

The repellency effect of different concentrations of fipronil insecticide on the reduction of bait attractiveness was evaluated. The tests had been designed to compare the attractiveness of bait containing effective dose of insecticide with cockroach diet and bait without insecticide. On the other hand, the results of this experiment determined the bait efficiency to attract different cockroach stages in the field where there are remaining foods and other attractive materials under cabinets and other cockroach shelters. We tested the fipronil repellency using the NMO. Ninety cockroaches including adult non-gravid females, males and nymphal stages (30 individuals per instar) were released together in the main chamber and starved for 24 h. Bait matrix (30% banana powder, 50% biscuit powder and 20% roasted peanut butter) impregnated with four fipronil concentrations at 0.01%, 0.02%, 0.04% and 0.08% were encoded as a-I, a-II, a-III and a-IV, respectively. The test was started by putting the 4 encoded bait concentrations and two controls (bait without insecticide, cockroach diet) into the test chambers while an empty chamber was used as a negative control. Then the test chambers were opened using the L-shaped bar after 2 h and the cockroaches were allowed to enter. The entrance of each test chamber was closed after 24 h and the trapped cockroaches were recorded based on their developmental stage and sex. The experimental layout was conducted in factorial arrangement in randomized complete design with three replications. The fipronil repellency tests were performed using fresh group of insects in each

replication. After each experiment, the olfactometer chambers were cleaned and washed with distilled water.

Data analysis

The data of this experimental study was subjected to Normality test using Minitab® Version 17.1.0 (Minitab 17, 2013). Analysis of variance (ANOVA) with the Honestly Significant Difference (HSD) or Tukey's Test was performed using SAS 9.1 or Graph pad Prism software programs. The significance level was considered at $P < 0.05$. The data was graphically presented using Microsoft Excel 2016.

Results

The set-up of the newly modified Olfactometer (NMO)

Schematic view of three steps of cockroach reaction from the beginning of the attractiveness test was presented in Fig. 2. The cockroach reaction include: I. The semiochemical odors of foods/baits reception II. Cockroach activation and orientation, and III. Chamber selection based on attractiveness of the food odors.

The advantages of the NMO include:

(1) Monitoring of cockroaches movement is possible without using a highly sensitive video camera. Therefore, the attractiveness of different food or commercial toxic bait formulations like gel, paste and powder can be compared.

(2) It was innovatively designed so that it allows one way movement of test insects from the main chamber towards the test chamber and prevents their return.

(3) Installation of a small fan (V12, mA 0.2) on transparent plastic lid of the main chamber creates low velocity airflow from the test chambers to the main chamber.

The results of food attractiveness tests showed good performance of the NMO. It produced precise data on cockroach attraction to different food compounds comparable to that obtained from direct observation in pans. The accumulation of trapped cockroaches in test chambers at increasing time intervals (3, 6, 12, 24 and 48 h) indicated the prevention of scape (Table 1). However, the following points were important in setting up the NMO for optimum performance:

(1) Cockroaches should be allowed to settle before beginning the test and opening of the test chambers should be done using L-shaped bar (Fig. 1i).

(2) The airflow velocity should be kept low to avoid falsified attractiveness results.

(3) Shelter should be provided by placing cardboards to keep the trapped cockroaches in place.

(4) The starvation period of German cockroach should be at least 24 h for better and purposeful attraction of cockroaches towards test food.

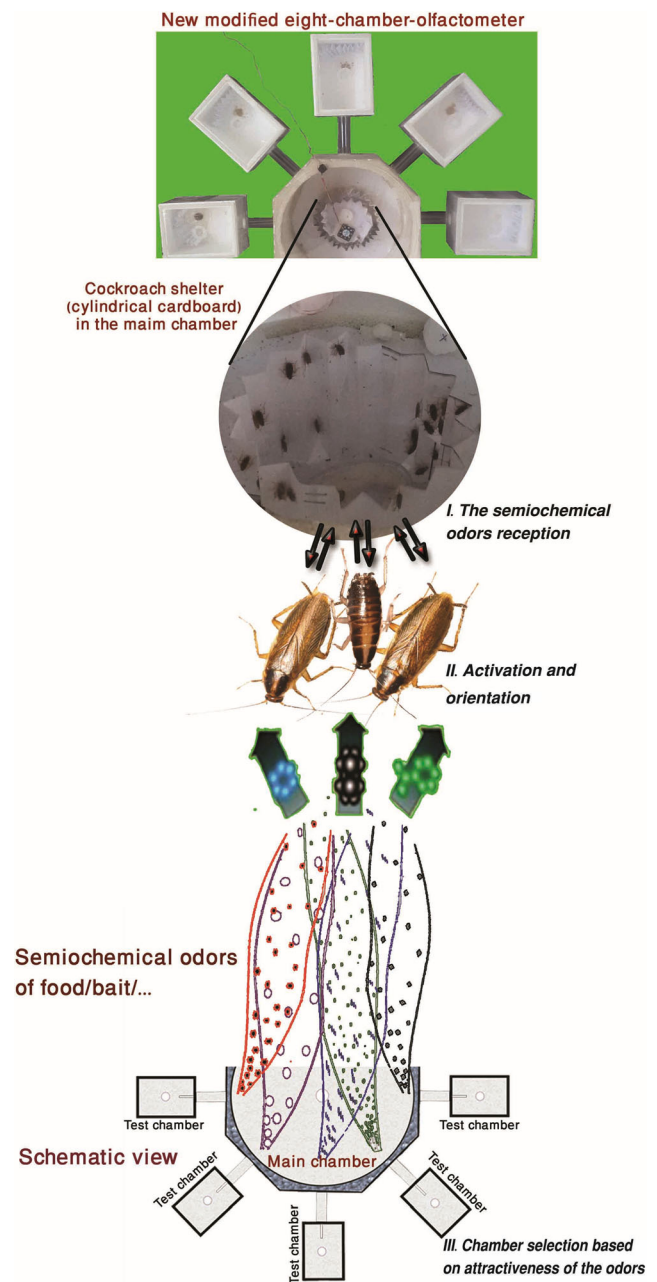


Fig. 2 Schematic view of cockroach reaction from the beginning of the attractiveness test

The food compounds attractiveness using new modified Olfactometer

We used a total number of ninety cockroaches including adult males, adult non-gravid females and last nymphal stages in each replication of experimental attractiveness test using olfactometer. The analysis of variance showed no significant difference among sex and life stages but there was significant difference among different foods ($p = 0.01$). Also the results of interaction between sex/stages of cockroach and foods indicate significant difference at $p = 0.01$ (Table 2).

Table 1 The attraction of German cockroaches to carbohydrate-rich and protein-rich foods at different time intervals using NMO

Food compounds	Frequency of attracted German cockroaches				
	3 h	6 h	12 h	24 h	48 h
Baguette Bread Powder	1	1	2	4	5
Peanut butter (roasted)	5	7	18	21	25
Biscuit powder	4	6	14	19	20
Peanut butter (not roasted)	0	1	1	1	2
Chicken liver powder	0	0	2	2	2
Banana powder	4	10	14	16	17
Potato puree powder	3	3	7	7	8
Control	0	0	0	0	0
Total	17	28	58	70	79

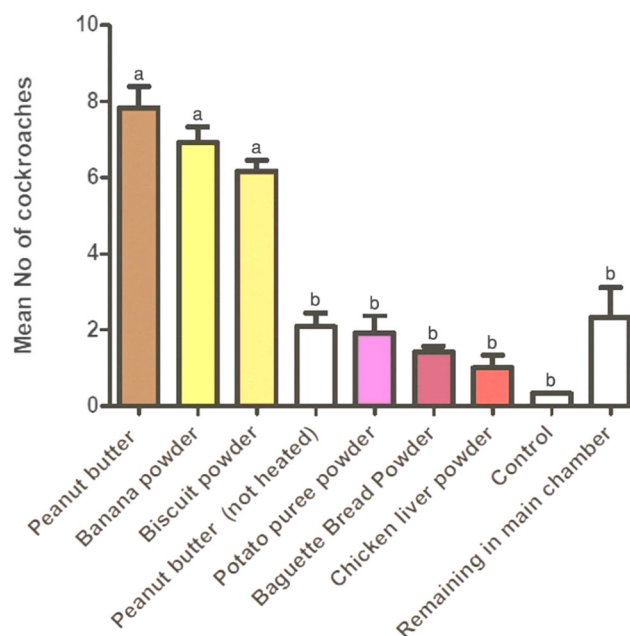
Generally, the number of attracted and trapped cockroaches into each test chamber (without considering the life stage and sex), showed that roasted peanut butter, banana powder and biscuit powder were the most attractive foods drawing 31.33%, 27.67% and 24.67% of the cockroaches, respectively. The attractiveness of these three foods was not significantly different ($p > 0.05$) from each other but was significantly different ($p = 0.001$) from other foods. Raw peanut butter, potato powder, baguette powder, and chicken liver powder at 8.33, 7.67, 5.67, and 4.00% respectively showed the least attractiveness (Fig. 3).

Also, the results of food attractiveness with considering the life stages and sexes showed the nymphs were mostly attracted to biscuit and banana powders (25%) with less attractiveness towards roasted peanut butter (12.5%). On the other hand, adult females were attracted to roasted peanut butter (38.3%), biscuit powder (24.1%) and banana powder (13.3%), while adult males had tendency towards banana powder (30.8%) and roasted peanut (27.5%) (Fig. 4). There

Table 2 Variance analysis of attracted German cockroaches (female (non-gravid), male and nymphal stages) to carbohydrate-rich foods (baguette powder, potato puree, banana powder, biscuit powder) and protein-rich foods (unheated peanut butter, chicken liver powder) using NMO

SOV	DF	MS
Sex/Stages	2	1.1 ^{N.S}
Foods	7	107.5**
Sex/Stages × Foods	14	15.8**
Error	72	2.42
CV	44.95	

N.S: non-significant, **: P value < 0.05, CV: coefficient of variation, SOV: source of variations, MS: Mean of the squares, DF: degrees of freedom

**Fig. 3** Attractiveness of German cockroaches to different foodstuff present in olfactometer. The error bars indicate S.E. ($n = 120$ in four replicates). In each column the same letters are not significantly different

was significant difference between males and females attraction towards banana and biscuit ($P \leq 0.05$) (Fig. 4). The females preferred biscuit whereas the males preferred banana and both were significant ($P \leq 0.05$). Also, the adult females differed from the nymphs in their attraction towards the roasted peanut butter rather than biscuits as depicted in Fig. 4.

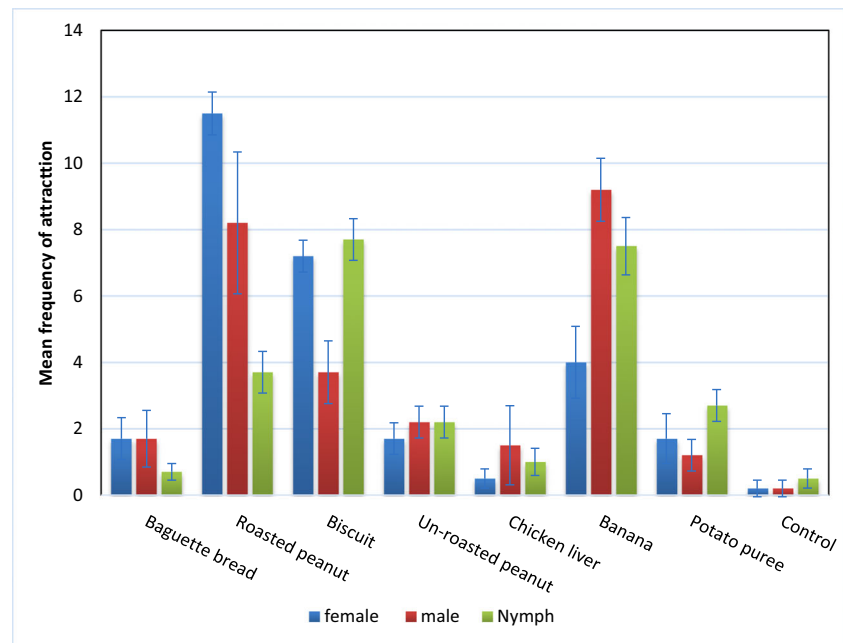
The food compounds attractiveness with direct observation

Variance analyses of pan experiments showed significant difference among sex and life stages of attracted cockroaches to foods and among different foods by direct observation. Furthermore, the interaction results between foods and sex/stages of cockroach indicate significant difference at $P < 0.01$ (Table 3). According to the mean comparison, the most attractive food for nymphal stage was biscuit powder. For gravid adult females, the most attractive foods were roasted peanut butter and biscuit powder ($P \leq 0.05$), while the most attractive food for non-gravid female adults was roasted peanut butter. The attractiveness of foods for male adults in order of preference were banana powder, roasted peanut butter, and biscuits powder respectively (Fig. 5). This result was similar to that obtained using the olfactometer.

Fipronil repellency using new modified Olfactometer

The result showed that fipronil had repellency effect at different concentrations. This repellency increased in direct correlation with increased concentrations of fipronil insecticide

Fig. 4 The mean frequency of German cockroach attraction to carbohydrate and protein-rich foods using NMO. The error bars indicate S.E. (Tukey' HSD; $P \leq 0.05$)



(Fig. 6). The analysis of variance of different sex and stages of attracted cockroaches to man-made baits and diet showed significant difference among man-made baits and diet at $P < 0.01$ (Table 4). Furthermore, there was significant difference between man-made bait without insecticide and impregnated man-made bait with fipronil insecticide. Also there was significant difference between impregnated bait at 0.01% fipronil insecticide and cockroach diet ($P \leq 0.05$) (Fig. 6).

Discussion

In this study, the attractiveness of seven food stuffs including carbohydrate-rich and protein-rich materials to German cockroach was evaluated and the repellent effect of fipronil in combination with the preferred foods was tested in order to

Table 3 Variance analysis of direct attracted German cockroaches (non- gravid female, Gravid female, male and nymphal stages) to carbohydrate-rich foods (baguette powder, potato puree, banana powder, biscuit powder) and protein-rich foods (unheated peanut butter, chicken liver powder) using pans during one hour experiment

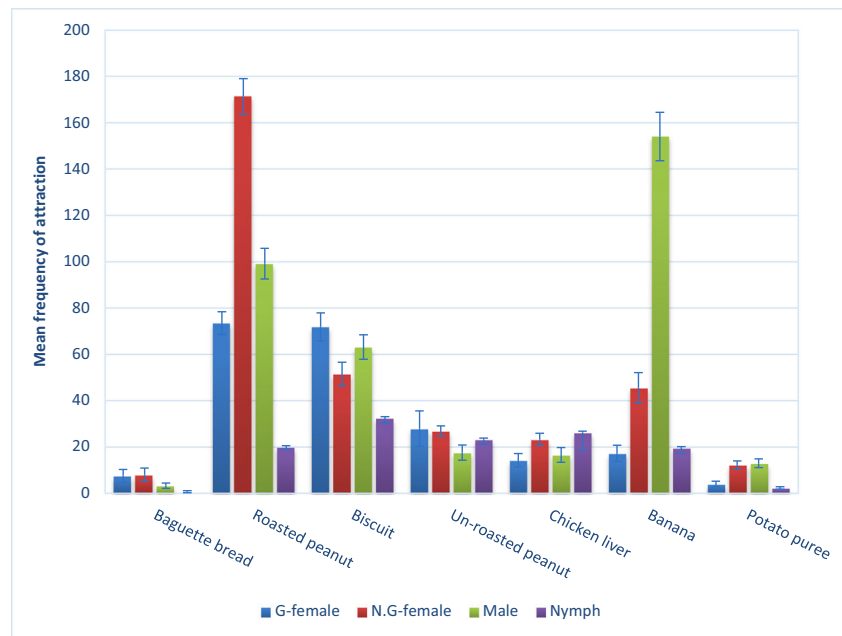
SOV	DF	MS
Sex/Stages	3	5427.3**
Foods	6	12,152.1**
Sex/Stages× Foods	18	3366.2**
Error	56	62.38
CV	21.26	

** : P value < 0.05 , CV: coefficient of variation, SOV: source of variations, MS: Mean of the squares, DF: degrees of freedom

introduce a new formulation of an attractive toxic bait against the cockroaches. Results of this study revealed that the attractiveness of German cockroach to the foods were life stage and sex dependent where nymphs preferred carbohydrate-rich materials such as biscuit and banana, while the most attractive food for adult females was protein-rich food (roasted peanut butter). However, the adult male cockroaches were attracted to both carbohydrate- and protein-rich foods such as banana powder and roasted peanut butter. This finding is very important in cockroach IPM programs utilization of ATBs (Wang and Bennett 2009; Gondhalekar 2019; Wang et al. 2019). In this regard ATBs with high attractive and palatable foods are useful for controlling cockroaches, though their attractiveness must be as high as other leftover foods to feed on (Ko et al. 2016).

A simple olfactometer with high accuracy which performed well and produced results comparable to those obtained with direct observation was designed. In other words, the result of the direct attraction of German cockroach to different foods was the same with the result of the food attractiveness obtained from the new modified olfactometer. This newly modified apparatus could be recommended for investigating cockroach behavior and attraction to various food stuff at different life or physiological stages and sex status. In addition, the olfactometer can be used to compare the attractiveness of various commercially ATB formulations like gel, paste and powder. The test chamber was selected randomly to put the foods/baits. The comparative results of attractiveness/repellency of different foods/baits in the randomly repeated tests show no noticeable difference between each replication test. These findings present a validation that the olfactometer is balanced and ensure that one or more chambers are not

Fig. 5 The mean frequency of German cockroach attraction to carbohydrate and protein-rich foods using pans during one hour experiment. The error bars indicate S.E. (Tukey' HSD; $P < 0.05$)



preferred over the other. However, the following points were important in setting up the NMO for optimum performance: (1) Cockroaches were allowed to settle before beginning the test and opening of the test chambers was done with the use of the L-shaped bar (Fig. 1i). (2) The airflow velocity was kept low to avoid falsified attractiveness result. (3) Shelter was provided by placing cardboards to keep the trapped cockroaches in place. (4) The starvation period of at least 24 h was observed for better and purposeful attraction of cockroaches towards the test food.

Cockroach infestation is seen usually at different life stages and with different sexes, hence considering a mixture of different attractive materials that recognize these differences is very important for maximum efficacy. This is in agreement with various dietary requirements of German cockroach at different developmental stage, physiological, and sex status (Service M 2012). It is known that female fecundity depends on the ingestion of proteins, especially for egg development whereas in male cockroaches, fertility does not highly depend on proteins. For example, the males of Madagascar hissing

Fig. 6 The mean frequency of different stages of German cockroach attracted to bait at 4 concentrations of fipronil insecticide - 0.01%, 0.02%, 0.04% and 0.08%, bait without insecticide (positive control), cockroach diet (bread powder, peanut butter and milk powder 10%) and chamber without any material (negative control) using NMO. The error bars indicate S.E. (Tukey' HSD; $P < 0.05$)

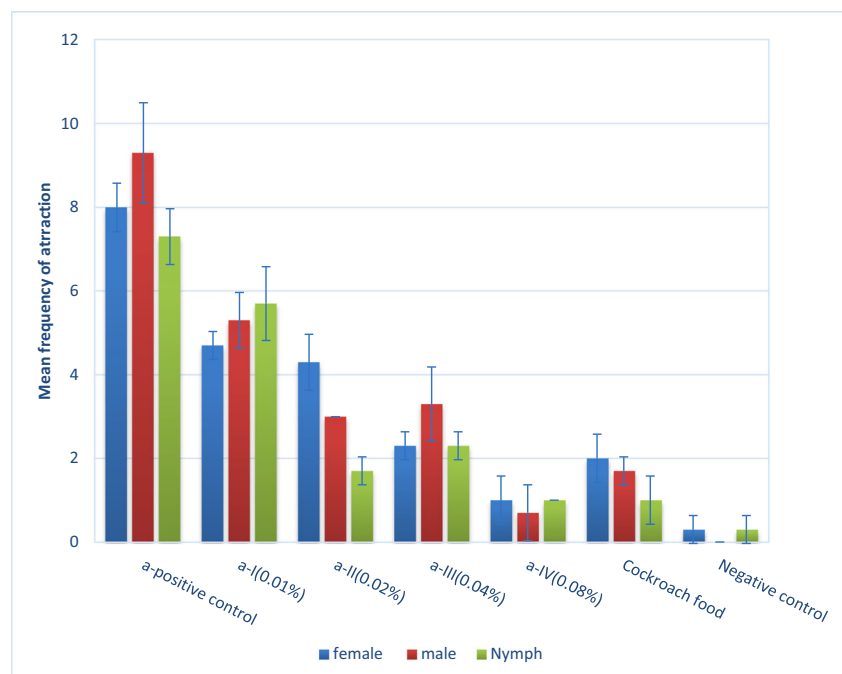


Table 4 Variance analysis of attracted German cockroaches (non-gravid female, male and nymphal stages) to bait at 4 concentrations of fipronil insecticide 0.01%, 0.02%, 0.04% and 0.08%, bait without insecticide, cockroach diet using NMO

SOV	DF	MS
Sex/Stages	2	1.97 ^{N.S}
Baits/Diet	6	69.74 ^{**}
Sex/Stages× Baits/Diet	12	1.54 ^{N.S}
Error	42	0.98
CV	31.89	

N.S: non-significant, **: P value<0.05, CV: coefficient of variation, SOV: source of variations, MS: Mean of the squares, DF: degrees of freedom

cockroaches *Gromphadorhina portentosa* (Dictyoptera: Blaberidae) preferred carbohydrate-rich foods but the females preferred protein-rich foods (Carrel and Tanner 2002). Moreover, Carrel and Tanner (2002) cited carbohydrates as nutrients that both sexes used as a primary energy source because of their disability to convert lipids to monosaccharides. We used a combined number of cockroaches including female, male and nymphal stages in different experimental tests using olfactometer. It was created in a way that resembles the cockroach population pattern in the field. It is more conducive to produce an attractive bait with high efficacy in the field condition. This study agrees with the study conducted by El-Sharabasy et al. (2014), where they reported that banana was the most attractive food for adult German cockroach among four different foods -banana, biscuit, luncheon and cooked cheese. Banana chips contain 16 volatile compounds mainly esters of acetates (Mui et al. 2002) and some were confirmed to be attractive compounds for German cockroach (Karimifar et al. 2011). Furthermore, banana powder is a preferred choice for cockroach because it has very pleasing and attractive odor, is rich in carbohydrate and can serve as a primary energy source, and its texture is soft and mushy (Reiersen 1995; Carrel and Tanner 2002; El-Sharabasy et al. 2014).

Results of this study showed that roasted peanut butter have an attractive odor which significantly attracted female German cockroach. Peanut butter is not only a protein-rich food, but also has a high lipid content, and its roasted form had more attractive odor than the raw peanuts. Although roasted peanuts are usually highly attractive, as confirmed here, nonetheless the quality of peanuts and the roasting process will dramatically affect the odors emanating from these foods. Karimifar et al. (2011) showed that extracts of peanut butter and malt attracted male German cockroach. Peanut butter and distiller's grain captured significantly more cockroaches than the GP-2 tablet or the Victor pheromone lure (Nalyanya et al. 2000). Peanuts are rich in oil and

semiochemicals like 1-hexanol which results from decomposition of lipids. This enhances the attractiveness of natural food sources and efficiency of insecticidal baits (Karimifar et al. 2011). Awad et al. (2000) reported that attractiveness of peanut butter was due to ingredients such as β -sitosterol. Ibrahim et al. (2017) showed that roasted peanut was the most attractive food to male and female cockroaches and a mixture composed of equal amounts of roasted peanut, coriander oil, fresh coriander leaves, and zucchini fruit attracted more cockroaches than peanut butter.

In addition to banana powder and roasted peanut, biscuit powder was found to be another very attractive food for German cockroach especially for the nymphs and females. Biscuit powder is a rich-carbohydrate source with a pleasant odor. However, El-Sharabasy et al. (2014) reported biscuit had a low attraction compared to banana, luncheon and cooked cheese. It should be noted that different brands of biscuits may vary in composition and therefore have different attractiveness and palatability.

Baits' agreeable odor is an essential factor in cockroach attraction. Each compound of bait formulation that creates repellency to pest can greatly reduce its efficiency. German cockroaches are repelled by most insecticides, especially when incorporated in bait forms and this lead to failed cockroach control (Durier and Rivault 2000). In this study, the repellency of the baits containing fipronil showed that cockroaches avoided baits mixed with higher concentrations of fipronil compared with the baits without or low concentrations of the insecticide. When fipronil was incorporated in the baits, a significant higher number (27.5%) of the cockroaches remained in the main chamber of the olfactometer compared with those (11%) that remained in the main chamber when fipronil free foods were tested. However, attractiveness of the bait mixed with 0.01% fipronil was significantly higher than the free-fipronil diets in this study ($P \leq 0.05$). This means that the baits with 0.01% fipronil are attractive as the foods in the field conditions. It has been shown that resistant strains of cockroaches to other group of insecticides were susceptible to low concentrations of fipronil and it killed German cockroaches in nanogram quantities per insect (Srinivasan et al. 2005; Sitthicharoenchai et al. 2006; Nasirian 2008). Therefore, the baits prepared with fipronil (0.01%) were useful for the control of German cockroaches and can be advocated. Durier and Rivault (2000) reported that fipronil gel was as attractive as bread for large nymphs and adults, but bread was more attractive than fipronil gel for small and medium-sized nymphs. Although, the baits containing 0.01% fipronil were significantly more attractive than cockroach diets alone, a high mortality was observed with baits containing 0.02% insecticide. Hence, 0.02% is recommended as an ideal concentration for an active ingredient in evaluating bait efficacy in field condition.

In conclusion, carbohydrate-rich and protein-rich foods with strong and pleasant odor such as banana, some biscuits and peanut are probably good components that could be introduced as attractive ingredients of cockroach baits. Moreover, the low repellency preparation of bait with low concentration of fipronil could compete with cockroach diet in laboratory and probably with leftover foods in the field condition. Ultimately, formulation of ATB with high efficiency necessitates the evaluation of active ingredient repellency, bait compositions attractiveness and preference/palatability in laboratory and field condition. The NMO introduced in this study can be recommended to researchers as useful instruments to study German cockroach behavior to obtain quality ATB formulation for roach control.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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