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Control *of Periplaneta* Americana using Boric Acid & Neem Tree leaflets powder

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Abstract

The American cockroach, *Periplaneta americana* is the largest of house-infesting cockroaches and causes' public health problems making it an strategic target for control.
Different concentrations of Boric acid and neem tree leaflets (old of young developmental stage, OY) powders were used in this study. The powders were incorporated into food mixtures designed here to produce baits and the lethal dose (LD50) was calculated for each treatment.
For both treatments, the toxicity was found to relate with the amount ingested by a cockroach (mg/ cockroach). Increased concentrations of Boric acid were found toxic to *P. americana* and mortality percentage increased in a positive correlation with concentration. LD50 was 2.0889±0.00136 g.
On the other hand, two effects were observed for neem OY leaflets: a) a toxic effect and increase in mortality for concentrations up to 3.75g with LD50 =4.345±0.07694 g; and b) a repellent effect as well as a decrease of mortality for baits with concentrations of 5g-6.667g.

Keywords: *Periplaneta americana*, control, lethal dose (LD50), boric acid, Neem tree leaflet powder.

Introduction

The American cockroach (*Periplaneta americana*) is a cosmopolitan species. It is the largest of house-infesting cockroaches with an average length of 4 cm. They are flattened and oval in shape with reddish-brown to dark brown colour.

Periplaneta americana have an incomplete metamorphosis lifecycle: egg, several of nymphal instars and adult. The egg case measures 8–10 mm and contains 16 eggs. Environmental factors such as temperature and humidity can increase or decrease the developmental time (Bell and Adiyodi, 1981). They eat a great variety of food, including those used for human consumption. They prefer starchy and sugary materials in addition to their own cast-off skins, dead and crippled cockroaches, fresh and dried blood, excrement, sputum, and the fingernails and toenails of babies and sleeping or sick persons.

American cockroaches are considered a public health problem due to their association with human waste and diseases. At least 22 species of pathogenic human microorganisms, as well as five species of flatworms, have been isolated from American cockroaches (Rust et al., 1991).

Management involves chemical insecticides and sprays which are effective in controlling cockroaches' populations but may be harmful to human and beneficial organisms and might cause ecological problems. In addition, they may develop resistance to commonly used compounds. Moreover, many insecticides are repellent to them and are therefore avoided (Wooster et al., 1989). Therefore, many conventional insecticides are to be replaced by biorational pesticides (i.e. pesticides of natural origin with limited or no adverse effects on the environment or beneficial organisms) or low risk insecticides (e.g. Boric acid).

Boric acid (H_2BO_3) is an inorganic low cost, white powder chemically derived from boron

and water. It has a long history as an insecticide in urban pest management. It has been shown as an effective alternative to usual neurotoxic insecticides (Gore and Schal, 2004) with a relatively low toxicity to mammals (Fail et al., 1998, Hubbard, 1998, Wester et al., 1998). It acts as a stomach poison affecting the insects' metabolism and the dry powder affects the exoskeleton cuticle. Ingested boric acid causes structural alterations in the mid-gut of German cockroach (Habes et al., 2006). Boric acid reduces the number of oocytes per paired ovaries and the size of basal oocytes as well (Kilani-Morakchi et al., 2009).

Neem tree (*Azadirachta indica*) typically grows in the tropical and subtropical parts of Asia, but because of their considerable climatic tolerance, are now cultivated in other warm regions of the world. All parts of *Azadirachta indica* contain several active volatile compounds that have biological insecticides effects. The parts with comparable effect as pesticides are seeds, which are scarce and available only from June to October and the old leaflets of young leaves developmental stage (OY); which are available all year round (Siddig and Baleela 2007). About 550 insect pest species are sensitive to the active compound azadirachtin (Debashri and Tamal, 2012). Azadirachtin was found to interfere with protein synthesis in several insects including the desert locust *Schistocerca gregaria* (Annadurai and Rembold 1993) and hemolymph of the oriental leafworm moth *Spodoptera litura* (Xiaodong et al., 1995).

In addition, azadrachtin is well documented as an antifeedant. Siddig and Baleela (2007) reported reduction in reproduction and development in addition to antifeeding behaviour on two important store pests (i.e. *Trogoderma granarium* Everts and *Tribolium castaneum* Herbs).

When American cockroaches were treated with azadirachtin (extracted from neem seeds) the activity of their midgut enzyme was reduced and their growth inhibited (Paranagama et al., 2001).
The most recent technological advances in cockroach control involve the use of bait formulations (Appel, 2004). Therefore we designed two types of baits in this study: 1) using different concentrations of boric acid and 2) using different concentrations of OY powder. Our objective was to determine usefulness of both compounds for cockroaches management, to compare between the effect and toxicity of the two compounds and to calculate the lethal dose for each in mg/cockroach.

Materials & Methods

Sample collection and maintenance

Five hundred (500) *P. americana* adults were collected from infested manholes of houses in Al-Azhri borough and University of Khartoum, Khartoum State.

For maintenance purposes, a plastic container covered by iron grid was used as a maintenance cage. In the maintenance cage, collected cockroaches were sheltered with carton cylinders (length 7cm, diameter 3cm) and commercial egg cartons. Cockroaches were supplied with food and water was provided in a piece of wet gauze and were allowed to adapt in the maintenance cage for at least 24 hours before the start of the experiments.

The experiment box design

The experiment box was designed as a rectangular cage 50cm length X 30cm width X 15cm high) with glass walls and iron grid cover. Ten centimeter of the lower inner side of each wall was coated with petroleum jelly mixed with gasoline (2:1) respectively to prevent cockroaches from escaping. The floor of the box was lined with disposable paper and commercial egg carton boxes (Figure 1a).

Poisoned food preparation

Food mixture was prepared by mixing powdered dried: carrot, lettuce, milk, sorghum flour and yeast as a supplement using the ratios 1:1:2:10:1 respectively weight/weight.
The total weight of any poisoned food mix was 25 g. Five different dosages boric acid (H_2BO_3, purity 99.5%) were used: 1) contained a concentration of 0.945g boric acid; 2) contained 1.26g boric acid; 3)contained

1.687g boric acid; 4) contained 2.25g boric acid and 5) contained 3g boric acid.

Neem OY leaflets were collected and dried in shade and then powdered using a blender. Five concentrations of OY poisoned food were also prepared: 1) contained 2.1g neem OY leaflets powder; 2) contained 2.8125g OY powder; 3) contained 3.75g OY powder; 4) contained 5g OY and 5) contained 6.667g OY leaflet powder.

Baits were prepared by mixing dry poisoned food with water, the mixture was then spread and divided into small rectangular and rhombus shapes and left to dry (Figure 1b). Baits' weights were measured and recorded and the baits were then frozen to prevent fungal and bacterial contamination.

Toxicity experiments

To determine the toxicity of boric acid, 40 to 45 cockroaches were transferred to an experiment box then cockroaches were starved for 24h before the beginning of experiment but were provided with water on gauze (adopted with modification from Buczkowski et al., 2001).

The cockroaches were fed on a single bait for 2h then the bait was collected and its weight measured again. The difference in weight represents cockroaches' consumption. Cockroaches' consumption was then used to calculate boric acid toxicity (mg of boric acid per body weight) and mortality rate was recorded continuously for 36h and the dead cockroaches were removed continuously.

For determining the Neem OY leaflets powder toxicity experiment, 40 cockroaches were used in each experiment and the same steps as described above were followed.

Statistical analysis

To determine insecticide toxicity; indication of significant differences were determined by non overlap of the 95% fiducial limits (FL). 95% fiducial limits (FL) = ±1.96 (95% confidence intervals) ×Standard deviation (SD).

Standard deviation (SD) was calculated (manually) by dividing one over b^2 (slope of the regression line squared).

$$SD=\frac{1}{b^2}$$

For performing probit analysis, mortality was corrected for control mortality (M) by using Abbott's formula (Abbott, 1925):

$$\text{Corrected mortality \%}=\left(\frac{M(observed)-M(control)}{100-M(control)}\right)x100$$

The regression equation (1) was calculated using MS Excel to determine the relationship between mortality percentages as a response to eating the shaped food and the concentration of boric acid or OY neem powder.

$$y = a + bx \qquad (1)$$

Whereas:

y ≡ response (percentage mortality),
x≡ concentration of boric acid or OY neem powder,
a≡ the intersect of the regression line with Y axis
b≡ the slope of the regression line.

The regression equation (2) was calculated using probit double transformation analysis to determine the relationship between mortality percentages as a response to eating the shaped food and the concentration of Neem powder.

$$y = ax^2 + bx+c \qquad (2)$$

Whereas:

y ≡ response (percentage mortality),
x≡ concentration of boric acid or OY neem powder, a≡ the intersect of the regression line with Y axis and b≡ the slope of the regression line. C ≡ constant.

Lethal dose 50% (LD50 or 50%mortality) was calculated by substituting y from equations (1&2) with 50 (mortality percentage). While a, b and c were calculated using MS Excel, therefore the only variable remained (x) which represent the concentration of boric acid or OY neem powder with probit analysis after finding x value it was converted to antilog and this value represent LD50.

a) b)

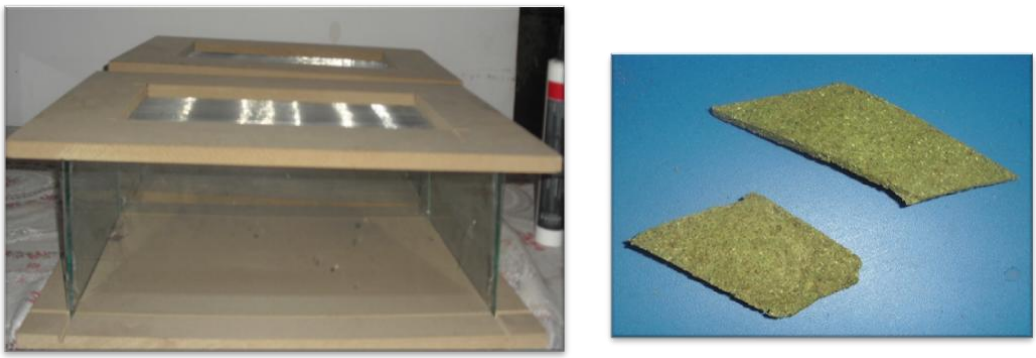

Figure 1a) The experiment box, b) Baits were prepared by mixing dry poisoned food with water

The average of one cockroach bait consumption was calculated manually by dividing the total bait consumption over the total number of cockroaches used in an experiment. The average bait consumption was then used to estimate the lethal dose (mg/cockroach) in each concentration.

The equation used was:

$$W\ dosage = Wc \times \frac{Wd}{Wt}$$

Whereas:

$W\ dosage \equiv$ lethal dose (mg/cockroach) .

$Wc \equiv$ average of bait consumption by one cockroach.

$Wd \equiv$ dose weight.

$Wt \equiv$ total weight of the bait (25 g).

Results

In this study, the toxicity and LD50 of boric acid and old leaflets of young neem leaves (OY) were estimated against *P. americana*. When boric acid concentration was increased in the bait, mortality percentage increased as well in a linear positive relationship (Table 1).

LD_{50} (50% mortality) was calculated as 2.0889±0.00136 g, whereas LD_{90} (90% mortality) was found to be 3.1435±0.00136 g and LD_{95} = 3.276±0.00136 g; with 95% fiducal limit FL (±0.00136) for boric acid.

Boric acid toxicity was related to the amount of boric acid ingested by cockroach. For the bait with a boric acid concentration of 3g and the average boric acid ingestion 2.1650 mg / cockroach, 80% mortality rate was observed indicating a high toxic effect. Whereas for the bait with the concentration of 0.965g and an average boric acid consumption of 0.7924mg/cockroach only 6.6% of the total number of cockroaches used in the experiment died indicating a low toxic effect (Table 2 and Figure 2).

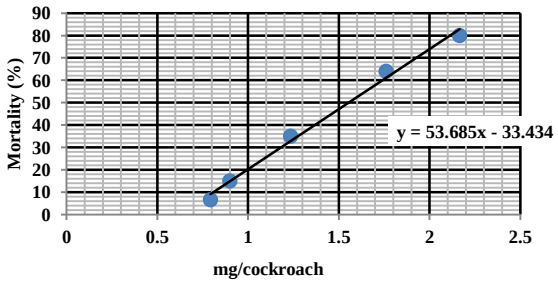

Figure 2: Dose (mg/cockroach) response relationship of boric acid against adult *Periplaneta americana*

Table 1: Relationship between boric acid concentration and mortality (%) of cockroaches:

Number of cockroaches	Boric acid conc.	Mortality (%)
45	0.945 g	6.6
40	1.260 g	15
40	1.687 g	35
45	2.250 g	64
40	3.000 g	80
40	0.0 g Control	2.5

LD50 (50% mortality) was calculated to equal1.552±0.00677 mg/cockroach, LD90 (90% mortality) = 2.297±0.00677 mg/cockroach and LD95= 2.392±0.00677 mg/cockroach with 95% fiducal limit FL (±0.00677).

Neem OY leaflets powder toxicity

Two effects were observed when OY neem leaflet powder was used for preparing baits: a) the toxicity of neem powder positively correlated with the amount of neem powder ingested by cockroach and b) an indication of a repellant effect for baits that contained 5g - 6.667g of neem powder.

LD50 (50% mortality) was calculated using the polynomial regression in Figure 3 and was found to equal4.1506±16.51496g. With 95% fiducal limit FL (±16.51496). y = probit 5 (LD50); x= 0.5515.; antilog 0.5515= 3.5604± 0.003664g. With 95% fiducal limit FL (±0.00677).

However, the observed relationship was better described by polynomial distribution rather than a linear one (Figures 4 and 5). The positive correlation obtained for mortality and concentration in Figure 6.

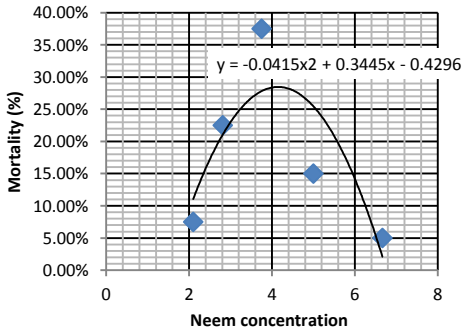

Figure 3: Dose (mg/cockrach) /response relationship of OY neem leaflets powder against adult *Periplaneta americana*

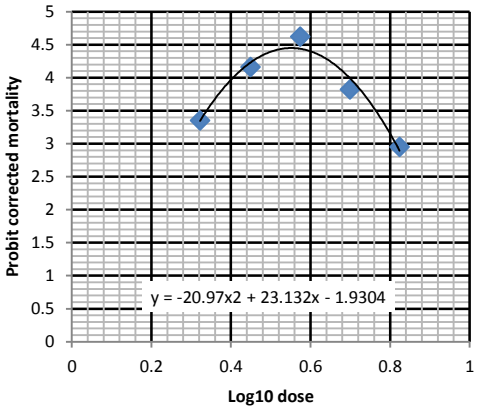

Figure 4: Log10 dose /probit mortality relationship of all OY neem leaflets powder doses against adult *Periplaneta americana*

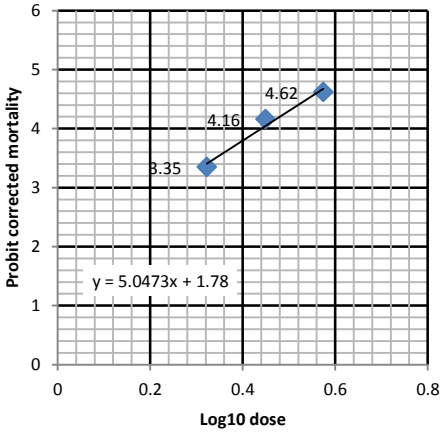

Figure 5: .Log dose /probit corrected mortality relationship of neem OY leaflet powder (first three doses) against adult *Periplaneta americana.*

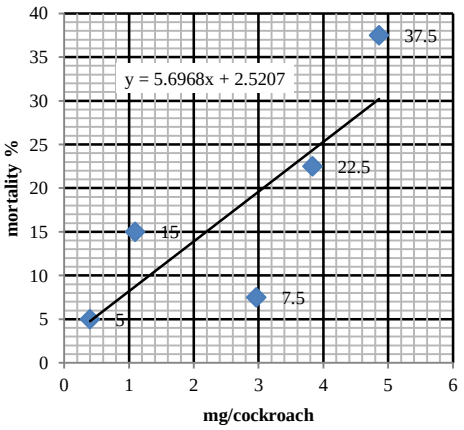

Figure 6: Dose (mg/cockroach) /response relationship of neem powder against adult *Periplaneta americana*

Table 2: Lethal dose of boric acid (mg/cockroach).

No.	Conc.**	B/g***	B/I****	Lethal dose (mg/insect)	M %
1	0.965	0.9238	0.02053	0.7924	6.6
2	1.260	0.7135	0.01784	0.8991	15
3	1.687	0.7316	0.01829	1.2340	35
4	2.250	0.8823	0.01960	1.7600	64
5	3.000	0.7190	0.01797	2.1650	80
6	Control	0.9861	0.02465	0.0000	2.5

** Boric acid conc.(g), *** Bait consumption/g, **** Average of bait consumption g/insect, M mortality

Toxicity of neem OY leaflet powder correlates positively with the amount of neem powder ingested by cockroaches (Figure 6). However, a negative correlation was observed between baits that contain 5g -6.667g of OY powder and mortality percentages (i.e. when OY concentration increased, mortality decreased) (Table 4). y= 5.047x+1.78; when y = probit 5(LD50); x= 0.638; antilog 0.638= 4.345g.

From above equation: r^2 (correlation coefficient) = 0.967.

From the above equation: standard deviation was calculated as = 0.0393 the LD50 (50% mortality) =4.345±0.07694 g. With 95% fiducal limit FL (±0.07694). (0.397&1.095 represent the ingested dose mg/cockroach which causes mortality5% &15% respectively). y = 5.6968x + 2.5207.
r^2 (correlation coefficient) = 0.809086, (Linear relationship).
SD= 0.03081.

Summary of both experiments outcome is in Table 5 and difference between the two treatments is in Table 6.

Table 3: The relationship between OY neem leaflets powder concentration and mortality.

Conc.		Mortality			No. of insects
Gram	**Log10**	**%**	**Abbott corrected %**	**Probit**	
0.0 control	_	2.5	-	_	30
2.1000	0.3222	7.50	5.0	3.35	40
2.8125	0.4491	22.5	20.0	4.16	40
3.7500	0.5740	37.5	35.0	4.62	40
5.0000	0.6990	15.0	12.5	3.82	40
6.6670	0.8239	5.00	2.5	2.95	40

Highlighted in grey are the highest mortality percentages.

Table 4: Lethal dose of Neem OY leaflets powder (mg/cockroach).

No.	Conc.	Bait (g)	B/I *	Lethal doses (mg/insect)	M % **
1	2.1000	1.4119	35.29	2.964	7.5
2	2.8125	1.3626	34.06	3.832	22.5
3	3.7500	1.2956	32.4	4.859	37.5
4	5.0000	0.2059	5.148	1.095	15
5	6.6670	0.0595	1.488	0.397	5
6	Control	1.2236	0.04078	0.000	0.000

* Average of bait consumption g/insect, **M mortality

Table 5 Summary of Boric acid and neem tree OY toxicity experiments outcome

Treatment	Regression	r^{2*}	SD **	LD*** ,			95% FL ****
				50%	90%	95%	
1. Dose (Boric acid)	y=37.925x-29.221	0.9757 linear	0.000695	2.0889±0.00136 g	3.1435±0.00136g	3.276±0.00136g	±0.00136
2. Dose response (mg/cockroach) (boric acid)	y=53.685x-33.434	0.9930 linear	0.000347	1.552±0.00677 mg/cockroach	2.297±0.00677 mg/cockroach	2.392±0.00677 mg/cockroach	±0.00677
3. Dose (mg/cockroach) (OY Neem leaflets powder)	$y = -0.041x^2 + 0.344x - 0.429$	0.6711 polynomial	8.426	4.1506±16.51496g	Nd	Nd	±16.51496
4. Log10 dose /probit mortality (OY Neem leaflets powder)	y= 5.047x+1.78	0.967polynomial	0.0393	y=probit 5(LD50); x= 0.638; antilog 0.638= 4.345g	Nd	Nd	±0.07694

* Correlation coefficient, ** Standard deviation, *** Lethal dose, **** Fiducal limit

Table 5: The differences between boric acid and OY neem experiment.

Experiment No.	Boric acid				OY powder			
	Concentration (g)	Average of bait consumption (g/insect)	Lethal dose (mg/insect)	Mortality %	Concentration (g)	Average of bait consumption (mg/insect)	Lethal doses (mg/insect)	Mortality %
1	0.965	0.02053	0.7924	6.6	2.1	35.29	2.964	7.5
2	1.26	0.01784	0.8991	15	2.8125	34.06	3.832	22.5
3	1.687	0.01829	1.234	35	3.75	32.4	4.859	37.5
4	2.25	0.0196	**1.76**	**64**	5	5.148	1.095	15
5	3	0.01797	**2.165**	**80**	6.667	1.488	0.397	5
6	Control	0.02465	0	2.5	Control	35.29	0	0

Highlighted in grey are the high mortality rates, lethal doses and the concentrations. The highest mortality rates are highlighted in grey and bold.

Discussion & Recommendation

Cockroaches are nasty pests which had lately infested lots of houses in Khartoum State. Use of chemical insecticides is hazardous and may prove fatal if consumed by chance by humans or pets and other useful/harmless insects.

In search of the best low cost, efficient and non-toxic insecticide for controlling cockroaches' infestation, a number of experiments were carried out here. In this study cockroaches were treated with two different compounds: boric acid was used as a low risk insecticide whereas neem old leaflets of young leaves powders (OY) were used as biorational insecticides.

This study reveals interesting comparisons between boric acid and neem OY powder for controlling the American cockroaches.

Boric acid solid baits were found toxic to *P. americana*. A direct positive correlation was observed between increase of boric acid concentration and mortality. This is in agreement with Gore and Schal (2004) who used boric acid in liquid bait which effectively killed cockroaches; however, baits used in this study have the advantage of being hard enough to carry around without fear of spilling.

Boric acid is an inexpensive inorganic insecticide with favorable safety recorded and no known cases of resistance in arthropods. So it can be used to control American cockroaches.

Neem OY powder was found toxic up to 3.75 g, however, higher concentrations did not affect mortality but had a great indication for a repellent effect (antifeeding behavior). However, boric acid was more efficient as an insecticide. Interestingly, few cockroaches ate from baits containing 5g - 6.667g OY neem powder. This can be due to either that the taste of food with high concentration of OY was still palatable for these insects or there is defect with their chemoreceptors.

For means of *P. americana* control, the developed OY neem bait can be used either as insecticides or as repellant to control American cockroach's population within households.

Recommendations

1. Boric acid can be applied as effective insecticide to control American cockroaches' population.
2. Neem OY powder also can be applied as botanical insecticide to control American cockroaches' population. As well it can be applied as repellent to American cockroaches, when it used at high concentration ≥5g.

Both of Neem and Boric acid can be applied in houses and other facilities due to their low toxicity to human and other mammals.

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