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## Effects of Ibuprofen and Caffeine Concentrations on the Common Bed Bug (*Cimex lectularius* L.) Feeding and Fecundity

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### Abstract

Bed bugs are ectoparasites of humans and require a blood meal for their growth and reproduction. Since humans consume Ibuprofen as pain medication and drink coffee (caffeine), bed bugs are likely to acquire these drugs through blood feeding. In this study, we determined the biological effects of Ibuprofen and caffeine on bed bug feeding, fecundity and egg hatch. Five concentrations of Ibuprofen and caffeine were incorporated into reconstituted human blood (RHB). Control treatment had no Ibuprofen or caffeine. Each treatment had six replications. Groups of 20 adult bed bugs (10 males: 10 females)/treatment/replication were pre-weighed, allowed to feed for 45 minutes, and then reweighed. After feeding, bed bugs were transferred into a glass jar equipped with harborage and all the jars were placed in an environmental chamber undisturbed for three 7-day intervals to determine fecundity and nymph emergence. Ibuprofen data showed that the mean mass of the 20 adult bed bugs increased by 0.1074 g (125.65%) in the control but by 0.1336 g (157.30%) at 200 ppm after feeding. After 7 days, 306 and 146 eggs were laid by the 60 female bed bugs fed on 0 and 200 ppm Ibuprofen, respectively. After 2 weeks, 94% eggs hatched with no significant differences amongst treatments. In the caffeine experiments, the bed bug mean mass increases were 0.1219 g (163.90%) in control and 0.0790 g (104.62%) at 50 ppm caffeine. After 7 days, 264 eggs were laid by 60 female bed bugs in control but only 81 eggs were laid at the 50 ppm. Nymph emergence was >80% for all caffeine concentrations after 2 weeks. These results demonstrated that increasing Ibuprofen concentrations had positive effects on mass gain but negative effects on egg laying capacity of bed bugs. Caffeine concentrations had negative effects on bed bug feeding, fecundity and egg hatch.

**Keywords:** *Cimex lectularius*; Bed bugs; Ibuprofen; Caffeine; Feeding; Fecundity

### Introduction

The bed bug (*Cimex lectularius* L., Hemiptera: Cimicidae) infestations have steadily increased and widely reported in North America, Western Europe, and Australia, since the 1990s, and are causing serious concerns to hotel/rental industry, public health professionals and the public at-large. Bed bugs indiscriminately infest buildings regardless of low- or high-income homes (98%), luxury hotels or motels (75%), College dorms (47%) or nursing homes (46%) [1]. The bed bug resurgence was attributed to an increase in international travel and trade, and use of less toxic insecticides [2-4]. The U.S. Department of Commerce reported that there were 51 million international tourists in 2000, a 15% increase over a period of 1995-2000 [4]. The bed bug management practices were affected from cancellation of chlorinated hydrocarbon insecticides, minimum use of organophosphates, carbamates, pyrethroids, use of less toxic insecticide-gel baits, and insect growth regulators [5]. The bed bug resurgence was also reported in other countries including Korea [4], Malaysia and Singapore [6], Italy [7] and China [8]. This recent bed bug resurgence has created a renewed interest to seek new information on bed bug biology, molecular based population genetics, and sustainable management techniques. Research on bed bug resistance to commonly used insecticides was conducted by numerous researchers [3,9-12].

Humans use various products containing Ibuprofen to relieve headache, minor aches, and pains. Humans also routinely consume coffee and various soft drinks containing caffeine. Since bed bugs principally feed on humans in residential environment, they can potentially acquire Ibuprofen and caffeine from blood stream. However, the biological impacts of these drugs on bed bugs have not been studied.

Ibuprofen (iso-butyl-propanoic-phenolic acid; (RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid) is a non-steroidal anti-

inflammatory drug. It is commonly used to reduce fever and treat pain or inflammation caused by headache, toothache, back pain, arthritis, menstrual cramps, or minor injury. Ibuprofen is the active ingredient in more than 50 brand names and generic medications, such as Advil® and Motrin® that humans consume. It is also used in combination with other drugs for control of other illness, such as flu (Advil® Flu and Body Ache Caplets), cold (Motrin® Children's Cold) [13,14].

Several studies were conducted on the use of Ibuprofen to treat insect bites and stings [15,16] and other illnesses in humans [17,18], but no research was conducted to determine the effect of Ibuprofen on insect biology. There were studies on the impact of Ibuprofen on aquatic life, such as in *Daphnia magna* Mueller, *Litopenaeus* spp. Farfante and *Planorbis carinatus* Muller [19-22]. Heckmanna et al. [19,20] revealed that the population growth rate in water fleas (*Daphnia* spp.) was significantly reduced when exposed to Ibuprofen, and the survival was affected at the highest concentration. Alfaro-Montoya [21] studied the impact of Ibuprofen on marine shrimps (*Litopenaeus* spp.) and found significant differences in treated females with developing ovaries (45 ± 19%) as compared with females in control (12 ± 8%). Pounds et al. [22] detected a reduction in the weight of mollusks (*P. carinatus*) exposed to Ibuprofen.

Caffeine (1,3,7-trimethylxanthine) is a bitter white crystalline

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alkaloid, a central nervous system stimulant and the most widely consumed addictive substance in the world [23]. Worldwide consumption was estimated around 70 to 76 mg/person/day [24]. The caffeine consumption in USA was estimated at 196 to 238 mg daily [23,25]. The common dietary sources of caffeine are coffee, chocolate, tea and some soft drinks. The amount of caffeine in food products varies, depending on the serving size, type of product and preparation method [26-28].

Fernandes et al. [29] reported that caffeine and chlorogenic acid (a phenolic compound from coffee) applied on coffee leaves stimulated the movement of the green scale insect (*Coccus viridis*, Green), thus reducing their feeding compared to untreated leaves. Caffeine, the phenolic, and the alkaloidic compounds derived from coffee leaves increased egg-laying by the coffee leaf miner, *Leucoptera coffeella* (Guérin-Ménéville) [30].

The biological effects of Ibuprofen and caffeine when consumed by bed bugs have not been previously studied. Therefore, this research was undertaken to gather data to fulfill this deficiency. We proposed the hypothesis that Ibuprofen and caffeine may negatively affect the bed bug biology. This hypothesis was tested with the following objectives: 1) determine the mass/body weight change in the bed bugs from blood feeding; 2) delineate the bed bug fecundity; and 3) ascertain nymph emergence.

## Materials and Methods

### Insects

The colony of bed bugs (Harlan strain) used throughout this study was established from live specimens obtained from the Department of Entomology, University of Minnesota, St. Paul, MN on March 2011. The Minnesota bed bug colony is descendants from the colony acquired from the Insect Control Research Center (Baltimore, MD) [31], with specimens collected from a natural infestation in Gainesville, FL sometime before 1983. In our laboratory, the bed bugs were confined in 500 mL Van Water and Rogers (VWR) short wide-mouth glass jar (VWR International, Radnor, PA), containing 9.0 cm circular VWR filter papers (Radnor, PA) with four folds for harborage and substrate for eggs. The glass jars containing bed bugs were covered with organza fabric (JoAnn's Fabric, Lincoln, NE) for ventilation and containment. All jars were maintained in a Percival Scientific environmental growth chamber (Perry, IA) at  $23 \pm 2^\circ\text{C}$ ,  $55 \pm 5\%$  relative humidity, and a photoperiod of 12:12 (Light: Dark with light on 10 AM) [32]. The bed bugs were fed weekly with expired reconstituted human blood (RHB) obtained from the Nebraska blood bank (Lincoln, NE).

### Treatments

This study included five concentrations of Ibuprofen (40, 80, 120, 160 and 200 ppm) and caffeine (10, 20, 30, 40 and 50 ppm) plus the control as individual treatments (Tables 1 and 2). A stock solution from each of Ibuprofen and caffeine standard was prepared (Ibuprofen: 200 ppm, Fluka PHR1004; caffeine: 50 ppm, Fluka PHR1009; Sigma-Aldrich, St. Louis, MO) and diluted further to attain desired concentrations as specified in Tables 1 and 2. The Ibuprofen concentrations were determined based on an average human having 5 liters of blood [33] and the maximum over the counter dose of  $6 \times 200$  mg Ibuprofen tablet within 24 hours [34]. The increase in Ibuprofen concentrations was equivalent to an additional dose of  $1 \times 200$  mg tablet. The caffeine concentrations were determined based on the amount of caffeine in a 355 ml Starbucks coffee (260 mg, [27,28]) and

an average human body having 5 liters of blood [33]. The Ibuprofen and caffeine were incorporated in the RHB.

### Experimental design

The experimental setup was a complete randomized design with feeding groups consisted of one randomly selected experimental unit (EU) of each treatment. The EUs consisted of 20 adult bed bugs (10 M:10 F) kept in 125 ml VWR straight side wide-mouth glass jars (Radnor, PA) and sealed with the organza fabric for the duration of the study. The bed bugs were provided with one 9.0 cm VWR filter paper with four folds for harborage. Each treatment (of Ibuprofen, caffeine and control) was replicated six times and was conducted at different time interval.

### Feeding experiment

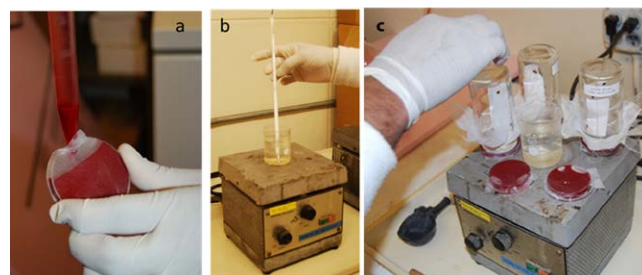
The bed bug feeding setups used for the treatments were modeled after the Chin-Heady et al. [35]. Six 50 mm petri dishes were filled with the appropriate concentration of treated blood (e.g., for the Ibuprofen, each 50 mm petri dish was filled with one of the concentrations (0, 40, 80, 120, 160 and 200 ppm) (Figure 1a). The petri dishes were labeled, sealed (with Nescofilm, LabShop, UK) and placed on a 316.7 cm<sup>2</sup> Sybron Thermolyne 1000 hotplate/stirrer (VWR, Radnor, PA) set at  $36 \pm 2^\circ\text{C}$ . One 125 ml beaker with 50 ml water was placed in the center of the hot plate to monitor the temperature (Figure 1b). After the blood was warmed up, the appropriate EU (0, 40, 80, 120, 160 or 200 ppm, one replication each) was placed upside down on the correspondingly labeled 50 mm petri dish to feed for 45 minutes (Figure 1c). After the 45 minutes, the petri dishes with blood were agitated to resuspend any settled blood cells. This process was repeated with each replication of EUs (0, 40, 80, 120, 160 or 200 ppm) until the bed bugs were fully fed. The 45-minute feeding regime was based on Johnson [36] who reported that bed bugs feed to repletion between 20 and 30 minutes if undisturbed.

### Mass gain/loss

The groups of 20 adult bed bugs in each experimental unit (EU) were pre-weighed on an Ohaus GA110 analytical balance (Parsippany, NJ) and the mass recorded. The bed bugs were allowed to feed for 45 minutes, removed from the EU and reweighed. The difference between the two masses is due to the amount of blood consumed. The EUs were then placed in the environmental chamber (at conditions described earlier) undisturbed for one week.

### Fecundity and nymph emergence

After one week in the environmental chamber, the adult bed bugs



**Figure 1:** The bed bug feeding unit: a) adding treated blood into a 50 mm petri dish; b) using 150 ml beaker for monitoring hot plate temperature; and c) feeding jars with bed bugs placed upside-down on the respective petri dish during bed bug feeding.

were removed from the EUs and the numbers of eggs laid on the filter paper in each EU were recorded. The EUs including the filter papers with eggs were returned to the environmental chamber undisturbed for two 7-day intervals to allow the bed bug nymphs to emerge. Emerged nymphs were counted twice, at 1 week and 2 weeks after eggs were laid. The percent nymph emergence was calculated by comparing the number of eggs laid versus the number of nymphs emerged.

### Statistical analysis

To determine the effect of Ibuprofen and caffeine on the bed bug feeding and fecundity, the data were analyzed using analysis of variance (ANOVA), SAS Enterprise Guide 4.3 [37]. The significant differences were further tested with the pairwise comparisons of the treatment levels using the generalized linear model (GLM) procedure with multiple comparisons corrected by the Tukey's test.

## Results

### Mass Gain/Loss and egg production

A significant effect ( $p < 0.05$ ) of the Ibuprofen concentrations

was observed on bed bugs mass percent increase ( $F=2.57$ ,  $df=5$ ,  $30$ ,  $P=0.047$ ), and eggs production ( $F=5.38$ ,  $df=5$ ,  $30$ ,  $P=0.0012$ ) (Table 1). The regression  $R^2$  and coefficient of variation were 0.2997 and 13.023 for the mass percent increase, and 0.4732 and 27.039 for bed bugs egg production, respectively. There was a significant increase in blood consumed by the bed bugs with 200 ppm Ibuprofen when compared to lower Ibuprofen concentrations and the control. Addition of Ibuprofen to the bed bug's diet also resulted in a significant reduction in egg production at 160 and 200 ppm concentrations (Table 3).

Significant differences ( $p < 0.05$ ) were observed in percent mass decrease ( $F=5.147$ ,  $df=5$ ,  $30$ ,  $P=0.0007$ ) and less egg production ( $F=7.02$ ,  $df=5$ ,  $30$ ,  $P=0.0001$ ) when bed bugs were fed on caffeine incorporated blood meals (Tables 2 and 4). Regression  $R^2$  and coefficient of variation were 0.4682 and 18.42 for the mass percent increase and 0.5461 and 31.09 for egg production, respectively. The analysis of variance determined that the addition of caffeine to the bed bugs' diet had a significant negative impact on the bed bugs feeding and fecundity.

Further observation revealed that the maximum mass increase was at the highest concentration of Ibuprofen (200 ppm) in the blood, 157.30

Treatment*	Ibuprofen (ppm)	N**	Bed Bugs Mean Mass (g) ( $\bar{x} \pm SEM$ )			Mean % Increase ( $\bar{x} \pm SEM$ )
			Before Feeding	After Feeding	Increase	
	0	120	0.0856 $\pm$ 0.0019	0.1930 $\pm$ 0.0043	0.1074 $\pm$ 0.0035	125.65 $\pm$ 4.59 a
	40	120	0.0831 $\pm$ 0.0022	0.2014 $\pm$ 0.0049	0.1183 $\pm$ 0.0038	142.67 $\pm$ 5.03 a
	80	120	0.0850 $\pm$ 0.0008	0.1939 $\pm$ 0.0064	0.1090 $\pm$ 0.0066	128.42 $\pm$ 8.12 ac
	120	120	0.0824 $\pm$ 0.0012	0.1992 $\pm$ 0.0036	0.1168 $\pm$ 0.0029	141.86 $\pm$ 3.16 a
	160	120	0.0849 $\pm$ 0.0023	0.2185 $\pm$ 0.0123	0.1096 $\pm$ 0.0111	131.88 $\pm$ 12.91 ae
	200	120	0.0849 $\pm$ 0.0016	0.2185 $\pm$ 0.0062	0.1336 $\pm$ 0.0054	157.30 $\pm$ 5.80 bd

\*Each treatment had six replications

\*\*Total numbers of bed bugs per treatment

**Table 1:** Average weight of the 20 bed bugs (10 M; 10 F) before and after feeding, plus mass and percent increase for each Ibuprofen concentration added to their blood meal. Significant differences ( $p < 0.05$ ) of the average mass percent increase is represented by the different letters.

Treatment*	Caffeine (ppm)	N**	Bed Bugs Mean Mass (g) ( $\bar{x} \pm SEM$ )			Mean % Increase ( $\bar{x} \pm SEM$ )
			Before Feeding	After Feeding	Increase	
	0	120	0.0745 $\pm$ 0.0015	0.1964 $\pm$ 0.0060	0.1219 $\pm$ 0.0058	163.9001 $\pm$ 8.81 a
	10	120	0.0823 $\pm$ 0.0028	0.2056 $\pm$ 0.0039	0.1233 $\pm$ 0.0043	151.2402 $\pm$ 8.81 ac
	20	120	0.0779 $\pm$ 0.0019	0.1919 $\pm$ 0.0042	0.1139 $\pm$ 0.0049	147.0597 $\pm$ 9.06 ae
	30	120	0.0822 $\pm$ 0.0032	0.1882 $\pm$ 0.0073	0.1060 $\pm$ 0.0091	131.2207 $\pm$ 14.58 a
	40	120	0.0779 $\pm$ 0.0035	0.1665 $\pm$ 0.0076	0.0885 $\pm$ 0.0044	113.7580 $\pm$ 3.43 bd
	50	120	0.0747 $\pm$ 0.0020	0.1538 $\pm$ 0.0148	0.0790 $\pm$ 0.0135	104.6169 $\pm$ 15.95 bd

\*Each treatment had six replications

\*\*Total numbers of bed bugs per treatment

**Table 2:** Average weight of the 20 bed bugs (10 M; 10 F) before and after feeding, plus mass and percent increase for each caffeine concentration added to their blood meal. Significant differences ( $p < 0.05$ ) of the average mass percent increase is represented by the different letters.

Treatment* Ibuprofen (ppm)	N**	Egg Production		Mean % Nymphal Emergence ( $\bar{x} \pm SEM$ )	
		Total	$\bar{x} \pm SEM$	Week 1	Week 2
0	60	306	51.0 $\pm$ 3.74 a	28.76 $\pm$ 7.66	97.06 $\pm$ 0.96a
40	60	300	50.0 $\pm$ 3.65 ac	30.33 $\pm$ 4.92	98.33 $\pm$ 0.95a
80	60	274	45.67 $\pm$ 2.42 ac	33.94 $\pm$ 3.01	99.27 $\pm$ 0.47a
120	60	268	44.67 $\pm$ 6.18 ac	33.58 $\pm$ 6.95	94.78 $\pm$ 2.17a
160	60	196	32.67 $\pm$ 5.88 b	60.71 $\pm$ 7.92	99.49 $\pm$ 0.79a
200	60	146	24.34 $\pm$ 0.80 bd	59.59 $\pm$ 12.72	97.26 $\pm$ 1.92a

\*Each treatment had six replications

\*\*Total numbers of bed bug females per treatment

**Table 3:** The number (and average per replication) of eggs laid by 60 female bed bugs in one week after consuming Ibuprofen in reconstituted human blood, plus the percent bed bug nymph emerged at week one and two after eggs were laid. Different letters represent significant differences ( $p < 0.05$ ) between the various test concentrations.



Treatment* Caffeine (ppm)	N**	Egg Production		Mean % Nymph Emergence ( $\bar{x} \pm$ SEM)	
		Total	$\bar{x} \pm$ SEM	Week 1	Week 2
0	60	264	44.00 $\pm$ 2.44 a	11.74 $\pm$ 5.06	94.70 $\pm$ 1.82a
10	60	170	28.34 $\pm$ 4.51 b	10.59 $\pm$ 5.27	91.76 $\pm$ 4.62a
20	60	176	29.34 $\pm$ 4.36 b	14.77 $\pm$ 6.94	97.16 $\pm$ 1.62a
30	60	164	27.34 $\pm$ 3.23 b	10.37 $\pm$ 5.20	93.29 $\pm$ 4.67a
40	60	127	21.17 $\pm$ 2.38 c	11.81 $\pm$ 10.78	92.13 $\pm$ 2.94a
50	60	81	13.50 $\pm$ 7.01 d	8.64 $\pm$ 6.81	81.48 $\pm$ 10.07a

\*Each treatment had six replications

\*\*Total numbers of bed bug females per treatment

**Table 4:** The number (and average per replication) of eggs laid by 60 female bed bugs in one week after consuming caffeine in reconstituted human blood, plus the percent bed bug nymph emerged at week one and two after eggs were laid. Different letters represent significant differences ( $p < 0.05$ ) between the various test concentrations.

$\pm$  5.80% ( $\bar{x} \pm$  SEM) and the least at the control, 125.65  $\pm$  4.59%. For all the concentrations tested, there were at least a doubling in the mass of the 20 bed bugs (Table 1) reflecting that bed bugs consumed the blood equivalent to their weight after allowed to feed for 45 minutes. The pairwise comparison of the percent mass increase showed a significant difference between the control and 200 ppm ( $p < 0.05$ ). In this case, the group of bed bugs fed with 200 ppm Ibuprofen consumed significantly more (20%) RHB, than the bed bugs in the control treatment. The control treatment did not differ significantly from any other Ibuprofen concentrations but there were significant differences between the 200 ppm vs. the 60 and 160 ppm (Table 1).

In terms of caffeine, the mean percent mass decrease for caffeine concentrations and the control varied between 104% (50 ppm caffeine) and 163% (control) (Table 2). The maximum increase was observed in the control (0 ppm caffeine), with 163.90  $\pm$  8.81% ( $\bar{x} \pm$  SEM) and the most decrease was at 50 ppm caffeine in the RHB at 104.62  $\pm$  15.95%. The bed bugs consumed blood meal equivalent to their body weight in all caffeine concentrations (Table 2). The pairwise comparison of the effect of the addition of caffeine in the RHB to percent mass increase showed a significant difference ( $p < 0.05$ ) in the control and in two lowest concentrations (10 and 20 ppm) vs. the two highest concentrations (40 and 50 ppm). There were no significant differences between the control vs. the three low caffeine concentrations (10, 20 and 30 ppm) (Table 2).

### Ibuprofen effect on fecundity and nymph emergence

The 60 female bed bugs fed with the highest concentration of Ibuprofen (200 ppm) in the RHB laid 146 eggs, at an average of 24.33  $\pm$  0.80 ( $\bar{x} \pm$  SEM) per replication (Table 3). However, the 60 female bed bugs in the control laid a total 306 eggs at an average of 51.00  $\pm$  3.74 per replication (Table 3). There was a significant decrease between the numbers of eggs laid by the 60 female bed bugs in the control, 40, 80 and 120 ppm Ibuprofen vs. the numbers of egg laid by bed bugs fed on 200 ppm Ibuprofen in the RHB. No significant differences were observed between the control vs. the bed bugs fed on 40, 80 and 120 ppm Ibuprofen. The result showed that there was a negative effect on bed bugs fecundity with the increasing concentration of Ibuprofen in RHB. When fed with increasing concentration of Ibuprofen, although the bed bugs fed to repletion, the number of eggs laid decreased by 35 and 52%, between the control vs. 160 and 200 ppm Ibuprofen in the blood, respectively.

In the Ibuprofen consumption study, of the 306 eggs laid by the bed bugs in the control, 28.76 and 97.06% of them hatched after week one and two, respectively (Table 3). A maximum of 60.71% of the eggs hatched after one week, this was at the 160 ppm Ibuprofen concentration. After the second week, greater than 94% of all the eggs laid in all the Ibuprofen concentrations hatched. For all treatments and

the control, only 35 eggs did not hatched, out of 1490 eggs laid (Table 3). The Ibuprofen may have affected the egg's development, which resulted in the 52% reduction in eggs laid at the 200 ppm Ibuprofen concentration, but it did not affect nymph emergence. After two weeks, there were no significant differences in nymph emergence among the treatments. There was a 97.26% hatch rate for the eggs (Table 3) in the Ibuprofen treatments.

### Caffeine effect on fecundity and nymph emergence

The number of eggs produced by the 60 female bed bugs in the control for the caffeine treatment was 264, with an average of 44.00  $\pm$  2.45 ( $\bar{x} \pm$  SEM) per replication (Table 4). There was a decrease in the number of eggs produce by the bed bugs fed with the increasing concentration of caffeine in the RHB. The least number of eggs were produced by the 60 female bed bugs fed with the highest concentration of caffeine (50 ppm) in the RHB reflecting a total of 81 eggs, 13.5  $\pm$  2.86 per replication (Table 4). The difference between the number of eggs laid by the bed bugs in the control vs. the highest caffeine concentration (50 ppm) was 64%. There were significant differences ( $p < 0.05$ ) in the number of eggs produced by the control vs. all concentrations of caffeine in the RHB (Table 4).

For the caffeine consumption study, of the 264 eggs laid by the bed bugs in the control treatment 11.7 and 94.7% hatched after week one and two, respectively (Table 4). After the first week, less than 15% of the eggs hatched per concentration, but after the second week >90% of eggs hatched in all treatments, except at 50 ppm caffeine treatment, which had only 81% nymph emergence (Table 4). Although low caffeine concentrations (<30 ppm) did not affect bed bugs feeding (Table 2), there was a significant reduction in the number of eggs laid (Table 4). After two weeks, there were no significant differences in nymph emergence among the treatments (Table 4).

### Discussion

This study revealed that Ibuprofen had positive effect on blood consumption but negative effect of on egg production. However, the percent nymph emergence was not significantly affected in various Ibuprofen concentrations. The bed bugs fed on caffeine incorporated RHB had negative effects on both mass gain and egg production. When fed with increasing levels of Ibuprofen, there was a significant decrease in the number of eggs laid by the bed bugs. A caution should be exercised because ibuprofen can have negative side effects in humans, such as increased risk of gastrointestinal bleeding [38,39], and should not considered as a management strategy for bed bugs.

When fed with increasing concentration of Ibuprofen, although the bed bugs fed to repletion, the number of eggs laid decreased by 47.7% between the control and the highest concentration of Ibuprofen

in the blood. One reason for the reduction in egg produced may be that Ibuprofen affected the egg development in the bed bugs. Alfaro-Montoya [21] has shown that Ibuprofen negatively affected marine shrimp ovaries. Tesh and Guzman [40] fed three mosquito species various concentrations of ivermectin in human blood and recorded mortality and effect on fertility. These authors reported that dying mosquitoes displayed signs of acute toxicity, such as incoordination, movement difficulty and lethargy. They also observed a noticeable decrease in both the number and viability of the mosquitoes' eggs. In another study conducted by Sheele et al. [41] to observe the effect of ivermectin on bed bugs, volunteers consumed 200 mg/kg orally and then had bed bugs feed on them, 50 and 63% (n=24) mortality was recorded, compared to 4 and 8% (n=24) in the control group, after 2- and 20-days, respectively.

The result from the caffeine study showed that with increasing caffeine concentrations in RHB, there were negative effects on bed bugs feeding and fecundity, but not on nymph emergence. There was a positive relation between the numbers of egg produced by the bed bugs vs. percent mass increase due to feeding. The more blood the bed bug consumed resulted in more egg production. A negative correlation was observed between increasing caffeine concentration in the blood vs. the number of eggs produced by and the amount of blood consumed by the bed bugs. When fed with increasing concentration of caffeine, bed bugs consumed significantly less (36%) blood and produced significantly fewer eggs, 64% between the control and the highest concentration (50 ppm) of caffeine in the blood. Wright et al. [42] found that honey bees rewarded with naturally occurring caffeine from *Coffea* and *Citrus* species, were three times as likely to remember the floral scent as was honey bees rewarded with sucrose alone. It also improves the honey bees' long-term memory for flowers.

The correlation between the number of eggs produced and the amount of blood consumed was explained by Johnson [36] who reported three interrelated variables, the weight of unfed female, a mass of blood meal and the number of eggs produced. Johnson [36] further reported a positive correlation between the weights of unfed females and a mass of blood meal with the number of eggs produced. Usinger [43] also, observed a correlation between food intake and egg production and longevity, although longevity was not studied in this research, a correlation between food intake and egg production was observed. Similar observations were recorded in other insect species; the checker-spot butterfly (*Euphydryas editha*) had increased egg production and egg mass with increased consumption of carbohydrates and nectar [44]. In previous studies, [45,46] consumption of caffeine was shown to cause mortality in insects. Although not tested in this study, further research is needed to confirm mortality in bed bugs.

In conclusion, Ibuprofen and caffeine negatively affected bed bugs fecundity, although nymph emergence was unaffected. When fed with increasing levels of Ibuprofen, there was an increase in blood consumed, but when fed with increasing levels of caffeine, there was a decrease in blood consumed by the bed bugs. There was a greater than 81% emergence of nymphs from the eggs laid by the bed bugs exposed to Ibuprofen and caffeine. At all caffeine concentrations, the eggs laid were significantly less than the control. A positive correlation was observed between the number of eggs produced and the amount of blood consumed. This was in agreement with other similar studies, which reported a positive association between a mass of blood meal with the number of eggs produced in previous bed bug studies as well as several other insects.

The method developed in this research may be used to study the effects of other compounds, humans consume on bed bugs or even other insects that feed on humans, such as mosquitoes and ticks. Future studies could address the effects of other compounds, such as nicotine or other medication, taken on a regular basis, on bed bugs feeding and fecundity. Some of these studies could be extended to test survival and effects on the nymphs, which were hatched from eggs developed during the exposure to the test compounds.

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