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A Promising Use for Potassium Alum as Controlling Agent Against *Blattella germanica* (L.) (Dictyoptra: Blattellidae)

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Abstract: This is the first study to clearly demonstrate the potential on the use of potassium alum as a friendly-insecticide against *Blattella germanica* (L.) as an oviparous insects. This German cockroach is in first appendance to American cockroach. In our laboratory, we tested the common potassium alum for controlling *B. germanica* (L.). Starved adult and nymph stages for three days (i.e. deprived from food but have access to water) were left to feed with crashed pellets of potassium alum. Gravid females were only shifted to eat from normal food to potassium alum. The mortality recorded as LT_{50}, the younger nymph third and fourth instars were died within 6 days after consuming 0.2mg of alum. Non- Gravid and gravid females were quit susceptible and decrease in their survival to high extent with LT_{50} 16 days and 14 days respectively. The wonderful effect was found that the effect of the potassium alum is extending effect to the egg capsules especially if the female started to eat at the beginning of the extrusion no nymphs could be emerged at all. The lipase activity of the female cockroaches that fed upon potassium alum was greatly decreased during her reproductive cycle which greatly reduced the percentage of emergency of nymphs especially if it starts feeding on potassium alum at the day 1 of the reproductive cycle i.e. the egg case start to protrude from the abdomen. These would be a great potential in increasing oocyte resorption with no chance for new offspring to live i.e. decrease the protein content of the oothecae to low level. The results revealed that the female and male need to ingest 1.0 mg, 3.0 mg of potassium alum respectively to induce 100% mortality after 16 days for female and 28 days to male after consuming potassium alum. In all the tested stages only males had a higher LT_{50} which was found to be 28 days. The potassium alum had to be ingested by the cockroaches to induce mortality. The potential applications of this novel technique will be discussed.
Keywords: Environmentally friendly insecticide, potassium alum, *Blattella germanica.*

Introduction

The German cockroach is the most successful of the species infesting buildings in everywhere. Cockroaches have successfully adapted to various habitats including rodent burrows, sand dunes, caves and human dwellings[1]. These roaches act as an active carrier of many pathogenic organisms [2-5] and also may cause allergic reactions in some people[6-8]. There are several reasons for this cockroach's persistence and the difficulty of controlling it. German cockroaches produce a larger number of eggs per capsule and they undergo the shortest time from hatching until sexual maturity, resulting in a rapid population growth. A greater number of nymphs hatch successfully because the female carries the egg capsule during the entire time the embryos are developing within the eggs. Also, and most importantly, German cockroaches are smaller than most other cockroaches and can conceal themselves in many places inaccessible to individuals of the larger species[9].

Cockroaches can be oviparous, ovoviviparous, or viviparous[10].Therefore, they serve as excellent models for comparative studies on mechanisms regulating female reproductive physiology and behavior, including such topics as the synthesis and release of regulatory hormones, synthesis and emission of pheromones, and sexual receptivity. *B. germanica* is an oviparous blattellidae with a unique pattern. Females emerge as sexually unreceptive adults, undergo several days of sexual maturation (which can be extended indefinitely if inappropriate conditions prevail), become sexually receptive, recruit nonspecific males with sex pheromones, mate, and oviposit their vitellogenin-laden fertilized basal oocytes into an oothecae. *B. germanica* females carry the ootheca externally for about 21 days, until the young hatch[11,12]. During this time, oocyte development is inhibited. Thus, *B. germanica* is similar to ovoviviparous (e.g., *Nauphoe tacinerea, Leucophae amaderae*) and viviparous (*D. punctata*) cockroaches in which oocyte development is restrained while females are internally brooding their young. While most oviparous females exhibit relatively uninterrupted cycles of oocyte development, *B. germanica* females experience discrete patterns of oocyte development interrupted by long pregnancies. Adult females can survive up to 250 days and produce up to nine broods. This complex reproductive life history differs from many insect species, which mate soon after emergence, do not feed, and have short adult lives. In Blattella, as in all cockroaches studied to date, vitellogenesis and cyclic maturation of oocytes depends upon JH III synthesis by the CA[13-15]**.** Thus, the size of basal oocytes in the panoistic ovarioles serves as a reliable predictor of relative CA activity[16,17]. JH III is the only JH homolog in *B. germanica* and JH titer in the hemolymph is largely determined by the rate of JH synthesis[18]. JH production increases as oocytes grow after the imaginal molt, declines just before ovulation (as the oocytes become chorionated), and remains low during pregnancy. The German cockroach has three developmental stages: egg, nymph, and adult. Females produce a light brown, purse-shaped egg capsule that is less than 1/4 inch long and contains two rows of eggs. Each capsule contains up to 48 eggs (usually 30 to 48), and adult females usually produce from four to eight egg capsules during their lifetime. At room temperature, one capsule is produced about every 6 weeks. Egg capsules are carried, protruding from the abdomen, until hatching time when they are deposited into crevices and other sheltered locations. It usually takes 28 days for the capsule to hatch from the time it begins to form. Formation of the next egg capsule usually begins within a couple of weeks. The length of the egg stage varies from 14 to 35 days, with six to seven nymphal stages (instars) occurring over a period of 6 to 31 weeks. The life span of the adult female is considered as the most perfect mother as a powerful reproductive capacity. The reproductive physiology features of the German cockroaches. *B. germanica* is unique among household pest cockroaches in having an "ovoviviparous-like" gonotrophic cycle. Vitellogenic females exhibit high levels of food intake, while gravid females feed little and sporadically during a 20– 22 day pregnancy[19-21]. Thus, a female feeds little or not at all for 60 to 75% of her reproductive life. This might, at least in part, explain why reductions in pest cockroach populations are slow with insecticidal baits. Juvenile hormone analogs disrupt the metamorphic molt of last instars, resulting in sterile adults, and they are used routinely in cockroach control[22] Moreover, since pregnancy is maintained by low titers of JH, exposure to high levels of exogenous JH might increase the frequency of abortions in gravid females. Removal of feeding inhibition, caused by abortion of the ootheca, together with high titers of JHA, should further stimulate the previously gravid female to feed. Clearly, cyclic production of JH has profound effects upon the reproductive biology of the German cockroach. Changes in JH titer, therefore, are likely to influence the behavioral ecology of this insect. For instance, cyclic feeding in adult females, which is related to cycles of JH production[23].

The cockroach is an omnivorous and opportunistic feeder. It consumes decaying organic matter but since the cockroach is a scavenger it will eat almost anything. It prefers sweets and has been observed eating paper, boots, hair, bread, fruit, book bindings, fish, peanuts, old rice, putrid sake, the soft part on the inside of animal hides, cloth and dead insects[24,25]. In addition to best review on potassium alum did by [25] about its uses in folk medicine for long time. Also as a larvicidal agent against *Anopheles stephensi* and *Aedes aegypti*.

The aim of the present work is to investigate the efficacy of potassium alum or Potash alum as a friendly-insecticide to be used against *Blattella germanica* (L.) adult and nymph stages under laboratory conditions and its impact on the protein contents of egg capsule and lipase activity.

Experimental

Chemicals used:

The chemicals used of analytical grade were obtained from Sigma, Aldrich and Fluka (England) otherwise will be stated. Potassium alum from Elnaser Pharmaceutical Chemicals Co. Abu Zaabal Compay

(Egypt) the biochemical activity of lipase enzymes was measured according to the methods described by Mikani et al. (2012)[26] with some modifications. Newly emerged adults (7 d old) of both sexes were used for the experimental for the biochemical studies after treatment (the potassium alum fed group and two controls: one control group with normal food the other control without food i.e., starved). All the cockroaches either normal, treated or under treatment were provided with water from a cotton pads socked with water.

Extraction:

Male and female cockroach from each of the three groups (that fed upon the potassium alum for 24 h, after starved for 48 h of starvation and after re-feeding with normal food, and with normal food as a control) had their mid-gut dissected in 50 m Tris-HCL (pH 7.4) or PBS and its contents were removed. After dissection, the male and female cockroach mid-gut tissues were homogenized and ultra-centrifuged at 16,000 rpm for 10 min at 4°C. The supernatant was placed in a centrifuge tube and kept at 4°C for enzymatic quantification. Each experiment was repeated 3 times. All the above solutions were filtered before use.

Test insect

Blattella germanica were collected from Benha city Qualyubia Governorate Egypt at 2011.This colony was successfully reared and maintained at 27±2°C, ambient relative humidity (60–65%), a photoperiod of 12 h:12 h L:D, and provided with shelter, water and food of white bread. Insects were reared in glass containers (55 x 25 x 30 cm) covered with voil in order to keep them from escaping and to allow for ventilation. Corrugated cardboard tube shelters were arranged in the bottom of the recipient, which was maintained at 27 ± 2^{o}C, 60 ± 5% RH, in a photoperiod of 12 h: 12 h L:D, in insectary room. Water in plastic drinking bottles and white bread. The recipients were cleaned daily to prevent mould contamination. When adults would be eliminated and only the immature would be kept in the rearing container. This colony provided us nymphs, adult males, non-gravid and gravid females, developmentally synchronous colony permitted the use of many newly enclosed females from a single cohort in most experiments. Newly enclosed (day 0) adult females of similar size and degree of sclerotization with intact wings were selected for each experiment. Experiments were conducted under the same conditions as described above: controlled temperature and photoperiod, with water and food available. Adults males and females (virgin or at the time of extrusion i.e. the egg case started to protrude from the abdomen) after emergence were transferred to transparent plastic cups (10.0cm diameter, 4.5 cm high). The cockroaches, containing a bottle filled with water to avoid desiccation, kept individually for 3days without food (starved), another group was left to feed with potassium alum and normal group as a control. The cockroaches were cooled on ice before dissection for subsequent experiments.

Newly moulted nymph (3rd &4th instars) and adult were collected for experimental tests from stock cultures and maintained in small containers (5 individuals per 2 kg Kilner jar) at 25°C±5 and 70%±5 Relative humidity for making the treatment with potassium alum with two types of control, one group with normal food the other control without food(starved). Mated females at the time of extrusion of *Blattella germanica* (L.) were collected for further testing procedure with potassium alum and were tested for protein content of the produced oothecae by these treated females (that had consumed potassium alum), comparing it with control (mated females group with normal food). The container containing cockroach was covered with muslin cloth and tied with rubber ring.

Toxicity Tests.

The German cockroaches (adult male; non-gravid female; gravid female or 3rd or 4th nymphs) were confined in a 0.95-liter glass jar with a piece of white bread as a food, a moistened cotton wick, and 140 cm2 of cardboard harborage configured as a cylinder according to Appel[27]. The upper inside surface of the jar was lightly greased with petroleum jelly to prevent escape. Approximately 5.0 g of crashed pellets of potassium alum was placed in a plastic weighing boat in each jar outside of the harborage. Control jars included only water, white bread, and harborage. Five replicates were conducted for each cockroach stage for a total of 100 cockroaches. Cockroach mortality was recorded as LT_{50} intervals for days. To investigate the effects of food deprivation on mortality, German cockroaches were placed into glass jars with harborage, water, and potassium alum as above, but without food. Sony video camera Model No CCD- TRV27E-0Lux, made in Japan was used to count the number of stages during feeding according to Salama[25].

Adults males and females (virgin or at the time of extrusion) after emergence were transferred to transparent plastic cups (10.0 cm diameter, 4.5 cm high). The cups contained a bottle filled with water to avoid desiccation, and the cockroaches were kept individually for 48 and 96 h with either (1) no food/starved for one control, (2) potassium alum, or (3) normal food for a second control.

Newly molted nymphs (3^{rd}& 4^{th} instars) and adult cockroaches were collected for experimental tests from stock cultures and were kept in small containers (5 individuals per 2 kg Kilner jar) at 25°C±5 and 70%±5 relative humidity during the experiment with potassium alum. Two types of control were used: one group with normal food and another group without food (starved). The LT_{50} values of cumulative mortality was plotted against the time of the all stages of roaches fed with potassium alum or with competitive food were estimated using probit analyses according to Finney[28]. Mated *Blattella germanica* (L.) females were collected when they were extorting on egg capsule for additional experiments. The oothecae produced by these treated females were compared for protein content to those of the control group (mated females group with normal food). The container containing cockroaches was covered with a muslin cloth and tied with a rubber ring. All the tested cockroaches were provided with water from a cotton pads socked with water.

Adult females were staged according to the time after egg capsule formation, with the time of egg capsule formation designated as day 1. Staged females were selected every 24 h after egg capsule formation. The amount of food consumption was estimated by weighing the potassium alum and before and after the 4 d of treatment.

The biochemical activity of the lipase enzymes was measured according to the methods described by Mikani et al.[26]. Newly emerged adults (7 d old) of both sexes were used for the experimental for the biochemical studies after treatment (the potassium alum fed group and two controls: one control group with normal food the other control without food i.e., starved). All the cockroaches either normal, treated or under treatment were provided with water from a cotton pads socked with water.

(i.e., a treatment with no mortality and no repellency). The mean daily mortality percentage each treatment was plotted over time. i.e.LT_{50}

Biochemical studies

Adult non gravid female's i. e. before becoming pregnant and gravid females were staged according to the time after the oothecae begins to form; the time of oothecae formations was designated as day 1. Staged females were selected every 24 hours after oothecae formation. The protein content of the oothecae were measured for the normal and potassium alum treated female. The food consumption was estimated by per weighting of potassium alum and subtracting it after feeding by the tested cockroach after 4days of treatment.

Note: The percentage of cockroaches' mortality was plotted against the recorded time and the LT_{50} values were determined. Mortality percentage was always corrected by Abbott's formula [29] if the mortality in control exceeds 5 %.

Extraction:

Each gravid female cockroach (that feed upon (treated with) Potassium alum for 4 hours, starved for 3d and with normal food as a control) mid-gut was dissected in (50 m MTris-HCL (pH 7.4) or PBS) and its contents were removed. After dissection of the female cockroach mid-gut tissues were homogenized and ultra-centrifuged at 16,000 rpm for 10 min at 4 °C. The supernatant was placed in a centrifuge tube and kept at 4 °C for enzymatic quantifications. Each experiment repeated 3 times.

Lipase activity of the cockroach midgut was measured using a lipase measuring kit (Quantichrom Lipase Assay Kit, Bio Assay System, Hayward, CA). The tissue extracted was incubated individually in cold PBS for 30 min. Lipase activity released was quantified. To prepare the working reagent, 5mg of color reagent was mixed in 140 ml of assay buffer and 8 ml of dimercapto- propanol tributyrate (BALB). The working reagent was freshly prepared and used within 1 h. Then, 140µ ml of working reagent was added to 10 ml of each sample. The assay was based on an improved BALB method, in which SH groups formed from lipase cleavage of 120 BALB react with 5, 5'-dithiobis (2-nitrobenzoic acid). The optical density at 412 nm was proportionate to the enzyme activity of the sample, which was read using a microplate reader (SH-9000, Corona

Electric, Ibaraki, Japan). One unit of enzyme catalyzed the cleavage of 1 ml of substrate per minute under the assay conditions. Protein content in the resulted fraction was determined by Lowry et al. [30] method.

Reagents used:

1. Alkaline sodium carbonate solution (20g/L Na_2 CO_3 in 0.1mol/ 1Na OH) 100/ 31.
2. Copper sulphate sodium potassium tartarate solution (5g/L $CuSO_4$.5H2O in 10 g/L Na, K tartarate). Prepared fresh by mixing stock solutions.
3. Alkaline solution was prepared on day of use by mixing 50 ml of (i) and 1ml of (ii).
4. Folin- ciocalteau reagent (Stock solution).

Sodium tungstate 100 g, Sodium molybdate 25 g, Distilled water 700 ml., Phosphoric acid (85%) 50 ml., Con. HCL 100 ml. This solution was gently refluxed for 10 hours. Then the following were added. Lithium sulphate 150 g, Distilled water 50 ml and few drops of Bromine. The mixture was boiled for 15 minutes without condenser to remove excess bromine. Then the solution was cooled, completed to one liter with distilled water and filtered. Finally the reagent was titrated against 1Na OH to phenolphthalein end point. Before use, the solution was diluted with distilled water (about two folds).

i. Standard protein (bovine serum albumin 0.2 mg/ml) as a standard, was used for determination of protein of each sample.

The statistical analysis

The statistical analysis was performed using SPSS version 20.

Results

The food consumption test showed that the potassium alum have a promising effect as a potential food poison for nymphs, gravid female, non-gravid female and males as presented in (Table 1).The potassium alum was toxic to all developmental stages of the German cockroach. Third nymph's mortality with LT_{50} 6-7days. Gravid adult females were probably killed more rapidly than non-gravid females with LT_{50} 14, 16 days respectively. This is because theses stages are physiologically sensitive for consuming more food in preparation for growth or producing oothecae as indicated in Fig 1. The LT_{50} value for small nymphs was smallest one than any of the other stages. Small nymphs are highly aggregative do not generally move great distances, and are strongly coprophagous. It is likely that some of the nymphs defecated near other individuals and essentially provided them with food and micro flora. No significance was found between normal food and potassium alum result in less consumption of the toxicant. The results indicated that only the males needs longer time to die in presence of competitive food with LT_{50} 39days, this may be because some pheromone activity suppress his need for food, reduced the quantity of food used, or may be looking for his mats of opposite sex and probably must forage resulting in low consumption of potassium alum. Gravid females when they were consumed potassium alum its effect was extended to her oothecae and the results was presented in (Table 3). The biochemical test for the protein of the normal oothecae and that had consumed potassium alum while the production of her oothecae, the total protein indicated a significant change than the normal one which resulted in ootheca resorption. This clearly indicated that the potassium alum had an extended effect, i.e. ovicidal effect. An increased LT_{50} value for males than other stages is seemingly anomalous; however, male German cockroaches are often more sensitive to toxicants and repellents than other developmental stages and given the relatively greater amount of a slightly repellent effect to potassium alum. The LT_{50} values were also showed the higher value in comparison with other stages. We may explain these behavior of adult males by the highly defined olfactory sense (which in need for further investigation) Table 2. The results in Table 4 indicated that mean cumulative days to normal food, water and potassium alum by the different stages of German roaches. However, the most wonderful observation was all stages of roaches after consuming potassium alum never seek water. This is a privilege for potassium alum as a toxicant which indicated that the roaches will die not only because of the toxic effect but also will suffer from dissection.

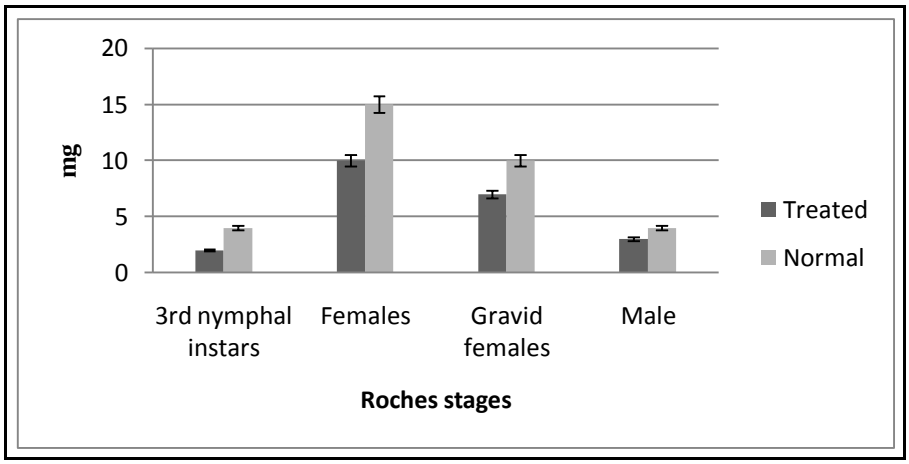

Fig.1: The potassium alum consumption in (mg) after 4days of feeding of different instars of *B. germanica* after ingestion (25 individual each) and normal one.

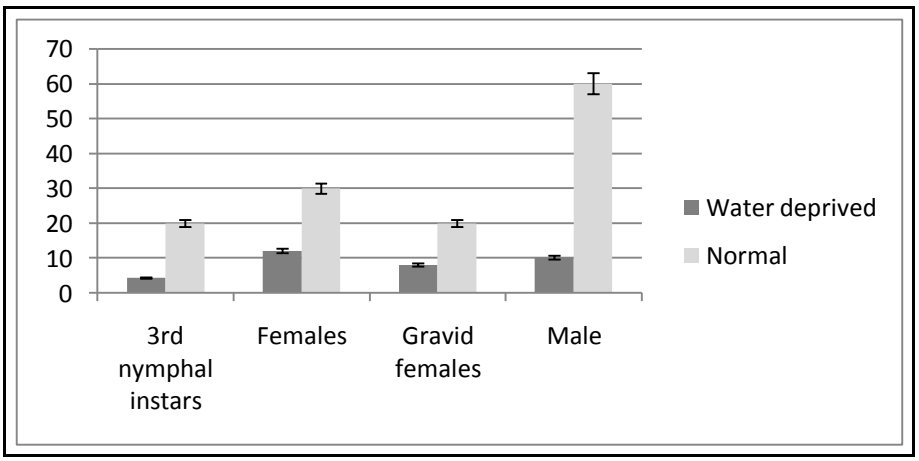

Fig. 2 Effects of water deprivation against time in days different stages of German cockroach.

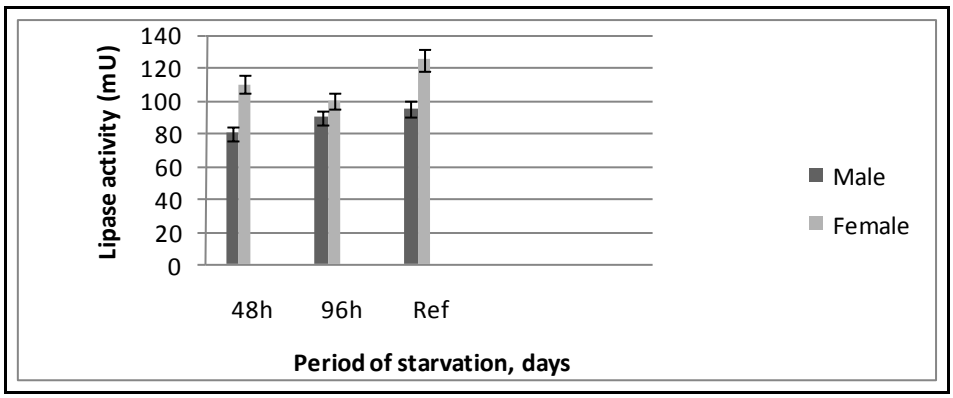

Fig. 3: Change in lipase activity in *B. germanica* cockroaches during 48h &96h of starvation and after re-feeding with normal food, compared with normal fed cockroach as a control each points represents 3 measurements± SE. The female was always with high enzymatic activity than male roaches ($p>0.05$).

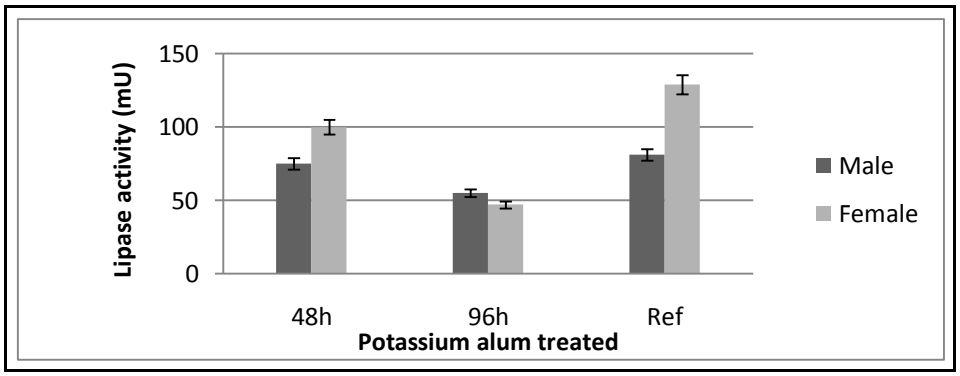

Fig 4: Effect of potassium alum feed male and female of *B. germanica* with reference with normal food and starved roaches on lipase enzyme activity. The activity of enzyme decreased in the starved from the fed in a time-dependent manner. The activity gradually decreased during 48h &96h of starvation and the potassium alum greatly affected the digestives enzyme by 17 % and 50% respectively after 48h 96h., each points represents 3 measurements± SE. The female enzymatic activity was greatly reduced than male roaches (p>0.01).

Table 1. Mean cumulative number of visits to normal food, water and potassium alum by male, female, gravid female and third instars nymph (each 20 individual).

Stages	**No.**	**Normal food (white bread)**	**Water visits**	**Potassium alum**
Adult ♂	20	10.0a (2.80)	7.0 (1.80)	5. 0b (1.10)
Adult ♀	20	12. 1c (1. 20)	6.4 (1.20)	11.2c (2. 45)
Gravid adult ♀	20	10,0a (1.0)	4.4 (1.60)	9.8a (0.20)
Nymph 3rd	20	10. 5d (1. 0)	6.9 (1.44)	9. 9d (1.44)

N.B. Cockroaches after feeding upon potassium alum never seek water.(75% RH+30 Temp.)
Means within the same row followed by the same letter are not significantly different (P > 0.05; Duncan multiple range test [SPSS version 20].

Table 2: Mortality of different *B. germanica stages* due to the effect of potassium alum based on the time LT_{50} of the average for 5 treatments (50 individual each). Most of the adult's cockroaches in control can live on water only for 60 days but nymphs died within 20 days. Cumulative mortality at the end of the experiment was analyzed using Tukey test.

B. germanica	LT_{50} With potassium alum alone (d.)	Confidence Interval 95%		SE±Slope	$\chi 2$	r	LT_{50} With competitive food (d.)	Confidence Interval 95%		SE±Slope	$\chi 2$	r
		Lower limits	Upper limits					Lower Limits	Upper limits			
Third nymphal instars	6.89a ±0.275	4.98	8.21	2.429±0.866	3. 458	0.856	7.2a ±1.90	6.83	8.16	0.882±0.42	3.972	0.926
Gravid females	14.0b ±0.547	13.16	15.23	2.575±0.560	3.827	0.962	15.0b±1.549	14.16	16.23	0.10±0.989	0.901	0.948
Females	16.00c±0.141	14.04	18.23	2. 369±0.4 63	3. 924	0.967	16.16c ±1.16	15.26	17.23	0.14±0.947	3.024	0.864
Males	28.12d ± 1.41	27.16	29.13	2.981±0.642	3.486	0.903	39.9 e± 0.817	35.84	43.95	0.28±0.946	0.802	0.986

N.B. Cockroaches after feeding upon potassium alum never seek water.

Table 3: The oothecae total protein produced by normal females of *B. germanica* and those fed with potassium alum at extrusion at different time of the reproductive cycle (the average of 20 oothecae ± SE). (P<0.05)

Time interval (days)	*B. germanica* oocyte protein increase per females with normal food (µg) ± SE	Fiducial limits		*B. germanica* oocyte protein decrease per females potassium alum fed (µg) ± SE	Fiducial limits	
		Lower limits	Upper limits		Lower limits	Upper limits
1-2	1540a ± 0.12	1451	1632	1305b ± 0.31	1245	1363
10-11	2470b± 0.13	2450	2530	1205c ± 0.45	1199	1207
14-20	1648 d± 0.12	1480	1810	1110e ± 0.45	1100	1150

** Vertically means bearing different letters are significantly different at P<0.001.

Values within a row followed by different lowercase letters were significantly different (P<0.001), while values within a column followed by the different lowercase letters were highly significantly different (P<0.001).

Table 4. Mean cumulative number of days to normal food, water and potassium alum by male, female, gravid female and third instars nymph (each 20 individual). N.B. Cockroaches after feeding upon potassium alum never seek water. (75% RH+30^{O}C Temp.)

Stages	No.	No food and no water in d. roaches died after	Water depravation in d. roaches died after	Food deprivation in d. roaches died after
Adult ♂	20	8.5 (1.70)	9.20 (1.90)	7.6 (1.60)
Adult ♀	20	44.7 (1.50)	7.2 (2.90)	10 (1.80)
Gravid adult ♀	20	28.4 (1.70)	5.2 (2.30)	7.9 (1.40)
Nymph	20	7.1 (1.60)	4.6 (2.70)	6.1 (0.90)

Discussion

The food consumption test showed that the potassium alum have a promising effect as a potential food poison for nymphs and female, only males showed repellency behavior which found to be in accordance with , Salama, [25].

Water deprivation

The time for which German cockroaches could survive without water observed in this experiment did not differ greatly from[31, 32]. They found that males could live for 8.8 or 20.4 days and females 11.9d ± 1.5 days without water. Female survival under conditions of water deprivation range between 5 and 9 days depending on their reproductive stage [33]. However, whether or not cockroaches have access to water after exposure to silica dust greatly affects efficacy of the dusts especially the unfluorinated silica (Gasil23D). A significant difference is usually found between the mortality of cockroaches with and without access to water [34]. Our observation showed that the roaches do not seek water after feeding on potassium alum. However, Caruba [35] they reported that cockroaches that suffer dehydration of about 30 percent of their body moisture, will immediately seek a water source and spend up to 50 percent of their time at the water source, by contrast, under normal conditions,

they spend less than 1 percent of their time obtaining water. Such changes in behavior in response to dehydration were also noted in this study. The roaches stop seeking water after feeding upon potassium alum.

The potassium alum was toxic to all developmental stages of the German cockroach this was found to be in accordance with Salama[25] Non-gravid and gravid adult females were probably killed more rapidly than males other stages because they consume more food in preparation for producing oothecae, this in accordance with [19, 25]. Also the nymphs was found to consume more food and the LT_{50} value for consuming the potassium alum were found highly significant. It is likely that some of the nymphs defecated near other individuals and essentially provided them food. However, the most repellent behavior was found with male's insects in consumption of potassium alum, the quantity of potassium alum eaten resulted in lower LT_{50} values i.e. longer time to induce mortality. So nymphs, non-gravid and gravid adult females when they were had eaten potassium alum is effective. However, in presence of competitive food there was the only change in LT_{50} value for males which had increased than the other tested stages of roaches. It is possible that nymphs periodically sample foods in their environment by consuming small amounts of many different foods. This would result in similar levels of consumption of potassium alum in jars. An increased LT_{50} value for males for potassium alum is seemingly anomalous; however, male German cockroaches are often more sensitive to toxicants and repellents than other developmental stages[36] and given the relatively greater amount of a slightly repellent effect to potassium alum. The LT_{50} values were also showed the higher value in comparison with other stages. We may explain these behavior of adult males by the highly defined olfactory sense (which in need for further investigation).

Females

B. germanica females was found to be the most effected stages by the potassium alum consumption than the males in comparison with LT_{50} (16, 28 respectively). They had cyclical feeding patterns as they feed intensively before mating and the time of extrusion (the production of an ootheca). However, as the ootheca protruded from her abdomen, the feeding is sporadic and the consumption of potassium alum is low. This was also documented by Cochran[19] for *B. germanica* females and other oviparous and ovoviviparous cockroaches,[37-38]and Salama[25] concerning feeding habits. The egg resorption was happened because as the females cannot gets her enough food as she consumed potassium alum, which resulted in cessation of feeding. The females gets her needs form the ootheca as it is still attached with her body, give no chance for nymphs to hatch. These results gives the consumption of potassium alum a privilege in control management's strategy over other controlling methods, as give no more offspring. This was also reported with in case of starvation as reported by [39,40]. In addition to Cruz et al.[41] they stated that in untreated females, vitellogenin production starts on day 1 after the imaginal molt, when corpora allata begin to synthesize JH III at rates doubling the maximal of larval stages. This coincidence suggests that the female reaches the threshold of JH production necessary to induce vitellogenin synthesis on day 1 of adult life. Their data lead to postulate that larvae do not synthesize vitellogenin simply because they do not produce enough JH, not because their fat body is incompetent. In our case roaches that had consumed potassium alum cannot get more food to synthesize more of fats and consequently no JH which lead to their death. Also the females consumed the potassium alum with low titer of JH lost their chance to be a mother i.e. they had to reabsorb her oothecae. We may add in explanation for the death of the oocytes that the female that had consumed the potassium alum lost their JH titer that keep her pregnancy, this will lead to the induction of the oocytes apoptosis. This was a good explanation with lower lipase activity with the potassium alum fed females. In our study we had noticed that the gravid female had consumed potassium alum which lead to her death with her oocytes. It may be explained by these females lost the mechanical stress in her abdomen which lead to abort her ootheca.

Lipids are the most suitable materials for storage of energy reserves. Compared to carbohydrates, lipids can supply as much as eight times more energy per unit weight [42]. In this study, potassium alum consumed roaches showed high significant decrease in lipid contents after 48 hrs of treatment than the control.

Our results showed that the non-gravid and gravid female and the immature stages were too sensitive to the effect of potassium alum. This may be explained by the capacity of the fat body to produce vitellogenin increases with time, possibly owing to the progressive structural development of the tissue, mainly related to polyploidy and proliferation of organelles involved in protein synthesis which occur in normal insect without consuming the poison. This suggests that nymphal fat body would need even more JH than the freshly emerged adult to start vitellogenesis. With more complex physiological event in insect bodies, we would also like to highlight that ecdysteroids are involved in the activation of apoptosis, whereas JH protects against cell death, especially in processes related to metamorphosis. Thus, a plausible explanation for the huge amounts of JH produced in the second half of the vitellogenic cycle is that JH prevents the onset of apoptosis in the follicle

cells and resorption of the growing oocyte. So the interruption occurs inside the body of the insect that had consumed the potassium alum will lead to their death. With the indication with the low level of proteins in the egg case and lipase titre in German cockroach body. We could simply say that the potassium alum affect both the life of the females roaches and leads to resorption of their growing oocytes which give an excellent reduction of the roaches population. Thus cockroach control programs could benefit from strategies that inhibit sexual receptivity and oocyte growth in virgin females, also induce gravid females to abort.

Nymphs of *B. germanica* was also highly effected by feeding on potassium alum with LT_{50}10 days, which was found in accordance with Salama[25]. As the insects feed greedily to grow up they need more food, this lead to consume bigger amount of potassium alum.

Male's results showed that it had the longest time i.e. with competitive food big LT_{50} (28-39d.). Males as being in adult form, they feed steadily i.e. the consumption of potassium alum is normal without cyclical feeding before or after matting. That is why it takes longer time to die. These was found to be in agreement with Salama[25] concerning males of *Periplaneta americana* and consumption of potassium alum. The repellency behavior of the males of the German cockroaches to potassium alum remains enigmatic. Thus, a plausible explanation for these behavior is their olfactory organ doing such wonderful job to be the most resistant stages in roach's community. Further investigation is needed to get more understanding concerning such behavior.

Female's *B. germanica* was found to be the most effected stages by the potassium alum consumption than the males in comparison with LT_{50}(16, 28 respectively). They had cyclical feeding patterns as they feed intensively before mating and the time of extrusion (the production of an ootheca). However, as the ootheca protruded from her abdomen, the feeding is sporadic and the consumption of potassium alum is low. This was also documented by Cochran [19] for *B. germanica* females and other oviparous and ovoviviparous cockroaches [25.38, 43-45]concerning feeding habits. The egg resorption was happened because as the females cannot gets her enough food as she consumed potassium alum, which resulted in stop feeding. The females gets her needs form the ootheca still attached with her body, give no chance for nymphs to hatch. These results gives the consumption of potassium alum a privilege in control management's strategy over other controlling methods, as give no more offspring. This was also reported with in case of starvation as reported by Engelmann & Rau[43].

Nymphs of *B. germanica* was also highly effected by feeding on potassium alum with LT_{50} 6 days, which was found in accordance with Salama[25]. As the insects feed greedily to grow up they need more food, this lead to consume bigger amount of potassium alum.

Male's results showed that it had the longest time i.e. with big LT50 (28-30d.). Males as being in adult form, they feed steadily i.e. the consumption of potassium alum is normal without cyclical feeding before or after matting. That is why it takes longer time to die. These was found to be in agreement with Salama[25] concerning males of *Periplaneta americana* and consumption of potassium alum.

Also the results of this study showed that treatment with potassium alum affects not only to the nymphs and adults of *B. germanica* (L.) but also the oothecae produced by gravid females that had ingested potassium alum at the time of extrusion. This is might be explained by the potassium alum kill the internal bacterial flora which lead to the death of the roaches. As many authors had reported the role of the associated microorganisms in roches[44–50]reported that the cockroaches host symbiotic bacteria in the ovary and in specialized cells (bacteriocytes) of the fat body. He added that these bacteria have the typical cell boundary profile of gram-negative bacteria and are enveloped in a vacuolar membrane produced by the host cell. Molecular sequence data of 16S rDNA of endosymbionts of five species of cockroaches and M. darwiniensis indicate that they are members of the Flavobacteria-bacteroides group and that the infection occurred in an ancestor common to cockroaches and termites probably after the end of the Paleozoic (250 Ma BP). He indicated that the symbiotic bacteria are transmitted transovarially and, during embryogenesis, they are integrated into the morphogenetic processes. In particular, they were able to demonstrate that the origin of the bacteriocyte should be looked for in the cells of the haemocyte line (embryonic plasmatocytes).

This is study showed the extended ovicidal effects of potassium alum on *B. germanica* (L.), and the result is nearly conclusive. After treatment with potassium alum, *B. germanica* (L.) offspring cannot build colonies in our homes. However, the results of this study also showed that potassium alum has no contact toxicity on the eggs of *B. germanica* (L.). One possible explanation for this result could be that this type of pianistic ovariole acts as a transferable link between the active ingredient and the ootheca when the ootheca is still attached to the female body, However, in addition to effects in nymphs and adults, the results showed that

the oothecae produced by gravid females that had eaten the potassium alum at the time of extrusion (the female carries an empty ootheca and oocyte development is inhibited during pregnancy; the produced ootheca and its contents were resorped. Additionally, no nymphs emerged from these eggs. This result may be due to the reduced water content of the ootheca caused by the potassium alum. The roches were stopped feeding as they suffer from paralysis. This happened to the ootheca as a results of female's interruption by feeding on potassium alum with no enough water for the eggs to develop without receiving additional water from the substrate [24]Normal females exhibited cyclical feeding patterns. They fed intensively prior to mating and the production of an ootheca, during ovulation and only minimally on ovi-position. After hatching of nymphs, females resume feeding until the production of the next ootheca, however, in the potassium alum treated group, this feeding pattern ceased. These females died soon after with no chance for her ootheca to hatch. The females had consumed potassium alum died with the oothecae attached with her abdomen with no premature dropping. This finding was different from that had reported by Wooster and Ross [50] whom reported the premature dropping of oothecae by gravid females by German cockroaches and American roaches by Salama [25], which leading to a lower survival rate of the oothecae due to the sub lethal effects of insecticides treatments. Similar results were observed by Bressan-Nascimento et al.[51]. They reported that extreme temperatures affected embryonic development, resulting in enviable ootheca. Our results were also in accordance with King[52] who evaluated the ovicidal activity of the benzoyl phenylurea noviflumuron in the laboratory on three adult groups (virgin females, virgin males, and fertilized, non-gravid females) of the German cockroaches, *Blattella germanica* (L.), through ingestion of treated bait. He reported that noviflumuron caused a significant ovicidal effect. Noviflumuron is an insect growth regulator (IGR) that prevents successful molting. Potassium alum presents more advantages as an insecticide as it induces death in adults at the non-molting stage and in the molting nymphs.

The high susceptibility of the nymphs, females and gravid females is due to their increased feeding habits, which leads to them ingesting more potassium alum. This observation is in accordance with the results of Woodruff [53] who concluded that insects (cockroaches) approximately double in weight between instars. Additionally, the non-gravid females in gonotrophic cycle require more food during their reproductive cycle.

A key finding when comparing the effect of potassium alum on *B. germanica* (L.) with the effects of other insecticides that are usually used to control cockroach infestation is that the usual insecticides lower the population number but do not affect the hardened egg cases of the cockroaches, which are impervious to most insecticides. Most conventional insecticides only reduce the population without affecting the ootheca, which allows *B. germanica* (L.) to build a new colony after treatment. The results of this study suggest that potassium alum can safely control nymphs, adults and the oothecae. In our attempt to provide an explanation for this phenomenon of death of the cockroaches treated with potassium alum, we might say that the potassium alum inside the insect gut leads to a loss of its own symbiotic flora, which help the insect to digest its food, resulting in reduced enzymatic release. In normal cockroaches, this leads to mortality of the insect. Lee et al.[54] also found that the alum astringency might be due the precipitation of salivary proteins.

The extended effect from the female at time of extrusion to the oothecae may be due to the reduced water content in the oothecae. As the roaches never seeks water after consuming potassium alum. This reduced water content also explains the reduced size of the ootheca and the reduced chance of nymph emergence. Female cockroaches fed potassium alum before mating and after the ootheca had protruded from the abdomen were highly affected. Comparison of these data revealed that high mortality due to potassium alum is most likely caused by the interaction of several factors. Digestive enzymes were detected in the mid- gut of *Blattella germanica* (L.), and the activity lipases was higher in the mid-gut of females than in that of males, a result also found by Oyebanji et al. [55] with *Periplaneta americana*. This result could explain the great susceptibility of the females with lower LT_{50} than the male cockroaches, which shows the highest LT_{50} among all the instars tested.

The digestive enzymes in insects are adapted to the diet on which they feed. Omnivorous insects such as the cockroach secrete protease, lipase, amylase, invertase and maltase, to digest hydrolysin natural proteins, fats, starch, cane sugar and maltose, respectively, as reported by Wigglesworth,[56] In our study, the cockroaches were inclined to eat potassium alum because of its sweet taste. Also Hamilton and Schal[20] stated that German cockroach females compensate for low dietary protein levels by elevating consumption rates and reproduce normally. Our results showed that potassium alum lead to inhibition of the digestive lipase enzymes, resulting in their death. Starvation for 3 days reduced lipase activities by approximately 17% and 50%, respectively, in the mid-gut of *Gryllus rubens*. Starvation reduced lipase activity in the mid-gut, as indicated in our study, which is in accordance with the results reported by Thomas and Nation[57]**.** However, the activities of these enzymes

drastically changed after re-feeding. The mechanism of enzymatic release comes from the brain of cockroaches, but feeding on potassium alum greatly inhibited the activity of these enzymes, which lead to cockroach mortality, i.e., irreversible damages.

We noticed that the German roaches like to eat the potassium alum as just like the white bread as a carbohydrate, a substance that is easily digested by cockroaches. The destruction of the gut flora form the effect of potassium alum in the present study may indicate that the symbiotic microorganisms in the mid-gut of German roaches get affected which lead to roaches death. This in accordance with[46-48] whom reported that the gut bacteria in *P. americana* played a functional role in the development and survival of the insect species. Additionally, their omnivorous, detritophagous feeding habit and association with symbiotic bacteria has contributed to their survival for at least 350 million years.

The higher lipase activity in the mid-gut of females with the great inhibition after potassium alum feeding was giving the answer for the early mortality happened to the non-gravid and gravid female during our study and accompanied by resorption of oothecae by these females, as it meet the explanation by Kawooya & Law, [58] and Briegel, [59] they reported that, it is possible that female wasps made maximum use of lipase substrate in the mid-gut in order to meet specific reproductive requirements. Lipids, mostly triacylglycerol (TAG), and smaller amounts of phospholipids (PL) and cholesterol, make up 30-40% of the dry weight of the insect oocyte. Our view meet with Weaver et al.[60] whom interpret that the rapid increase in JH biosynthesis and JH titre was required to support reproductive processes such as vitellogenin production by the fat body, vitellogenin uptake by the oocytes and synthesis of oothecal proteins by the left colleterial gland. Also, lipids are the main sources of energy for the developing embryo [42] and the PL is needed for the formation of membranes. Insect oocytes synthesize TAG using fatty acids (FA) [60, 61]but since the ability of oocytes to synthesize FA *de novo* is very limited, Ziegler and Van Antwerpen[62] concluded that nearly all the lipids must be imported, especially from ingested food. This requirement might have necessitated maximal digestion of lipase substrate in the female midgut. We will publish a detailed account of the effect Orlistat which is a specific and long-acting lipase inhibitor against females of *Bellatella germanica*. It exerts its activity by bonding to gastric lipases and in turn no hydrolysis and no absorption to fats in our next paper. Additionally, the effect of nanoparticles of potassium alum against *Bellatella germanica* as a coating for the newly emerged nymphs is currently under investigation in our laboratory. These results are agreement with those obtained by Ismail et al [63] .

In conclusion, the results of this study confirmed the insecticidal efficiency of potassium alum on *Bellatella germanica* adults and nymphal instars. It would be the best option for eliminating infestations caused by the fast reproductive potential of female's cockroaches. The potassium alum was easy to apply and was readily fed upon by German cockroaches. In fact, we observed cockroaches feeding on the cadaver of their own species that had died from potassium alum previously and died after these coprophagous habits. In conclusion, the potassium alum was toxic to all developmental stages of the German cockroach. In general, potassium alum provided a good control of German cockroach infestations. This study showed the importance of controlling the insect's species through the controlling of the weak gravid female. It would be of great importance in eliminating the pests especially in household insects.

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