

Boric Acid Toxicity Trials on the Wood Borer *Heterobostrychus aequalis* Waterhouse (Coleoptera: Bostrychidae)

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Abstract: Problem statement: The *Heterobostrychus aequalis* Waterhouse was a serious pest lumber of timber heveawood. The *H. aequalis* infest and tunnel along the wood grain, depositing eggs, which concomitantly turn into larvae and reduces the wood starch to a soft powder. The objectives of the study were to establish a suitable method for preservative trials on the wood borer and access the toxicity levels of boric acid for controlling the *H. aequalis* infestation. **Approach:** The voracious *H. aequalis* was collected from infested heveawood, mated and cultured on cassava. The adult beetles were exposed to boric acid treated cassava blocks as pilot trials. The boric acid concentration used was 1-5% including a control. Each treatment consists of five replicates. The voracious of *H. aequalis* generation was cultured for obtaining its freshly new emerged adult by exposing a number of *H. aequalis* directly from infested heveawood into artificial diet block and cassava. The mortality, weight loss of the test blocks, frass discharge duration and numbers of holes caused by *H. aequalis* were observed. **Results:** The results showed that the *H. aequalis* infested tended to penetrate the film after wrapping the heveawood with a layer of transparent cling film for 24 h experiment. The *H. aequalis* larvae stock cultured collected from infested heveawood exhibited differences in colour, activeness and aggression among males and females. The suitable test block size used was (2.0×5.0×1.5) cm³. The freshly emerged voracious of *H. aequalis* adults was more suitable than the larvae for boric acid toxicity trials. The 2% concentration was sufficiently killed the 4% boric acid treated test blocks. There was no hole observed on 5% boric acid treated test block and hence these concentrations were not infested by *H. aequalis* due to absence of frass in exposed blocks. **Conclusion/Recommendations:** There are noticeable physical and behavioural differences between voracious of *H. aequalis* collected from infested heveawood. The freshly emerged of *H. aequalis* adults were found suitable than the larvae as an indicator for determining the boric acid toxicity trials. The 5% of boric acid concentration was sufficient to control the infection of *H. aequalis* on heveawood. Further studies focusing on interaction between larvae, adults and population growth of *H. aequalis* with different hosts need to be carried out towards clarifying the predisposing factors that induce the infestation processes.

Key words: Boric acid concentration, cassava block, heveawood, *H. aequalis*, toxicity trials

INTRODUCTION

Malaysia, undeniable is still one of the richest country with biodiversity of flora and fauna (Akbar *et al.*, 2010; Leng *et al.*, 2009; Saga *et al.*, 2010; Satrio *et al.*, 2009). It's also a home for various species of insects such as *Heterobostrychus aequalis* Waterhouse. The *H. aequalis* also known as false powderpost beetles or lesser auger beetle a serious pest of lumber especially heveawood. The adults as well as the larvae

infest and bore on dried or seasoned wood and cause excessive damages to the wood structure (Creffield, 1991; Ivie, 2002; Peters *et al.*, 2002). Previous studies found the distribution of *H. aequalis* in Andaman and Mariana Island, Malaysia, Phillipines, Sri Lanka Papua New Guinea and Cuba to name a few (Woodruff, 1967). Wong *et al.* (2005) and Akhter (2005) examined that heveawood have low durability and hence prominent to fungi attack and insect infestation due to high composition of starch. *Hevea brasiliensis* wood or

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normally known as heveawood is one of Malaysia biggest commodity products in particular furniture both for local and international. Lawrence and Britton (1991) studied that *H. aequalis*, infest, bore and tunnels along the wood grain, depositing eggs, which then emerges to larvae and reducing the wood starch to a fine, soft powder, earning them the name powderpost beetles (Peters *et al.*, 2002).

Anatomically, the body length of *H. aequalis* is about 6-13 mm with the apical declivity of elytra bituberculate on each side while the pronotum is lobed with teeth on the anterior border of pronotum broadly separated (Ho and Hashim, 1993). In addition, Woodruff (1967) described that *H. aequalis* has a broad tooth-like projection on its anterior of their sides. Furthermore, its sides are strongly deflexed on apical half with the projection of the posterior angles. The *H. aequalis* apical declivity of elytra arcuately deflexed. In order to differentiate the male and female of *H. aequalis*, the male has inner two stout tubercles pointing obliquely upwards. On the other hand, female *H. aequalis* has smaller pronotum, teeth on anterior apical border of pronotum shorter and the apical declivity of elytra without marginal tubercles (Ho and Hashim, 1993).

In general, pest control technician must identify the insects by examining the wood itself and by using the existence of emergence holes because larvae and adults of wood infesting beetles are hardly seen when damage occurred. Creffield (1996) found that for some wood boring beetles such as the longhorn beetles, only the insect larvae tunnel within the wood while all stages including larvae and adults of beetles such as pinhole borers tunnels in the wood. However, the insects which in turn decompose the woods could enhance the recycling nutrients and preventing the forest floor from being littered dead wood (Peters *et al.*, 2002). Furthermore, the insect usually do not attack healthy trees and do not infect timbers after they have been milled.

Bostrychidae attack individual pieces of trim or flooring and rarely cause significant damage in framing lumber. Holscher and Wintersteen (1998) found that *Dinoderus minutus* is common in bamboo and rarely attack and infest seasoned wood. As for *H. aequalis*, the female bores into the wood and deposit its eggs in the tunnels that their made (Ho and Hashim, 1993). De Angelis (1995) examined that the female *H. aequalis* wills first "taste" the wood to determine whether it contains enough starch to nourish its offspring. Archer and Lebow (2007) stated that by coating the wood surface with paraffin wax, varnish shellac, or paint, it helps to prevent "tasting" and thus eggs will not be laid.

However, to our knowledge, there is a lack of information and studies for developing a standard method for preservative trials of wood borer of *H. aequalis*. Moreover, many wood preservation studies and methods only focus on how to eliminate 100% insect infestation on it rather than accurate studies on how to avoid pest attack by using a correct and standardize level of preservative. Therefore, the objective of this study is to project and establish a correct and suitable test method to study the behaviour or biology of *H. aequalis* together with the correct percentage of wood preservative concentration need to be used to prevent the infestation of this pest. Boric acid was used as the treatment to determine the toxicity trials on *H. aequalis*. Boric acid is a common type of preservative or pesticide applied to controls insects infestation, spiders, algae and fungi (Environmental Protection Agency, 1993). The boric acid is white solid, odourless and inert at room temperature and usage in food, beverages and non-food products. Furthermore, boric acid are found toxic to insects when it ingest and also very abrasive especially towards the exoskeleton of the insects (Environmental Protection Agency, 1993). In addition, boric acid is a common water-borne preservative use to treat heveawood; although, no studies carried out to determine the sufficient dose of boric acid needed to control the *H. aequalis* infestation on heveawood. Aydin and Colakoglu (2007) studied that borates compound are likely to be used more to treat solid and engineered wood for indoor purposes. Therefore, the present study conducted aims to establish a suitable method for preservative trials on the wood borer and access the toxicity levels of boric acid for controlling the *H. aequalis* infestation.

MATERIALS AND METHODS

The toxicity trials of boric acid, adult's beetles were collected from infested heveawood and *Dyera costulata* wood obtained from Wood Composite Laboratory, Faculty of Forestry. Purposes of using beetles collected from infested wood source were to obtain voracious freshly emerged adults to obtain accurate result of toxicity trials test. The lateral view of male and female adults is shown in Fig. 1 and Fig. 2, respectively. The adults of *H. aequalis* were kept in Petri dish lined with black filter paper and covered for one night without any nourishment to achieve successful mating among them. After that, the mated male and female were exposed to cassava blocks and maintained for 8 weeks until the emergence of the F1 phenotypes generation.



Fig. 1: Lateral view of male adult (magnification: $\times 1.26$)



Fig. 2: Lateral view of female adult (magnification: $\times 1.26$)

The cassava block was chosen as the test blocks instead of heveawood because it gives faster results. Hodges *et al.* (1985) reported that Bostrychidae is an important pest that infests cassava chips in tropical area in Africa. Furthermore, *Dinoderus* spp. and *P. truncates* also found to be infesting farm stored products in Ghana (Rees, 1991). The size of cassava blocks stated by Abood *et al.* (1994) was modified slightly to form test blocks. The sizes of test block used are $(2.0 \times 5.0 \times 1.5) \text{ cm}^3$ and was used as the basis for the assessment of boric acid toxicity trials. The cassava blocks were dried in the oven at 60°C for 48 h. This must be done continuously in order to obtain a 12% moisture content of cassava. In order to avoid water vapour been trapped in the plastic bag filled with the oven dried cassava blocks, the dried cassava block was air dried before kept in plastic bag.

Preparation of boric acid: Boric acid is used as the treatment in order to establish the exact dose of boric acid concentration to preserve the heveawood. In the laboratory, method of study of the effects of boric acid

equivalent on termite, boric acid equivalent used was in the range of 1.1-1.2%. Hence, for toxicity trials of boric acid on *H. aequalis*, five different level of boric acid ranging from 1-5% were used. The calculations of boric acid concentration are as follows:

Volume, $V = 1000 \text{ mL}$

Weight of Boric acid powder, $g = X$

$(X/V) \times 100\% = \text{Boric acid concentration percentage } (\%)$

Akhter (2005) found that heveawood can be treated satisfactorily by soaking and pressure process with acceptable penetration and retention. Hence, soaking method was used to preserve the cassava test blocks with 5 different concentrations which were 1-5 respectively. Dhamodaran and Bhat (1992) mentioned that net average of 0.4% borax-boric acid is adequate to give protection against fungi and borers of wood species. Moreover, in the study of efficacy of borax-boric acid preservatives on heveawood, Borax-Boric Acid and Sodium-Pentachlorophenate (NaPCP) mixture of the treatment by dipping in mixture of required concentration for a specific time given by the equation (Narayanan *et al.*, 2005):

$$T = 3.2 (D/C)^2$$

Where:

D = Thickness of the specimen, cm

T = Immersion time, h

C = Concentration of the solution in percentage

The thickness of cassava test block, $D = 1.5 \text{ cm}$

Exposing treated test blocks to *H. aequalis* adults:

The voracious *H. aequalis* culture in heveawood medium was used. Two pairs of *H. aequalis* adults with equal female and male were exposed to the test blocks. The mortality, number of holes formed, duration of frass discharge and the behaviour were observed through this experiment on five different concentrations of treated test blocks. This observation was done daily for two weeks. The data were analysed to evaluate the mean mortality of *H. aequalis* adults, mean number of holes, duration of frass discharge and mean weight loss of boric acid treated test blocks and suitable boric acid concentration to control *H. aequalis* attacks. The calculation of the data used to determine the significance level and mean differences by using Analysis of Variance (ANOVA). The Tukey's (HSD) were used to detect any significant differences between

mean values among treatments. The Statistical Analysis System (SAS Ver. 9.2) was used for the statistical analysis.

RESULTS

Table 1 shows the mean mortality of *H. aequalis* adults on boric acid treated test blocks over 14 days. The numbers of dead adults obtained from the treated test block with 1-5% concentration within two weeks were higher compared to the control test. Also according to the result, 1-2% boric acid is enough to kill *H. aequalis* adults due to the preservative action as a stomach poison when the insects digest it. Mean mortality of adults on control test block was 0.6 ± 0.40 while mean mortality of adults on 1 and 2% boric acid treated test blocks were 3.6 ± 0.25 and 3.0 ± 0.32 . Moreover, mean mortality of adults on 3, 4 and 5% boric acid treated test blocks were 4.0 ± 0.00 , 4.0 ± 0.00 and 3.8 ± 0.20 , respectively.

Table 2 shows the mean number of holes made by *H. aequalis* adults on boric acid treated test blocks with different concentrations. Furthermore, 0% boric acid concentration test block showed the highest number of holes followed by 1-4% boric acid concentration treated test blocks. The mean number of holes made by *H. aequalis* adults on 0% boric acid treated test block was 8.4 ± 0.98 . The mean number of holes on 1% and 2% boric acid treated test blocks were 1.4 ± 0.25 and 1.0 ± 0.45 , while the 3, 4 and 5% boric acid treated test blocks were 0.6 ± 0.40 , 0.2 ± 0.20 and 0.0 ± 0.00 . From the observation, there were no holes made by *H. aequalis* adults on the test block which were treated with 5% boric acid, indicating a sufficient amount to control the infestation of this wood borer on heavewood.

Table 3 shows the mean duration of frass discharge by the *H. aequalis* adults on boric acid treated test blocks. The duration of frass discharge were higher on 0% boric acid treated test block with a mean value of 60.0 ± 1.64 followed by 1-5% with 9.6 ± 1.60 , 6.6 ± 1.66 , 4.4 ± 1.17 , 3.0 ± 0.00 and 0.0 ± 0.00 , respectively.

Table 4 shows the mean weight loss of boric acid treated test blocks. The mean weight loss of control test block showed the highest value of 2.25 ± 0.45 at 0% concentration while the 5% treated test block had no weight loss recorded. This result could be due to the absence of boring activities done by *H. aequalis* on 5% boric acid treated test blocks. The mean weight loss for 1 and 2 boric acid treated test blocks are 1.81 ± 0.10 and 0.54 ± 0.21 , while for 3 and 4% boric acid treated test blocks are 0.60 ± 0.09 and 0.44 ± 0.04 .

Table 1: The mean mortality of *H. aequalis* adults over 14 days

Boric acid conc. (%)	Mortality (%)
0	15±0.40c
1	90±0.25ab
2	75±0.32b
3	100±0.00a
4	100±0.00a
5	95±0.20a

Means followed by the same letter are not significantly different at $P \leq 0.05$ as determined by Tukey's HSD test.

Table 2: The mean number of holes made by *H. aequalis* adults over 14 days

Boric acid conc. (%)	Number of holes (%)
0	8.4±0.98a
1	1.4±1.25b
2	1.0±0.45b
3	0.6±0.40bc
4	0.2±0.20bc
5	0.0±0.00c

Means followed by the same letter are not significantly different at $P \leq 0.05$ as determined by Tukey's HSD test.

Table 3: The mean duration of frass discharge by *H. aequalis* adults over 14 days

Boric acid conc. (%)	Duration
0	60.0±1.64a
1	9.6±1.60b
2	6.6±1.66b
3	4.4±1.17bc
4	3.0±0.00bc
5	0.0±0.00c

Means followed by the same letter are not significantly different at $P \leq 0.05$ as determined by Tukey's HSD test.

Table 4: Mean weight loss of boric acid treated test blocks

Boric acid conc. (%)	Weight loss (g)
0	2.20±0.45a
1	1.81±0.10a
2	0.54±0.21b
3	0.60±0.09b
4	0.44±0.04b
5	0.0±0.00c

Means followed by the same letter are not significantly different at $P \leq 0.05$ as determined by Tukey's HSD test.

DISCUSSION

The cassava pellets were found to be very suitable for collecting *H. aequalis* larvae instar by first, second, third, fourth, fifth and sixth instar. Moreover, *H. aequalis* eggs also can be obtained easily by using cassava pellet without any physical injuries to the beetle's egg. This particular technique described by Abood *et al.* (1994) as a standard technique with great potential for retrieval of eggs and different stages of larvae instars.

The method of collecting a newly emerged voracious *H. aequalis* adults are by keeping a number of vigorous *H. aequalis* adults collected directly from

infested heveawood for 24 h without any nourishment. This is to allow successful mating among the female and the male adults. After that, *H. aequalis* adults were exposed to a new cassava block and maintained for about two months respectively. Then, the newly emerged adults were used for the preservative trials test.

The results showed that the *H. aequalis* adults can also be collected or forced out from the infested wood by wrapping the wood with a thin and transparent layer of cling film for 24 h. Through this method, the *H. aequalis* adults tended to penetrate out from the holes through the cling film. These techniques were able to determine the present of insects on a particular infested wood. Hazarika *et al.* (2005) described that the female of Bostrychidae tends to insert their eggs in host plant tissues which protect it offspring from danger and extreme conditions. In addition, the adult larvae and eggs in the wood will not be harmed or killed upon the wood has been cut. Moreover, we can keep the infested wood for longer periods instead of cutting the wood into slices that may kill the *H. aequalis*. Similarly, Abood (2008) proved that infestation of *H. aequalis* will keep on going until the entire inside structure of the wood become smooth and eventually leaving a fine powdery frass.

There are several prominent differences observed throughout the research about *H. aequalis* that was collected directly from infested heveawood. The larvae of *H. aequalis* collected from infested heveawood are yellowish brown in colour compared to culture of *H. aequalis* which are yellowish white in colour. Moreover, larvae collected from infested heveawood also have a visible black line on its back which could not be found on culture larvae. The black line formed was due to the fact that the larvae has been consuming lignin from the heveawood while a laboratory culture larva does not get any lignin supply in cassava. In addition, Cymorek (1968) clarified that wood-boring insects were forced to ingest the wood because this particular pest due to unable to digest cellulose as a source of carbohydrate.

In addition, when the voracious *H. aequalis* adults from infested heveawood were exposed to new culture medium or cassava block, the insects will actively move around the blocks. On the other hand, when a cultured of *H. aequalis* adults with an artificial diet were transferred to the new cassava block for starting new culture, however, some of it does not bore or become inactive due to the new environment exposure. Furthermore, a number of the culture adults died when exposed to new cassava due to the weakness of *H. aequalis* could not penetrate or bore through the hard

cassava block wall. However, this situation was not observed on cassava blocks that had been exposed to the voracious adults. After a few hours of exposure, there were of frass discharge indicating that the insects are trying to bore the cassava blocks (Woodruff and Fasulo, 2006).

The aggression among the female and male of voracious *H. aequalis* adults was observed when kept in a petri dish lined with filter paper for 24 h without any nourishment. This was done to achieve successful mating among them and fertilise female adults before being cultured. The activeness and aggressive behaviour among the voracious *H. aequalis* adults had caused many of its members to lose its limb concomitantly died. On the other hand, there was a difference in behaviour observed on group of *H. aequalis* collected from the maintained culture of artificial diet in the laboratory. Slow movement and less interaction among adults indicated that a successful mating would not be achieved if the cultured of *H. aequalis* was used for preservative trials.

On the first day of exposure to the 1% boric acid treated test block, all the *H. aequalis* adults were actively moving around the blocks and leaving gnawing marks. Moreover, there was frass discharge noticed on the treated heveawood indicating that the adults bore into the blocks. Furthermore, some of the adults tend to be in a crack on the test blocks even though the blocks were impregnated with boric acid. However, after one day of exposure to the treated test blocks, some of the adults started moving away from the blocks. After the second day of exposure, some of *H. aequalis* still bore, but most of it had become weak and sluggish. This symptom indicates that the boric acid ingested by *H. aequalis* was acting as a stomach poison. The first mortality of *H. aequalis* was noticed on the second day of exposure. In addition, a number of holes made by *H. aequalis* were recorded on all of the five replicates of 1% boric acid treated test blocks on the very first day of exposure.

Earliest frass discharge noticed was on the first day of exposure but there were less number of holes made by *H. aequalis* compared to the number of holes in 1% boric acid treated test block. The mortality of *H. aequalis* was also higher on 2% boric acid as compared to 1% boric acid treated test blocks. Furthermore, the mortality duration rate was faster than the mortality duration rate of 1% boric acid treated test block where the first mortality of *H. aequalis* recorded on the first day of exposure. The mortality of the first day was observed on the second and third replicate while fewer holes were found on the third, fourth and fifth replicate. Moreover, the behaviour of *H. aequalis* in

terms of activeness was becoming less strong and also being unable to climb or to grip on the treated test block.

The 3% boric acid treated test blocks exhibited the earliest mortality was on the first replicate in the first day of exposure. Two mortalities of *H. aequalis* were recorded during the first day. Furthermore, the earliest holes made by *H. aequalis* were recorded on the fourth replicate with only one hole. Total of three holes were made on the 3% boric acid treated test blocks. On the other hand, no holes were made on the first, second and the fifth replicate. Some of the adults liked to hide in the cracks of the test blocks. This situation may be due to the tendency of *H. aequalis* to bore the softer inner part of the crack compared to the harder outer part of the block. At the 10 day of exposure, all adults on the first and third replicates were dead. The exoskeleton colour of the adults also became darker and drier due the contact of boric acid being very abrasive on the *H. aequalis* exoskeleton.

The 4% boric acid treated test block, only one hole was made which was noticed on the third replicate test block. The earliest mortality was recorded on the first day, in the fourth and fifth replicate, respectively. In addition, all the adults on the second replicate died on the ninth day of exposure. Furthermore, no visible holes were noticed on first, second, fourth and fifth replicates. At the beginning of exposure, the *H. aequalis* adults were actively moving around the treated test blocks. Then, *H. aequalis* started showing abnormal behaviour such as suddenly became hyperactive, before slowing down and then stop moving completely. The hyperactive behaviour movement showed that the boric acid act as a stomach poison when it ingested. The ingestion of the poison starch by *H. aequalis* can be detected by the presence of gnawing marks on the treated test blocks.

The mortality duration at 5% boric acid treated test block was slower compared to the mortality duration rate recorded at 2% boric acid treated test block. The earliest mortality was observed on the second day of exposure. The second replicates had the earliest complete mortality of *H. aequalis* by the eighth day of exposure. From the study, the mortality duration was relatively slow in each replicate. In the first replicate, after the first mortality, the next mortality only happened on the sixth and twelfth days of exposure. On the second replicate, earliest mortality was observed on the sixth day with two mortalities of *H. aequalis*. Most of the adults on 5% boric acid treated test block could not actively move around the test blocks during the first day of exposure. Moreover, there was no sign of frass discharge and holes.

From the observation done on the preservative trials of the wood borer of *H. aequalis*, the 5% boric acid concentration was found to be adequate to prevent attack of *H. aequalis* because there were no infestations noticed on each replicate. In addition, Abood (2008) recommended that dipping treatment is suitable to be implemented for logs at logging area or factory in order to preserve the wood for long-term use. Moreover, there was no frass discharge seen on the test block. Furthermore, no gnawing marks had been done by *H. aequalis*, hence none were detected on the cassava test block surface.

CONCLUSION

The presence of *H. aequalis* adults in infested wood can be detected by the existence of hole made by the insects through a thin layer of cling film that was used to wrap the infested wood. In addition, there were differences in physical and behavioural observed between *H. aequalis* cultured in rich starch diet with the one collected from infested wood. Furthermore, freshly emerged *H. aequalis* adults have been found to be more suitable to be used as indicator to examine the boric acid toxicity trials compared to the larvae because the adults of *H. aequalis* gave visible and earlier notification of mortality, number of holes made and discharge of frass on test blocks. The 5% boric acid concentration was found to be sufficient to control the activeness of *H. aequalis* due to no holes and frass discharge here detected on test blocks treated with that particular concentration. There is a necessity to study the relationship between the *H. aequalis* and its food sources especially wood along with population growth of *H. aequalis* larvae and adults on different hosts. Through such a study, the effects on *H. aequalis* can be discovered if certain substrates are removed from the host. Moreover, the predisposing factors affecting infestation by *H. aequalis* on the hosts can be established. Moreover, study of toxicity trials of boric acid on *H. aequalis* by using heveawood and other potential hosts of *H. aequalis* should be established.

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