Survival of A. Ahngerianus (Isoptera: Hodotermitidae) Under the Influence of External Factors (Relative Humidity and Temperature) and Use of Different Water Sources

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Abstract: - *A.ahngerianus* Jacobs (Hodotermitidae) is a species of subterranean termite causing damage in Central Asian cities. Despite its economic importance, there has been no research on its basic biological aspects for laboratory management and control strategies. The aim of this study was to evaluate relative humidity, temperature and to identify other water sources that *A. ahngerianus* may use for best survival under laboratory conditions. Other water sources that termites can use were identified using a range of six relative humidity levels (10 to 100%) and three temperatures (25, 30, and 35 °C). These included metabolic water from food decomposition, fat body water, free liquid water, groundwater, and water bound to various substrates (e.g., wood, soil, and chick carcasses). The results of this study showed a significant influence of all factors on termite survival or termite preference. At 100% humidity and 25 °C, *A.ahngerianus* had the highest survival rate, reaching 89.62% after three days of observation. Based on these preliminary analyses, it was concluded that with the appropriate percentage of humidity, further studies using biologically relevant conditions can be conducted to investigate different aspects of *A.ahngerianus* biology.

Key-Words: - Isoptera, Hodotermitidae, relative humidity (RH), Exterra, *A.ahngerianus*, stabilized materal SM, Juma Mosque Museum, carbon cycle

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1 Introduction

Coptotermes Wasmann (Isoptera: Rhinotermitidae) is a genus of subterranean termites with 21 validated species [8], of which 16 are classified as pests of economic importance in various parts of the world [10]. In Uzbekistan, termites of the genus *Anacanthotermes*, mainly *A.turkestanicus* and *A.ahngerianus*, are widespread in both natural and urbanized ecosystems, [1]. Termites destroy all wooden elements in various constructions, such as architectural and cultural monuments, strategically important constructions, hydraulic structures, and residential and administrative buildings. A termite family of 25,000 individuals, occupying a space of 100 cm3, consumes an average of 50,000 cm3 of different types of cellulose. At the same time, they significantly impact on the global carbon cycle, increasing the concentration of carbon dioxide and methane in the atmosphere. According to Sapunov (2009), the ability to digest cellulose, which results in the emission of CO2, is the outcome of combining termites' digestive secretions with enzymes of symbionts and the consequential biochemical processes, [1], [11].

Due to their small size and partially sclerotized bodies, water loss is a common problem for all termite species. They must find and use water resources to avoid or tolerate desiccation. Termites can use water resources like free water, groundwater, wet food, or wet soil. Termites live in various habitats and microhabitats with differing levels of ambient humidity and availability of water, and water management among termite species has been the subject of many studies, [5], [9]. Some Kalotermitidae termites exhibit behavior and survival effects that indicate high humidity is toxic to them, [7]. Different termite species can tolerate a wide range of (sometimes extreme) humidity and temperature conditions; however, there are certain conditions that termites prefer more. Whereas dry wood termites depend heavily on metabolically derived water, damp wood and subterranean termites that live in high RH environments obtain water from their surroundings, [6], [12].

The secret life of termites, strong resistance to environmental factors, the functional specialization of their castes, and their ability to restore populations within short periods complicate the control. Due to the economic importance of *A.ahngerianus* as a pest, it is necessary to find strategies for its management. However, the environmental requirements to keep populations of *A.ahngerianus* alive in the laboratory for an extended period, which allows the establishment of bioassays to evaluate their control, have not been reported to date.

However, the establishment of live subterranean termite colonies in the laboratory is logistically complicated, time-consuming, and challenging, [7] due to factors such as temperature, [5], [18], humidity, [16] and the food source [7]. In particular, desiccation is a determining factor for termite survival, so it is essential in laboratory survival studies to consider not only relative humidity but also the substrate moisture and food, [3], [5].

In order to provide preliminary information of the basic biological requirements for the survival and maintenance of *C.testaceus* in laboratory conditions, the aim of this study was to evaluate the survival of *C.testaceus* at various levels of relative humidity, temperature, substrate moisture, and preference for different substrates, to obtain the appropriate conditions that allow establishing live termite colonies in the laboratory, to ultimately carry out bioassays to study the control of *C.testaceus*.

Termites’ hidden life and strong resistance to environmental factors, high humidity, as well as the functional specialization of their castes and their ability to restore populations within short periods, complicate the control.

With this in mind, determining the environmental factors that influence their activity and limiting them to a certain extent, preventing termite infestations and developing control measures are current challenges.

Two objectives were taken in the study to determine the effect and viability of humidity (RH) and temperature (0C) on the termite species *A.ahngerianus*. The first objective of this study was to investigate the survivorship of *A.ahngerianus*, which inhabits distinctly different microhabitats and live in high relative humidity (RH) environments. In contrast, they are found in dry timber and can be wet or dry. The second objective of this study was to investigate the RH preferences of the A. as mentioned above *A.ahngerianus*. Termites can obtain water from sources other than humidity. These include metabolic water accepted through the breakdown of food from the fat body, free liquid water and water bound to various substrates (e.g., wood, soil, and nest mate cadavers). Body fat is an essential organic compound that performs vital bodily functions. They serve as an additional water source for various life cycles.

2 Materials and Methods

The area is a lowland located in the north-western part of Uzbekistan, along the lower reaches of the Amudarya River, between 60’-61’ longitude and 41’-42’ latitude, at 113-138 m above sea level. The vegetation period of plants is 200–210 days. The climate is extremely continental, with an average annual precipitation of 80–90 mm. The average temperature in January is -50C and in July, it is +300C. Meadow, meadow marshy, marsh-sandy, and traditional alkali soils predominate, [13]. The deserts of Kyzyl-Kum and Kara-Kum greatly influence on the climate of the oasis. The region is in the steppe zone, in the western part of the Khorezm oasis and the southern part of the Aral Sea, 100 m above sea level. The relief consists of a low plain. It is the old Amu-Darya delta and consists of river sediments. The western and southwestern parts of the region connecting with Kara-Kum are covered with sand. Some minerals include limestone, sand, clay, and other building materials, [2]. The study of the route covered all districts of the Khorezm region. The insects were collected yearly in spring, summer, autumn, and winter. As shown in figure 1, the distribution of termites in the natural and urban zones of the Khorezm region (Uzbekistan) was determined using GPS, [14].

Fig. 1: Distribution of termites belonging to the genus *Anacantotermes* in the Khorezm region (Uzbekistan)

Individual termites from colonies of *A.ahngerianus* were collected from Exterra, cardboard paper, and bucket traps as described by Su and Scheffrahn, [15] (Figure 2).

Fig. 2: Apparat Exterra

During the research, more than 5230 termite individuals (larvae, nymphs, soldiers, workers, and imagos) were washed from the termite nest and its galleries, termite mud tunnels, and soil surface. Collected termites were stored in a special termite nest and regularly steamed with water at 25°C to climate them to laboratory conditions >95% RH. In this study, a total of 3740 workers were used in a single colony (Figure 3).

Fig. 3: Special termite nest

2.1 Survival of *A.ahngerianus* at Different Percentages of Relative Humidity (RH) and Temperature

The methodology was proposed by Zukowski and Su, [5] with modifications. Desiccative humidity boxes consisted of six environmental boxes (EB) conditioned using plastic containers with a lid (8.4 × 31.01 × 23.02 cm) with a 2.7 cm diameter hole in the central part of the lid for inserting a digital thermo-hygrometer HTC-2 (Figure 4).

Fig. 4: Special termite nest

The RH inside the EB was stabilized with six RHSM: two saturated salt solutions ($MgCl_2$, NaCl), salt, $CaCl_2$, silica gel, water (H2O), and wet wipes in various amounts as in [5] (Table 1).

Table 1. RHSM and amounts used to obtain different RH

RHSM & RH obtained (%)		
Wet wipes	**Water**	**NaCl**
200 ml	100 ml	100 g
99.96±0.02	86.32 ± 0.02	68.48 ± 0.06

RHSM & RH obtained (%)		
Silica gel	**$MgCl_2$**	**$CaCl_2$**
100 g	100 g	100 g
51.48 ± 0.09	35.68 ± 0.07	10.1 ± 0.01

[a]Mean (M) ± Standard Error (SE)

To achieve a high RH, the water and wet wipes were placed inside the plastic containers EB; at moderate humidity, the salts and the silica gel were placed in the plastic containers described above, and the $CaCl_2$ was used to obtain the lowest RH in EB. The RH in the EB was assessed for three days with a digital hygrometer.

The average RH (± standard error of the mean SEM) of the boxes and their associated stabilizing material were as follows (n = 15): 99.96 ± 0.02% (wet wipes), 86.32 ± 0.02% (H_2O), 68.48 ± 0.06% (NaCl), 51.48 ± 0.09% (Silica gel), 35.68 ± 0.07% ($MgCl_2$) and 10.1 ± 0.01% ($CaCl_2$). The temperature was stabilized at 25, 30 and 35 °C in an incubator (Dry-air thermostat DBO-200).

The daily number of live termites and the survival percentage were calculated. Dead termites were removed daily. For the six RH at the three temperatures tested, the bioassay required five repetitions with fifteen workers in each repeat (a total of 1350 workers).

Statistical analysis was carried out using JMP statistical software, [4]. All data were analyzed using analysis of variance (ANOVA) in a one-by-6 factorial arrangement. *A.ahngerianus* (1) and RH (6) were the factors, and percentage survival was the response variable. Percentage survival values were arcsine-square root transformed before analysis. At = 0.05, post hoc Tukey honestly significant difference tests were used to evaluate all pairwise differences.

2.2 Termite Survival in Various Types of Water Sources

The bottoms of transparent plastic containers (with a diameter of 52 mm and a height of 53 mm) were scratched with sandpaper to provide traction for termite movement. Temperature and humidity levels inside the jars were measured as described previously. Twenty-five worker termites were placed in each plastic box with small holes drilled in the box's lid to allow air movement and prevent termites from escaping. We used six boxes that provided various water sources (or lack thereof) in the experiment. These include six plastic containers containing a piece of dry wood (*Ferula assa-foetida*), a part of wet wood (*Ferula assa-foetida*), dry paper, wet paper, wet soil, and dry soil (Table 2).

Table 2. RHSM and amounts used to obtain different RH

Substrates		
dry wood	**wet wood**	**dry paper**
humidity %		
58.24 ± 0.08	58.28 ± 0.05	58.46 ± 0.06
temperature°C		
24.5 ± 0.03	24.5 ± 0.03	24.52 ± 0.05

Substrates		
wet paper	**dry soil**	**soil + water**
humidity %		
58.5 ± 0.06	58.6 ± 0.05	58.42 ± 0.05
temperature°C		
24.52 ± 0.05	24.32 ± 0.03	24.46 ± 0.04

Silica gel was used to maintain medium humidity in plastic containers in a dry and wet environment. The purpose of this experiment was to find out where the termites get their moisture in dry conditions. The mean RH (±SEM) of the chambers and their associated water sources were as follows: 51.48 ± 0.09% (a piece of dry wood, Ferula assa-foetida), and 51.56 ± 0.07% (a piece of wet wood, Ferula assa-foetida). 51.52 ± 0.08% (dry paper), 51.48 ± 0.09% (wet paper), 51.42 ± 0.05% (dry soil), 51.44 ± 0.03% (soil + water).

The average temperature (± SEM) of the chambers and their associated water sources were as follows: 24.5 ± 0.03°C (a piece of dry wood Ferula assa-foetida), 24.5 ± 0.03 °C (a part of wet wood Ferula assa-foetida), 24.52 ± 0.05°C (dry paper), 24.52 ± 0.05°C (a damp paper), 24.32 ± 0.03°C (dry soil), 24.46 ± 0.04% (soil + water). Experiments were recorded every 24 hours. The daily number of live termites was recorded for 14 days. Studies were conducted to investigate the significant reduction in body mass of *A.ahngerianus* worker individuals. At the end of the twelve-day study, worker individuals of *A.ahngerianus* species that survived the different RH were weighed. To solve several issues related to the vital activity of the termites, *Anacanthotermes ahngerianus* generally accepted methods were used.

3 Results

3.1 Survival of *A.ahngerianus* at Different Percentages of Relative Humidity (RH) and Temperature

Table 3 shows the survival rate of worker termites of *A.ahngerianus* for 72 hours after being exposed to six RH and three temperatures. The RH of 99% presents the highest percentage of survival at 72 hours of observation for the three temperatures evaluated. Likewise, it was observed that as the RH decreases, the termite survival decreases gradually, too, while at higher temperatures, there's a lower survival.

Table 3. Percentage of survival (M ± SE) of *A.ahngerianus* 72 hours after being exposed to six RHs and three temperatures

	SM and humidity (%)					
H / C	**Wet wipes** 99.96±0.02	**Water** 86.32 ± 0.02	**NaCl** 58.24 ± 0.08	**Silica gel** 51.48 ± 0.09	**$MgCl_2$** 35.68 ± 0.07	**$CaCl_2$** 10.1 ± 0.01
25	89.62 ± 0.03	70.44 ± 0.08	62.52 ± 0.05	39.6 ±0.03	30.4 ± 0.03	20.48 ± 0.08
30	75.34 ± 0.07	59.34 ± 0.07	38.28 ± 0.06	8.58 ± 0.03	3.56 ± 0.04	0.0 ± 0.0
35	65.68 ± 0.09	23.44 ± 0.04	9.38 ± 0.05	6.6 ± 0.03	0.0 ± 0.0	0.0 ± 0.0

Note: SM- stabilizing material

With 20.48 ± 0.08 termite survival, 25 °C and 10% (CaCl2) RH combination provided the lowest termite survival. Termite survival has been reported to fall to 0% as temperature rises (30°C, 35°C) and humidity falls. As a result of studies on the viability of the *A.ahngerianus* in six different humidity levels (H) and three different temperatures (°C), we found that the termites in water and wet wipes survived better than those in other substrates (NaCl, $MgCl_2$, and $CaCl_2$). According to Figure 5, the termite viability in containers with wet wipes of 100% humidity is (99.96), 89.6% at 25°C, 75.3% at 30°C, and 65.7% at 35°C above zero.

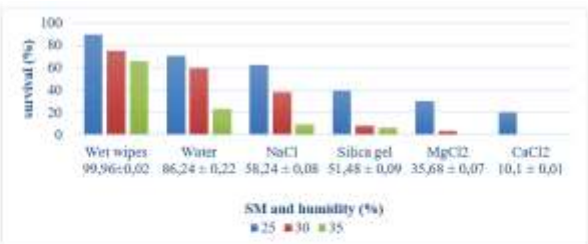

Fig. 5: Survival percentage of *A.ahngerianus*

It was noted that in containers filled with water only, it is 86.32% at 25°C humidity, 70.4% at 25 °C, 59.3% at 30°C, and 23.4% at 35°C above zero. Moreover, it was also found that the termite survival at 10.1% humidity in $CaCl_2$ was 20.5% at 25°C, and the termites ultimately died at 30-35°C above zero.

3.2 Termite Survivorship with Various Types of Water Sources

The termites displayed statistically different responses to RH for two weeks. It was studied in 6 plastic containers (dry wood, wet wood, dry paper, wet paper, dry soil, soil + water) stabilized to 58% humidity with NaCl salt that different water sources cause the survival of the termites. According to the results of the study, it was noted that the survival rates of *A.ahngerianus* worker termites in plastic containers with wet paper, soil and water were 65% and 56%, respectively, and the lowest survival rate in dry soil containers was 6% (Table 4).

Table 4. Survival of termites in different types of water sources

Substrates and humidity %					
dry wood 58.24 ± 0.08	**wet wood** 58.28 ± 0.05	**dry paper** 58.46 ± 0.06	**wet paper** 58.5 ± 0.06	**dry soil** 58.6 ± 0.05	**soil + water** 58.42 ± 0.05
25.56 ± 0.04	43.46 ± 0.04	36.4 ± 0.04	65.3 ± 0.07	6.62 ± 0.07	56.34 ± 0.07

According to the statistics in Figure 6, on the 14th day of the experiment, out of 25 worker termites in each plastic container, the survival of termites was 43.46% (11 workers)in plastic boxes placed in a piece of wet wood *Ferula assa-foetida*, 65.3% (16 termites)in boxes with wet paper, and the survival of termites was 56.34% (14) in containers with soil+water, while the survival of worker termites was 23% (17 worker termites) out of 75in plastic containers with dry substrates, i.e., the survival range was 25.56% (6) in a piece of dry wood *Ferula assa-foetida*, 36.4% (9) in the dry paper, and 6.62% (2) in plastic containers with dry soil.

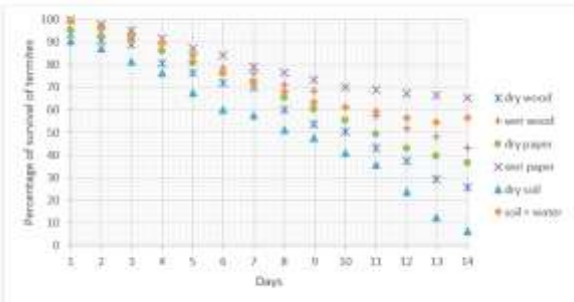

Fig. 6: 14-day survival of termites in different types of water sources

In dry paper and dry food plastic containers, the survival of termites may be due to using the decomposition of body fat and cannibalism of dead termites as the water source.

Except for wet wood, *Ferula assa-foetida*, wet paper, soil+water plastic containers, worker termites in all dry wood *Ferula assa-foetida*, dry paper, and dry soil plastic boxes were significantly smaller at the end of the experiment (on the 14th day) than the beginning, that is, termite mass decreased (5.3–116.6 mg loss per 10 termites). Thirteen of the 17 *A.ahngerianus* workers survived at the various RH and were then placed in a dish and provided with food and water. These 13 workers exhibited a total weight gain of approximately 351 mg. The remaining four workers were not accounted for, indicating that nest mates might have cannibalized them for food and water source. The ten workers exhibited a decrease in mass of approximately 43.2% of their weight, which could be attributed to the loss of body water or body fat.

4 Discussion

The conducted research and the analysis of the obtained results show that the increase in humidity in the buildings, increase in damage levels and the temperature around 25 °C create favorable conditions for working termites. On the contrary, the increase in the temperature of the environment and the decrease in the relative humidity of the air have harmful effects on the termites, decrease their immunity and may cause death.

According to the study's findings, *A.ahngerianus* is less capable of tolerating desiccation. This agrees with preliminary studies on *C.formosanus* species in [3] and [12]. The physiological mechanisms involved in desiccation tolerance remain to be elucidated, but they are probably related to cuticular permeability and total body water percentage. The results illustrate the preference of *A.ahngerianus*; despite living in arid environments, it also dwells in different environments with high moisture levels. For regular activity and survival, *A.ahngerianus* requires not only high RH but also other sources of water, [9]. The tunneling activity of *A.ahngerianus* revealed that this species tunneled more in moister substrates (Figure 7).

Fig. 7: Termite tunnels in wooden columns

Many termites are more likely to be found, survive more prolonged, and consume food resources at higher rates in environments where water resources are more likely to persist, [3]. In our study, *A. ahngerianus* lives longer and consumes food resources at a higher rate due to different water sources (groundwater, rainwater and increased humidity in the building), even though it lives in an environment with scarce water resources. The geographical habitat nature of *A. ahngerianus* includes areas with extended wet and dry seasons; tolerance to a wide range of RH and water availability is beneficial, [1]. In this study, *A.ahngerianus could* tolerate conditions in which water sources were lacking and utilize water sources when they were present. This is similar to the findings of the study mentioned above. Another group of behavior involved in desiccation tolerance is cannibalism, which is an additional means of obtaining water and food resources under water-stressed conditions, [3].

Our research has proven that high humidity in buildings in dry conditions leads to the development of termites and damage to buildings. When the humidity level in the buildings is low, termites use groundwater or soil moisture. Also, in the absence of a water source, cannibalism (using the liquid of a dead termite) among termites has been confirmed in our research.

Although *A.ahngerianus* requires access to water sources, excess moisture can be detrimental to them. In [17], the authors found evidence of water toxicity in dry wood termite species. However, such cases were not observed in our research. In our study, the humidity in the Juma Mosque Museum was beneficial for termite activity and affected moisture preservation in the wooden columns. Despite the change in air temperature and relative humidity, the difference in wood moisture occurs very slowly. But wood moisture eventually stabilizes at equilibrium humidity determined by the average relative humidity. It was found that there are strong symbiotic relationships between termites and the genera of fungi *Termitomyces* and *Hilaria* in the building. It was proved that the digestion coefficient of termites significantly changes when they feed on rotten wood, the number of individuals in their family increases rapidly, and additional sexual individuals are formed quickly.

The results of this study highlight the importance of water for the survival of *A.ahngerianus* and RH preference, desiccation and RH tolerance, and moisture exposure tolerance.

Based on the result of the present study, we can conclude that one of the main factors providing the mechanical activity of termites is body fat, which is easily included in the metabolic cycle as a source of energy and water.

5 Conclusion

High humidity and temperatures of 20–25°C had a positive effect on termite development; conversely, high or low temperatures and low humidity hindered termite survival. It is established that when humidity in a building is lowered, the termite feeds on ground water and nutrients in the body as a source of water. It is proved that increased humidity in buildings leads to the multiplication of fungi of the genera *Termitomyces* and *Hilaria*, resulting in termite development and damage to buildings. In conclusion, humidity reduction, dehumidification, and ventilation systems in historic buildings, residences, and other structures are vital to preventing and controlling termite damage to buildings. Research results and recommendations on the use of waterproofing materials in building practice and measures to prevent the ingress of moisture are also of great importance.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Ikram Abdullaev and other authors conceived of the presented idea. Ikram Abdullaev developed the theory and performed the computations. Other authors verified he analytical methods. All authors discussed the results and contributed to the final manuscript. All authors contributed to the article and approved the submitted version.