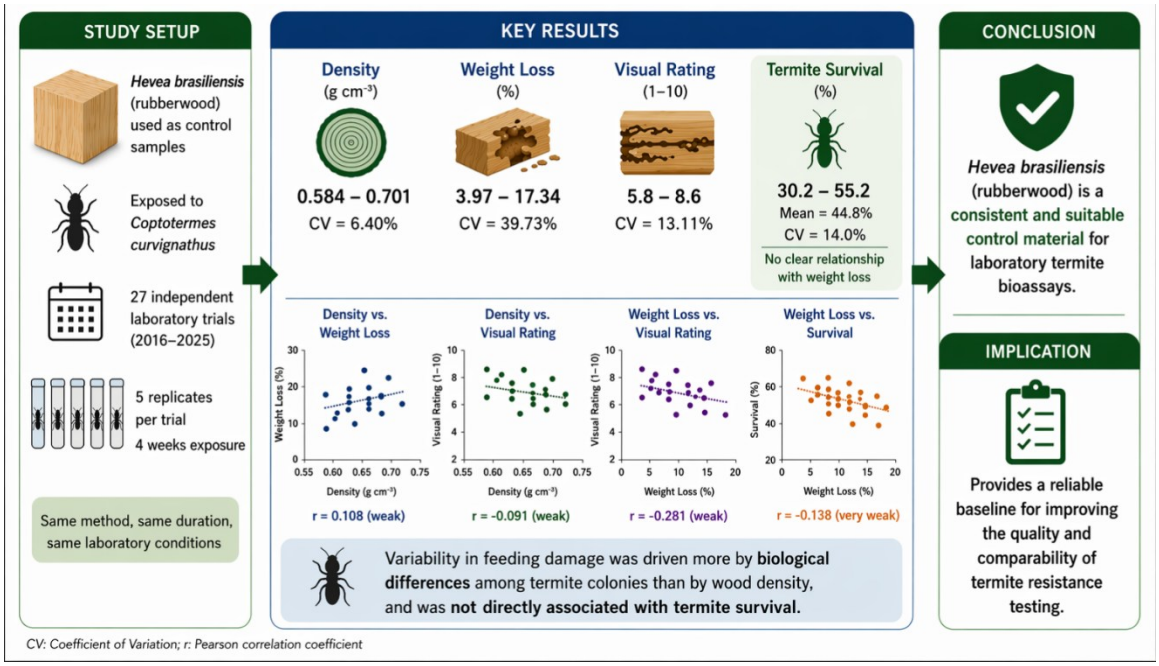
Evaluating the Stability of *Hevea brasiliensis* (Rubberwood) as a Control Material in Subterranean Termite Bioassays

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DOI: 10.15376/biores.21.4.9528-9540

GRAPHICAL ABSTRACT



Evaluating the Stability of *Hevea brasiliensis* (Rubberwood) as a Control Material in Subterranean Termite Bioassays

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Subterranean termites are major biological agents causing wood deterioration in tropical regions. Laboratory bioassays commonly use susceptible reference materials as controls to ensure termite feeding activity; however, information on the long-term variability of such materials remains limited. This study evaluated the variability and repeatability of *Hevea brasiliensis* (rubberwood) as a control material in laboratory tests involving *Coptotermes curvignathus*. Data from 27 independent trials conducted between 2016 and 2025 were analysed, with five replicates per trial exposed for four weeks using a standardized Forest Research Institute Malaysia in-house method. The mean density ranged from 0.584 to 0.701 g/cm³, while mean weight loss varied from 3.97% to 17.34%. The coefficients of variation for density, weight loss, and visual rating were 6.40%, 39.73%, and 13.11%, respectively. Weak correlations were observed between density and weight loss (r = 0.108), density and visual rating (r = −0.091), and weight loss and visual rating (r = −0.281). The results indicate that variability in feeding damage was primarily influenced by biological differences among termite colonies rather than wood density. *Hevea brasiliensis* was confirmed as a consistent and suitable control material for laboratory termite bioassays.

DOI: 10.15376/biores.21.4.9528-9540

Keywords: Hevea brasiliensis; Rubberwood; Coptotermes curvignathus; Termite bioassay; Weight loss; Visual rating; Repeatability

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INTRODUCTION

Subterranean termites are among the most destructive biological agents affecting wood and wood-based materials in tropical regions. In Southeast Asia, termite infestations are responsible for substantial structural damage and economic losses, particularly in buildings and wooden infrastructures (Kim and Chung 2022). Among the various termite species occurring in the region, *Coptotermes curvignathus* is widely recognised as one of the most aggressive subterranean termites in Malaysia (Ab Majid and Ahmad 2013). This species readily attacks untreated wood and has therefore been widely used as a test organism in laboratory evaluations of wood durability and wood preservative efficacy (Messaoudi and Wong 2023; Roszaini and Mohamad Nasir 2026).

Laboratory bioassays are essential tools for assessing the resistance of wood species and evaluating the performance of wood protection systems against termite attack (Leroy *et al*. 2023). Standardised testing procedures typically include the use of susceptible reference materials as control samples to ensure adequate termite feeding activity during

experiments. The presence of a reliable control material is important not only to confirm termite activity but also to allow comparison among experiments conducted under similar testing conditions. Despite its importance, limited attention has been given to the long-term consistency and variability of control materials used in termite bioassays (Lenz 2009).

Although several wood species are susceptible to subterranean termite attack, *Hevea brasiliensis* is widely used as a control or reference material in laboratory termite bioassays due to its commercial availability and consistent susceptibility to termite feeding. (Roszaini and Mohamad Nasir 2026).

In tropical wood durability studies, *Hevea brasiliensis* (rubberwood) is commonly used as a reference material due to its well-documented susceptibility to subterranean termites. *Hevea brasiliensis* is widely available in Malaysia and is frequently employed as a control specimen in laboratory feeding tests involving subterranean termites. Although its susceptibility to termite attack has been well established, information on the variability of termite feeding damage on *H. brasiliensis* across repeated laboratory trials remains limited. Such information is essential for evaluating the repeatability of laboratory bioassays and confirming the suitability of *H. brasiliensis* as a reference material in long-term experimental programmes.

Variability in termite bioassays may arise from several sources, including differences in termite colony vigour, worker–soldier composition, environmental conditions during testing, and intrinsic properties of wood specimens such as density, moisture content, and extractive composition.

Various susceptible wood species have been used as reference or control materials in laboratory termite bioassays, including southern yellow pine, poplar, spruce, and rubberwood, depending on regional availability and testing standards (Lenz 2009; Grace and Yamamoto 1994; França et al. 2022). However, the long-term consistency and repeatability of such control materials have received relatively limited attention, particularly under tropical testing conditions involving *Coptotermes* species.

Therefore, the objective of this study was to evaluate the variability and repeatability of *H. brasiliensis* control samples used in laboratory termite bioassays over an extended period. Data obtained from repeated experiments conducted between 2016 and 2025 were analysed to examine the relationships among wood density, weight loss, and visual rating following exposure to subterranean termites. The results provide insights into the stability of *H. brasiliensis* as a reference material and contribute to improving methodological reliability in laboratory termite resistance testing. This study provides one of the few long-term evaluations of control material performance in termite bioassays, offering new insights into the stability and repeatability of laboratory testing.

EXPERIMENTAL

Wood Materials

Hevea brasiliensis Muell. Arg (rubberwood) specimens were used as control samples in the laboratory termite bioassays. The *H. brasiliensis* specimens used in this study were obtained from commercially processed mature plantation-grown materials maintained as laboratory control specimens. Detailed information such as tree age, DBH, and exact harvesting coordinates was not consistently available because the study utilised archived control materials collected over multiple years. The specimens consisted

predominantly of sapwood, which is widely recognised as more susceptible to termite attack than heartwood.

All specimens were prepared from the same batch of *H. brasiliensis* to minimise variability in wood properties. In this study, the term "same batch of wood" refers to commercially processed *H. brasiliensis* materials prepared and maintained under similar laboratory storage conditions for use as control specimens throughout the experimental period. The specimens were obtained from mature plantation-grown *H. brasiliensis* commonly used for laboratory termite bioassays.

The wood blocks were cut to uniform dimensions [25 mm (L) × 25 mm (R) × 6 mm (T)]. Five replicates were used for each experimental trial according to the requirements of the Forest Research Institute Malaysia (FRIM) in-house termite testing method (AK/4 2014), which is based on ASTM D3345 (2022) and AWPA E1 (2023), with minor modifications.

Density Measurement

Prior to testing, the density of each specimen was determined before conditioning. Density was calculated based on the oven-dry weight (m) and volume (v) of each specimen and expressed in g/cm³ (Eq. 1):

$$\text{Density} = m / v \quad (1)$$

This parameter was included to examine the potential influence of wood density on termite feeding behaviour.

Termite Testing Method

Subterranean termites used in the experiments belonged to the species *Coptotermes curvignathus* Holmgren. Termites were collected from several collection points within a large area of the Forest Research Institute Malaysia (FRIM) campus to ensure sufficient numbers of individuals for testing. Different colonies were used for different trials, although all termites were obtained from the same general locality.

For each experimental unit, approximately 400 termites were introduced into the test container. The termites consisted primarily of workers (360) with a smaller proportion (40) of soldiers, reflecting the natural caste composition typically used in laboratory termite bioassays.

A total of 27 independent laboratory trials were conducted between 2016 and 2025 using the same FRIM in-house testing method (AK/4 2014). Each trial consisted of five replicate specimens of rubberwood, resulting in a total of 135 test specimens.

The exposure period for each trial was four weeks, during which the termites were allowed to feed on the wood specimens under controlled laboratory conditions (22 ± 2 °C temperature and 60 ± 5% relative humidity (RH). At the end of the exposure period, the specimens were removed, cleaned, and prepared for subsequent measurements.

Evaluation of Termite Damage

Termite damage was evaluated using two commonly applied indicators: weight loss and visual rating.

Weight loss was determined by comparing the oven-dry weight (g) of the specimens before and after termite exposure. The percentage of weight loss was calculated to quantify the extent of wood consumption by termites (Eq. 2) below:

$$\text{Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{ x } 100 \quad (2)$$

Visual damage was assessed using a numerical rating scale based on the FRIM in-house method (AK/4 2014), as presented in (Table 1) after exposure.

Table 1. Visual Rating System Based on FRIM In-house Method (AK/4 2014)

Rating	Description
10	Sound
9.5	Trace, surface nibbles permitted
9	Slight attack up to 3% of cross-sectional area affected
8	Moderate attack, 3 to 10% of cross-sectional area affected
7	Moderate/severe attack, penetration, 10 to 35% of cross-sectional area affected
6	Severe attack, 30 to 50% of cross-sectional area affected
4	Very severe attack, 50 to 75% of cross-sectional area affected
0	Failure

At the end of each test, the number of surviving termites was recorded to determine termite survival rate. Survival was calculated as the percentage of live termites remaining from the initial number introduced at the beginning of the experiment.

Statistical Analysis

Descriptive statistics were calculated for density, weight loss, and visual rating across all trials. The variability of each parameter was evaluated using the coefficient of variation (CV) using Microsoft Excel.

Pearson correlation analysis was performed to examine the relationships among wood density, weight loss, and visual rating. All statistical analyses were conducted using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSION

Descriptive Statistics of Wood Density, Weight Loss and Visual Rating

A total of 27 independent laboratory trials were conducted between 2016 and 2025 using *H. brasiliensis* control samples exposed to subterranean termites. The mean density of the *H. brasiliensis* specimens ranged from 0.584 to 0.701 g/cm³ across the trials (Table 2). The relatively narrow range of density values indicates that the wood specimens used in the experiments were generally uniform, which is consistent with previously reported density ranges for *H. brasiliensis* (Teoh *et al.* 2011). The use of specimens obtained from the same batch of wood likely contributed to this consistency and minimised variability arising from differences in intrinsic wood properties.

Mean weight loss varied among trials, ranging from 3.97% to 17.34%, with most trials recording values between 8% and 13% (Table 2; Fig. 1). This range of mass loss

indicates active termite feeding on the *H. brasiliensis* specimens and is comparable to values commonly reported for susceptible wood species under laboratory conditions (Chotikhun *et al.* 2024). Within-trial variability of weight loss was generally moderate, with standard deviations typically below 3%, suggesting consistent feeding behaviour within each experimental unit. Despite the relatively uniform wood material, the variations in weight loss across trials reflect differences in termite feeding activity among colonies.

Visual ratings ranged from 5.8 to 8.6, reflecting moderate to severe termite damage on the *H. brasiliensis* specimens after the four-week exposure period. The observed range of visual ratings is consistent with previous studies reporting substantial surface damage in susceptible wood species subjected to termite attack (Grace and Yamamoto 1994; Arango *et al*. 2006). The relatively consistent visual rating values observed across trials indicate that visual assessments were reasonably stable despite variations in weight loss.

Termite survival ranged from 30.2% to 55.2% across the trials, with an overall mean of 44.8% (Table 2). The moderate survival rates indicate that a substantial proportion of termites did not survive until the end of the four-week exposure period. However, the observed survival range remained sufficient to sustain active feeding on the *H. brasiliensis* specimens throughout the test duration. Similar levels of termite survival have been reported in laboratory bioassays, where mortality may occur due to handling stress and laboratory conditions despite continued feeding activity (Evans *et al.* 2013). Although some individual specimens exhibited relatively low weight loss values, the overall mean weight loss and visual damage ratings confirmed that termite feeding activity remained sufficiently active throughout the laboratory trials. Similar variability has also been reported in laboratory bioassays involving susceptible reference wood species. The visual ratings presented in this study represent mean values derived from replicate assessments (Fig. 2) and therefore may include decimal values. The occurrence of visible damage and measurable weight loss confirms adequate termite activity during the bioassays.

Table 2. Summary Statistics for *H. brasiliensis* Control Samples Across 27 Laboratory Trials

Parameter	Mean	SD	Min	Max	CV (%)
Density (g/cm³)	0.636	0.041	0.478	0.731	6.402
Weight loss (%)	9.887	3.928	1.495	20.085	39.730
Visual rating	7.130	0.934	4.000	10.000	13.105
Survival (%)	44.811	6.280	30.200	55.230	14.015

Values represent overall mean, standard deviation (SD), minimum, maximum, and coefficient of variation (CV)

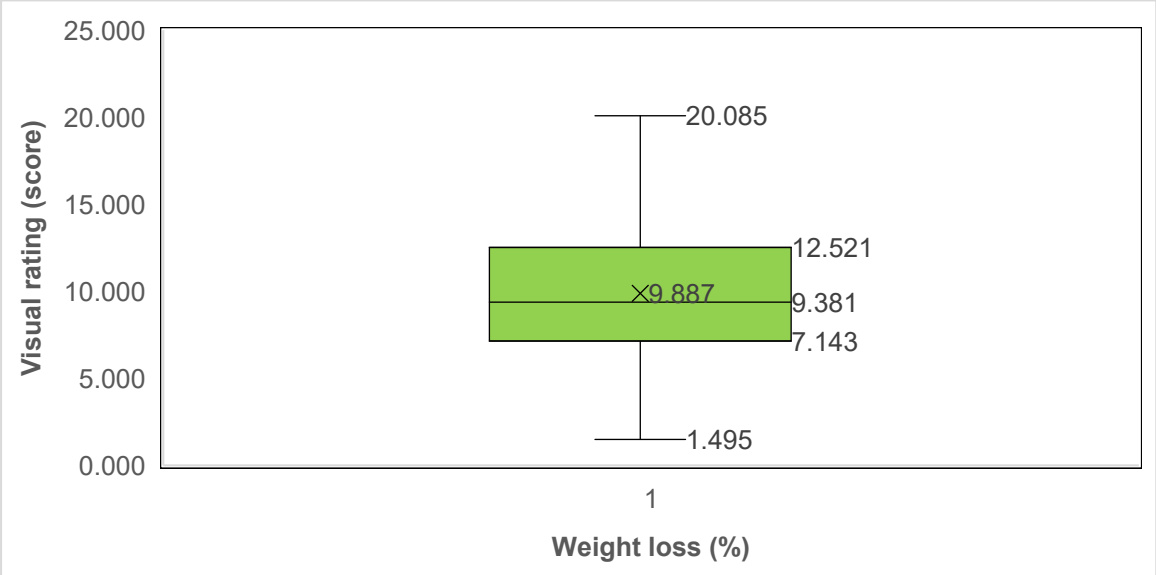


Fig. 1. Distribution of weight loss (%) of *H. brasiliensis* reference samples across 27 independent laboratory trials conducted between 2016 and 2025. The boxplots represent the median, interquartile range, and variability of termite feeding damage within each trial.



Fig. 2. Representative termite damage on *H. brasiliensis* specimens after four weeks of exposure to *C. curvignathus*: (A) slight attack, (B) moderate attack, and (C) severe attack

Overall Variability of Measured Parameters

The overall variability of the measured parameters was evaluated using the CV. The CV values for density, weight loss, and visual rating were 6.4%, 39.7%, and 13.1%, respectively. The relatively low CV value for density indicates that the *H. brasiliensis* specimens were relatively consistent, confirming the consistency of the material used.

In contrast, weight loss exhibited higher variability across trials, which is commonly observed in termite bioassays. The variability in weight loss is likely influenced by the uniformity of the wood samples, the inherently irregular feeding behaviour of termites, and differences in colony vigor (França *et al.* 2022). Subterranean termites are known to exhibit considerable variation in feeding intensity depending on colony condition, caste composition, and environmental adaptation (Evans *et al.* 2013). Therefore, the observed variability in weight loss is more likely to reflect biological differences among termite colonies rather than inconsistencies in the experimental method.

Visual rating showed moderate variability, suggesting that the visual assessment method provided relatively consistent results across repeated trials. This indicates that visual rating remains a useful rapid screening tool, although it may not fully capture the actual extent of wood consumption.

The variability in weight loss observed across the 27 laboratory trials is most likely associated with biological differences among termite colonies, including variations in colony vigor, caste composition, and feeding intensity. Although the *H. brasiliensis* specimens were prepared using similar procedures and exhibited relatively consistent density values, termite feeding behaviour is inherently variable under laboratory conditions. Similar observations have been reported in previous termite bioassays involving susceptible wood species, where differences in feeding activity were attributed primarily to colony-related biological factors rather than wood density alone (Grace and Yamamoto 1994; Lenz *et al.* 2003).

Despite the observed variability, the overall weight loss and visual rating values consistently demonstrated active termite attack on the *H. brasiliensis* specimens throughout the study period. This finding supports the suitability of *H. brasiliensis* as a practical and susceptible reference material for laboratory termite bioassays in tropical regions. Comparable use of susceptible control materials has also been reported for species such as southern yellow pine, spruce, and poplar in standard laboratory evaluations (França *et al.* 2022; Sugahara *et al.* 2024).

Relationship Between Density and Termite Damage

Pearson correlation analysis showed a weak positive correlation between wood density and weight loss ($r = 0.108$) (Fig. 3). Similarly, the relationship between density and visual rating was also weak ($r = -0.091$) (Fig. 4). These results indicate that variations in *H. brasiliensis* density within the observed range had minimal influence on termite feeding damage.

This observation suggests that once a wood species is highly susceptible to termite attack, moderate variations in density have minimal influence on feeding preference or consumption rate. Similar findings have been reported in other studies, where susceptible wood species exhibited substantial termite feeding regardless of density differences (Martin and Lôpez 2023; Etcheverry *et al*. 2025).

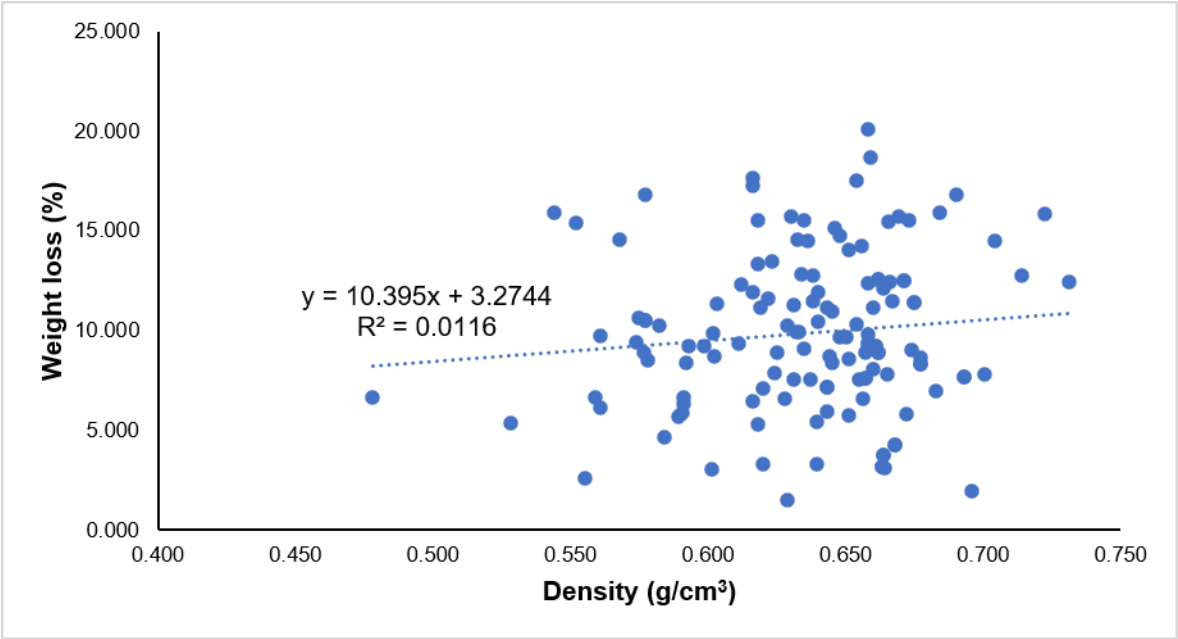


Fig. 3. Relationship between wood density (g/cm³) and weight loss (%) of *H. brasiliensis* reference material after four weeks of exposure to subterranean termites. The scatter plot indicates a weak relationship between density and termite-induced mass loss across the 27 laboratory trials

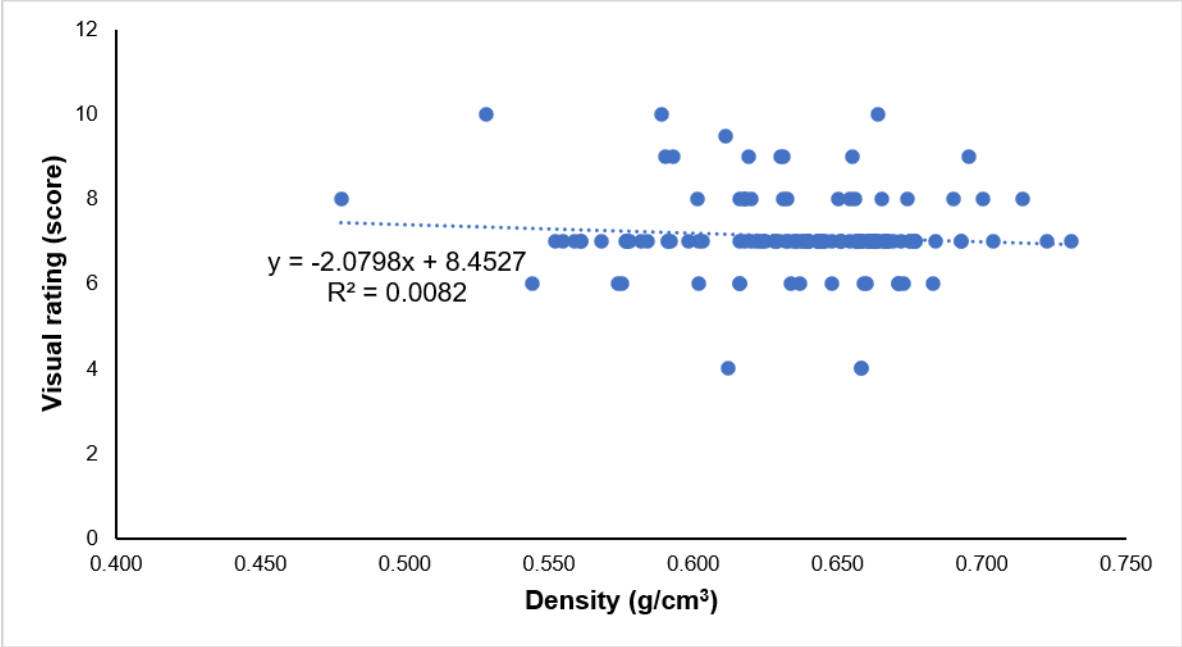


Fig. 4. Scatter plot showing the relationship between wood density (g/cm³) and visual rating of *H. brasiliensis* reference material following a four-week laboratory exposure to subterranean termites. The results indicate that variations in density had minimal influence on the visual assessment of termite-induced damage.

Relationship Between Weight Loss and Visual Rating

The correlation between weight loss and visual rating was negative but weak (r = −0.281) (Fig. 5). This result indicates that specimens with higher weight loss generally tended to receive lower visual ratings, reflecting greater visible damage. However, the relatively low correlation suggests that visual rating alone may not fully represent the actual amount of wood consumed by termites.

Visual rating methods are inherently subjective and primarily assess surface damage, whereas weight loss provides a quantitative measure of wood consumption (Shirmohammadi *et al.* 2023). Therefore, weight loss remains a more reliable indicator of termite feeding intensity in laboratory tests.

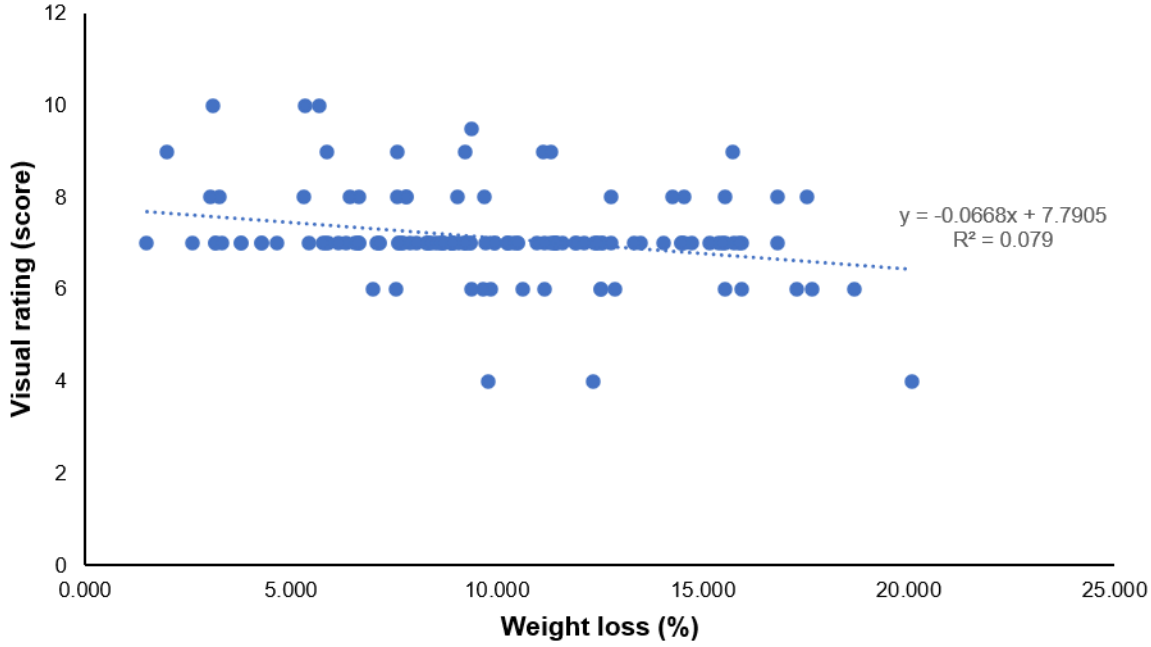


Fig. 5. Relationship between weight loss (%) and visual rating of *H. brasiliensis* specimens after four weeks of exposure to subterranean termites. The scatter plot illustrates the weak negative correlation between gravimetric mass loss and visual assessment of damage.

Relationship Between Weight Loss and Survival

The relationship between weight loss and termite survival after four weeks of exposure is presented in Fig. 6. Termite survival ranged approximately from 30% to 56% across the trials, while weight loss varied between 4.0% and 17.3%. No clear linear relationship was observed between weight loss and termite survival. This suggests that feeding activity and mortality are not directly linked in laboratory termite bioassays. Termites may continue feeding even under suboptimal conditions, while mortality may be influenced by environmental stress, handling, and colony health (Evans *et al.* 2013). Therefore, termite survival alone may not be a reliable indicator of feeding performance in laboratory tests.

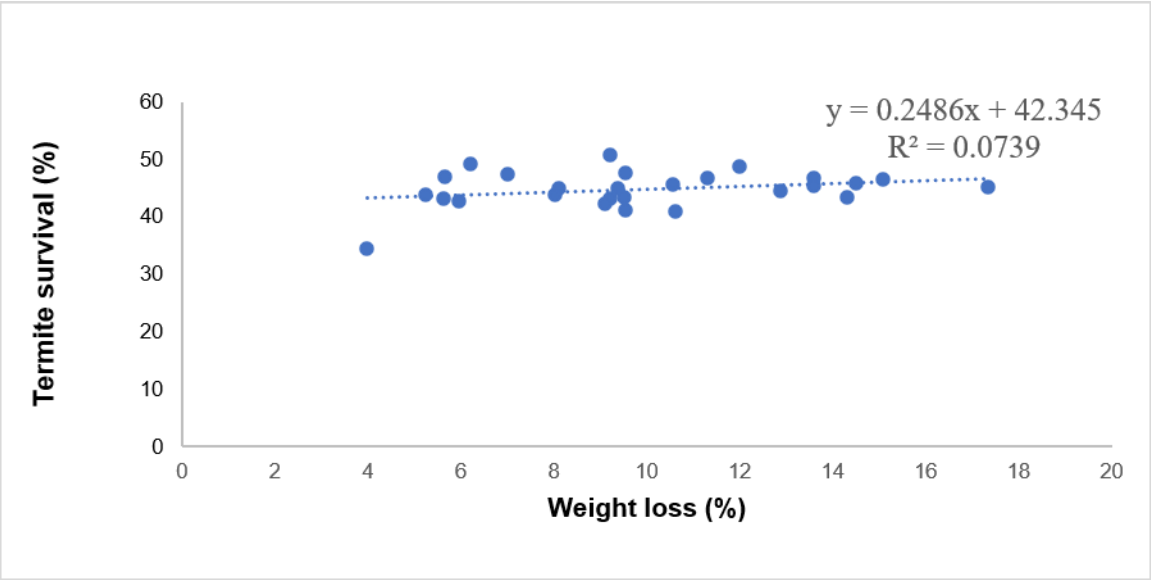


Fig. 6. Relationship between weight loss (%) and termite survival (%) after four weeks of exposure. The scatter plot illustrates the association between feeding activity and termite mortality across the laboratory trials.

Variation in Weight Loss Across Trials

The variation in mean weight loss across the 27 laboratory trials is shown in Fig. 7. Mean weight loss values fluctuated between approximately 4.0% and 17.3%, with most trials falling within the range of 8% to 13%. No consistent increasing or decreasing trend was observed across the trials conducted between 2016 and 2025. These fluctuations are most likely attributable to natural variability in termite feeding behaviour rather than systematic differences in experimental conditions. As all trials were conducted using the same testing method, wood material, and exposure duration, the observed variation is best explained by biological differences among termite colonies. Subterranean termites, particularly *C. curvignathus*, are known to exhibit variability in feeding intensity depending on colony vigor and environmental factors (Evans *et al.* 2013).

Despite these fluctuations, the absence of a clear temporal trend indicates that termite feeding activity remained relatively stable over time. This supports the reliability of *H. brasiliensis* as a reference material in long-term laboratory bioassays.

Although all experiments were conducted using the same testing protocol and similar wood materials, biological variability among termite colonies likely contributed substantially to differences in feeding intensity across trials.



Fig. 7. Variation in mean weight loss (%) of *H. brasiliensis* reference material across 27 laboratory trials conducted between 2016 and 2025, showing fluctuations in termite feeding activity over time.

CONCLUSIONS

1. The density of *H. brasiliensis* specimens remained relatively consistent across the 27 trials.
2. Weight loss exhibited greater variability, reflecting biological differences in termite feeding activity among colonies.
3. Weak correlations between density and termite damage parameters indicate that density had minimal influence on feeding behaviour within the observed range.
4. The findings support the suitability of *H. brasiliensis* as a suitable and practical reference material for laboratory termite bioassays.

ACKNOWLEDGMENTS

The authors are grateful for the support of the Wood Entomology Laboratory staff for their assistance in termites' testing.

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Article submitted: April 19, 2026; Peer review completed: May 9, 2026; Revisions accepted: May 17, 2026; Published: August 10, 2026.
DOI: 10.15376/biores.21.4.9528-9540