The Termitidae, otherwise known as white ants, are a subfamily of the Isoptera order and are characterized by their ability to construct large subterranean nests. These insects are known for their damage to wood structures and are considered a major pest in many regions. In the New World, Nasutitermes corniger is the most important pest species of this genus, known for its ability to masticate wood and feces, and other local materials cemented with salivary secretions (Boulogne et al. 2017). The feeding habits of these insects lead to the decomposition and cycling of nutrients (Jouquet et al. 2011), which is crucial for maintaining the health of ecosystems. Despite their ecological importance, the study of Nasutitermes corniger is often challenging due to their cryptic lifestyle, which makes it difficult to observe their behavior and ecological interactions in the wild. This has led to the development of laboratory studies to better understand the survival and ecology of these insects.

In the present study, the effect of food resource and carton nest material on group survival of Nasutitermes corniger was investigated in laboratory conditions. The hypothesis tested was that the combination of a food resource and carton nest material in the experimental arenas would increase the survival of termite groups. The study was carried out using five colonies collected at the Universidade Federal Rural de Pernambuco (UFRPE) campus SEDE (8°04'03" S, 34°55'00" W), in Recife, Pernambuco, Brazil. The bioassays were conducted using Petri dishes (80 x 15 mm) with a filter paper. The following conditions were established: (1) termite groups (seven workers and three soldiers) alone (control), (2) termite groups with 3 g of carton nest material (NW), (3) termite groups with 5 g of freshly cut sugarcane baits (a surrogate of food resource) (SC), and (4) termite groups with 3 g of carton nest material and 5 g of freshly cut sugarcane baits (NW+SC). Nest material used in the bioassays was collected from the central part of the nest and it was kept whole without any water. All pieces of nests used were recently sampled. The choice of sugarcane as food resource was based considering previous studies with Nasutitermes corniger that used sugarcane as bait in laboratory experiments (Scheffrahnn et al. 2005; Silva et al. 2021). The caste proportion in the group was chosen to account for the natural proportion of soldiers in Nasutitermes corniger colonies (i.e., 30%) (Haverty 1977). Bioassays were conducted in laboratory under controlled conditions (25 °C, 70% r.h.), kept in the absence of light. The number of dead individuals was determined at one-hour intervals for the first eight hours, and then daily until the death of all individuals. Three repetitions per treatment were performed for each of the five colonies tested, totaling 60 repetitions/bioassay.

Data were subjected to censored survival analysis under Weibull distribution, using R software (R Development Core Team 2019). Statistical analysis was used to check whether treatments (control, NW, SC and NW+SC) would affect the group survival of Nasutitermes corniger. Contrast analysis was conducted to check differences among treatments.

To confirm whether the death of termites occurs by fungal pathogen, dead termites were placed in a humid chamber in a biochemical oxygen demand (B.O.D.) incubator (25 °C, 12h photoperiod). The fungus sporulation was assessed after five days to allow sufficient time for sporulation to occur.
termite corpses to sporulate. The proportion of termites killed by fungi was assessed using Generalized Linear Model (GLM) with Binomial error distribution. Models were validated by inspecting residuals and testing for overdispersion. Differences among treatments were tested by Analysis of Deviance (ANODEV; a maximum-likelihood equivalent of ANOVA) followed by Contrast Analysis.

In general, the mean time to death of termites was 121.30 hours. The survival of termite groups was significantly affected by treatments (Likelihood= 5,983.9, d.f.= 600, P < 0.001). The survivorship of termite was lower in the treatments without sugarcane baits (control and NW) compared to treatments with sugarcane baits (SC and NW+SC) (Tab. 1; Fig. 1). A higher survival of groups kept with sugarcane compared to others food resources (decomposed wood, dry wood, and wood paper) was already observed for N. coxipoensis (Albuquerque et al. 2008). After a few days from the beginning of our bioassays, however, sugarcane baits showed a fungal appearance. Our study showed that proportion of termites killed by fungi was significantly affected by treatments (Deviance= 416.9, d.f. = 20, P < 0.001). The proportion of termites dead by fungi in the control and NW significantly differ from those in the SC and NW+SC groups. Termite mortality reached 68% and 72% due to the fungal pathogen in SC and NW+SC, respectively (Tab. 2). Such result can indicate that the presence of sugarcane baits as food resource in laboratory bioassays impaired the experiment because death occurs due to the presence of the fungus in the sugarcane and not because natural factors. Thus, to conduct experiments with sugarcane baits we suggest that sugarcane must be replace every two days to avoid proliferation of fungi or even used some antifungal.

### Table 1. Mean time to death of termite groups among treatments (control, carton nest material [NW], sugarcane baits [SC] and carton nest material + sugarcane baits [NW + SC]).

| Treatments   | Mean time to death (hours) |
|--------------|----------------------------|
| Control      | 63.36                      |
| NW           | 72.89                      |
| SC           | 189.92                     |
| NW + SC      | 213.16                     |

### Table 2. Proportion of termites dead by fungi among treatments (control, carton nest material [NW], sugarcane baits [SC] and carton nest material + sugarcane baits [NW + SC]).

| Treatments   | % of termites dead by fungi       |
|--------------|-----------------------------------|
| Control      | 0.00±0.00 a                       |
| NW           | 0.00±0.00 a                       |
| SC           | 0.72±0.04 b                       |
| NW + SC      | 0.68±0.02 b                       |

Mean followed by the same letter did not differ significantly by ANODEV followed by Contrast Analysis.

Even though the nests of *N. corniger* are constructed from stercoral carton, and other local materials cemented with saliva, thus, incorporating antimicrobial substances, and provide a significant benefit to termites (*Noiriot 1970; Thorne & Haverty 2000; Bulmer et al. 2009*). Our results indicate that the presence of carton nest material did not increase the termite survival compared to the control. This absence of significant variation can be explained with two factors: i) the size of carton nest material used in the present study did not provide enough chemical signal to increase group cohesion, and, therefore, increase survival; or ii) for *N. corniger* carton nest material did not enhance survival.

In conclusion, the results of this study may contribute to the establishment of bioassay protocols, maintaining adequate conditions for the survival of *N. corniger* under laboratory condition. Future studies must test the alternative food sources which can keep termite alive without proliferation of fungi. Another point that can be improved is use nest material in different conditions and the combination of temperature and substrate moisture (mL of water/g of nest material).

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### Authors’ Contributions

PFC conceived the experiment. LFF, CRS and RECS collected the colonies in the field and performed the bioassays. LFF and PFC performed the statistical analyses. All authors wrote the manuscript.

### Conflict of Interest Statement

The authors declare no conflicts of interest.

### References

Albuquerque, A. C.; Cunha, F. M.; Oliveira, M. A. P.; Veiga, A. F. S. L.; Luma-Alves Lima, E. A. (2008) Análise de substratos para teste de sobrevivência com *Nasutitermes coxipoensis* (Holmgregn) (Isoptera: Termitidae). *Arquivos do Instituto Biológico*, 75: 529-532. doi: [10.1590/1808-1657v75p5292008](https://doi.org/10.1590/1808-1657v75p5292008)

Boulbogne, I.; Constantino, R.; Amusant, N.; Falkowski, M.; Rodrigues, A. M. S.; Houël, E. (2017) Ecology of termites from the genus *Nasutitermes* (Termitidae: Nasutitermitinae) and potential for science-based development of sustainable pest management programs. *Journal of Pest Science*, 90(1): 19-37. doi: [10.1007/s10340-016-0796-x](https://doi.org/10.1007/s10340-016-0796-x)

Bulmer, M. S.; Bachelet, I.; Raman, R.; Rosengaus, R. B.; Sasisekharan, R. (2009) Targeting antimicrobial effector function in insect immunity as a pest control strategy. *Proceedings of the National Academy of Sciences of the United States of America*, 106(31): 12652-12657. doi: [10.1073/pnas.0904063106](https://doi.org/10.1073/pnas.0904063106)

Constantino, R. (2002) The pest termites of South America: taxonomy, distribution and status. *Journal of Applied Entomology*, 126(7-8): 355-365. doi: [10.1046/j.1439-0418.2002.00670.x](https://doi.org/10.1046/j.1439-0418.2002.00670.x)

Donovan, S. E.; Eggleton, P.; Bignell, D. E. (2001) Gut content analysis and a new feeding group classification of termites. *Ecological Entomology*, 26(4): 356-366. doi: [10.1046/j.1365-2311.2001.00342.x](https://doi.org/10.1046/j.1365-2311.2001.00342.x)
Ferreira, D. V.; Cruz, J. S.; Sacramento, J. J. M.; Rocha, M. L. C.; Cristaldo, P. F.; Araújo, A. P. A. (2019) Effect of temperature and substrate moisture on group survival of Constrictotermes sp. (Isoptera: Termitidae) under laboratory conditions. *Revista Brasileira de Entomologia*, 62: 9-11. doi: 10.1016/j.rbe.2018.12.004

Haverty, M. I. (1977) The proportion of soldiers in termite colonies: a list and a bibliography (Isoptera). *Sociobiology*, 2(3): 199-216.

Jouquet, P.; Traoré, S.; Choosai, C.; Hartmann, C.; Bignell, D. (2011) Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *European Journal of Soil Biology*, 47(4): 2150-222. doi: 10.1016/j.ejsobi.2011.05.005

Loreto, R. G.; DeSouza, O.; Elliot, S. L. (2009) Colored glue as a tool to mark termites (*Cornitermes cumulans*; Isoptera. Termitidae) for ecological and behavioral studies. *Sociobiology*, 54(2): 351-360.

Marins, A.; Cristaldo, P. F.; Paiva, L. R.; Miramontes, O.; DeSouza, O. (2023) A new approach to mark termites (*Cornitermes cumulans* (Kollar) Blattodea: Isoptera) for laboratory bioassays. *Brazilian Journal of Biology*, 83. doi: 10.1590/1519-6984.03316

Noirot, C. (1970) The Nests of Termites. In: Krishna, K.; Weesner F. M. (Eds.), *Biology of Termites*, pp. 73-120. New York: Academic Press.

R Development Core Team 2019. (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [https://www.R-project.org/](https://www.R-project.org/)

Sacramento, J. J. M.; Cristaldo, P. F.; Santana, D. L.; Cruz, J. S.; Oliveira, B. V. S.; dos Santos, A. T.; Araújo, A. P. A. (2020) Soldiers of the termite *Nasutitermes corniger* (Termitidae: Nasutitermitinae) increase the ability to exploit food resources. *Behavioral Processes*, 181: 104-272. doi: 10.1016/j.beproc.2020.104272

Silva, A. N. F.; Silva, C. R.; Santos, R. E. C.; Arce, C. C. M.; Araújo, A. P. A.; Cristaldo, P. F. (2021) Resource selection in nasute termite: The role of social information. *Ethology*, 127(3): 278-285. doi: 10.1111/eth.13125

Scheffrahn, R. H.; Krecek, J.; Szalanski, A. L.; Austin, J. W. (2005) Synonymy of neotropical arboreal termites *Nasutitermes corniger* and *N. casta* (Isoptera: Termitidae: Nasutitermitinae), with evidence from morphology, genetics, and biogeography. *Annals of the Entomological Society of America*, 98(3): 273-281. doi: 10.1603/0013-8746(2005)098[0273:SONATN]2.0.CO;2

Thorne, B. L.; Haverty, M. I. (2000) Nest growth and survivorship in three species of neotropical *Nasutitermes* (Isoptera: Termitidae). *Environmental Entomology*, 29(2): 256-264. doi: 10.1093/ee/29.2.256

Zukowski, J.; Su, N. Y. (2017) Survival of termites (Isoptera) exposed to various levels of relative humidity (RH) and water availability, and their RH preferences. *Florida Entomologist*, 100(3): 532-538. doi: 10.1653/024.100.0307