FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 6 No. 4, August, 2022, pp 20 - 24
DOI: https://doi.org/10.33003/fjs-2022-0604-1053

COMPARATIVE LETHAL IMPACT OF GLYPHOSATE AND PARAQUAT ON AFRICAN MOUND-BUILDING TERMITES (*Macrotermes bellicosus* Smeathman)

***Otabor J. Itohan, Okrikata Emmanuel and Bulus Shalom**

Department of Biological Sciences, Federal University Wukari, Taraba State

*Corresponding authors' email: missjaneotabor@gmail.com

ABSTRACT

In Nigeria, glyphosate and paraquat are common herbicides employed for weed control because they are cost-effective in controlling weeds. It has been shown that, herbicides released into the environment can indirectly affect non-target species, often in harmful ways. We thus investigated the lethal effects of Force up® (glyphosate) and ParaQ® (paraquat) on workers of African mound-building termites (*Macrotermes bellicosus* Smeathman). The worker termites were treated with 30µl of glyphosate and paraquat at different concentrations viz; 25, 12.5, 6.25, 3.13 and 1.56 ml per 500 ml of water, while distilled water was used as control. Mortality of termites was recorded for 12 and 24 hrs after exposure. Data collected were subjected to variance analysis and probit regression. Results showed that glyphosate caused 48 - 88% mortality, while paraquat caused 36 - 96% mortality after 24 hrs exposure. The mortality was not dose-dependent as 12.5ml of glyphosate and paraquat exerted higher percentage of mortality than 25ml concentration. Conversely, the mortality increased after 24 hrs indicating time-dependent mortality. The toxicity index, i.e. (LC_{50} and LC_{90}), was 16.82ml and 14.87ml for glyphosate and 2.63ml and 2.196ml for paraquat after 24 hrs of exposure. This apparently shows that paraquat is highly toxic to *M. bellicosus* at concentrations lower than the recommended rate. These findings calls for the re-evaluation of the use of paraquat-based herbicides in weed control as it negatively impacts termites populations which are key beneficial non-targets.

Keywords: Glyphosate, Herbicides, *Macrotermes bellicosus*, Paraquat, Toxicity

INTRODUCTION

Macrotermes bellicosus Smeathman (Isoptera: Termitidae), also known as the African mound-building termite, is a fungus growing and mound-building polymorphic social insect that employs swarm intelligence to exploit food sources and environments (Bandiya *et al*., 2013). They play vital roles in ecological systems, including plant decomposition, nutrient cycling, soil microbial activity, and modification of soils' chemical and physical structure; hence, referred to as "ecosystem engineers" (Holt and Lepage, 2000; Jouquet *et al*., 2013). The mound of *M. bellicosus* affects the tree flora of several ecosystems, being a source of heterogeneity in the landscape (Traore *et al*., 2008). In Nigeria, *M. bellicosus* is ubiquitous, and a mound is known to shelter approximately 360,000 neuters (Ekpo and Onigbinde, 2007). They are used as food by various animals and humans and are known to have high nutritional value (Katayama *et al*., 2008). In agro-ecosystems, *M. bellicosus* contribute to soil fertility and thus enhance productivity in agriculture. Their burrowing activities significantly improve soil infiltration, creating tunnels and galleries to allow rainwater to soak in deeply, reducing runoff and, consequently, soil erosion. Besides promoting aeration, drainage and penetration of roots, they contribute to soil formation processes via their hypogeal lifestyle (Tan and Wong, 2013).

Aside negative human health impacts; pesticides are globally considered one of the major causes of deleterious effects on natural and semi-natural ecosystems (Okrikata and Ogunwolu, 2017; Okrikata *et al*., 2022). Of all the classes of known pesticides, herbicides are the most widely used in agriculture and non-crop systems (Freemark and Boutin, 1994). Herbicides effectively control weeds in agricultural systems. In tropical countries like Nigeria, they kill many unwanted plants in agriculture, forestry, canals, lakes, fish ponds, and slow running water (Ogeleka *et al*., 2016).

Glyphosate is a broad spectrum and slow action herbicide used in agriculture, municipalities and home gardens. It kills weeds by blocking the action of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, an enzyme that aids the production of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan. These amino acids are required for protein synthesis, plant growth and maintenance. The systemic ability of the leaves to easily absorb glyphosate and be transported from the application point to the meristematic tissues of the plant makes it an effective herbicide against perennial crops (Duke, 2018).

Paraquat on the other hand, is a fast action, non-selective and contact herbicide used against weeds in orchards, plantation crops and various tillage systems (Li *et al*., 2013). It is a low cost and highly efficient herbicide that prevents photosynthesis when applied to weeds. It impairs photosynthesis by accepting electrons from photosystem I (PSI), which prevents Nicotinamide Adenine Dinucleotide Phosphate Hydrogenase (NADPH) formation, resulting in the production of reactive oxygen species in chloroplasts, thereby attacking the plant's bio-membranes (Li *et al*., 2013).

The advent of Herbicide Resistant Crops (HRCs) makes herbicides the most efficient and cost-effective weed management option in most agricultural landscapes. Its efficiency and affordability have resulted in its indiscriminate use, causing weed resistance and harmful effects on non-target organisms. The environmental fate of the active ingredients in the commonly used herbicides and the damage inflicted on different classes of non-target organisms have been reported (e.g. Aronzon *et al*., 2011; Abraham *et al*., 2018; Ejomah *et al*., 2020). In arthropods (e.g. bees, beetles, dragonflies, spiders, and wasps), herbicides have been reported to cause increased mortality, decreased abundance, reduced fecundity and altered behavioural responses (Mirande *et al*., 2010; Abraham *et al*., 2018). It has been shown, for example, that glyphosate has the capacity to alter growth in toad larvae exposed to the herbicide and the functionality of crucial genes in mussels (Baier *et al*., 2016; Milan *et al*., 2018). It has also been reported that, exposure to glyphosate disrupts the gut microbiota and decreased cognitive behaviours in *Apis mellifera* (Herbert, 2014; Motta *et al*., 2018). Furthermore, Ejomah *et al*. (2020) revealed that atrazine and 2,4-D resulted in 100% mortality in *M. bellicosus* while paraquat-induced low fertility in *Anisopteromalus calandrae* (Lacoume *et al*., 2009). All these buttress the deleterious impactz of herbicides on non-target organisms.

The foregoing showcases that; although herbicides in agriculture have tremendously improved yield and food security globally and particularly in Nigeria, there are concerns about herbicides' lethal and sub-lethal effects on non-target organisms, especially insects. Majority of studies on the deadly impact of herbicides on arthropods focus on insects in the order Hymenoptera (for example, bees and wasps) and Coleoptera (e.g. beetles) and less commonly Diptera, Neuroptera, Odonata, Hemiptera and Heteroptera (Kale *et al*., 1995; Abraham *et al*., 2018; Faita *et al*., 2020; Odemer *et al*., 2020). Also, there are many reports on the lethal effect of glyphosate and paraquat on various amphibians, reptiles and humans (Gill *et al*., 2018; Peillex and Pelletier, 2020). However, there are few reports on the effect of herbicides on the African mound termites (*M. bellicosus*) (Ejomah *et al*., 2020; Ekaye *et al*., 2022). Therefore, this study investigated the lethal effect of commonly used herbicides in Nigeria (glyphosate and paraquat) on *M. bellicosus.*

MATERIALS AND METHODS

Collection of Termites

Samples of *M. bellicosu*s were collected from a termite mound in a field within Wukari, Taraba State, Nigeria, and placed in a 10L plastic bucket. It was transported to the laboratory of the Biological Sciences Department, Federal University Wukari, Taraba State and placed in a dark room for about 6 hours until the termites gets acclimatized. The sand was seperated from worker termites using a sieve and placed in petri dishes before commencement of the assays.

Herbicides

Force up® is a herbicide made of glyphosate (360g/L) and isopropylamine salt. It is a non-selective systemic foliar-applied agricultural herbicide to control annual and perennial grasses and broad-leaf weeds applied before or after planting. ParaQ® is a contact, post-emergence herbicide constituted by 200g/L of paraquat dichloride. Force up® and ParaQ® are used to control weeds around human habitations and crop farms such as in maize, soybeans, cassava, melon, groundnut, and sugarcane farms. The manufacturer's recommended herbicide concentration is 6.25 ml in 500 ml of water. For this study, we used five concentrations (25.00, 12.5, 6.25, 3.13 and 1.56 ml of herbicide per 500 ml of water) that fall within the range of herbicides applied by farmers, sometimes indiscriminately to control weeds (personal observation). Distilled water was used as a control. Freshly prepared solutions in distilled water were used in the assays at a temperature of 25±2°C and 65±10% relative humidity.

Mortality Bioassay

Worker termites were divided into different groups of five and placed in small petri dishes (diameter 90 mm) lined with a disc of moist filter paper (Whatman No. 1). Less than an hour before the experiment, fresh dilutions of test compounds were mixed and 30µl droplet of each of the herbicide in various concentrations was applied dorsally with a micropipette on each insect. A control was set up whereby a 30µl droplet of distilled water was applied to each insect. Five replicates of five insects were used for each of the herbicide treatment and control group. A total of 150 worker termites were used for each of glyphosate and paraquat (5 worker termites x 6 herbicide treatments x 5 replicates). All treated termites were kept in a dark chamber at a temperature of 25±2˚C and relative humidity of 65±10%. Insects were monitored every 12 hours for up to 24 hours after treatment with herbicides. A worker termite was considered dead if it does not move when touched lightly with a forceps. The number of dead insects was recorded and percentage mortality was calculated using the model below:

$$Mortality\ (\%) = Number\ of\ dead\ termites\ x\ 100/Total\ number\ of\ termites$$

Statistical Analysis

The mortality data collected were transformed to $\log_{10}$ and analyzed using a generalized linear model. The differences among treatments were separated using Student Newman Keuls (SNK) test. Probit regression was used to estimate the toxicity index i.e. concentrations of each herbicide estimated to cause 50% and 90% mortality (LC_{50} and LC_{90}). All analyses were performed using SPSS statistical software, version 23.0.

RESULTS

Table 1 shows the index of toxicity (LC_{50} and LC_{90}) of *M. bellicosus* when exposed to different concentrations of glyphosate and paraquat for 12 and 24 hrs at 95% confidence level. The LC_{50} and LC_{90} values of glyphosate increased after 24 hrs (16.82ml and 14.87ml), while that of paraquat decreased after 24 hrs exposure. The low toxicity index of paraquat after 24 hrs (2.63 and 2.14ml) indicates that very low concentrations result in the mortality of 90% of the test organism.

Table 1: Comparative Lethal Effect (LC_{50} and LC_{90}) of Glyphosate and Paraquat on *M. bellicosus*

Herbicides	Exposure time (hr)	LC_{50} (ml)	LC_{90} (ml)
Glyphosate	12	10.83	8.27
	24	16.82	14.87
Paraquat	12	2.72	2.22
	24	2.63	2.14

Figure 1 shows that the percentage mortality exerted by each of glyphosate and paraquat was not dose-dependent. Results indicates that 12.5 ml of glyphosate caused 72% mortality after 12 hrs and 88% mortality after 24 hrs, whereas 25 ml concentration resulted in 12% and 48% mortality after 12 and 24 hrs exposure, respectively. Also, it was observed that, 12.5 ml of paraquat caused 88% and 96% mortality after 12 and 24 hrs, respectively, while 88% mortality was recorded after 12 and 24 hrs exposure to glyphosate.

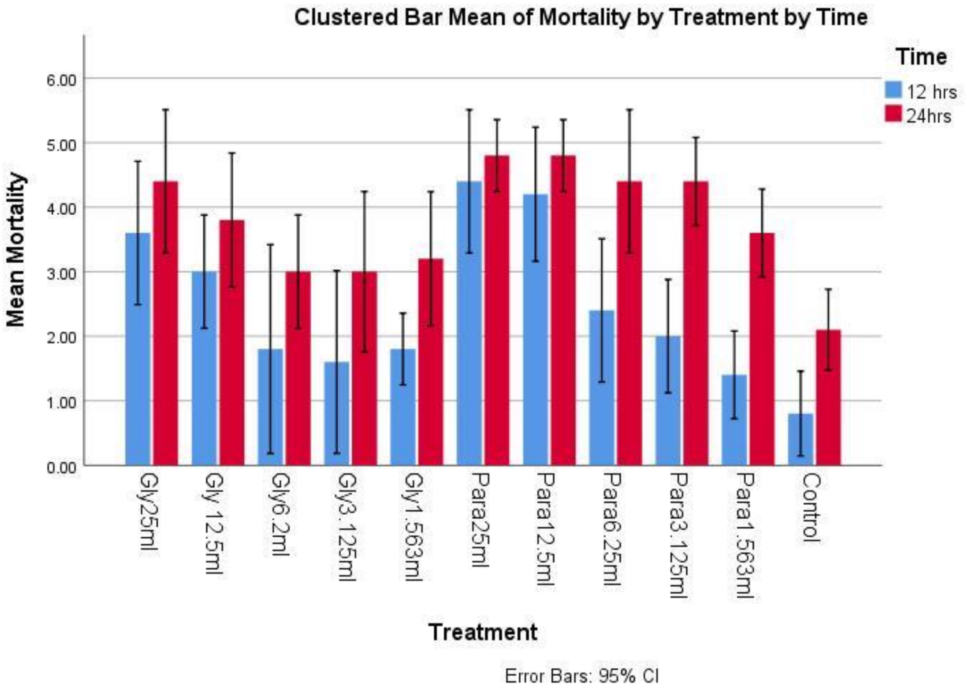

Figure 1**:** Log_{10} transformed mean (±SE) mortality of *M. bellicosus* exposed different concentrations of glyphosate and paraquat herbicides at 12 and 24 hrs exposure time (n = 5; replicates = 5 termites each)

DISCUSSION

Herbicides have become an indispensable component of agriculture for eradicating weeds in farming systems. It provides the most effective and inexpensive weed control option in most agroecological systems. In many sub-Saharan African countries, Nigeria inclusive, agricultural land preparation increasingly depends on herbicides, and the high demand for these herbicides is likely driving their increased production (Ezebuiro *et al*., 2021). The rapidly rising utilization of pesticides and their associated problems have triggered efforts to minimize the use of herbicides in agriculture without reducing yield. Also, reports of weed resistance to herbicides have resulted in the repeated application by farmers to eliminate weeds, consequently leading to harmful effects on non-target organisms (Duke, 2018).

Despite the nutritional and ecological values of termites, there are limited studies on the adverse impact of herbicides on them (For exception, see Ejomah *et al*., 2020; Ekaye *et al*., 2022). Thus, in this study, we applied five concentrations of commercially manufactured glyphosate (Force up®) and paraquat (ParaQ®) on termites. The results demonstrated that the manufacturer's recommended application rate of glyphosate caused 70% mortality while paraquat caused 80% mortality after 24 hrs exposure. In a similar study by Ekaye *et al*., (2022), glyphosate resulted in 100% mortality of worker termites after 48 hrs exposure. It can be implied therefore, that the time of exposure influenced mortality as more insects died after 48hrs exposure. Also, Straw *et al*. (2021) reported that the recommended concentration of commercially formulated glyphosate resulted in 96% mortality in honey bees, *Apis mellifera* L. (Hymenoptera: Apidae) whereas in this study, the recommended concentration of glyphosate resulted in 70% mortality of *M. bellicosus*. Perhaps the difference in test organisms could have accounted for the variation in percentage mortality.

In this study, we observed that the toxicity of paraquat increased with time whereas that of glyphosate decreased with time indicating that, glyphosate is less toxic than paraquat against *M. bellicosus*. Comparing the LC_{50} and LC_{90} values of paraquat obtained in this study with the manufacturer's recommended concentration, paraquat was highly toxic to termites as the lethal concentration values fall below the recommended dosage range at 12 and 24 hrs. This is similar with the studies on the sub-lethal effects of paraquat on fertility in males of *Anisopteromalus calend*ar (Hymenoptera: Pteromalidae) which indicated that low doses of paraquat resulted in hypo-fertility in *A. calendax* (Krucek *et al*., 2015). A similar finding was reported by Lacoume *et al*. (2009) with respect to *Drosophila melanogaster* when exposed to paraquat.

CONCLUSION

Given the lethal effect demonstrated by glyphosate (Force up®) and paraquat (ParaQ®) against termites in this study, the potential of herbicides to contribute to global decline of non-target beneficial insects is buttressed. More so, the observed higher lethal impact of paraquat vis-à-vis glyphosate calls for a re-evaluation of the use of paraquat-based herbicides in weed management.

REFERENCES

Abraham, J., Benhotons, G. S., Krampah, I., Tagba, J., Amissah, C. and Abraham, J. D. (2018). Commercially formulated glyphosate can kill non-target pollinator bees under laboratory conditions. *Entomologia experimentalis et applicata*, 166(8): 695 - 702.

Aronzon, C. M., Sandoval, M. T., Herkovits, J. and Pérez-Coll, C. S. (2011). Stage-dependent toxicity of 2, 4-dichlorophenoxyacetic on the embryonic development of a South American toad, Rhinella arenarum. *Environmental Toxicology*, 26(4): 373 - 381.

Baier, F., Gruber, E., Hein, T., Bondar-Kunze, E., Ivanković, M., Mentler, A. and Zaller, J. G. (2016). Non-target effects of a glyphosate-based herbicide on Common toad larvae (Bufo bufo, Amphibia) and associated algae are altered by temperature. *PeerJ*, *4*, e2641.

Bandiya, H. M, Majeed, Q, Ibrahim, D, Yahaya, M. M and Yahaya M. A (2013). Intra-colonial Population of *Macrotermes bellicosus* (Smeathman) [Isoptera: Termitidae] in Sokoto, Semi-Arid Zone of North-Western Nigeria. *Nigerian Journal of Basic and Applied Science*, 21(1): 55 - 59

Chaves, A., Faita, M. R., Ferreira, B. L., Poltronieri, A. S. and Nodari, R. O. (2020). Effects of glyphosate-based herbicide on royal jelly production of Apis mellifera (Hymenoptera: Apidae) in field conditions. *Journal of Apicultural Research*, 60(2): 277 - 279.

Duke, S. O. (2018). The history and current status of glyphosate. *Pest Management Science*, 74(5): 1027 - 1034.

Ejomah, A. J., Uyi, O. O. and Ekaye, S. O. (2020). Exposure of the African mound-building termite, *Macrotermes bellicosus* workers to commercially formulated 2,4-D and atrazine caused high mortality and impaired locomotor response. *PLoS ONE* 15(3): 1 - 14.

Ekaye, S.O., Uyi, O. and Ezugwu, C. H. (2022). High Mortality and Impaired Locomotor Response of Organophosphates Herbicide, Glyphosate on the African Mound Termite, Macrotermes bellicosus Workers . *European Journal of Science, Innovation and Technology*, *2*(1): 149 - 159.

Ekpo, K. E. and Onigbinde, A. O. (2007). Characterization of Lipids in Winged Reproductives of the Termite *Macrotermes bellicosus*. *Pakistan Journal of Nutrition*, 6(3): 247 - 251.

Ezebuiro, P. U., Yakubu, A. I. and Audu, M. (2021). Evaluation of three pre-emergence herbicides for weed control in soya bean [*Glycine max* (L.) Merr.] in Dutsinma and Sokoto, Sudan Savanna. *FUDMA Journal of Sciences*, 5(4): 368 – 374.

Freemark, K. and Boutin, C. (1994). Nontarget-plant risk assessment for pesticide registration. *Environmental Management*, 18(6): 841 - 854.

Gill, J. P. K., Sethi, N., Mohan, A., Datta, S. and Girdhar, M. (2018). Glyphosate toxicity for animals. *Environmental Chemistry Letters*, 16(2): 401 - 426.

Herbert, L. T., Vázquez, D. E., Arenas, A. and Farina, W. M. (2014). Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour. *Journal of Experimental Biology*, 217(19): 3457 - 3464.

Holt, J. A., and Lepage, M. (2000). Termites and soil properties. In T. Abe, M. Higashi, & D. E. Bignell (Eds.), Termites: Evolution, sociality, symbiosis, ecology, pp. 389 – 407.

Jouquet, P., Bottinelli, N., Kerneis, G., Henry-des-Tureaux, T., Doan, T. T., Planchon, O. and Tran, T. D. (2013). Surface casting of the tropical Metaphire Posthuma increases soil erosion and nitrate leaching in a laboratory experiment. *Geoderma*, 204: 10 - 14.

Kale, P. G., Petty Jr, B. T., Walker, S., Ford, J. B., Dehkordi, N., Tarasia, S. and Sohni, Y. R. (1995). Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environmental and Molecular Mutagenesis*, 25(2): 148 - 153.

Katayama, H., Yamamoto, A., Mizushima, N., Yoshimori, T., and Miyawaki, A. (2008). GFP-like proteins stably accumulate in lysosomes. *Cell Structure and Function*, 33: 1 – 12.

Krůček, T., Korandová, M., Šerý, M., Frydrychová, R. Č., Krůček, T., Korandová, M. and Szakosová, K. (2015). Effect of low doses of herbicide paraquat on antioxident defense in Drosophila. *Archives of Insect Biochemistry and Physiology*, 88(4), 235 - 248.

Lacoume, S., Bressac, C. and Chevrier, C. (2009). Male hypofertility induced by Paraquat consumption in the non-target parasitoid Anisopteromalus calandrae (Hymenoptera: Pteromalidae). *Biological control*, 49(3): 214 - 218.

Li, J., Mu, J., Bai, J., Fu, F., Zou, T., An, F. and Zuo, J. (2013). Paraquat Resistant, a Golgi-localized putative transporter protein, is involved in intracellular transport of paraquat. *Plant Physiology*, 162(1): 470 - 483.

Milan, M., Dalla Rovere, G., Smits, M., Ferraresso, S., Pastore, P., Marin, M. G. and Matozzo, V. (2018). Ecotoxicological effects of the herbicide glyphosate in non-target aquatic species: Transcriptional responses in the mussel Mytilus galloprovincialis. *Environmental Pollution*, 237: 442 - 451.

Mirande, L., Haramboure, M., Smagghe, G., Pineda, S. and Schneider, M. I. (2010). Side-effects of glyphosate on the life parameters of eriopis connexa (Coleoptera: Coccinellidae) in Argentina. *Communications in agricultural and applied biological sciences*, 75(3): 367 - 372.

Motta, E. V., Raymann, K. and Moran, N. A. (2018). Glyphosate perturbs the gut microbiota of honey bees. *Proceedings of the National Academy of Sciences*, 115(41): 10305 - 10310.

Ogeleka, D. F., Okieimen, F. E., Ekpudi, F. O. and Tudararo-Aherobo, L. E. (2016). Short-term phyto-toxicity consequences of a non-selective herbicide glyphosate (Roundup^{TM}) on the growth of Onions (*Allium cepa* Linn.). *African Journal of Biotechnology*, 15(18): 740 – 744.

Okrikata, E., Agere, H., Malu, S. P., Adepoju I. O. and Ahmed, M. (2022). Determination and health risk assessment of pesticide residues in watermelon (*Citrullus lanatus*) fruit samples in Wukari, Nigeria. *The Bioscientist Journal*, 10(1): 41 – 54.

Okrikata, E. and Ogunwolu, E. O. (2017). Farmers' perceptions on arthropod pests of watermelon and their management practices in the Nigerian Southern Guinea Savanna. *International Journal of Agricultural Research,* 12(4): 146 – 155.

Peillex, C. and Pelletier, M. (2020). The impact and toxicity of glyphosate and glyphosate-based herbicides on health and immunity. *Journal of Immunotoxicology*, 17(1): 163 - 174.

Pereira, A. G., Jaramillo, M. L., Remor, A. P., Latini, A., Davico, C. E., da Silva, M. L. and Nazari, E. M. (2018). Low-concentration exposure to glyphosate-based herbicide modulates the complexes of the mitochondrial respiratory chain and induces mitochondrial hyperpolarisation in the Danio rerio brain. *Chemosphere*, 209: 353 - 362.

Straw, E. A., Carpentier, E. N. and Brown, M. J. (2021). Roundup causes high levels of mortality following contact exposure in bumble bees. *Journal of Applied Ecology*, 58(6): 1167 - 1176.

Krůček, T., Korandová, M., Šerý, M., Frydrychová, R. and Szakosová, K. (2015). Effect of low doses of herbicide paraquat on antioxidant defense in Drosophila, *Archives of Insect Biochemistry and Physiology*, 88(4): 235 – 248.

Tan, A., and Wong, N. (2013). Parameterization studies of solar chimneys in the tropics. *Energies*, 6: 145 – 163.

Traore, S., Nygard, R., Guinko, S. and Lepage, M. (2008). Impact of *Macrotermes termitaria* as a Source of Heterogeneity on Tree Diversity and Structure in a Sudanian Savannah Under Controlled Grazing and Annual Prescribed Fire (Burkina Faso). *Forest Ecology and Management*, 255: 2337 - 2346.

Vázquez, D. E., Latorre-Estivalis, J. M., Ons, S. and Farina, W. M. (2020). Chronic exposure to glyphosate induces transcriptional changes in honey bee larva: A toxicogenomic study. *Environmental Pollution*, 261: 114148.