Oxidative stress induction by crude extract of *Xylaria* sp. triggers lethality in the larvae of *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

Background: *Aedes aegypti* is currently controlled with synthetic larvicides; however, mosquitoes have become highly resistant to these larvicides and difficult to eradicate. Studies have shown that insecticides derived from fungal extracts have various mechanisms of action that reduce the risk of resistance in these mosquitoes. One possible mechanism is uncontrolled production of reactive oxygen species (ROS) in the larvae, which can cause changes at the cellular level. Thus, the crude extract of *Xylaria* sp. was evaluated to investigate the oxidative effect of this extract in *A. aegypti* larvae by quantifying the oxidative damage to proteins and lipids.

Methods: The larvicidal potential of the crude extract of *Xylaria* sp. was evaluated, and the extract was subsequently tested in human lung fibroblasts for cytotoxicity and ROS production. ROS level was quantified in the larvae that were killed following exposure to the extract in the larvicide test.

Results: The crude extract of *Xylaria* sp. caused cytotoxicity and induced ROS production in human lung fibroblasts and *A. aegypti* larvae, respectively. In the larvicide trial, the extract showed an LC50 of 264.456 ppm and an LC90 of 364.307 ppm, and was thus considered active. The extract showed greater oxidative damage to lipids and proteins, with LC90 values of 24.7 µmol MDA/L and 14.6278 × 10⁻³ nmol carbonyl/mg protein, respectively.

Conclusions: Crude extracts of *Xylaria* sp. induced oxidative stress that may have caused the mortality of *A. aegypti* larvae.

Keywords: Cytotoxicity. Carbonyl. Dengue. TBARS. Endophytic fungus.
pathogens, nematode parasites, and fish that feed on larvae. Chemical control is currently carried out by synthetic larvicides derived from organophosphorus compounds, carbamates, and pyrethroids; however, they cause great environmental damage, are costly, and have been causing high resistance rates in A. Aegypti.

Owing to the resistance of mosquitoes and the low effectiveness and high cost of programs to control the vectors of these diseases, new strategies are needed to combat vectors using mechanisms that are less polluting, less toxic, and pose less risk to human health. Some studies involving natural bioactive products made from endophytic fungi have revealed promising alternatives. Endophytic fungi are microorganisms that inhabit the internal parts of host plants in all or part of their life cycle and produce a vast amount of bioactive metabolites.

According to Aury, larvicides and insecticides from endophytic fungal extracts are widely used today and have an advantage over synthetic compounds, given their various action mechanisms, which reduces the risk of resistance in mosquitoes. According to the literature, an example of the action mechanism of endophytic fungi is the release of reactive oxygen species (ROS), which results in cellular oxidative damage in insects and larvae because ROS are free radicals that can cause cellular toxicity when antioxidant enzymes are produced in less quantity than ROS.

Although many studies on the metabolites of fungal endophytes have been conducted, only one has been reported, in which endophytes of the genus Xylaria showed a larvicidal activity against A. Aegypti. Xylaria endophytes belong to the family Xylariaceae (Sordariomycetes, Xylariales), which has at least 85 genera and probably more than 1,000 species, and can produce metabolites with a wide spectrum of biological activities. This study aimed to evaluate the larvicidal activity of the raw extract of Xylaria sp. against third-instar larvae of A. aegypti, and to verify the possible entomotoxic effects of this extract.

**METHODS**

**Plant material**

The endophytic fungus Xylaria sp. was isolated from the plant species Passovia Stelis (L.) Kuijt (Loranthaceae), which was collected from the campus of the Federal University of Amazonas (3°08’57”S, 58°26’38”W), in the city of Manaus, Amazonas, Brazil. A voucher specimen (No. 11422) was deposited in the herbarium of the university.

**Isolation and identification of the fungus**

The endophytic fungus was isolated according to the method described by Maier and Souza. Fungal isolates were identified by grouping their macromorphological and micromorphological similarities. The endophytic fungi were preserved in distilled water.

**Preparation of Xylaria sp. raw extract**

Xylaria sp. was harvested and transferred to 35 Erlenmeyer flasks containing 300 mL of potato dextrose broth and maintained at a controlled temperature of 28 °C for 28 days. After fermentation, the medium was filtered to remove the visible mycelium and subjected to liquid/liquid partition with ethyl acetate evaporated in a rotary evaporator. The resulting raw extracts were subsequently subjected to larvicide testing.

**Larvicidal test**

**Mosquito breeding**

The eggs of A. aegypti were obtained from a colony kept in an insectarium at the Laboratory of Malaria and Dengue of the National Amazon Research Institute. The eggs were then immersed in water until hatching. For bioassays, the resulting larvae were fed crushed larval food until the third instar phase under the growth conditions described by Medeiros et al.: temperature, 26 ± 2 °C; humidity, approximately 80%, photoperiod, 12 h.

**Selective larvicidal bioassay**

Five disposable cups with a capacity of 50 mL containing 9.8 mL of water were used for the selective bioassay. Briefly, 10 third-instar larvae of A. aegypti were introduced into each cup. Next, 100 µL of Xylaria sp. extract diluted in dimethyl sulfoxide (DMSO) was pipetted separately into each cup at concentrations of 500, 250, 125, 62.5, and 31.25 ppm, followed by 100 µL of crushed larval food. Each bioassay was performed in triplicates. DMSO (1%) was used as a negative control. The final volume of each cup was 10 mL. Bioassay readings were taken at 24, 48, and 72 h after the exposure of the larvae to the extract.

**Dose-response bioassay**

Dose–response bioassays were performed according to the WHO protocol. The five concentrations used in this assay were developed from the lowest concentration that caused at least 50% mortality of the larvae in the selective bioassays. The test was performed in quintuplicates. Data were analyzed by one-way ANOVA (p ≤ 0.05) using GraphPad Prism software (version 6.0; San Diego, CA, USA) and expressed as mean (%) ± standard deviation. Lethal concentrations (LC50 and LC90) and confidence intervals (CI = 95%) were calculated using Probit analysis in the Poloplois software version 1.0 (LeOra Software, Berkeley, CA).

**In vitro cytotoxicity**

Cytotoxicity testing of Xylaria sp. extract was performed according to the methodology described by Ahmed et al. Resazurin (AlamarBlue®) and MRC-5 cells (ATCC-CCL-171-human lung fibroblasts) were used to evaluate the toxicity of the extract in humans if used as a larvicide. Doxorubicin (20 µg.mL−1) was used as a positive control, and DMSO (0.1%) was used as a negative control. Fluorescence was measured at 570 nm using an Elisa Microlate reader (DTX-800; Beckman Coulter). Data were analyzed by a two-way ANOVA test (p ≤ 0.05) using GraphPad Prisma version 6.0, and were used to calculate the IC50 of the extract.

**Determination of ROS levels using DCFH-DA**

ROS levels in MRC-5 cells following treatment with Xylaria sp. extract were determined using 2′,7′- dichlorohydrofluorescein diacetate (DCFH-DA) according to the method described by Eruslanov and Kusmartsev. Paclitaxel (256 µg.mL−1) and hydrogen peroxide (17 µg.mL−1) were used as positive controls, and buffered saline solution (PBS) was used as a negative control. Cell fluorescence was determined at 570 nm using an Elisa Microlate reader (Dx800 multimode detector; Beckman Coulter). Data were analyzed by two-way ANOVA (p ≤ 0.05) using GraphPad Prisma version 6.0.
**Lipid and protein oxidative damage assay**

*Homogenisation of larvae*

Larvae of *A. aegypti* subjected to the larvicide test at lethal doses of 50% and 90% were cold-macerated in a pestle and mortar with 10 mL of phosphate buffer (pH 7.3, 75 mM for each 1 g of larvae). The homogenate was centrifuged at 15,000 rpm for 30 min at 4 °C. The supernatant was used to quantify oxidative damage to proteins and lipids.

*Lipid oxidative damage*

Lipid oxidative damage was measured using malondialdehyde (MDA), in accordance with the technique described by Ohkawa and et al. The total MDA level in the samples was calculated as the ratio of sample absorbance to that of a standard MDA solution (1,1,3,3-tetrahydroxypropane) multiplied by the standard solution concentration (concentration curve). Larval homogenate and phosphate buffer (75 mM) were used as negative controls. The results are expressed in μmol/L. The data were subjected to statistical analysis by two-way ANOVA with Dunnett’s test for the control using GraphPad Prism (version 6.0). All experiments were performed in triplicate.

*Oxidative damage to proteins*

The protein oxidative damage test was performed according to the methodology initially described by Levine et al. (1990) and Lowry (1951), in which oxidative damage to proteins was measured by quantifying carbonyl groups based on reaction with 2,4-dinitrophenyl hydrazine (DNPH) prepared in HCl 2.5 M. Protein content was determined using the Lowry method with BSA as a standard. Larval homogenate and HCl 2.5 M were used as negative controls. All experiments were performed in triplicates. Statistical tests were performed using the two-way ANOVA test with Dunnett’s test concerning the control using GraphPad Prism version 6.0. All experiments were performed in triplicate.

**RESULTS**

**Larvicidal test: selective and dose-reactive bioassays**

According to the results of the selective bioassay, *Xylaria* sp. extract resulted in a satisfactory mortality rate of over 50% in *A. aegypti* larvae, but only concentrations between 250 and 500 ppm exhibited larvicidal activity. Thus, the concentrations used in the subsequent dose test were in the range of 250-500 ppm (400, 375, 350, and 325 ppm) (Table 1).

The mortality rates at 24, 48, and 72 h after exposure revealed that the highest mortality rate occurred at 24 h after treatment of *Xylaria* sp. extract a concentration of 400 ppm, with a mortality rate of 91% (Table 1). This finding revealed a satisfactory larvicidal activity of *Xylaria* sp. extract against the of *A. aegypti* larvae, as according to the WHO, an extract is considered active if the mortality rate is greater than 80%.

According to the probit analysis, the LC50 value of *Xylaria* sp. extract was 264.456 ppm, and the LC90 value was 364.307 ppm, with a significance level of 95% (Table 2). The parametric test (χ2) showed no significant difference between the two doses (LC50 and LC90), as both concentrations caused mortality in the larvae. Thus, the concentrations corresponding to the LC50 and LC90 are expected to cause 50% and 90% mortality of the larvae, respectively.

**In vitro test in cells: cytotoxicity and ROS production**

The *Xylaria* sp. extract was cytotoxic at a concentration of less than 50 µg.mL−1 (Figure 1), capable of inhibiting the growth of more than 50% of cells, compared with the standard drug doxorubicin at a concentration of 20 µg.mL−1. Considering the mass death of MRC-5 cells, ROS level was also evaluated in these cells to verify whether the death of these cells was due to increased ROS production.

ROS assay was also performed using different concentrations (50, 25, 12.5, 6.25, 3.12, and 1.56 µg.mL−1) of *Xylaria* sp. extract (Figure 2). The results revealed that *Xylaria* sp. extract at all tested concentrations induced ROS production compared to the negative control. However, the highest ROS level was observed in cells treated with *Xylaria* sp. extract at 50 and 25 µg.mL−1; moreover, ROS production decreased as the extract concentration decreased.

| Treatment | Concentration (ppm) | n (samples) | 24 h | 48 h | 72 h |
|-----------|--------------------|-------------|------|------|------|
| DMSO      | 0 (%)              | 100         | 0    | 0    | 0    |
| Xylaria sp.| 400                | 100         | 91   | 5    | 0    |
|           | 375                | 100         | 80   | 9    | 2    |
|           | 350                | 100         | 69   | 17   | 0    |
|           | 325                | 100         | 61   | 13   | 3    |
|           | 300                | 100         | 49   | 25   | 2    |
|           | 275                | 100         | 35   | 13   | 7    |

Table 1: Results of dose-reactive bioassays of different concentrations of *Xylaria* sp. extract in *A. aegypti* larvae.

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TABLE 2: LC₉₀ and LC₉₀ values of Xylaria sp. extract against A. aegypti larvae after 72 h of treatment. No mortality was observed in the negative control (DMSO) group.

| Extracts  | Concentration (ppm) | Mortality (%) ± SD | LC₉₀ (ppm) (LCL-UCL) | LC₉₀ (ppm) (LCL-UCL) | Regression equation | χ² |
|-----------|---------------------|-------------------|----------------------|----------------------|---------------------|-----|
| Xylaria sp. | 400                | 96.7 ± 0.0        |                      |                      |                     |     |
|           | 375                | 93.3 ± 1.7        |                      |                      |                     |     |
|           | 350                | 82.2 ± 2.3        |                      |                      |                     |     |
|           | 325                | 77.8 ± 2.3        | 264.456 (245.835-277.284) | 364.307 (350.9-384.617) | y = -17.315x + 9,212 | 3.263 ns |
|           | 300                | 76.7 ± 2.6        |                      |                      |                     |     |
|           | 275                | 53.3 ± 4.6        |                      |                      |                     |     |
|           | 0                  | 0.0 ± 0.0         |                      |                      |                     |     |

Concentration in ppm; SD: Standard deviation; LC₉₀: Lethal concentration that kills 50% of the larvae; LC₉₀: Lethal concentration that kills 90% of the larvae; Chi-square; n.s: Not significant (α = 0.05) (means that the data were fitted to the software without the need for adjustments.

**Evaluation of oxidative damage in A. aegypti larvae**

Statistical analysis revealed damage to the lipids of the larvae treated with Xylaria sp. extract, as the MDA levels were significantly (p < 0.0001) different between the larvae treated with Xylaria sp. at the LC₉₀ and the control (macerated larvae not exposed to the extract), with MDA levels almost twice as high (24.7 μmol/L) as that in the negative control (12.5 μmol/L). Larvae subjected to Xylaria sp. extract at the LC₉₀ showed no significant difference from the control, with a confidence limit of 95% (**Figure 3A**). These results were corroborated by the results obtained in the ROS production test of the in vitro extract, which showed an increase in total ROS (**Figure 2**).

Protein oxidative damage was also examined in A. aegypti larvae, and the results were also subjected to statistical analysis. Carbonyl levels were significantly (p <0.0001) different between the larvae exposed to the extract at LC₉₀ (14.6278 ×10⁻³ nmol carbonyl/mg protein) and the control (2.4491 ×10⁻¹ nmol carbonyl/mg protein). Carbonyl levels were also significantly (p < 0.0001) different between the larvae treated at the LC₉₀ (4.2893 ×10⁻¹ nmol carbonyl/mg protein) and the control. These values had a confidence limit of 95%, as shown in **Figure 3B**. Thus, these findings corroborate the protein oxidative damage assay results.

**DISCUSSION**

According to previous studies, endophytic fungi are considered potential producers of bioactive chemicals, especially those of the genus Xylaria, which includes several species of fungi found in plants. Moreover, they produce a wide variety of metabolites with different chemical structures, such as cytochalasins, terpenoids, alkaloids, coumarins, and benzoquinones, with a range of biological activities, such as phytotoxic, antifungal, antimalarial, and antibacterial activities.

The results of the present study support that Xylaria sp. extract can be an alternative source of larvicide for A. aegypti control, showing comparable efficacy to other extracts previously reported (6.6% mortality at a concentration of 250 μg.mL⁻¹).
Bar graph illustrating lipid peroxidation in A. aegypti larvae treated with Xylaria sp. extract at the lethal dose 50 (LD$_{50}$), Xylaria sp. extract at the lethal dose 90 (LD$_{90}$), and 1% DMSO (control). Data are expressed as MDA levels in mmol/L in the samples.

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**REFERENCES**

1. Brasil M da S. Boletim Epidemiológico Secretaria de Vigilância em Saúde – Ministério da Saúde. 2015;48(29).
2. Costa IMP, Calado DC. Incidência dos casos de dengue (2007-2013) e distribuição sazonal de culicídeos (2012-2013) em Barreiras, Bahia. Epidemiol e Serv Saude. 2016;25(4):735-44.
3. BRASIL M da S. Boletim Epidemiológico Secretaria de Vigilância em Saúde Vigilância em Saúde no Brasil 2003. 2019. Available from: http://portalarquivos2.saude.gov.br/
4. Rose RI. Pesticides and public health: Integrated methods of mosquito management. Emerging Infectious Diseases. CDC. 2001;7(1):17-23.
5. Para E, Braga IA, Valle D. Aedes Aegypti: Insecticides, Mechanisms of Action and Resistance. Artigo de Revisão. Rev Bras Epidemiol. 2007;16(4).
6. Zara ALS, Santos SM, Fernandes-Oliveira ES, Carvalho RG, Coelho GE. Estratégias de controle do Aedes aegypti: uma revisão. Epidemiol Serv Saude. Ministério da Saúde do Brasil. 2016;25:391-404.
7. Cardenas A, Orozco PJ, Rodriguez CE, Moneriz PC, Díaz CF, Mendez CDM. Aproximación al estudio del daño oxidativo causado por larvicidas naturales y temenos sobre proteomas de larvas del mosquito Aedes aegypti. Cienc y Salud Virtual. 2013;5(1):17-23.
8. Taui PL. Critical aspects of dengue control in Brazil. Cad saude pública / Ministério da Saúde, Fundação Oswaldo Cruz, Esc Nac Saúde Pública. 2002;18(3):867-71.
9. Penna MLF. A challenge for the public health system in Brazil: dengue control. Cad saúde pública / Ministério da Saúde, Fundação Oswaldo Cruz, Esc Nac Saúde Pública. 2003;19(1):305-9.

10. Barreto ML, Teixeira MG. Dengue no Brasil: Situação epidemiológica e contribuições para uma agenda de pesquisa. Estud Avancados. 2008;22(64):53-72.

11. Pinheiro JB, Polonio JC, Orlandelli RC, Pamphile JA, Gollas HC. Atividade larvicida de fungos endofíticos: uma revisão. Brazilian J Dev. 2020;6(6):3576:74.

12. Costa MBS, de Oliveira CM. Endophytic Fungi in The Fight Against Neglected Tropical Diseases. Mini-Reviews Med Chem. 2020;20(16):1683-93.

13. Ayumi TB, Gabriela BST, Daiane OM, Gustavo BL, Yasuo OJ, Rafaela SRMA. Larvicidal and ovicidal effects of Crotalaria pallida extracts on the vector Aedes aegypti. Brazilian J Dev. 2020;6(5).

14. Abutaha N, Mashaly AMA, Al-Mekhlafi FA, Farooq M, Al-shami M, Wadaan MA. Larvicidal activity of endophytic fungal extract of Cochliobolus spicer (Pleosporales: Pleosporaceae) on Aedes caspius and Culex pippis (Diptera: Culicidae). Appl Entomol Zool. 2015;50(3):405-14.

15. Tian J, Liu XC, Liu ZL, Dai Z, Zhou L. Larvicidal spirobisopranaphthelal of the endophytic fungus Berkleasmium sp. against Aedes albopictus. Pest Manag Sci. 2016;72(5):961-5.

16. Marques AM, Kaplan MAC. Active metabolites of the genus Piper against Aedes aegypti: natural alternative sources for dengue vector control. Univ Sci. 2015;20(1):61-82.

17. Ray S, Singh V, Singh S, Sarma BK, Singh HB. Biochemical and histochemical analyses revealing endophytic Alcaligenes faeicalis mediated suppression of oxidative stress in Abelmoucos sculentus challenged with Sclerotium rolfsii. Plant Physiol Biochem. 2016;109:430-41.

18. Ahmed AM. Lipid Peroxidation and Oxidative Protein Products as Biomarkers of Oxidative Stress in the Autogenous Mosquito, Aedes caspius, Upon Infection with the Mosquitocidal Bacterium, Bacillus thuringiensis kurstaki. undefined. Pak J Zool. 2012;44:525-36.

19. Thongwat D, Pimolsri U, Somboon P. Screening for mosquito larvicidal activity of thai mushroom extracts with special reference to steccherinum sp against Aedes aegypti (L) (Diptera: Culicidae). Southeast Asian J Trop Med Public Health. 2015;46(4):586-95.

20. Lee SR, Kreuzenberg NB, Jang M, Oh T, Ko S, Ahn JS, et al. Xyloneside A: A New Glycosylated Incisterol Derivative from Xylaria sp. FB. ChemBioChem. 2020;21(16):2253-8.

21. Caño ERP, Albuquerque MP, Alves RP, Pereira AB, Victoria FDC. Morphological and molecular characterization of three endolichenic isolates of Xylaria (Xylariaceae), from cladonia curta ahti & marcelli (cladoniaceae). Plants. 2019;8(10).

22. Ibrahim A, Tanney JB, Fei F, Seifert KA, Cutler GC, Capretta A, et al. Metabolomic-guided discovery of cyclic nonribosomal peptides from Xylaria ellisi sp. nov., a leaf and stem endophyte of Vaccinium angustifolium. Sci Rep. 2020;10(1):1-17.

23. Maier W, Hammer K, Dammann U, Schulz B, Strack D. Accumulation of sesquieterpenoid cyclohexenone derivatives induced by an arbuscular mycorrhizal fungus in members of the Poaceae. Planta. 1997;202(1):36-42.

24. Queiroz, FMM, Oliveira KW, Cunha MMF, Schwarz A. Evaluation of (anti)genotoxic activities of Phyllanthus niruri L. in rat bone marrow using the micronucleus test. Braz J Pharm Sci. 2013;49(1):135-48.

25. Simões SS, Costa MBS, Souza AQL, Sousa WC, Oliveira CM. Brazilian Applied Science Review. Braz Ap Sci Rev. 2020;4(3):1262-70.

26. Medeiros E, Rodrigues IB, Litaiff-Abreu E, Da AC, Pinto S, Tadei WP. Larvicidal activity of clove (Eugenia caryophyllata) extracts and eugenol against Aedes aegypti and Anopheles darlingi. African J Biotechnol. 2013;12(8):836-40.

27. Robertson, JL, Preiser, HK, Russell RM. Polo Plus Probit and Logit Analysis, User’s Guide. 2003.

28. Ansar Ahmed S, Gogal RM, Walsh JE. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H]thymidine incorporation assay. J Immunol Methods. 1994;170(2):211-24.

29. Eruslanov E, Kusmartsev S. Identification of ROS using oxidized DCFDA and flow-cytometry. Methods Mol Biol. 2010;594:57-72.

30. Sofic E, Sapcanin A, Tahirovic I, Gavranksapetanovic I, Jellingr K, Reynolds GP, et al. Antioxidant capacity in postmortem brain tissues of Parkinson’s and Alzheimer’s diseases. J Neural Transm Suppl. 2006;(71):39-43.

31. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyst Biochem. 1979;95(2):351-8.

32. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of Carboxyl Content in Oxidatively Modified Proteins. Methods Enzymol. 1990;186(C):464-78.

33. Lowry OH, Rosebrown NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.

34. Organização Mundial da Saúde – OMS. Orientações técnica para utilização do larvicida pyriproxyfen (0,5 G) no controle de Aedes aegypti. 2013. Available from: http://www.cvs.saude.sp.gov.br/

35. Zhao J, Shan T, Mou Y, Zhou L. Plant-Derived Bioactive Compounds Produced by Endophytic Fungi. Mini-Reviews Med Chem. 2011;11(2):159-68.

36. Sánchez-Ortiz BL, Sánchez-Fernández RE, Duarte G, Lappe-OLiveras P, Macias-Rubalcava ML. Anti-fungal, anti-oomycete and phytotoxic effects of volatile organic compounds from the endophytic fungus Xylaria sp. strain PB3 from isolated from Haematoxylon brasiliense. J Appl Microbiol. 2016;120(5):1313-25.

37. Tüberk A, Tüberk NCF, Souza AQL, Gama AM, Rodrigues-Filho E, Costa FM et al. Larvicidal effects of endophytic and basidiomycete fungus extracts on Aedes and Anopheles larvae (Diptera, Culicidae). Rev Soc Bras Med Trop. 2013;46(4):411-419.

38. Moro AM, Brucker N, Charão M, Bucão R, Freitas F, Bailer M, et al. Evaluation of genotoxicity and oxidative damage in painters exposed to low levels of toluene. Mutat Res - Genet Toxicol Environ Mutagen. 2012;746(1):42-8.

39. Barbosa KBF, Costa NMB, Afenas CGR, Paula SO, Minim VPR, Bressan J. Estresse oxidativo: Conceito, implicações e fatores modulatórios. Revista de Nutrição. 2010;23:629-43.

40. Stadtman ER, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids. Amino Acids. 2003;25:207-18.

41. Cong S, Dong W, Zhao J, Hu R, Long, Y, Chi, X. Characterization of the Lipid Oxidation Process of Robusta Green Coffee Beans and Shelf Life Prediction during Accelerated Storage. Molecules 2020;25(5):1157.

42. Zhang J, Ahmad S, Wang LY, Han Q, Zhang JC, Luo YP. Cell death induced by α-terthienyl via reactive oxygen species-mediated mitochondrial dysfunction and oxidative stress in the midgut of Aedes aegypti larvae. Free Radic Biol Med. 2019;137:87-98.