UNIVERSIDADE FEDERAL DE ALFENAS

LUANA DE PAULA FERREIRA

EFEITOS ALELOPÁTICOS DE EXTRATOS FOLIARES DE *Syzygium cumini* (L.) EM *Lactuca sativa* L.: UM ESTUDO SOBRE GERMINAÇÃO, CRESCIMENTO INICIAL E INTEGRIDADE CELULAR

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Dissertação apresentada como parte dos requisitos para obtenção do Título de Mestre em Ciências Ambientais pela Universidade Federal de Alfenas/UNIFAL-MG.
Orientador(a): Prof. Dr. Sandro Barbosa
Coorientador: Dr. Antônio Rodrigues da Cunha Neto
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LUANA DE PAULA FERREIRA

“ Efeitos alelopáticos de extratos foliares de *Syzygium cumini* (L.) Skeels em *Lactuca sativa L.* : um estudo sobre germinação, crescimento inicial e integridade celular ”

A Banca examinadora abaixo-assinadaaprova a Dissertação apresentada como parte dos requisitospara a obtenção do título de Mestre em Ciências Ambientais pela Universidade Federal de Alfenas. Área de concentração: Ciências Ambientais.

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Dedico este trabalho aos meus pais, Diva e Paulo. A todas as pessoas que me ajudaram e ao meu filho, Victor.

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“O sucesso é a soma de pequenos esforços repetidos dia após dia.”

(Rober Collier)

RESUMO

O uso de herbicidas químicos na agricultura traz diversas consequências para a saúde humana e ao meio ambiente. Os aleloquímicos tem sido considerados uma estratégia potencial para o desenvolvimento de bioherbicidas. Dentre inúmeras espécies pertencentes à família Myrtaceae, a *Syzygium cumini* (L.) Skeels, popularmente conhecida por jambolão, tem se destacado neste contexto por apresentar propriedades antioxidantes e antimicrobianas, sendo uma espécie rica em compostos fenólicos e metabólitos secundário. Ao que se refere a seus constituintes fitoquímicos, são encontradas nesta espécie algumas substâncias químicas, produzidas naturalmente pelas plantas para se protegerem do ataque de pragas e doenças, ajudando também a suportar condições adversas do ambiente. A presença destes constituintes torna o jambolão numa espécie com potencial alelopático. O presente trabalho tem como objetivo analisar o efeito alelopático de extratos aquoso e hidroetanólico obtidos de folhas de *Syzygium cumini* (L.) Skeels aplicados em biotestes com *L. sativa*. Para avaliar o efeito alelopático da espécie, foram realizados estudos dos efeitos fitotóxico e citogenotóxico na germinação e crescimento inicial de *L. sativa* em contato com os extratos obtidos. O delineamento experimental do teste fitotóxico foi realizado em blocos casualizados, com 4 repetições e 5 concentrações (5, 10, 20, 40 mg. mL^{-1}) e água destilada como controle (0%). Para o cálculo do índice mitótico foi feito uma regressão em CE50 do controle, com 3 tratamentos (aquoso, hidroetanólico e controle) em 4 repetições. Os parâmetros avaliados foram: Protrusão Radicular (PR), porcentagem de germinação no 4° (G4) e 7° (G7) dia, Índice de Velocidade de Germinação (IVG), Número de Plântulas Normais (NPN) e Anormais (NPA), Índice de Efeito Alelopático (RI), Comprimento de Parte Aérea (CPA), Alongamento de Raiz (AR), Biomassa Fresca (BF), Índice Mitótico (IM) e Frequência de Anormalidades Cromossômicas (FAC). Foi realizada a análise da atividade enzimática e da peroxidação lipídica. A análise estatística consistiu da análise de variância - ANOVA ($p < 0,05$), e para os demais resultados foi feita comparação de médias pelo teste de *Scott-Knott*. Para todos parâmetros morfológicos, os extratos se mostraram eficientes a partir de 10 mg. mL^{-1}. Para todos os parâmetros germinativos houve atraso da germinação nas concentrações de 20 e 40 mg. mL^{-1}. As anormalidades incluíram micronúcleos, pontes em anáfase e telófase, c-metáfases, *stickiness*, cromossomos perdidos e cromossomo atrasado em anáfase e telófase. Além disso, os extratos também causaram alterações oxidação das sementes de alface. Isso sugere que o jambolão pode ser fonte de prospecção de aleloquímicos.

Palavras-chave: jambolão; alface; bioensaios; fitotoxicidade; citotoxicidade.

ABSTRACT

The use of chemical herbicides in agriculture has several consequences for human health and the environment. Allelochemicals have been considered a potential strategy for the development of bioherbicides. Among numerous species belonging to the Myrtaceae family, *Syzygium cumini* (L.) Skeels, popularly known as jambolão, has stood out in this context for presenting antioxidant and antimicrobial properties, being a species rich in phenolic compounds and secondary metabolites. Regarding its phytochemical constituents, some chemical substances are found in this species, naturally produced by plants to protect themselves from attack by pests and diseases, also helping to withstand adverse environmental conditions. The presence of these constituents makes jambolão a species with allelopathic potential. The present work aims to analyze the allelopathic effect of aqueous and hydroethanolic extracts obtained from *Syzygium cumini* (L.) Skeels leaves applied in biotests with *L. sativa*. To evaluate the allelopathic effect of the species, studies were carried out on the phytotoxic and cytogenotoxic effects on the germination and initial growth of *L. sativa* in contact with the extracts obtained. The experimental design of the phytotoxic test was carried out in randomized blocks, with 4 replications and 5 concentrations (5, 10, 20, 40 mg. mL^{-1}) and distilled water as control (0%). To calculate the mitotic index, a regression was performed on the EC50 of the control, with 3 treatments (aqueous, hydroethanolic and control) in 4 replications. The parameters evaluated were: Root Protrusion (PR), percentage of germination on the 4th (G4) and 7th (G7) day, Germination Speed Index (IVG), Number of Normal (NPN) and Abnormal (NPA) Seedlings, Allelopathic Effect Index (RI), Shoot Length (CPA), Root Elongation (AR), Fresh Biomass (BF), Mitotic Index (MI) and Frequency of Chromosomal Abnormalities (FAC). Analysis of enzymatic activity and lipid peroxidation was carried out. Statistical analysis consisted of analysis of variance - ANOVA ($p < 0.05$), and for the other results, means were compared using the Scott-Knott test. For all morphological parameters, the extracts were efficient from 10 mg. mL^{-1}. For all germination parameters, there was a delay in germination at concentrations of 20 and 40 mg. mL^{-1}. Abnormalities included micronuclei, bridging in anaphase and telophase, c-metaphases, stickiness, lost chromosomes, and delayed chromosome in anaphase and telophase. Furthermore, the extracts also caused oxidation changes in lettuce seeds. This suggests that jambolão can be a source of prospecting for allelochemicals.

Keywords: jambolão; lettuce; bioassays; phytotoxicity; cytotoxicity.

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1 INTRODUÇÃO

Os defensivos agrícolas têm sido utilizados na agricultura por vários anos e, a cada período, novos compostos são produzidos e registrados com a finalidade de garantir um bom controle de pragas e uma produção alimentar satisfatória (BRASIL, 2020). Entre as categorias de defensivos amplamente comercializados, destacam-se os herbicidas (IBAMA, 2020b) que, por sua vez, são compostos bioativos com capacidade de impedir o desenvolvimento vegetal por provocar diferentes efeitos nas plantas, comumente usados para matar as ervas daninhas ao interromper o equilíbrio dos processos bioquímicos e fisiológicos das plantas (Ervin *et al.*, 2019).

De acordo com o balanço de vendas de defensivos agrícolas no Brasil, divulgado pelo Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), em 2021 foram comercializadas mais de 720,87 mil toneladas de defensivos agrícolas no Brasil, o que representa um aumento de 5,03% em relação ao ano anterior (IBAMA, 2022a). Desses, aproximadamente 56% herbicidas, seguido de fungicidas e inseticidas (IBAMA, 2022b).

Segundo o Sindicato Nacional da Indústria de Produtos para a Defesa Vegetal (SINDIVEG), o estado de Minas Gerais movimenta um setor bilionário e ocupa o 2° lugar no *ranking* de consumo de agroquímicos (SINDIVEG, 2021). Diante disso, a comunidade científica tem buscado métodos alternativos para o controle de problemas fitossanitários como as plantas daninhas.

A maioria dos estudos investigam alelopatia em espécies vegetais (Barbosa *et al*., 2018; Nunes *et al*., 2014; Silveira *et al*., 2014;). Atualmente as substâncias alelopáticas são utilizadas para controle de plantas daninhas, pois interferem positivamente no controle de plantas infestantes e contribuem para o manejo ecológico livre dos compostos nocivos dos herbicidas sintéticos (Ervin *et al.*, 2019).

Todo órgão da planta pode ser fonte de substâncias alelopáticas, suas sementes, frutos, flores, folhas, caules e raízes contêm aleloquímicos, cuja composição e quantidade variam de espécie para espécie (Amâncio *et al*., 2020). Segundo Barbosa *et al.* (2018) as substâncias aleloquímicas podem interferir nas plantas superiores, inibindo a germinação, causando injúrias durante o crescimento da raiz e dos meristemas, inibindo assim o desenvolvimento inicial da planta.

Dentre as espécies pertencentes à família Myrtaceae, a *Syzygium cumini* (L.) Skeels, comumente conhecida como jambolão, é uma espécie que vem sendo bastante estudada por suas propriedades (Carvalho *et al*., 2016). Além de ser uma planta rica em compostos fenólicos,

apresenta propriedades antioxidantes e antimicrobianas (Freita *et al.,* 2021). Assim, a espécie tornou-se uma planta de grande interesse em pesquisas alelopáticas.

Ressalta-se que na revisão bibliográfica realizada para construção desta proposta, não foram encontrados estudos sobre a fitotoxicidade e/ou citogenotoxicidade de extratos foliares (ou de outros órgãos) da espécie *Syzygium cumin*i (L.) Skeels sobre a dinâmica da germinação e crescimento inicial de plântulas.

O objetivo deste trabalho foi analisar os efeitos fitocitotóxico dos extratos foliares de *Syzygium cumini* (L.) Skeels obtidos por diferentes formas de extração e secagem por liofilização e *spray drying* em biotestes de *L. sativa*, bem como a análise da atividade enzimática superóxido dismutase (SOD) e da peroxidação lipídica. Além disso, este trabalho poderá introduzir conhecimentos sobre o desenvolvimento de bioherbicidas eficazes, menos invasivos ao meio ambiente, que possam ser úteis à agricultura de baixo impacto.

2 DESENVOLVIMENTO

A seguir, a revisão de literatura atualizada acerca dos temas abordados nesta dissertação, com intuito de gerar embasamento teórico para a análise e discussão dos resultados obtidos.

2.1 CARACTERIZAÇÃO BOTÂNICO-AGRONÔMICA DO JAMBOLÃO E SUA CONSTITUIÇAO FITOQUÍMICA

Segundo Carvalho *et al.* (2016), o jambolão pertence à família Myrtaceae de origem Asiática, especificamente da Índia. No Brasil encontra-se em diferentes regiões do país como uma árvore ornamental, conhecida como jamelão, azeitona preta, ameixa preta e amora silvestre indiana. Cientificamente, é conhecida pelas nomenclaturas *Syzygium cumini* e *Syzygium jambolanum*.

A espécie é descrita como uma árvore que mede cerca de 10 metros de altura e de 3 a 4,5 metros de diâmetro de projeção da copa, com folhagem abundante, e ramos de coloração acinzentada-claro, de caule aéreo e ereto. Apresenta folhas simples, pecioladas, lanceoladas ou lanceoladas oblongas a elípticas, com margens onduladas, ápices cuspidados e bases cuneadas. Suas flores estão dispostas em inflorescências, de coloração branca a creme (Prato, 2013).

Com formato de bagas elipsoidais, o fruto jambolão possui comprimento de 3 a 5 centímetros, polpa esbranquiçada ou rosada e uma semente localizada no centro da fruta. Esse dispõe de coloração verde e quando atinge a maturação apresenta uma coloração de roxa a preta (Singh *et al*., 2018).

Figura 1 – Árvores, frutos e folhas de jambolão

Fonte: Autoria própria, 2022.

No Brasil, a floração do jambolão inicia entre os meses de setembro a novembro e a maturação da fruta entre dezembro e fevereiro. São frutas não climatéricas, sendo necessário atingir o tempo de maturação completa para colheita (Sabino *et al.*, 2018), além disso, é uma

fruta altamente perecível, devido à fragilidade da polpa e do epicarpo, provendo assim baixa proteção contra injúrias físicas e agentes infecciosos, deste modo não pode ser armazenada por longo tempo (Baraiya *et al.*, 2015).

O jambolão tem se destacado por ser rico em vários constituintes benéficos à saúde, como compostos fenólicos, e por apresentar propriedades antioxidantes e antimicrobianas (Freita *et al.*, 2021). As diferentes partes da planta do jambolão são amplamente utilizadas na medicina popular. Seu uso intenso estimulou a pesquisa científica, identificando indicações terapêuticas devido às inúmeras ações farmacológicas (Rodrigues *et al*., 2015).

Um dos compostos fenólicos que existe no jambolão são as antocianinas, mas também taninos hidrolisáveis, flavonóis e flavonoides (Lestario *et al*., 2017). A produção de extratos naturais com altos níveis de antocianinas do jambolão podem ser considerada uma abordagem muito útil para as indústrias alimentícia, farmacêutica, cosmética e para produção de corantes naturais (Brito *et al.*, 2017). Além das propriedades antioxidantes, a espécie apresenta efeito antimicrobiano no crescimento de *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus* e *E. coli enterotoxigênica*, principais patógenos responsáveis por doenças transmitidas por alimentos (Haque *et al.*, 2017).

Ao que se refere a seus constituintes fitoquímicos, são encontradas algumas substâncias químicas, como flavonoides, antocianinas, quercetina, rutina, mirecetina e seus glicosídeos (açúcares) e taninos hidrolisáveis, produzidas naturalmente pela planta para proteção contra o ataque de pragas e doenças, ajudando também a suportar condições adversas do ambiente (Priya *et al.*, 2017). As concentrações desses compostos variam de acordo com o *habitat* e alterações climáticas às quais estão expostas as plantas (Tavares *et al*., 2017).

2.2 FITOQUÍMICA APLICADA AO POTENCIAL ALELOPÁTICO

A interação entre as plantas ocorre não apenas por competição, mas muitas plantas invasoras possuem vantagens adaptativas baseadas na produção e liberação de biomoléculas no ambiente. Essas substâncias são produzidas em sua maioria via metabolismo secundário e denominadas de aleloquímicos, que podem influenciar estimulando ou inibindo o crescimento e o desenvolvimento de outras plantas e organismos, num fenômeno conhecido como alelopatia (IAS, 2019) e representam alguma vantagem contra a ação de microrganismos, vírus, insetos, e outros patógenos ou predadores, interferindo na conservação, dormência, germinação de sementes e no crescimento de plântulas (Li *et al*., 2020).

A espécie *Syzygium cumini* (L.) Skeels tem sido estudada quanto aos seus efeitos protetores contra o estresse oxidativo e a formação de radicais livres. Segundo Pessoa (2021), a partir da prospecção fitoquímica foi evidenciado que as folhas de *S. cumini* possuem uma rica composição de metabólitos secundários, taninos e saponinas, nos quais as empresas tem um grande interesse.

Todas as plantas produzem metabólitos secundários, que variam em qualidade e quantidade de espécie para espécie, de um local de ocorrência ou ciclo de cultivo para outro, pois muitos desses metabólitos tem sua síntese desencadeada por eventuais circunstâncias em que as plantas estão expostas. Derivados como terpenos/esteroides, flavonoides e taninos sofrem variação sazonal (Pessoa, 2021).

Dentre os metabólitos secundários estão os taninos hidrolisáveis e condensados, cumarinas, lignanas, ligninas, alcaloides derivados dos aminoácidos aromáticos, fenilpropanoides, flavonoides, antraquinonas, aminoácidos alifáticos e os alcaloides derivados destes terpenoides, esteroides e ácidos graxos (Bitencourt *et al*., 2021).

A resistência ou tolerância aos metabólitos secundários que funcionam como aleloquímicos é mais ou menos específica, existindo espécies mais sensíveis que outras, como por exemplo *Lactuca sativa* L. (alface), por isso muito utilizada em biotestes de laboratório (Bitencourt *et al*., 2021).

2.3 BIOENSAIOS APLICADOS A PROSPECÇÃO DE SUBSTÂNCIAS COM EFEITOS ALELOPÁTICOS/ALELOQUÍMICOS

Os bioensaios vegetais têm se tornado ferramentas frequentemente utilizadas em laboratórios, com fins de verificar a atividade de diferentes compostos sobre os parâmetros fisiológicos e citogenéticos de diferentes biotestes. Essa técnica é caracterizada por apresentar baixo custo, eficiência e resultados rápidos de pesquisa, razão pela qual a torna muito utilizada pelos pesquisadores (Amâncio *et al*., 2020). Além disso, a presença de fitotoxicidade para alguns vegetais de importância econômica foi verificada em bioensaios envolvendo extratos foliares de plantas (Alves *et al.*, 2019).

A literatura descreve que nos estudos sobre os efeitos de aleloquímicos podem ser utilizadas como bioensaios de fitotoxicidade e citogenotoxicidade as sementes nativas ou de espécies cultivadas, entre elas se destacam a alface.

De acordo com trabalhos, como o de Barbosa *et al*. (2019), a alface contempla diversas peculiaridades, dentre elas, rápida germinação, onde sofrem mudanças fisiológicas e tornam-se

altamente sensíveis ao estresse ambiental. Além disso, apresenta crescimento linear em ampla faixa de variação de pH, baixa sensibilidade aos potenciais osmóticos, grande número de células em divisão na zona meristemática radicular.

Testes de toxicidade com alface permitem que os efeitos fitotóxicos possam ser analisados por diferentes variáveis como germinação, alongamento de raiz e biomassa vegetal. Além de ser padronizada, internacionalmente, como planta bioindicadora (Gonçalves *et al*., 2016).

2.4 PROCESSOS DE SECAGEM POR LIOFILIZAÇÃO E *Spray Dryer*

A liofilização é um processo de secagem comumente utilizado em laboratórios de pesquisa e instituições de conservação biológica (Meyer *et al*., 2022). O método envolve a remoção da água das células da planta por meio da sublimação sob pressão, interrompendo as reações químicas e atividades biológicas, permitindo o armazenamento seguro e prolongado, com a possibilidade de reativação futura (Corrêa, 2013).

Atualmente, o sistema Nano *Spray Dryer* B-90 HP apresenta alto desempenho, que pode produzir pequenas quantidades de partículas submicrométricas a partir de soluções diluídas, emulsões e suspensões. A técnica é realizada com o objetivo de melhorar as propriedades de manuseio dos produtos e melhorar a estabilidade à oxidação, protegendo os compostos bioativos (Bürki *et al*., 2011). O princípio básico deste método consiste em dissolver os materiais do núcleo/parede em água para preparar uma emulsão na forma líquida e, em seguida, alimentar esta emulsão em um meio quente (100–300 °C) para evaporar o etanol. O produto seco final pode ser coletado na forma de pó ou como partículas aglomeradas, dependendo do projeto de operação do secador e das condições de operação. A alta temperatura da câmara de secagem facilita a evaporação do etanol em gotas (Bourbon *et al*., 2020).

2.5 TESTE DE CITOTOXICIDADE

Os testes de citotoxicidade são muito explorados, devido a sua competência em investigar a viabilidade celular. São utilizados comumente para identificar se as substâncias de interesse exibem efeitos citotóxicos diretos, indicativo fundamental para compreensão quanto aos mecanismos de atuação para determinados genes, proteínas e vias relacionadas à morte celular após exposição a agentes tóxicos (Fagundes *et al*., 2017).

O índice mitótico (IM), caracterizado pelo número total de células em divisão no ciclo celular, tem sido utilizado como parâmetro para avaliar a citotoxicidade de diversos agentes (Fernandes *et al*., 2017). Segundo Hoshina (2022), IM significativamente inferiores ao controle negativo podem indicar alterações, decorrentes da ação química no crescimento e desenvolvimento dos organismos expostos. Por outro lado, IM superiores ao controle negativo são resultados de um aumento na divisão celular, o que pode ser prejudicial às células, levando a uma proliferação celular desordenada. A avaliação de IM e da frequência de anormalidades cromossômicas (FAC) no teste com *L. sativa* pode ser realizada tanto em células meristemáticas quanto em células de raízes (Leme, 2018). Além de avaliar efeitos mutagênicos, a análise permite investigar os mecanismos de ação de agentes químicos e pode ser considerada um método confiável para determinar a presença de agentes citotóxicos no ambiente e, portanto, um teste sensível para estimar os níveis de poluição. Vários estudos utilizam a avaliação do IM para detectar citotoxicidade e a maioria deles apresentou resultados satisfatórios às análises propostas (Fagundes *et al*., 2017; Fernandes *et al*., 2017; Leme, 2018).

2.6 ESTRESSE OXIDATIVO E ATIVIDADE ANTIOXIDANTE (SOD)

O estresse oxidativo decorre de um desequilíbrio entre a geração de compostos oxidantes e a atuação dos sistemas de defesa antioxidante. A geração de radicais livres e/ou espécies reativas é resultante do metabolismo de oxigênio. A mitocôndria da planta, por meio da cadeia transportadora de elétrons, é a principal fonte geradora (Eleutherio *et al*., 2021). O sistema de defesa antioxidante tem a função de inibir e/ou reduzir os danos causados pela ação deletéria dos radicais livres e/ou espécies reativas não radicais. Esse sistema, usualmente, é dividido em enzimático (superóxido dismutase, catalase e glutationa peroxidase) e não-enzimático. No último caso, é constituído por grande variedade de substâncias antioxidantes, que podem ter origem endógena ou dietética (Nguyen *et al*., 2020).

A Superóxido dismutases (SOD) são enzimas que participam do controle de espécies reativas de oxigênio no corpo (ROS), que quando em excesso podem causar lesões celulares importantes. As enzimas da SOD dão início na conversão de radicais superóxidos em oxigênio e peróxido de hidrogênio para que então possamos excretar o excesso de ROS (Eleutherio *et al*., 2021; Nguyen *et al*., 2020).

A produção de peróxido de hidrogênio é uma das principais características do metabolismo celular, notadamente sob condições adversas, como nos casos de estresses bióticos ou abióticos.

3 JUSTIFICATIVA

O uso indiscriminado de defensivos agrícolas na produção de alimentos no Brasil, bem como o grande número de substâncias registradas nos últimos anos tem acarretado grandes problemas ambientais e impactos à saúde humana. Na perspectiva de reduzir esses impasses, o estudo de extratos de plantas com atividade alelopática surge como uma opção para o manejo integrado de pragas e que, pode contribuir para a redução de doses e aplicações de herbicidas químicos sintéticos.

A *Syzygium cumini* (L.) Skeels, conhecida como jambolão, é uma espécie que produz naturalmente constituintes fitoquímicos para se proteger do ataque de pragas e doenças, ajudando também a suportar condições adversas do ambiente. A planta ainda produz metabólitos secundários com efeito alelopático, como os flavonoides, que podem se mostrar menos agressivos ao meio ambiente quando comparados a herbicidas comerciais.

No entanto, a atividade alelopática das folhas de *Syzygium cumini* (L.) Skeels em bioensaios vegetais com *L. sativa* ainda não foram verificadas em estudos anteriores.

Assim, o presente estudo justifica-se por ser composto por bioensaios científicos com o intuito de averiguar o potencial fitocitogenotóxico e bioquímico das substâncias existentes nas folhas da espécie *Syzygium cumini* (L.) Skeels. Além disso, o estudo alelopático gera a possibilidade de introduzir conhecimentos para a elaboração de bioherbicidas que possam colaborar com atividades agronômicas de baixo impacto, novas perspectivas para a agricultura sustentável e a preservação da biodiversidade.

4 OBJETIVOS

A sessão a seguir trata dos objetivos gerais e específicos deste trabalho.

4.1 OBJETIVO GERAL

Analisar o efeito alelopático de extratos obtidos das folhas de *Syzygium cumini* (L.) Skeels por método de liofilização *Spray Dryer*, aplicados em bioensaios com *L. sativa*.

4.2 OBJETIVOS ESPECÍFICOS

O presente trabalho se propôs a:

a) Verificar o efeito fitocitogenotóxico de extratos foliares de *Syzygium cumini* (L.) Skeels;
b) Realizar análise da atividade enzimática (SOD), peroxidação lipídica e danos associados ao efeito citotóxico da espécie;
c) Colocar esse trabalho na vanguarda dos estudos como fonte de aleloquímicos para produção de herbicidas naturais.

5 CONSIDERAÇÕES FINAIS

Os aleloquímicos são substâncias produzidas naturalmente pelo metabolismo secundário da planta, participando da atividade alelopática da espécie. Teoricamente, todas as plantas são potencialmente capazes de sintetizar metabólitos secundários.

A espécie *Syzygium cumini* (L.) Skeels tem se tornado uma planta de grande relevância para estudos alelopáticos, considerando que há muitos benefícios em suas aplicações, sendo uma espécie rica em compostos fenólicos e metabólitos secundário.

Em meus estudos, os extratos da espécie apresentaram efeito fitocitotóxico, sugerindo sua viabilidade como fonte de aleloquímicos, sendo uma alternativa viável e sustentável para a agricultura de baixo impacto, com benefícios tanto para o meio ambiente quanto para a saúde humana.

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Phyto- and cytogenotoxicity of leaf extracts from Syzygium cumini (L.) Skeels in Lactuca sativa L. assays

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	Editor-in-chief Dear Editor Please, find enclosed an original manuscript entitled “Phyto- and cytogenotoxicity of leaf extracts from Syzygium cumini (L.) Skeels in Lactuca sativa L. assays” by Ferreira et al. submitted for publication in this journal. On behalf of all authors, I declare that this paper has not been published previously, including in electronic form, in English or in any other language, and it is not under consideration for publication elsewhere. No part of the research has been published in any form elsewhere, unless it is fully acknowledged in the manuscript. The work is an original research carried out by the authors. All authors have approved the publication of the article in its present form and declare that they have no conflicts of interest. All authors agree with the contents of the manuscript and its submission to the journal. All authors listed have contributed significantly to the work and agree to be in the author list. The work was carried out at the Universidade Federal de Alfenas and the institution has approved the submission of the paper for possible publication. The study was supported financially in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through the scholarships, grants to a number of the authors and other fundings. The funding sources were not involved in the design of the study, in the collection, analysis or interpretation of data, in writing the report, or in the decision to submit the paper for publication. We look forward to hearing from you concerning our submission in due course. Yours sincerely, Authors
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Phyto- and cytogenotoxicity of leaf extracts from *Syzygium cumini* (L.) Skeels in *Lactuca sativa* L. assays

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Abstract

Syzygium cumini (L.) Skeels is a tropical, perennial fruit tree commonly known as jambolan. Its leaves are notable for containing metabolites with potential phyto- and cytotoxic properties, which are associated with allelopathic activity. In this context, the objective of this study was to investigate the effects of aqueous and hydroethanolic extracts, dried by lyophilization and spray drying, prepared from the leaves of *Syzygium cumini*. For this purpose, 30 *Lactuca sativa* seeds were used per treatment group (5, 10, 20 and 40 mg mL^{-1}), with distilled water serving as a negative control. Germination and early growth parameters were evaluated, including radicle protrusion, initial and final germination percentages, germination speed index, allelopathic effect index, shoot length, root elongation, number of abnormal seedlings, fresh biomass, mitotic index, frequency of chromosomal abnormalities, lipid peroxidation, hydrogen peroxide content, and superoxide dismutase activity. Regarding germination parameters, delayed germination was observed at concentrations of 20 and 40 mg mL^{-1}. The extracts exhibited phytotoxicity starting at 10 mg mL^{-1} in terms of growth parameters. A progressive increase in extract concentration led to changes in enzymatic activity and cell division. Based on these findings, the phytotoxic potential of *Syzygium cumini* leaf extracts was confirmed.

Keywords: Aqueous extracts; hydroethanolic extracts; jambolan; lettuce; chromosomal abnormalities

Introduction

Syzygium cumini (L.) Skeels (Myrtaceae) is a tropical, perennial fruit tree commonly known as Indian blackberry, black plum, jambolan, or jambolao. It is native to South and Southeast Asia, particularly Pakistan, India, Afghanistan, Myanmar, Indonesia, the Philippines, Hawaii, and Australia (Qamar et al., 2022). The species is widely distributed in various parts of the world, especially in tropical regions. Used in traditional medicine, it exhibits anticancer, antimutagenic, antioxidant, antihyperglycemic, antihyperlipidemic, anti-inflammatory, and cardioprotective properties (Rahma et al., 2023). The bioactive molecules present in *S. cumini* include phenolic acids, terpenes, alkaloids, tannins, and saponins. Polyphenols are reported to be the major constituents of its leaves and are considered primarily responsible for its biological potential (Kumari et al., 2023).

The leaves of *Syzygium cumini* are notable for containing a wide range of phytochemicals, including kaempferol, myricetin, quercetin, caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, gallic acid, tannins, nilocetin, α-pinene, α-cadinol, pinocarvone, pinocarveol, α-terpineol, myrtenol, eucarvone, muurolol, myrtenal, cineole, and geranylacetone (Ahmed et al., 2019; Kumari et al., 2023). Studies have demonstrated its cytotoxic potential in both *in vitro* and *in vivo* models, using different parts of the plant: seeds (Ruthurusamy et al., 2015); fruits (Ezhilarasan et al., 2019); and leaves (Artanti et al., 2019; Fiqri et al., 2020). Among the biological activities of *S. cumini*, its phytotoxicity remains relatively unexplored. The ability of its phytochemicals to interfere directly or indirectly with the life cycle of other plants, causing morphological, biochemical, and cytogenetic damage, a phenomenon known as allelopathy, has yet to be thoroughly investigated.

The use of plant-based models is effective for detecting phyto-cytotoxic agents (Santos et al., 2017). The combined assessment of morpho-germination variables (germination rate and early seedling development), cytogenetic parameters (mitotic index and cell cycle aberrations), and biochemical markers is essential for understanding the impact of phytotoxic phytochemicals (Govêa et al., 2020; Amâncio et al., 2021; Santos et al., 2024; Moreira et al., 2024). This methodology is considered reliable, as cytogenetic and

biochemical variables influence one another and are directly related to morphological parameters. For instance, the growth of an organ such as the root is intrinsically linked to both an increase in cell number and cell elongation during developmental and differentiation stages (Ribeiro et al., 2013; Carvalho et al., 2019; Amâncio et al., 2020; Calvelli et al., 2023).

Given the above, this study aimed to investigate the effects of aqueous (decoction) and hydroethanolic (1:1) extracts from *Syzygium cumini* leaves, both dried by lyophilization and spray-drying. The phytotoxic, cyto-genotoxic, and biochemical activities were described, and their effects were classified using allelopathic and genotoxic synthesis effect methods. For this purpose, *Lactuca sativa* L. was used as the test system, and germinative, morphological, biochemical, and cytogenetic variables were evaluated.

Materials and methods

Extraction method

Syzygium cumini leaves were collected in the region of Alfenas, Minas Gerais, Brazil (21°24'54.73"S; 45°57'38.63"W) (Figure 1). A voucher specimen was deposited in the herbarium of the Federal University of Alfenas – MG under registration number 3251.

Figure 1. *Syzygium cumini* individuals at the collection site.

The leaves were dried in a forced-air circulation oven (Solab® SL 102) at 45 °C until a constant weight was reached. They were then ground using a knife mill (Cienlab® CE 430) and stored in amber glass containers.

a) Aqueous extract: The aqueous extract was obtained by the decoction method at 20% concentration (Brasil, 2019). It was subsequently lyophilized using a freeze-dryer

(Liotop® L101) under a pressure of 198 μHg and a temperature of −54 °C, and stored at −20 °C.

b) Hydroethanolic extract: Obtained by exhaustive percolation method (Brasil, 2019). The percolates were concentrated by rotary evaporation using a Buchi system (Rotavapor R-100, Heating Bath B-100, and Vacuum Pump V-100) at 45 °C. The total solid content was determined (Brasil, 2019), and adjusted to 11% solid residue by adding a drying adjuvant, colloidal silicon dioxide, followed by spray drying (BUCHI B-290).

Sample preparation: Both extracts were solubilized in distilled water at concentrations of 5, 10, 20, and 40 mg mL^{-1} using a magnetic stirrer for 5 minutes, then centrifuged at 1500 rpm for 15 minutes at 24 °C. After centrifugation, only the supernatant was used in the bioassays.

Phytotoxicity bioassay

The bioassays were conducted in 70 mm Petri dishes containing two sheets of Germitest® paper moistened with 3 mL of the previously described solutions, with distilled water used as a negative control. Thirty seeds of *Lactuca sativa* cv. Babá de Verão (ISLA PAK Ltda, lot 54053) were uniformly distributed in each dish and incubated in a biochemical oxygen demand (BOD) growth chamber (Ethik 411 FPD) at 24 °C under a 12-hour photoperiod.

Root protrusion was evaluated after 24 hours, while germination percentage was assessed on the 4th and 7th days (Brasil, 2019). The germination speed index was determined by counting germinated seeds every 6 hours during the first 48 hours and every 12 hours until the 7th day, calculated and adapted according to Maguire (1962).

On the 7th day, the seedlings were scanned using a professional scanner (Epson Expression 10000 XL, Epson America Inc., Long Beach, CA, USA) to measure root elongation and shoot length using ImageJ software. The number of abnormal seedlings was also recorded. Fresh biomass was weighed using an analytical balance.

The allelopathic effect synthesis (LI) was calculated by summing the response index (RI) values obtained in the experiment, according to Williamson and Richardson (1988).

$$RI = 1\text{-}C/(T(T>C)$$

Where: C = control response; T = treatment response.

$$LI = [\sum(RI)]/N$$

Where: N = number of variables analyzed.

Cytotoxicity bioassay

Cytotoxicity assessment was performed using the inhibitory concentration for root length (IC50) of each extract, established from a regression curve (Carvalho et al., 2019). *Lactuca sativa* seeds were exposed to aqueous and hydroethanolic extracts at IC50 concentrations, with distilled water used as a control. Root tips were collected after 24 hours of exposure, fixed in Carnoy's solution 3:1 (ethanol:acetic acid), and stored at -18 ± 2 °C. Slides were prepared using the squash method (Silva Souza et al., 2025). The mitotic index (MI) and the frequency of chromosomal abnormalities (FCA) were calculated based on the evaluation of 8000 cells per treatment, 2000 per replicate (MI = cells in mitosis / total number of cells counted; FCA = total abnormalities / total number of cells counted).

The synthesis of the cytogenotoxic effect was calculated as follows: mitotic cytogenotoxic effect (MCE); clastogenic alterations (CA); and aneugenic alterations (AA). Where: MCE = chromosomal abnormalities during mitosis / number of dividing cells (MCE = FCA/MI); CA = chromosomal bridges and breaks / number of anaphase and telophase cells analyzed; AA = stickiness, c-metaphase, lost and lagging chromosomes / total number of metaphase, anaphase, and telophase cells (Silva Souza et al., 2025).

Biochemical Analysis

a) Hydrogen peroxide (H_2O_2): 0.2 g of fresh seedling tissue obtained on the 7th day was ground in liquid nitrogen with 20% polyvinylpyrrolidone (PVP) (w/v), homogenized in 1.5 mL of 0.1% (w/v) trichloroacetic acid (TCA), and centrifuged at 12,000 rpm for 15 minutes at 4 °C. H_2O_2 concentration was determined by measuring absorbance at 390 nm in a reaction medium containing 100 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide (Velikova et al. 2000).

b) Lipid peroxidation: Assessed by quantifying thiobarbituric acid reactive substances (TBARS). 0.2 g of fresh seedling tissue obtained on the 7th day was ground in liquid nitrogen

with 20% PVP (w/v) and homogenized in 1.5 mL of 0.1% TCA (w/v). The homogenate was centrifuged at 12,000 rpm for 15 minutes at 4 °C. Aliquots of 250 µL of the supernatant were added to the reaction medium containing 0.5% (w/v) thiobarbituric acid (TBA) and 10% (w/v) TCA, and incubated at 95 °C for 30 minutes. The reaction was stopped by rapid cooling on ice, and absorbance was measured at 535 nm and 600 nm using a spectrophotometer (Buege and Aust, 1978).

c) Superoxide dismutase (SOD): Enzymatic extracts were obtained according to Biemelt et al. (1998). 0.2 g of seedlings were ground in liquid nitrogen with insoluble polyvinylpolypyrrolidone (PVPP), and 1.5 mL of extraction buffer was added (400 mM potassium phosphate, pH 7.8, 10 mM EDTA, and 200 mM ascorbic acid). The homogenate was centrifuged at 13,000 rpm for 10 minutes at 4 °C, and the supernatant was collected for enzymatic activity analysis. SOD activity was estimated based on the enzyme's ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries, 1977). Absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT photoreduction by 50% under the assay conditions.

Statistical Analysis

The experimental design was completely randomized (CRD) in a 2 × 5 factorial scheme, with two extraction methods and five concentrations (0, 5, 10, 20, and 40 mg mL^{-1}). Based on the root development inhibition data, the IC50 was determined through regression analysis to characterize cytotoxicity, comparing only the IC50 concentration of both extracts with the distilled water control. Both experiments were conducted with four replicates of 30 seeds each. All experimental data were subjected to ANOVA, and means were compared using the Scott-Knott test at a 5% significance level, with the aid of the Sisvar software version 5.4, as described by Ferreira (2019).

Results

As a result of the centrifugation process, the hydroethanolic extract was separated into two phases: the supernatant, referred to as the hydroethanolic extract, and the precipitate, characterized by the presence of Aerosil. Despite its intense coloration, the precipitate did not exhibit phytotoxic effects in the bioassay. This result confirms that the spray-drying

method using Aerosil as an adjuvant, although it may have carried some compounds during dilution and centrifugation, did not compromise the bioavailability of the phytochemicals involved in the phytotoxicity process.

In response to the *Syzygium cumini* extract, *Lactuca sativa* seeds exhibited changes in germination patterns, with root protrusion and germination speed index being the most affected parameters. Root protrusion at 24 hours (Fig. 2A) was inhibited starting at a concentration of 10 mg mL^{-1} when seeds were exposed to the aqueous extract. In contrast, the hydroethanolic extract caused inhibition only at 20 mg mL^{-1}, a concentration 60% higher than that of the aqueous extract, indicating lower toxicity. This trend is also observed in the germination speed index, where all concentrations of the aqueous extract increased the mean germination time of the bioassay (Fig. 2D) and differed statistically from the corresponding concentrations of the hydroethanolic extract.

For initial germination (recorded on day 4) and final germination (recorded on day 7), only the 40 mg mL^{-1} concentration of the aqueous extract showed significant effects, inhibiting 87% of initial germination (Fig. 2B) and 54% of final germination (Fig. 2C) of *L. sativa* seeds. In contrast, the hydroethanolic extract yielded values equivalent to the control at all concentrations for both germination assessments.

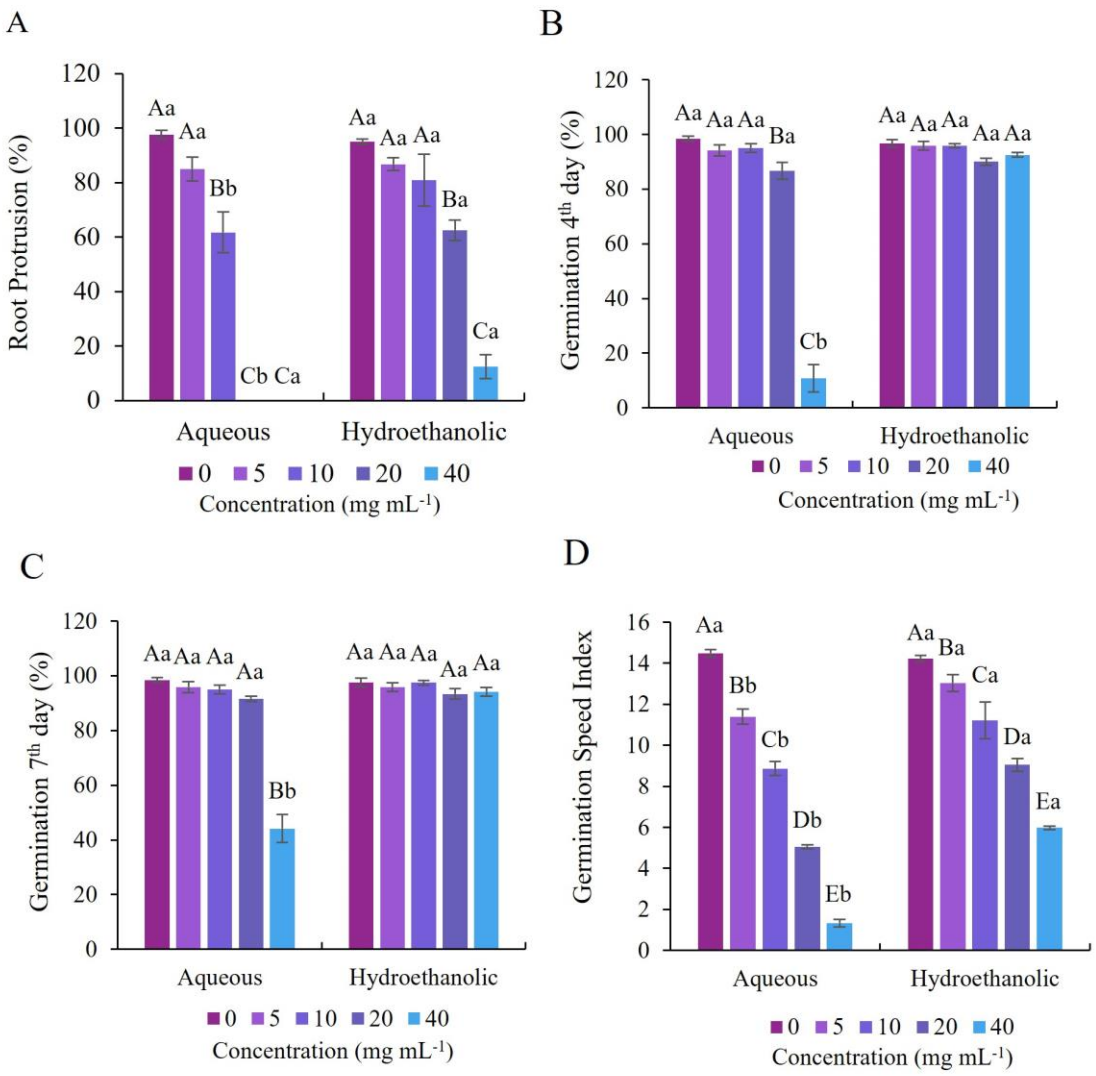


Figure 2. (A) Percentage of root protrusion, germination percentage at (B) 4 and (C) 7 days, and (D) germination speed index of *Lactuca sativa* exposed to different concentrations of aqueous and hydroethanolic leaf extracts of *Syzygium cumini*. Error bars represent standard error. Uppercase letters indicate no significant difference among concentrations, and lowercase letters indicate no significant difference between extraction methods, according to the Scott-Knott test at 5% significance.

The extracts significantly increased the number of abnormal seedlings starting at the concentration of 10 mg mL^{-1} (Fig. 3A). However, the aqueous extract at 40 mg mL^{-1} did not

yield any developed seedlings. Seedling fresh mass was also affected, indicating a directly proportional effect to the applied concentration (Fig. 3B).

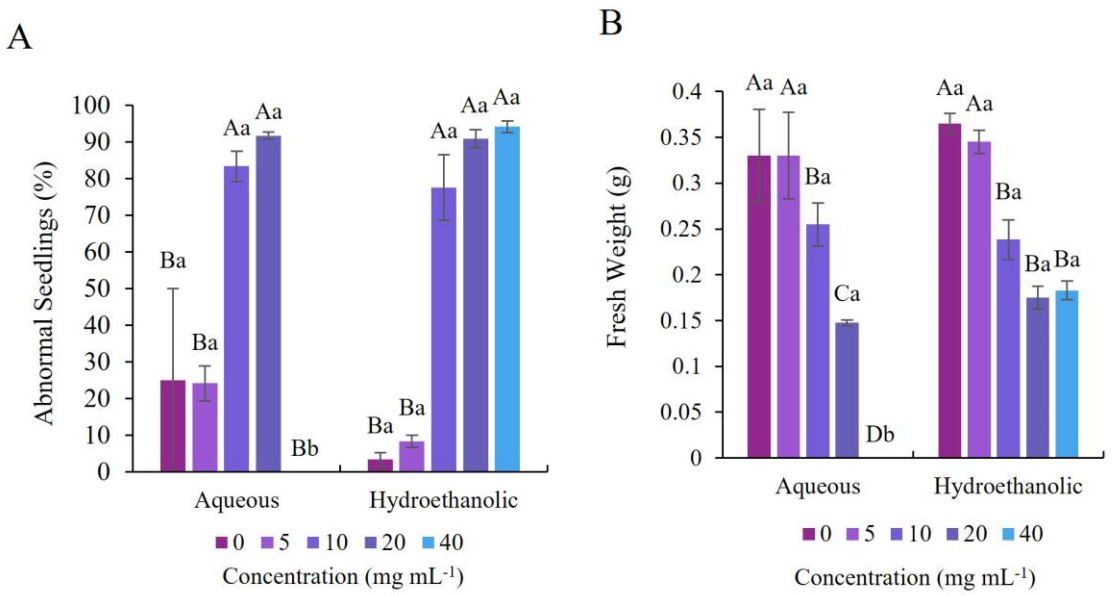


Figure 3. (A) Percentage of abnormal seedlings and (B) fresh mass (g) of *Lactuca sativa* exposed to different concentrations of aqueous and hydroethanolic leaf extracts of *Syzygium cumini*. Error bars represent standard error. Uppercase letters indicate no significant difference among concentrations, and lowercase letters indicate no significant difference between extraction methods, according to the Scott-Knott test at 5% significance.

Both extraction methods caused significant deleterious effects on the morphological parameters of *Lactuca sativa*, inhibiting the growth of the plant axis from the concentration of 10 mg mL^{-1} onwards. In contrast, the 5 mg mL^{-1} concentration stimulated root elongation in the hydroethanolic extract and shoot length in the aqueous extract, respectively (Fig. 4A). It is important to note that the 40 mg mL^{-1} concentration resulted in complete inhibition of shoot length in seedlings exposed to the aqueous extract. In addition to physiological alterations, such as germination inhibition, delayed development, and reduced growth, morphological changes were also observed, including phenotypic alterations in the seedlings such as chlorosis and deformation of the cotyledonary leaves (Fig. 4B and C).

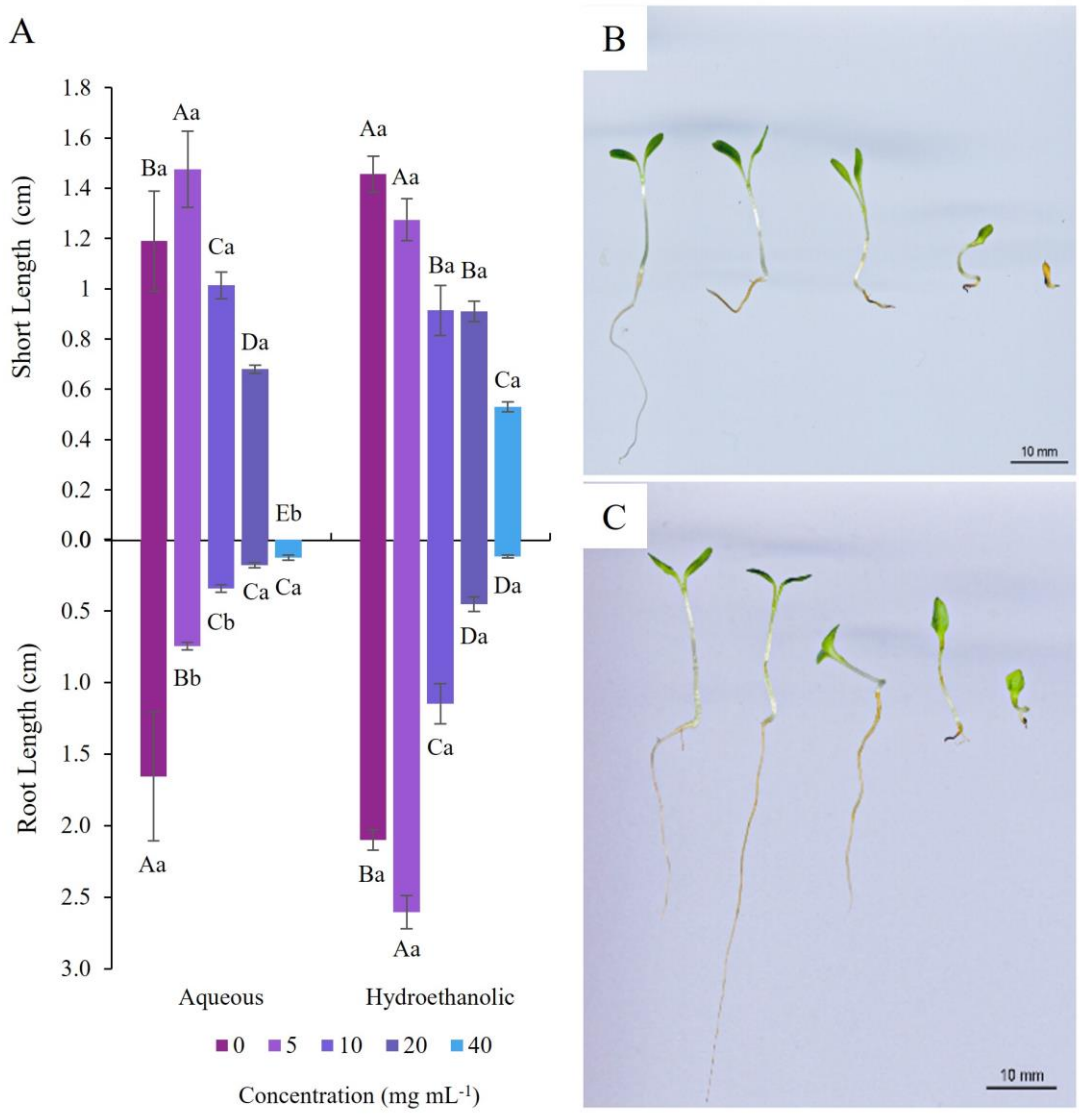


Figure 4. (A) Shoot and root length of *Lactuca sativa* exposed to different concentrations of (B) aqueous and (C) hydroethanolic leaf extracts of *Syzygium cumini*. Error bars represent standard error. Uppercase letters indicate no significant difference among concentrations, and lowercase letters indicate no significant difference between extraction methods, according to the Scott-Knott test at 5% significance.

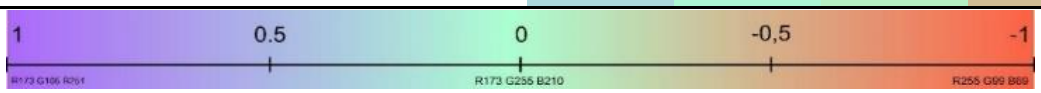
The response index serves as a summary of the allelopathic effects observed on the germinative and morphological parameters of *Lactuca sativa*, where inhibition is represented by values approaching –1 and stimulation by values approaching +1. Accordingly, Table 1 shows that the allelopathic effect reached maximum inhibition at the concentration of 40 mg

mL^{-1} for most parameters in the aqueous extract, while a stimulatory effect was observed only at 5 mg mL^{-1} for shoot and root length. Overall, the aqueous extract exhibited a stronger inhibitory effect, with a total value 34.5% higher compared to the hydroethanolic extract. Moreover, the concentration of 5 mg mL^{-1} was the only one to exhibit a stimulatory effect on *L. sativa* seeds when exposed to the hydroethanolic extract, specifically for root length and the proportion of normal seedlings.

Table 1. Allelopathic response indices of aqueous and hydroethanolic extracts obtained from *Syzygium cumini* leaves in bioassays using *Lactuca sativa* seeds.

Extract	Responsive Index	Concentration (mg mL^{-1})			
		5	10	20	40
Aqueous	Root Protrusion	-0.10	-0.35	-1.00	-1.00
	Germination 4th day	-0.03	-0.03	-0.11	-0.89
	Germination 7th day	-0.02	-0.03	-0.06	-0.55
	Germination Speed Index	-0.21	-0.38	-0.65	-0.91
	Short Length	0.08	-0.23	-0.49	-1.00
	Root Length	-0.60	-0.82	-0.91	-0.93
	Fresh Biomass	-0.08	-0.28	-0.58	-1.00
	Normal Seedlings	-0.15	-0.86	-1.00	-1.00
Hydroethanolic	Root Protrusion	-0.08	-0.14	-0.34	-0.87
	Germination 4th day	-0.02	-0.02	-0.08	-0.05
	Germination 7th day	-0.02	-0.03	-0.06	-0.55
	Germination Speed Index	-0.09	-0.22	-0.37	-0.58
	Short Length	-0.04	-0.31	-0.31	-0.60
	Root Length	0.28	-0.39	-0.76	-0.94
	Fresh Biomass	-0.01	-0.32	-0.50	-0.47
	Normal Seedlings	0.03	-0.76	-0.97	-1.00

Allelopathy synthesis effect	$\sum LI$	$\overline{LI}$			
		5	10	20	40
Aqueous	-2.02	-0.14	-0.37	-0.6	-0.91
Hydroethanolic	-1.32	0.006	-0.27	-0.42	-0.63



Colors in the RGB scale correspond to the following values: 1 (R173 G105 B251); 0 (R173 G255 B210); –1 (R255 G99 B69).

Based on regression analysis, root elongation inhibition equations were established for the aqueous extract ($y = 0.0021x^2 – 0.1173x + 1.4705$, $R^2 = 0.9105$) and the hydroethanolic extract ($y = 0.0017x^2 – 0.1283x + 2.4871$, $R^2 = 0.8356$). The aqueous extract resulted in an IC_{50} of 6 mg mL^{-1}, indicating a phytotoxicity 2.25 times greater than that of the hydroethanolic extract (IC_{50} = 13.5 mg mL^{-1}).

Both extracts reduced the mitotic index compared to the control, with cytotoxic effects of 30.1% and 41.46% for the aqueous and hydroethanolic extracts, respectively. Among the mitotic phases, a significant reduction ($p < 0.01$) in metaphase was observed with the hydroethanolic extract. An opposite effect was observed for chromosomal aberrations, with a marked increase in their frequency. The total frequency of chromosomal abnormalities increased by 3.22-fold for the aqueous extract and 2.24-fold for the hydroethanolic extract (Table 2).

Table 2. Mitotic index of aqueous and hydroethanolic extracts obtained from *Syzygium cumini* leaves in bioassays using *Lactuca sativa* seeds.

Treatments	IC_{50} (mg mL^{-1})	Total dividing cells	MI (%)	Mitotic phase (%)				CAF
				P	M	A	T	
Control	-	745	9.31±1.08	2.95±0.93	2.67±0.32	1.62±0.18	2.06±0.26	0.45±0.05
Aqueous	6	521	6.51±0.12*	0.92±0.24	1.65±0.50	1.91±0.36	2.09±0.33	1.45±0.04*
Hydr.	13.5	436	5.45±0.84*	1.26±0.39	1.02±0.12*	1.31±0.15	1.85±0.34	1.01±0.1*

(*) Treatment means differ statistically from the control according to the Scott-Knott test at 5% significance. MI = mitotic index; P = prophase; M = metaphase; A = anaphase; T = telophase; CAF = chromosomal abnormality frequency.

Chromosomal aberrations of clastogenic and aneugenic nature were recorded, with no nuclear changes (micronuclei or lobulated nuclei) detected. Only anaphase bridges were significantly increased in both extracts. Distinct patterns in the frequency of chromosomal aberrations were observed: the aqueous extract led to an increase in lost and lagging chromosomes during telophase, while the hydroethanolic extract induced an increase only in C-metaphase. Stickness was the only aberration significantly reduced compared to the control in the aqueous extract (Table 3). Details of the chromosomal abnormalities observed are presented in Figure 5.

Table 3. Chromosomal abnormalities in meristematic cells of *Lactuca sativa* induced by aqueous and hydroethanolic extracts from *Syzygium cumini* leaves.

Treatments	P.ANF	P.TEL	C-M	STICK	CP	CAA	CAT
Control	0.03±0.01	ND	0.01±0.01	0.19±0.05	ND	0.18±0.03	0.04±0.02
Aqueous	0.25±0.04*	0.01±0.01	0.01±0.01	0.04±0.01*	0.73±0.08*	0.16±0.03	0.25±0.07*
Hydro.	0.08±0.01*	ND	0.19±0.02*	0.21±0.04	0.21±0.11	0.29±0.06	0.04±0.01

(*) Treatment means differ statistically from the control according to the Scott-Knott test at 5% significance. (P.ANF) bridges in anaphase and telophase (P.TEL); (C-M) C-metaphases; (STICK) stickiness; (CP) lost chromosome; (CAA) lagging chromosome in anaphase; (CAT) lagging chromosome in telophase. ND = not detected.

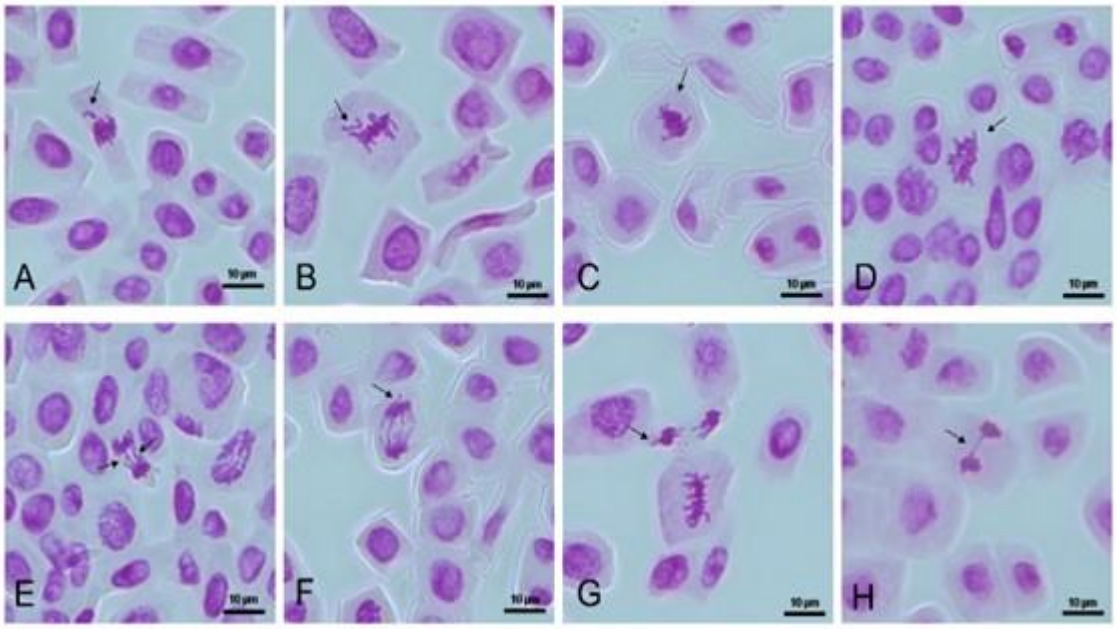


Figure 5. Chromosomal abnormalities identified in the root tips of *Lactuca sativa*. (A–B) lost chromosome in metaphase; (C) stickiness; (D) C-metaphases; (E) anaphase bridge; (F) lagging chromosome in anaphase; (G) lagging chromosome in telophase; (H) telophase bridge.

To determine the genotoxic potential of the extracts, Table 4 presents the classification profile of chromosomal abnormalities according to their respective mitotic phases. It is evident that the total cyto-genotoxic effect on mitosis i.e., the frequency of

chromosomal abnormalities divided by the mitotic index, was significantly affected by both extracts. The cytotoxicity profile of the aqueous extract was both clastogenic and aneugenic. The clastogenicity of the aqueous extract was 9.87 times higher than that of the negative control. On average, both extracts increased aneugenic abnormalities by 324.5%.

Table 4. Summary of the cyto-genotoxic effects in meristematic cells of *Lactuca sativa* induced by aqueous and hydroethanolic extracts from *Syzygium cumini* leaves.

Treatments	Mitotic dividing cells				Efect cito-genotoxic (%)		
	P	M	A	T	ECG	Clastogenic	Aneugenic
Control	236	214	130	165	5.01	0.71	6.70
Aqueous	69	132	153	167	22.29**	7.01*	21.06**
Hydroetanolic	101	82	105	148	19.13**	2.58	22.42**

(*) Treatment means differ statistically from the control according to the Scott-Knott test at significance levels: * $p \leq 0.05$; ** $p \leq 0.001$. P = prophase; M = metaphase; A = anaphase; T = telophase; ECG = mitotic cyto-genotoxic effect; ND = not detected.

There was a significant interaction between the extracts and concentrations for all biochemical variables. In the presence of the highest concentrations of the aqueous extract, a 15% increase in H_2O_2 accumulation was observed compared to the control (Fig. 6A), indicating a higher presence of reactive oxygen species (ROS). An increase in lipid peroxidation (Fig. 6B) was detected at all concentrations of both extracts, with an average effect of 223% at the higher concentrations (10, 20, and 40 mg mL^{-1}). The 5 mg mL^{-1} concentration caused a 6.5-fold increase in lipid peroxidation compared to the control. Superoxide dismutase (SOD) activity was significantly increased only in response to the aqueous extract, starting at the lowest concentration, reaching a maximum level 110% higher than the control (Fig. 6C).

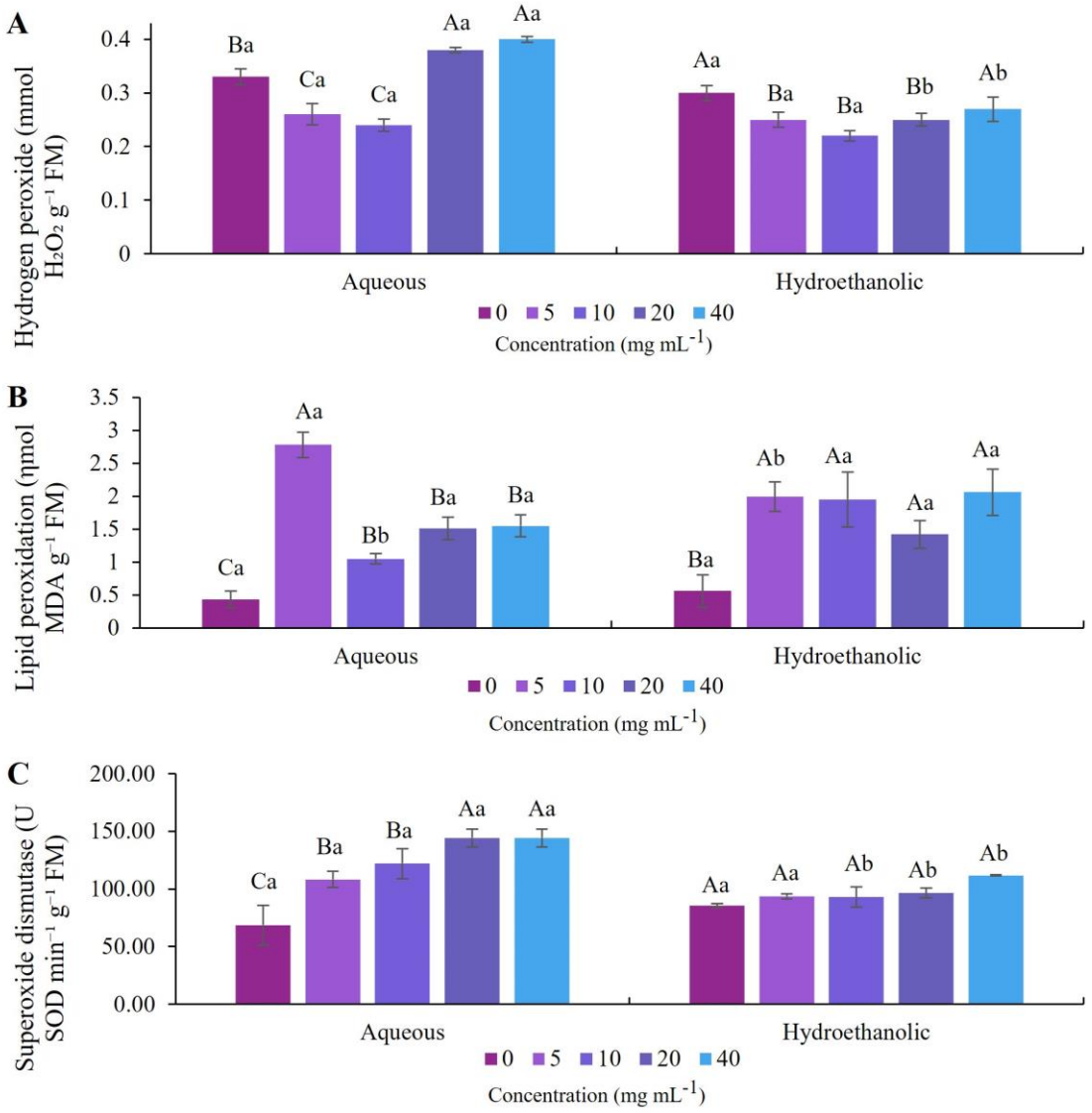


Figure 6. Biochemical analysis of *Lactuca sativa* exposed to aqueous and hydroethanolic leaf extracts of *Syzygium cumini*. (A) Hydrogen peroxide content (mmol H_2O_2 g^{-1} fresh mass); (B) Lipid peroxidation (ηmol MDA g^{-1} fresh mass); (C) Superoxide dismutase activity (U SOD min^{-1} g^{-1} fresh mass). Identical letters indicate no significant difference according to the Scott-Knott test at 5% significance. Uppercase letters compare concentrations within each extract, while lowercase letters compare concentrations between extracts.

Discussion

Preliminary studies on the phytotoxic effects of *Syzygium cumini* leaves demonstrate a selective response that depends on the extraction method and the target plant species. The

aqueous extract of dried *S. cumini* leaves exhibited limited inhibitory effects on the germination of *Phalaris minor*. This discrepancy in results may be associated with plant management practices and phenological stage, which, together with the extraction method, influence the quality and quantity of phytochemicals available in each bioassay (Calvelli et al., 2023; Moreira et al., 2024).

The morphological effects observed on the shoot and root development of *Lactuca sativa* are intrinsically linked to two fundamental processes governing growth and development in multicellular organisms: cell division and differentiation (Calvelli et al., 2023). Imbalances between these processes lead to premature termination of organogenesis and potentially abnormal growth, while the regulation of the transition from cell proliferation to elongation during early differentiation stages is influenced by the maintenance of reactive oxygen species (ROS) homeostasis (Bhattacharjee, 2019).

In this context, the evaluation of the mitotic index, in addition to determining the cytotoxicity observed in meristematic cells (Leme and Marin-Morales, 2009), has proven to be one of the main factors associated with root elongation in plant models. Accordingly, depending on the cytotoxicity level, a compound may cause uncontrolled cell proliferation, resulting in an increased mitotic index and potentially leading to tumor formation (Pinheiro et al., 2015). Conversely, a reduction in the mitotic index indicates a halt in cell division and the death of interphase nuclei, thereby decreasing the number of cells undergoing mitosis (Calvelli et al., 2023). Although both extracts exhibited mitodepressive activity in *L. sativa* meristematic cells, Santos et al. (2024) and Fiskesjö (1985) consider this effect significant when the mitotic index drops by more than 50%. Despite the low mitodepressive effect, both extracts induced an increase in chromosomal abnormality frequency, indicating high genotoxic activity (Amâncio et al., 2021). The presence of such abnormalities is considered a cytogenetic indicator of cytotoxicity (Santos et al., 2024).

The genotoxic effect is characterized by two main groups of chromosomal alterations. According to Leme and Marin-Morales (2009), clastogenic aberrations, responsible for genotoxicity, damage DNA structure, leading to modifications in chromosome integrity. In contrast, aneugenic aberrations affect the orientation and segregation of chromosomes, altering chromosome numbers and resulting in cyto-genotoxic effects. Both extracts promoted an increase in aneugenic events, including the induction of c-metaphase, lagging

chromosomes, and chromosome loss. These anomalies stem from improper polymerization of the mitotic spindle fibers, which prevents correct chromosomal alignment during the cell cycle. C-metaphase was the most significant abnormality observed in the hydroethanolic extract, resulting from mitotic spindle disruption and chromosomal structural damage, potentially leading to the formation of polyploid cells (Amâncio et al., 2021). This loss of sister chromatid cohesion may be linked to pericentromeric region inactivation caused by the absence of histone H3 phosphorylation at serine 10, resulting in abnormal chromosome orientation during cell division (Freitas et al., 2016). According to dos Santos et al. (2024), the origins and classification of chromosomal stickiness are not yet fully understood, but this abnormality may involve both aneugenic and clastogenic effects.

Another hypothesis for the formation of stickiness involves chromatid and chromosomal adhesion due to the high chromosomal contraction observed in polyploid cells (Fernandes et al., 2009), although no polyploidy was observed in any of the treatments. Compounds present in the extracts may disrupt the balance of structural proteins, leading to chromosomal aggregation and condensation, resulting in stickiness (Amâncio et al., 2021). Additionally, this protein imbalance, particularly involving histones, may lead to an increase in chromosomal bridges (Pinheiro et al., 2015), as observed with the aqueous extract, which was the only treatment to increase the occurrence of aneugenic abnormalities. Reactive oxygen species (ROS), especially hydroxyl radicals, can target DNA and DNA-binding proteins, leading to protein-DNA cross-linking (Choudhary et al., 2020). On the other hand, the significant reduction in stickiness formation caused by the aqueous extract suggests that other mechanisms may be involved in its formation, considering that changes in protein expression patterns are proposed as a causal pathway for both types of abnormalities.

Although the presence of micronuclei was expected as a result of such abnormalities, the current investigation did not detect them. This absence may be attributed to the disintegration of chromosomes and their fragments within the cytoplasm (Ribeiro et al., 2013). This phenomenon underscores the complex mechanisms involved in cell division and highlights the repercussions of chromosomal segregation abnormalities on the morphology and genetic composition of daughter cells upon exposure to *S. cumini* leaf extracts.

The reduction in the mitotic index and the increase in chromosomal abnormalities may be associated with elevated levels of reactive oxygen species (ROS). The production of

ROS has the potential to induce damage to microtubule structures and DNA, thereby compromising genetic stability and disrupting the cell cycle, potentially leading to apoptosis (Çavuşoğlu et al., 2024). ROS attacks result in DNA strand fragmentation, as well as the removal and/or modification of nucleotides, along with deoxyribose oxidation. These oxidative processes typically lead to various mutagenic aberrations. Regardless of the DNA's origin, such impairments cause abnormalities in the resulting proteins, thereby influencing multiple aspects of cellular physiology (Choudhary et al., 2020).

Accordingly, the observed increase in superoxide dismutase (SOD) activity and elevated levels of lipid peroxidation caused by the aqueous extract of *Syzygium cumini* in *Lactuca sativa* seedlings may be associated with the presence of compounds that promote the production of reactive oxygen species (ROS). Although ROS play a crucial role in regulating various processes associated with plant growth and development, excessive levels can exert deleterious effects on plant cells, leading to oxidative damage to essential cellular components such as proteins, DNA, and lipids, ultimately triggering programmed cell death (Heivachi et al., 2023). Cell membrane damage, resulting in excessive production of malondialdehyde due to lipid peroxidation, is considered a primary event mediating the toxicity of broad-spectrum pesticides (Chen et al., 2022).

The increased synthesis of superoxide dismutase (SOD) aims to inactivate the excess reactive oxygen species (ROS) in cells in order to achieve oxidative homeostasis (Çavuşoğlu et al., 2024). This is due to the fact that, unlike atmospheric oxygen, ROS possess an inherent capacity to continuously oxidize a wide range of cellular components, leading to oxidative cell death (Choudhary et al., 2020). Therefore, DNA is highly vulnerable to oxidative damage, and phytotoxic phytochemicals have the potential to induce DNA strand breaks either directly or through the abundant generation of ROS, resulting in lasting DNA damage and ultimately culminating in cell death.

These phytochemicals affect plants in different ways depending on their concentrations. In extract-based evaluations, this relationship becomes even more complex, with possible synergistic and antagonistic interactions among active compounds depending on the extraction method (Calvelli et al., 2023). Thus, the ability of cells to adapt to oxidative stress induced by these compounds is intrinsically linked to the species' inherent capacity to effectively scavenge free radicals. This highlights the critical role of endogenous radical-

scavenging mechanisms in mitigating the impact of allelopathic stressors (Çavuşoğlu et al., 2024). An imbalance in reactive oxygen species (ROS) levels in plant cells can disrupt redox homeostasis and trigger a cascade of reactions that lead to oxidative stress and damage, underscoring the delicate balance required for proper cellular function.

Among the main bioactive compounds of *Syzygium cumini*, high levels of phenolic compounds are noted for their antioxidant properties (Oliveira et al., 2022), also exhibiting cytoprotective effects as demonstrated by the XTT assay (Ahmed et al., 2019). However, the leaves are particularly notable for containing kaempferol, myricetin, quercetin, caffeic acid, chlorogenic acid, ellagic acid, ferulic acid, gallic acid, tannins, nilocetin, α-pinene, α-cadinol, pinocarvone, pinocarveol, α-terpineol, myrtenol, eucarvone, muurolol, myrtenal, cineole, and geranylacetone (Kumari et al., 2023). These compounds have been associated with antiproliferative and cytotoxic activities, suggesting that the anticancer properties of *S. cumini* seed extract may be attributed to these constituents (Ezhilarasan et al., 2019; Fiqri et al., 2020; Qamar et al., 2022).

Based on the results obtained in this study, it can be observed that *Syzygium cumini* exhibits a range of cytotoxic effects, depending on the plant part used and the extraction method applied. This divergence in cyto-genotoxic and mutagenic activity may be attributed to the use of different biological models and the specific types of target cells analyzed. Cytogenetic assays using *Lactuca sativa* have proven to be an appropriate system for assessing the cytogenotoxicity of various samples, which is directly related to the phytotoxicity of the root system (Amâncio et al., 2021; Cunha Neto et al., 2023; Calvelli et al., 2023).

Conclusion

Both extracts were effective at concentrations starting from 10 mg mL^{-1} for morphological parameters. Regarding germination parameters, a delay in germination was observed at higher concentrations, with the aqueous extract being more effective. These findings suggest that *Syzygium cumini* (L.) Skeels is a potential source of allelochemicals. Furthermore, oxidative stress was evident, inhibiting seedling development and enzyme homeostasis. The data revealed high levels of lipid peroxidation and elevated superoxide dismutase (SOD) activity.

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Author Contribution:

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Consent to Participate: Not applicable.

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Figure

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Figure

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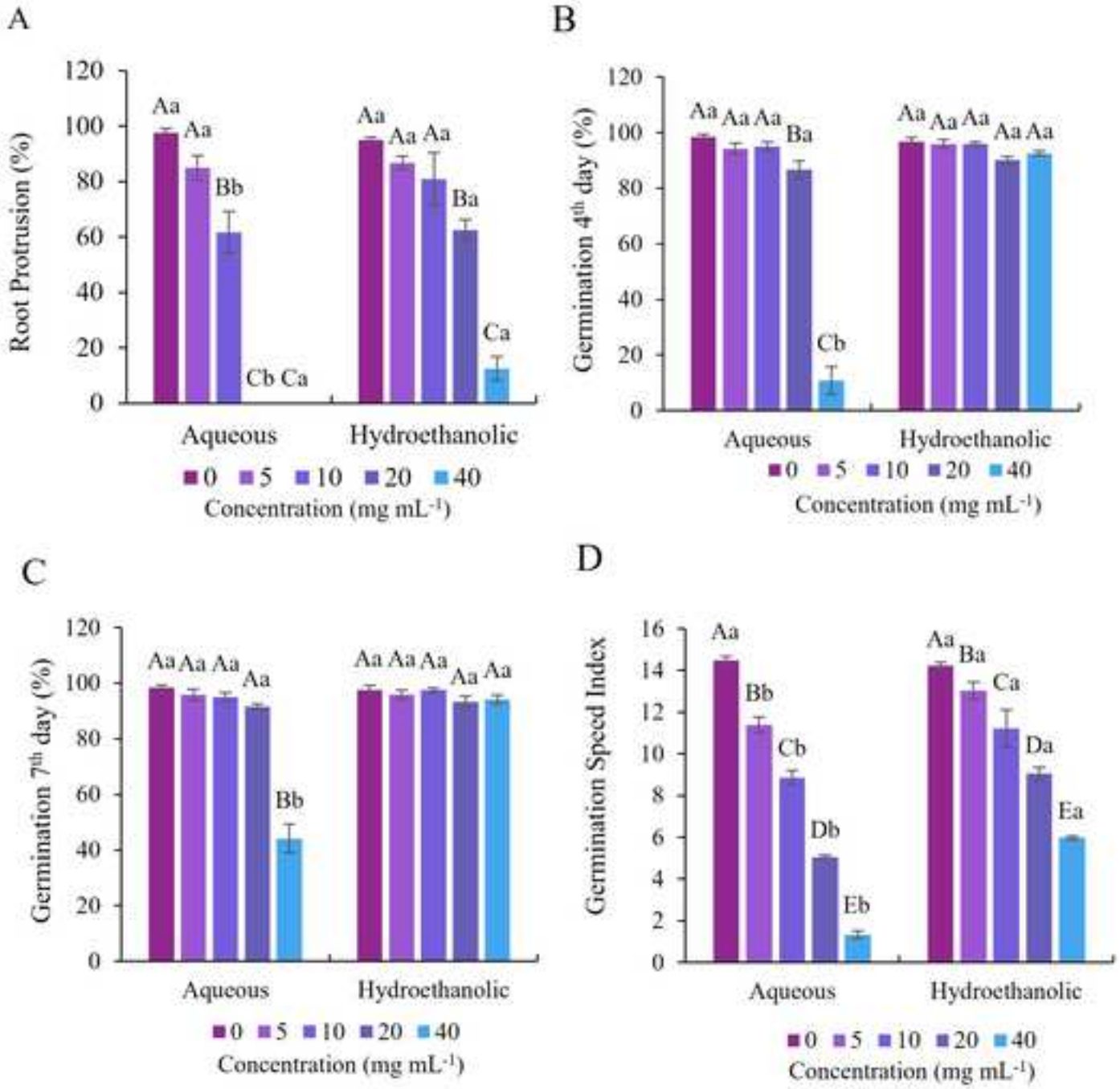

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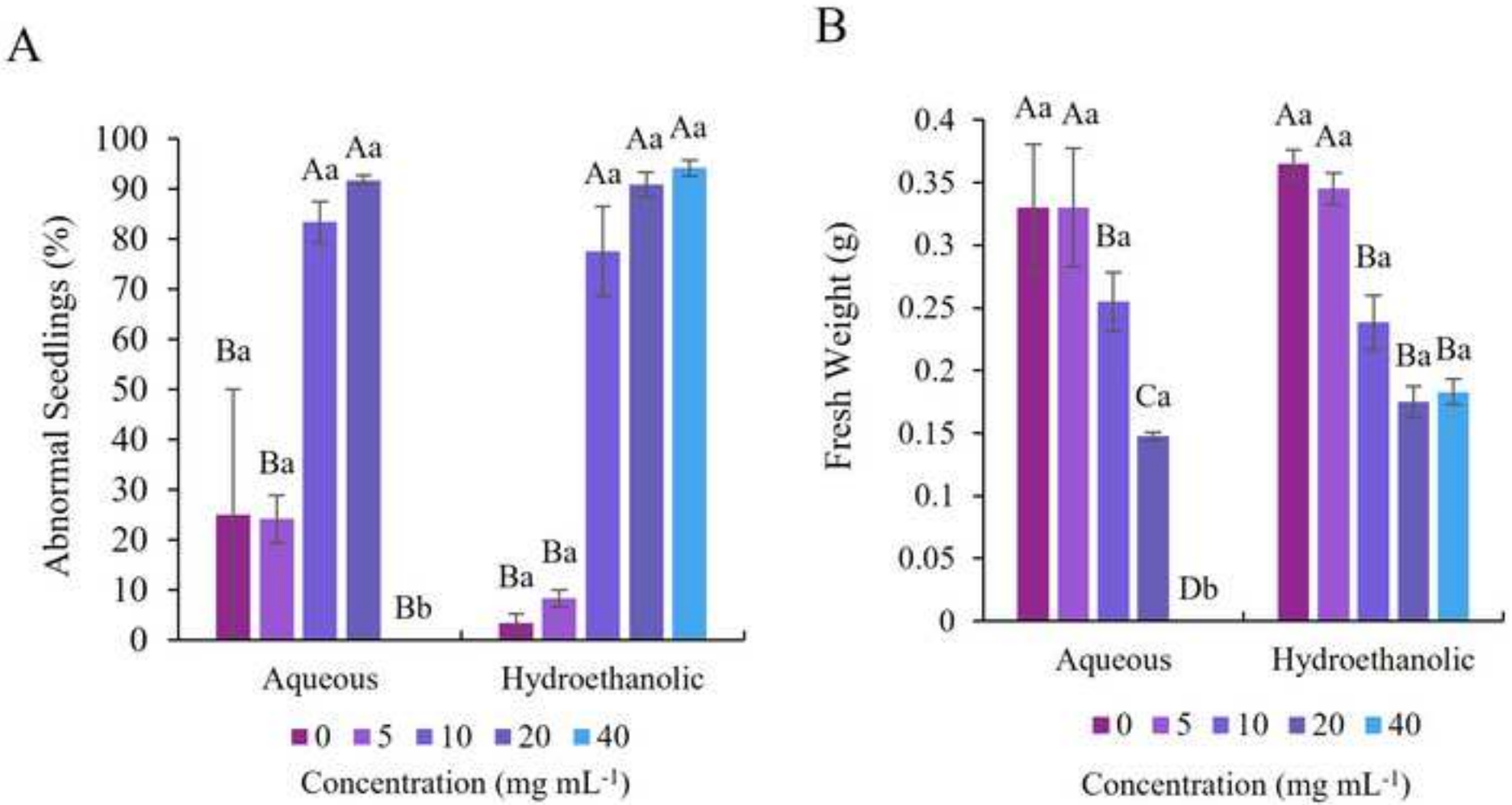

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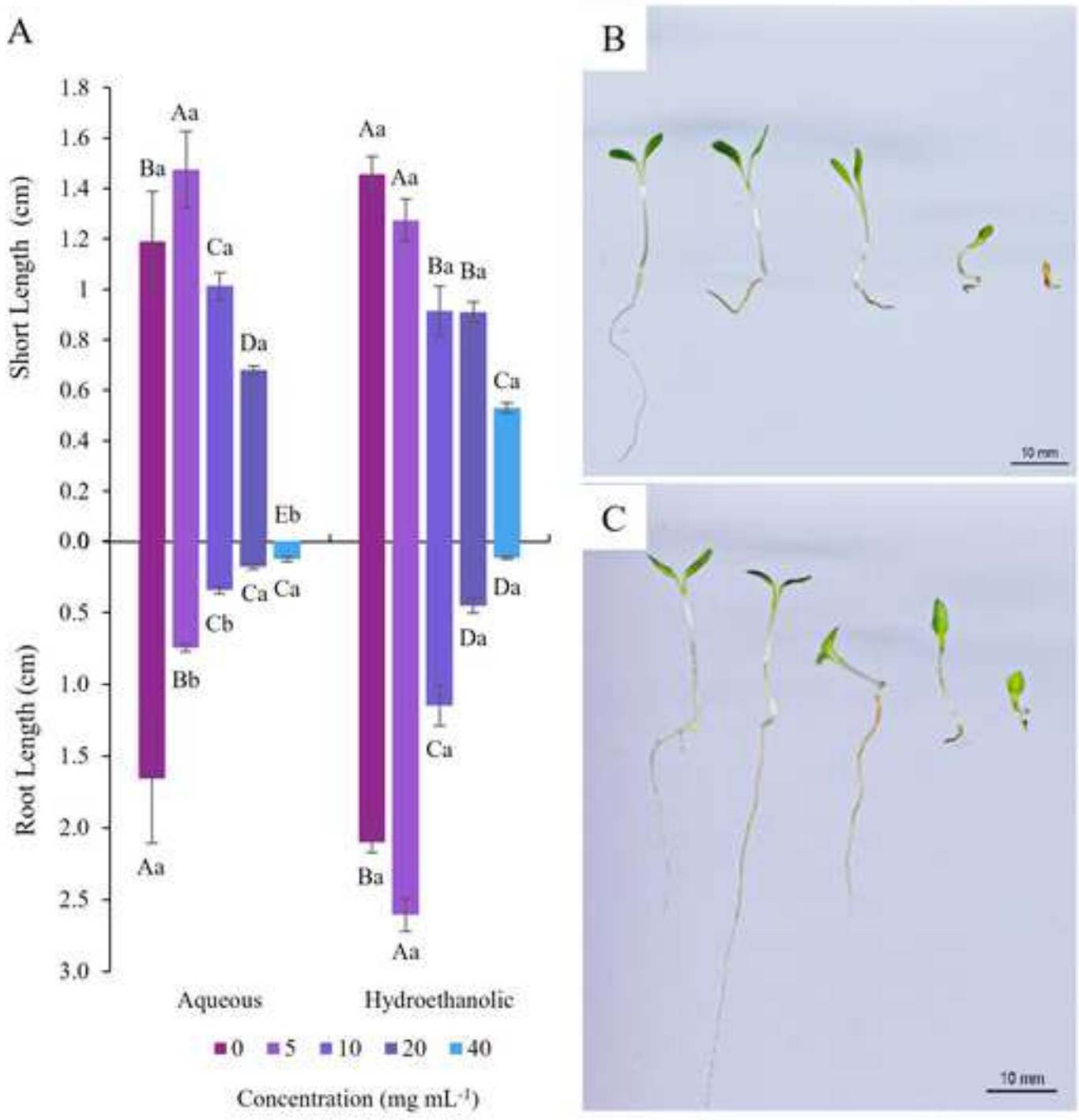

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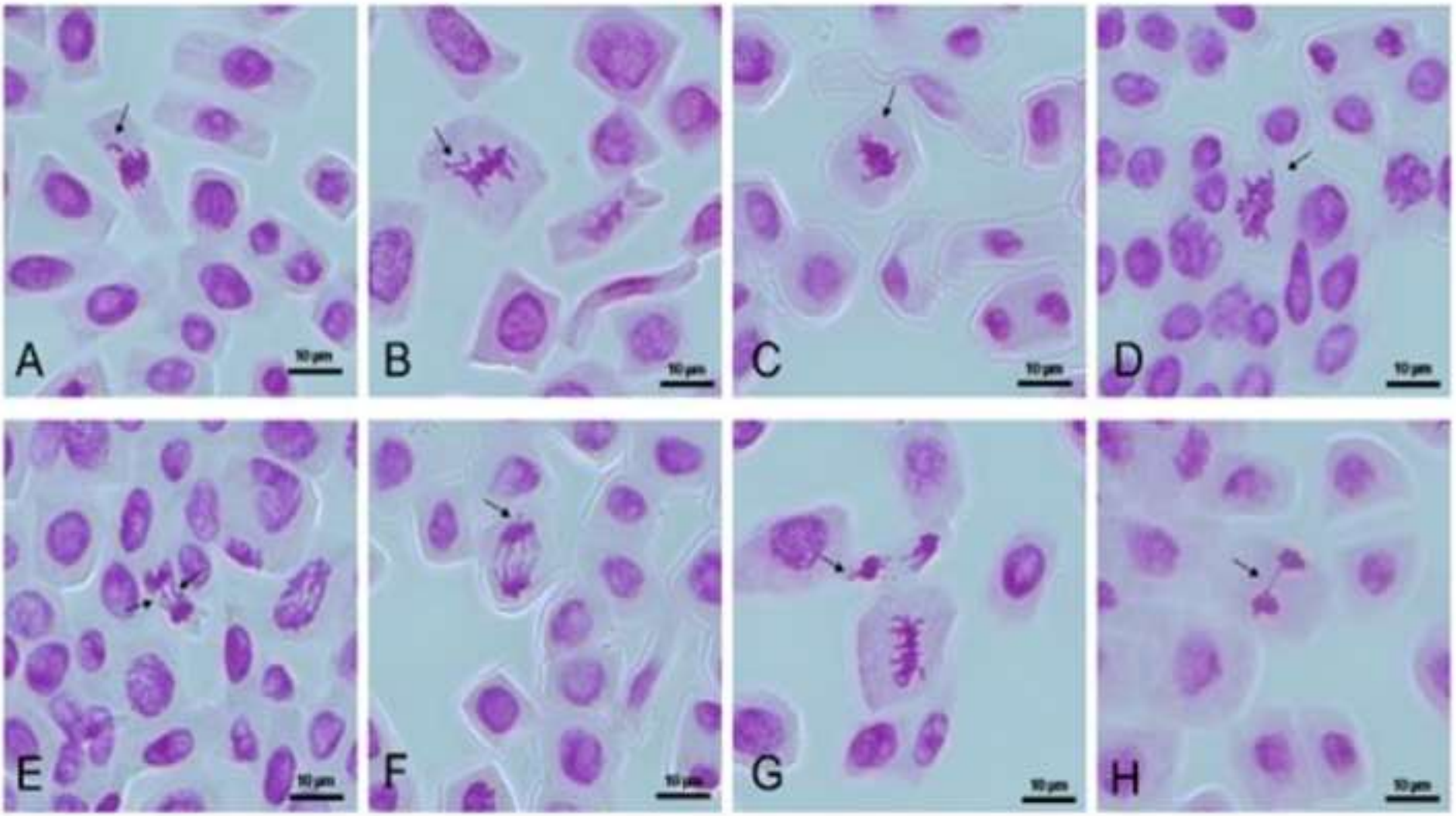

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