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PROGRAMA ASSOCIADO DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA MARINHA

JÉSSICA CARNEIRO OLIVEIRA

SEED PRIMING COM *ULVA LACTUCA* L. EM SEMENTES CULTIVADAS EM ANÁLOGOS DE REGOLITO LUNAR E MARCIANO

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Dissertação apresentada ao Instituto de Estudos do Mar Almirante Paulo Moreira e à Universidade Federal Fluminense, como requisito parcial para a obtenção do grau de Mestre em Biotecnologia Marinha.

Orientador: Prof. Dr. Renato Crespo Pereira
Co-orientador: Dr. Rafael Loureiro

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Dedico esta dissertação aos meus pais, Rita e Jales e ao meu amor Rick.

“A ciência ainda não nos provou se a
loucura é ou não o
mais sublime da inteligência”.

Edgar Allan Poe

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RESUMO

Com a realidade iminente do estabelecimento humano em bases lunares e marcianas é premente a necessidade de implantação de cultivo de alimentos nestes ambientes através da otimização de recursos disponíveis, como o regolito lunar e o marciano a fim de compatibilizar os sistemas biológicos com estes recursos locais. Além disso, a economia marciana e a lunar dependerão do cultivo e da comercialização de recursos alimentares de baixo custo obtidos através de biotecnologias sustentáveis. Extratos de macroalgas são considerados promissores para serem usados como *seed priming* antes da semeadura para germinação mais rápida e uniforme. O presente trabalho avaliou o efeito de diferentes concentrações de farinha de *Ulva lactuca* L. na germinação e no crescimento de *Capsicum annuum* L., *Lactuca sativa* L., *Cicer arietinum* L. e *Pisum sativum* L. em simuladores de regolito marciano e lunar. As sementes foram cultivadas em câmaras de ambiente controlado e irrigadas com solução de farinha de *U. lactuca*. A análise qualitativa de *U. lactuca* foi realizada para fins de conhecimento de seus bioativos, pela presença de alcalóides, terpenóides, fenol, saponinas, flavonoides, quinina e esteroides que estão presentes no metabolismo secundário de *Ulva* e são importantes vias metabólicas para a formação de fitormônios. No regolito marciano, o melhor tratamento para germinação de sementes de ervilha e grão de bico foi de 0,2 g. L^{-1} (p =< 0,001; p= 0,006), bem como na emergência de plântulas. No regolito lunar, as sementes de ervilha apresentaram picos de germinação em ambos os tratamentos (0,2 g. L^{-1} p= < 0,001 e 0,4 g. L^{-1} p= 0,001) em relação ao controle, assim como as sementes de grãos de bico (0,2 g.L^{-1} p= 0,002 e 0,4 g. L^{-1}; p= 0,008). A porcentagem de germinação de sementes de alface cultivadas em regolito lunar foi melhor em 0,2 g.L^{-1} em relação ao controle (p= 0,010). Para as demais sementes, não houve diferença significativa. Indicamos o uso de farinha de *U. lactuca* como bioestimulante, pela presença de reguladores de crescimento de plantas (PGRs) que melhoram a germinação e emergência de plântulas sob condições estressantes.

Palavras-chave: regolith simulant; plants in space; seed priming; regolith-based agriculture; biostimulant.

Abstract

Due to the imminent nature of human settlement on lunar and/or Martian bases, there is an urgent need to implement and make compatible food production in these environments by optimizing available lunar and Martian regolith resources. In addition, the Martian and lunar economy will depend on the cultivation and marketing of low-cost food resources obtained through sustainable biotechnologies. Macroalgae extracts are considered promising low-cost resources that can be used in seed priming before sowing to promote faster and more uniform germination. The present work experimentally evaluated the effect of different concentrations of the green macroalgae *Ulva lactuca* L. powder on the germination and growth of the cultivars *Capsicum annuum* L. (pepper), *Lactuca sativa* L. (lettuce), *Cicer arietinum* L. (chickpea) and *Pisum sativum* L. (pea) in Martian and lunar regolith simulators. The germination and emergence of the seeds were evaluated under controlled greenhouse conditions with the addition of two concentrations of *U. lactuca* powder (0.2 and 0.4 g.L^{-1}). The qualitative chemical analysis of *U. lactuca* was performed for the purpose of knowledge of its bioactives (alkaloids, terpenoids, phenol, saponins, flavonoids, quinine, and steroids) important as metabolic pathways for the formation of phytohormones. The speed of germination and emergence of the seeds of the cultivars in lunar regolith were higher than those observed in Martian regolith ($p < 0.05$). In Martian regolith, the best treatment for germination of pea and chickpeas seeds was 0.2 g.L^{-1} (p= < 0.001; p= 0.006), as well as seedling emergence. In lunar regolith, pea seeds germination peaks were obtained with both treatments (0.2 g.L^{-1} p= < 0.001 and 0.4 g.L^{-1} p= 0.001), as well as beak grain seeds (0.2 g.L^{-1} p= 0.002 and 0.4 g.L^{-1}; p= 0.008). The germination percentage of lettuce seeds grown in lunar regolith was better in 0.2 g.L^{-1} compared to the control (p= 0.010). For the other seeds, there was no significant difference. From the results obtained, we propose using *U. lactuca* powder as a biostimulant for the studied cultivars, due to the presence of plant growth regulators (PGRs) that improve germination and seedling emergence under stressful conditions.

Keywords: regolith simulant; plants in space; seed priming; regolith-based agriculture; biostimulant.

SUMÁRIO

1. INTRODUÇÃO

A Astrobotânica (*Astrobotany*) compreende o estudo da vida e as interações vegetais em ambientes espaciais. Esta área de estudos pressupõe entender a resposta das plantas para voos espaciais humanos, bem como a possibilidade de vida vegetal em outros planetas (MEDINA et al. 2021). Microgravidade, estresse oxidativo, estresse gerado pelo frio e radiação ionizante são apenas alguns dos fatores adversos do espaço que impactam a biologia vegetal e podem afetar o crescimento radicular, a captura de nutrientes e, posteriormente, alterar a maneira como a planta deve ser manuseada (MANZANO et al. 2022). Os estudos espaciais podem confiar em nossa compreensão da Astrobotânica, a partir do momento em que se compreende os mecanismos da fisiologia de plantas em condições extremas (OLUWAFEMI et al. 2018).

Essa linha de pesquisa começou entre os anos 1950 e 1960, por meio dos trabalhos de Jack Myers e outros, que estudaram o uso de algas para produção de O_2 e remoção de CO_2 para a Força Aérea dos Estados Unidos e a Administração Nacional de Aeronáutica e Espaço (NASA). Além disso, em 1970 a pesquisa agrícola baseada em regolitos já era um desafio, e assim permanece até os dias atuais (WALKINSHAW et al. 1972; MING & BRADY, 1989; EICHLER et al. 2021). Com os resultados desses estudos para a agricultura espacial, novas tecnologias e descobertas também foram produzidas e implementadas para a agricultura terrestre (WHEELER et al. 2010)

Descobrir como viver em Marte e/ou na Lua é o teste final de sustentabilidade da agricultura, e aprender a cultivar plantas em um ambiente hostil poderá maximizar a alimentação e minimizar o uso de recursos valiosos e nocivos, como água e fertilizantes, respectivamente (DE MICCO et al. 2009; EICHLER et al. 2021). O tema da agricultura para o espaço contribuiu e beneficiou a agricultura de ambiente controlado terrestre e continuará a fazê-lo no futuro (POULET et al. 2022).

1.1 A AGRICULTURA BASEADA EM REGOLITOS

O regolito é definido como substrato não consolidado sobre rocha e pode incluir poeira, rochas quebradas, solo e outros materiais relacionados (FACKRELL et al. 2021) e, para minimizar custos, este substrato não precisa ser enviado da Terra. A agricultura baseada em regolitos se aproveita de amplos dados científicos agrícolas já publicados e implementados na agricultura terrestre (EICHLER et al. 2021)

Todos os macros- e micronutrientes essenciais para o crescimento das plantas estão presentes, tanto no regolito lunar quanto no marciano, em quantidades suficientes, com exceção do nitrogênio reativo. O nitrogênio nas formas reativas (NO_3 e NH_4) é um dos minerais essenciais necessários à quase todo o crescimento das plantas (STEVENS et al. 2011). A principal fonte de nitrogênio reativo na Terra é a mineralização da matéria orgânica e a fixação do nitrogênio por meio de associações mutualísticas entre as plantas e o microbioma do solo (TAIZ et al. 2015).

A ausência de nitrogênio reativo suficiente pode ser resolvida usando espécies fixadoras de nitrogênio, como bactérias promotoras de crescimento de plantas (LORENTE et al. 2018). Sendo assim, é imprescindível adaptar o potencial dos microrganismos para transformar os regolitos em substratos mais adequados ao crescimento das plantas.

O estabelecimento da agricultura espacial será um processo decisivo para a sobrevivência do ser humano fora da Terra, principalmente por ser uma fonte alimentícia viável e de geração de renda, mas ainda há muitos obstáculos a serem superados (CANNON & BRITT 2019). Por exemplo, a luminosidade, crucial para o desenvolvimento de plantas, é consideravelmente menor comparada à Terra, tornando necessário que seja subvencionada e totalmente controlada (MING et al. 1989; WAMELINK et al. 2014; WHEELER 2017). Além disso, a radiação cósmica que atinge a superfície marciana diminui o crescimento das plantas, pois é 17 vezes maior do que na Terra, e poderá diminuir drasticamente a biomassa vegetal (TACK et al. 2021).

Para nutrição interplanetária, no futuro, é importante escolher culturas que não exijam processamento e tempo mínimo de preparo, forneçam vitaminas essenciais (B1, K, C) e não se degradem como a maioria dos compostos bioativos em sistemas de alimentos armazenados (JOHNSON et al. 2021; POULET et al. 2022). Além disso, a produção de alimentos frescos poderá fornecer informações nutricionais e psicológicas importantes para missões de longa duração e/ou para futuros colonos na escolha ideal de vegetais com propriedades organolépticas (MASSA & WHEELER, 2015; ZHANG et al. 2022).

Em sistema baseado em regolitos, pesquisas estão sendo realizadas com o intuito de otimização da utilização de recursos *in-situ* (*In-Situ Resource Utilization- ISRU*) (EICHLER et al. 2021) com o propósito de estabelecer um consenso de escolhas de plantas que preencham os requisitos nutricionais para futuros cultivos agrícolas fora da Terra (WHEELER et al. 2004; WHEELER, 2017; DURI et al. 2022), além de compreender como afetará o crescimento, a produtividade das plantas e as propriedades do substrato (DURI et al., 2022).

Kasiviswanathan et al. (2022) utilizaram alimentos como nabo, rabanete e alface em simulado marciano e, como suplemento adicional ao substrato, utilizou a alfafa. A rúcula, em simulado marciano, foi cultivada juntamente com minhocas e excrementos de porco que estimularam significativamente o crescimento das plantas (WAMELINK et al. 2022).

WAMELINCK et al. (2021) utilizaram tecidos de brotos cortados de *Lolium perenne* como fonte de nitrogênio reativo misturado aos simuladores com o propósito de incrementar nutrientes em agrião, rúcula, tomate, rabanete, centeio, quinoa, espinafre, cebolinha, ervilha e alho-poró cultivadas em regolito marciano e lunar.

Espécies vegetais como *Arabidopsis thaliana* e *Lactuca sativa* (alface), por seus rápidos crescimentos e fácil preparo, foram cultivadas em regolito marciano, com a adição de perclorato de cálcio, tóxico ao ser humano, em concentrações destinadas a imitar aquelas observadas na superfície de Marte e suplemento nutritivo (EICHLER et al. 2021). Nesse tipo de agricultura, os desafios a serem superados compreendem a garantia de um crescimento eficiente e a produção de ciclos de culturas bem-sucedidas ou que possam produzir alto teor de nutrientes (WAMELINK et al. 2019).

No beneficiamento de sementes, técnicas ou tratamentos específicos antes da semeadura propiciam a germinação mais rápida e uniforme e a geração de mudas mais tolerantes aos estresses abióticos, maior produtividade e qualidade das culturas (SIVRITEPE & SIVRITEPE 2016; DELIAN et al. 2017).

Espécies da macroalga verde marinha cosmopolita *Ulva* spp. vêm sendo indicadas como bioestimulantes pela presença de substâncias bioativas (JAULNEAU et al. 2010; HERNÁNDEZ-HERRERA et al. 2014; MIZIBRA et al. 2020), bem como macro- e microelementos, aminoácidos, vitaminas, fitohormônios, betaínas e esteróis que atuam no estímulo da germinação de sementes (KHAN et al. 2009; CRAIGIE 2011; SHUULUKA ET et al. 2013; DYVIA et al. 2015).

O melhoramento no desenvolvimento vegetal de plantas que crescem sob condições extremas propiciará não somente aumento de biomassa, mas também acrescentará ao seu metabolismo, fitormônios e bioativos essenciais à alimentação humana (LLORENTE et al. 2018).

1.2 A AGRICULTURA COM ESPÉCIES DE *ULVA*

A partir da constatação de bioativos das espécies de *Ulva*, inúmeras pesquisas foram desenvolvidas para identificar os diversos usos comerciais, como as propriedades bioestimulantes dessa macroalga (KHAN et al. 2009)

As macroalgas possuem macro- e microelementos, aminoácidos, vitaminas, fitohormônios e polissacarídeos que atuam no crescimento da planta (AMANO & NODA, 1994; STIRK et al. 2003; KHAN et al. 2009; SHUULUKA et al. 2013).

Os fitohormônios regulam o crescimento vegetal (TAIZ & ZEIGER, 2010), como a auxina e citocina regulam o desenvolvimento de raízes e caules enquanto a giberelina e o ácido abscísico controlam a germinação e atuam no estímulo à germinação e indução a dormência (TUHY et al. 2013). Além disso, os hormônios diminuem a ação nociva dos estresse ambientais causados em plantas (KHAN et al. 2009). Porém, o resultado da aplicação de espécies de *Ulva* em cultivares varia de acordo com o local e a época do ano em que a macroalga foi coletada (WANG et al. 2007; FELÍCIO et al. 2012).

O uso de *Ulva fasciata* poderia suprimir o crescimento do *Stemphylium solani* Weber (um patógeno fúngico comumente infectando tomates) atrasando sua melanização e, consequentemente, reduzindo a penetração do hospedeiro (REIS et al. 2018). Esta espécie aumentou a resistência sistêmica dos cultivares de feijão contaminados pelo fungo *Colletotrichum lindemuthianum*

Saccardo e Magnus, causador da antracnose (Paulert et al. 2009) e pelo fungo *Uromyces appendiculatus* F. Strauss (Borsato et al. 2010), assim como em macieiras infectadas pelo fungo *Botrytis cinerea* De Bary (Montealegre et al. 2010).

O uso de espécies de *Ulva* também tem sido empregado no tratamento de sementes e no crescimento de plantas. O extrato líquido de *U. lactuca* favoreceu a germinação de sementes do tomateiro *S. lycopersicum* (HERNÁNDEZ-HERRERA et al. 2014). Pela presença de promotores de crescimento, como ácido indol-3-acético (IAA) e ácido indol-3-butírico (IBA), giberelinas, citocinas, micronutrientes, vitaminas e aminoácidos, pequenas concentrações de *Ulva reticulata* Forsskal aumentaram o percentual de germinação e crescimento da lentilha *Vigna mungo* L. (SELVAM et al. 2013).

Foi observado maior desenvolvimento de radícula e de sementes de *Arabidopsis* quando tratadas com baixas concentrações de extratos de *U. intestinalis* L. Porém, este efeito foi prejudicial quando usadas altas concentrações desta macroalga, que inibiram a germinação das sementes e o crescimento de mudas (Ghaderiardakani et al. 2019).

Extratos de *U. lactuca*, em pequenas quantidades (0,0001 g/L), promoveu um aumento na germinação de sementes de soja comparado ao controle e, em paramentos morfológicos, melhorou o crescimento da planta além de induzir um aumento no teor de clorofila a, b, clorofila total e carotenoides nas folhas (RAMARAJAN et al. 2012).

Extratos de espécies de *Ulva* também auxiliam as plantas na tolerância ao estresse abiótico, como o salino. Estudos constataram maior germinação de sementes de trigo (*Triticum aestivum* L.) submetidas ao estresse salino, quando embebidas em diferentes concentrações de *U. lactuca* (IBRAHIM et al. 2014). Estes autores atribuíram o aumento do crescimento do trigo à presença de ácido ascórbico, prolina e glutationa encontrados em *U. lactuca*. Sendo assim, essa macroalga poderá ser usada no benefício de cultivares estressados por salinidade.

O uso de macroalgas marinhas na agricultura é considerado um insumo orgânico, por não ocasionar danos ambientais e por ser seguro para a saúde animal a humana e, desta maneira, as algas marinhas apresentam potencial de aplicação comercial (KHAN et al. 2009).

Entender os mecanismos de atuação dos extratos de macroalgas marinhas no desenvolvimento e proteção de plantas é um aspecto fundamental para estabelecer protocolos de aplicação delas em cultivos de plantas e no tratamento de sementes. Isto permitirá outro tipo de tecnologia agrícola para o cultivo orgânico, que apesar de diferente da convencional, também propicia uma colheita contínua, minimiza doenças e melhora o rendimento do cultivo, porém sem causar danos ambientais e a saúde humana e animal (CHARLIER 2008; SMETACEK & ZINGONE 2013).

Estes resultados confirmaram o uso de macroalgas do gênero *Ulva* como bioestimulantes para fins de agricultura orgânica. Porém, mais investigações devem ser focadas na atuação destes bioativos nas vias metabólicas da planta através de estudos multidisciplinares (GUPTA et al. 2011).

Portanto, com as comprovações dos efeitos benéficos de *Ulva* spp. em cultivares, deve ser avaliado o efeito do uso de farinha dessas espécies na germinação de sementes para melhora agrícolas sob condições estressantes. O uso não se limita apenas a substratos de regolito lunar e marciano simulado como neste estudo, e poderá ser utilizado em outras espécies que sofrem com o estresse e que possuem importância econômica na agricultura terrestre.

1.3 USO DE BIOESTIMULANTE DE *ULVA LACTUCA* COMO TÉCNICA DE *SEED PRIMING* NO MELHORAMENTO VEGETAL SOB ESTRESSE ABIÓTICO

A agricultura sofre com vários efeitos adversos externos e, principalmente, estresse biótico e abiótico que determinam como será a produtividade e o desempenho fisiológico dessas plantas (BORGES et al. 2014). Existem várias técnicas para melhorar a produção, principalmente as que visam diminuir o estresse (REIS et al. 2020). Dentre estas, para a produção orgânica que prioriza produtos que não degradem o ambiente, o uso de extratos de macroalgas marinhas produziu resultados promissores (REIS et al. 2020; HUSSEIN et al. 2021; HAMOUDA et al. 2022). Como exemplo, técnicas para embeber as sementes que sofrem estresse podem estimular a germinação, aumentar o rendimento de biomassa e a produção de pigmentos fotossintéticos (HERNÁNDEZ-HERRERA et al. 2014, 2016; SIVRITEPE & SIVRITEPE, 2016).

Técnicas de *seed priming* com fitormônios são descritas como um complemento eficaz para minimizar os efeitos de fatores estressantes em vegetais (BENÍTEZ GARCÍA et al. 2020; EL BOUKHARI et al. 2021; HAMOUDA et al. 2022). *Seed priming* consiste em pré-embeber sementes com a ajuda de substâncias bioativas, como os fitormônios, e que auxiliam na quebra de dormência e no estímulo metabólico da semente (RHAMAN et al. 2020). Há a melhora do comprimento da parte aérea e massa fresca da raiz e plântulas de trigo, além de maior porcentagem de clorofila a, b e total, carotenoide e de carboidrato do grão (SHAHBAZI et al. 2015).

DU JARDIN (2015) definem bioestimulante vegetal como qualquer substância ou microrganismo que, quando aplicado à planta, aumenta sua eficiência nutricional, tolerância ao estresse abiótico e/ou características de qualidade do cultivar, independentemente do conteúdo de nutrientes. Porém, YAKHIN et al. (2017) define que os bioestimulantes são produtos de origem biológica que melhoram a produtividade de uma planta devido a algum componente que está atuando sobre a fisiologia daquele organismo. Além disso, também propõem que os bioestimulantes devem ser classificados de acordo com as funções das moléculas encontradas no composto (YAKHIN et al. 2017). São considerados eficientes ao serem aplicados em mínimas quantidades para promover o crescimento de plantas e muitos desses são derivados de macroalgas marinhas (DU JARDIN 2015).

Espécies de *Ulva* spp. podem ser indicadas como bioestimulantes ao serem usadas em técnicas de *seed priming* por hidratação (RHAMAN et al. 2021), por conter em sua composição hormônios essenciais para o desenvolvimento e estímulo à germinação (EL BOUKHARI et al. 2020; RHAMAN et al. 2021).

Resultados de pesquisas recentes ajudaram a esclarecer as elaboradas redes de sinalização e o sofisticado *crosstalk* que ocorre entre as diferentes vias de sinalização de hormônios e os papéis dos principais hormônios vegetais na regulação das respostas ao estresse abiótico e biótico (MIRANSARI et al. 2014; VERMA et al. 2016; TUAN et al. 2018;).

Os hormônios analisados neste estudo foram citocinina (tZ ou cZ), auxinas (IAA), etileno, ácido abscísico (ABA), ácido jasmônico (JA), ácido salicílico (SA), giberelinas (formas GA_4) de todas as sementes germinadas de *C. annuum* (pimenta), *L. sativa* (alface), *C. arietinum* (grão-de-bico) e *P. sativum* (ervilha). Essa técnica permite que as sementes sejam preparadas para receber esse bioestimulante antes da germinação e melhorar os processos metabólicos.

Utilizar bioestimulantes de extratos de *Ulva* spp. como em técnicas de preparação de sementes (*seed priming*) é uma alternativa promissora para melhorar a sua capacidade metabólica e o desenvolvimento fisiológico de plântulas quando estas estão sob condições estressantes (El BOUKHARI et al. 2021). Essa técnica é uma alternativa viável no que se refere a biodisponibilidade de nutrientes para o cultivo em sistema regolítico.

1.4 O GÊNERO *ULVA* L. (ULVALES, CHLOROPHYTA)

Macroalgas marinhas do gênero *Ulva* são oportunistas, de crescimento rápido e se enquadra morfologicamente no grupo das macroalgas foliáceas (STENECK & DETHIER,1994). A morfologia do talo, cor, textura, forma e arranjo das células superficiais, estrutura basal, número de pirenoides são parâmetros para a correta identificação das espécies (REIS 1992). Cortes anatômicos do talo são examinados através microscopia óptica e verificado o tamanho da célula, a relação altura/largura da célula e a espessura do talo (Figura 1) (REIS 1992; COTO & PUPO 2009).

Figura 1. Corte de *U. lactuca* vista através do microscópico estereoscópico com aumento de 40x para identificação da espécie neste estudo, no Jardim Botânico do Rio de Janeiro, RJ.

Neste estudo, propomos compreender o uso de *U. lactuca* e sua possível interação de metabólitos secundários e seus precursores de fitohormônios como reguladores de crescimento vegetal, no melhoramento do vigor de sementes de pimentão (*Capsicum annuum* L.), alface (*Lactuca sativa* L.), grão de bico (*Cicer arietinum* L.) e ervilha (*Pisum sativum* L.). Poucos estudos foram realizados a fim de verificar indicadores de estresse, assim como nenhum estudo foi relacionado com o uso de macroalgas marinhas como bioestimulante para sementes que se desenvolvem nesse tipo de sistema.

É viável compreender como essa biotecnologia advinda de produtos naturais marinhos será capaz de ser promissora como bioestimulante, além de discutir como os metabólitos das sementes/plântulas (ácidos graxos, aminoácidos, alcaloides, taninos e flavonoides) são diretamente induzidos pela resposta a aplicação de *Ulva*. Sobretudo, avaliar o conteúdo fitoquímico dessa macroalga e compreender o possível *crosstalk* entre diferentes fitormônios na geração de uma resposta ao estresse sob cada tratamento realizado.

2. HIPÓTESE

O bioestimulante de *U. lactuca* e sua possível interação de metabólitos secundários e seus precursores de fitohormônios como reguladores de crescimento vegetal poderá ser implementado como técnica de *seed priming* no melhoramento do vigor de sementes de pimentão, alface grão de bico e ervilha pois essas sementes são diretamente induzidas pela resposta a aplicação de *U. lactuca.*

3. OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar o efeito do uso de farinha de *Ulva lactuca* em diferentes concentrações na germinação e emergência de plântulas de pimentão (*Capsicum annuum*); alface (*Lactuca sativa)*; grão de bico (*Cicer arietinum*), e ervilha (*Pisum sativum*), em simuladores de regolito marciano e lunar.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar a eficiência do uso de farinha de *U. lactuca* no percentual de germinação e emergência de plântulas;
- Quantificar os indicadores de estresse biótico e abiótico através de análise hormonal de sementes cultivadas a fim de estabelecer uma relação quando submetidos ao tratamento com *U. lactuca*;
- Analisar o perfil químico qualitativo de *U. lactuca.*

Desta maneira, o presente documento está organizado em consonância aos objetivos específicos estabelecidos para o estudo, que são reunidos em formato de artigo, cujas formatação segue a norma do periódico para o qual será submetido.

4. DESENHO EXPERIMENTAL

Espécimes de *U. lactuca* L. foram coletados na Praia do Arpoador, Município do Rio de Janeiro, RJ (22º 59’S - 43º 11’W) em 09 de junho de 2021. No laboratório do Jardim Botânico do Rio de Janeiro, RJ, Brasil, foram triadas para remoção das epífitas e de organismos associados e identificadas de acordo com Koeman (1985). Posteriormente, as amostras foram congeladas e liofilizadas para serem trituradas em moinho de bola (SOLAB-SL38 Solab Científica), por dois minutos até 0,90 mm, através de peneira de crivo, seguindo o protocolo descrito por REIS et al. (2020).

Sementes (n= 10) de pimentão (*C. annuum*), alface (*L. sativa)*, grão de bico (*C. arietinum*), e ervilha (*P. sativum*) foram semeadas em placas de Petri (n= 4) contendo simulador de solo lunar e marciano, conforme a Figura 2 e embebidas em 5mL de água destilada (autoclavada por 40 minutos, a 120 °C) contento diferentes concentrações (0.2 g. L^{-1}; 0.4 g. L^{-1} de solução de farinha de *U. lactuca*) e água destilada como controle. Os resultados dos testes de germinação foram registrados diariamente até o décimo dia.

Estas quantidades foram adaptadas a partir dos resultados obtidos por REIS et al. (2020) que também utilizaram *U. lactuca.*

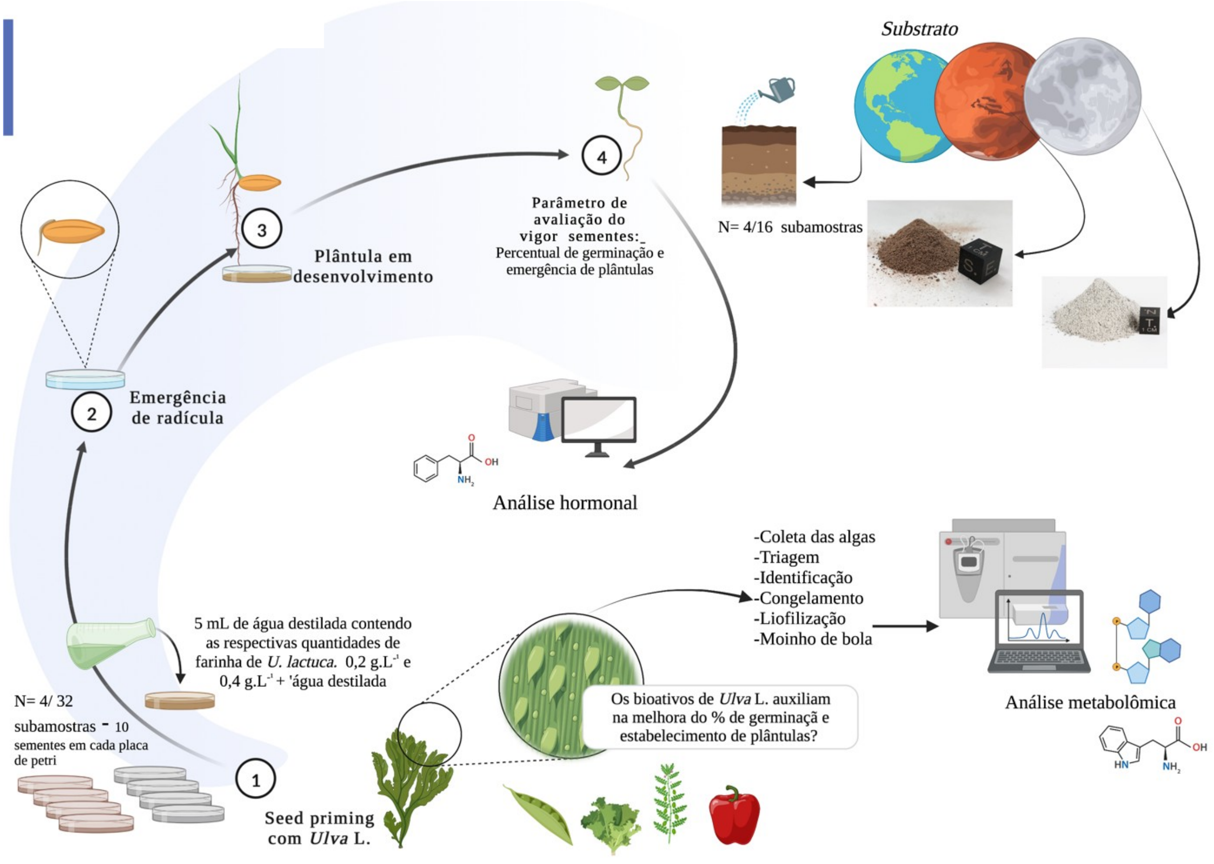


Figura 2. Desenho experimental para alcançar os objetivos propostos: a avaliação de germinação e emergência de plântulas de *Capsicum annuum*, *Lactuca sativa*, *Cicer arietinum* e *Pisum sativum* submetidas ao tratamento com farinha de *U. lactuca*.

5. ANÁLISE DE DADOS

Para avaliar o efeito da germinação das sementes quando submetidas aos tratamentos com *U. lactuca* (0,2 e 0,4 $g.L^{-1}$), utilizou-se análise estatística para estimar o grau de significância entre essas diferentes concentrações utilizando o software IBM® SPSS® Statistics. Os testes de homogeneidade da variância dos dados foram previamente testados para análises paramétricas dos dados pelo teste de Shapiro-Wilk. Análise de variância unifatorial (ANOVA) foi realizada para avaliar diferenças significativas entre cada parâmetro dos diferentes tratamentos em experimentos de sementes. Para avaliar as diferenças significativas entre as médias dos grupos a partir da ANOVA, foi utilizado o teste pos-hoc de Tukey. Os dados foram definidos como média ± desvio padrão, e o intervalo de confiança para a diferença dos testes foi de 95% (p= 0,05).

6. GERMINAÇÃO DAS SEMENTES

Os testes de germinação foram realizados no Jardim Botânico do Rio de Janeiro (protocolo) em estufa incubadora (tipo B.O.D. Eletrolab, modelo 122 FC), sob temperatura alternada de 20-30 °C e fotoperíodo de oito horas, e iluminação de 120 $\mu mol.m^{-2}.\ s^{-1}$ de acordo com as Regras para Análise de Sementes (BRASIL, 2009). As sementes foram previamente tratadas com solução de hipoclorito de sódio (5%) durante 5 minutos. A semeadura foi realizada em placa de Petri (10 cm de diâmetro) contendo como substrato para germinação os respectivos simulados lunar e marciano com 5 mL de água destilada em meio de cultivo. A placa foi forrada com filme plástico para evitar a evaporação do meio.

7. PARÂMETROS DE AVALIAÇÃO DO VIGOR DAS SEMENTES

O vigor das sementes foi determinado de acordo com as Regras para Análise de Sementes (Brasil, 2009) e os resultados dos testes de germinação foram registrados diariamente até o décimo dia. Quatro repetições foram utilizadas com 10 sementes em cada repetição. Diariamente, as sementes foram analisadas quanto à protrusão da raiz primária e a formação de plântulas normais. As sementes germinadas foram aquelas que apresentaram a protrusão da raiz primária com geotropismo positivo (1 mm) e as plântulas normais aquelas que apresentaram estruturas essenciais da plântula em perfeito estágio de desenvolvimento, conforme descrição nas Regras para Análise de Sementes (Brasil 2009). Os parâmetros de avaliação de vigor da semente foi o percentual de germinação e o percentual de emergência de plântula, os quais foram calculados com as fórmulas proposta por LABOURIAU & PACHECO (1978).

8. RESULTADOS DOS DADOS OBTIDOS NO JARDIM BOTÂNICO DO RIO DE JANEIRO (JBRJ) – PROTOCOLO

8.1 EFEITOS DA FARINHA DE *U. LACTUCA* NA GERMINAÇÃO E EMERGÊNCIA DE PLÂNTULAS

8.1.2 ANÁLOGO DO REGOLITO MARCIANO *MGS-1 MARS GLOBAL SIMULANT*

O percentual de germinação e de emergência de plântula de sementes de pimentão, grão de bico, ervilha e alface em tratamentos com adição de farinha e de *U. lactuca* (g.L^{-1}) após 10 dias de cultivo *in vitro* em substrato de análogo marciano são reunidos na Tabela I. Os dados foram definidos como média ± desvio padrão.

Tabela I. Percentual de germinação e de emergência de plântula de sementes de pimentão, grão de bico, ervilha e alface em tratamentos com adição de farinha e de *U. lactuca* (g.L^{-1}) após 10 dias de cultivo *in vitro* em substrato de análogo marciano

Regolito marciano

Germinação %	**Controle (água destilada)**	***U. lactuca* 0.2 g. L-¹**	***U. lactuca* 0.4 g. L-¹**
Pimentão	57 ± 5,8	80 ± 20,0	47 ± 25,2
Grão de bico	83 ± 5,8	90 ±10,0	73 ± 37,9
Ervilha	70 ± 10,0	63 ±15,3	67 ± 23,1
Alface	60 ± 0,0	70 ±26,5	60 ±20,0

Emergência de plântula %	**Controle (água destilada)**	***U. lactuca* 0.2 g. L-¹**	***U. lactuca* 0.4 g. L-¹**
Pimentão	40 ± 0,0	40 ± 26,5	7 ± 11,5
Grão de bico	70 ± 11,5	77 ± 20,8	63 ± 35.1
Ervilha	53 ± 5,8	43 ± 25,2	67 ± 23,1
Alface	47 ± 5,8	57 ± 25,2	60 ± 20,0

8.1.3 ANÁLOGO DE REGOLITO LUNAR FARSIDE LUNAR HIGHLANDS SIMULANT

O percentual de germinação e de emergência de plântula de sementes de pimentão, grão de bico, ervilha e alface em tratamentos com adição de farinha e de *U. lactuca* (g.L^{-1}) após 10 dias de cultivo *in vitro* em substrato de análogo lunar são reunidos na Tabela II. Os dados foram definidos como média ± desvio padrão.

Tabela II. Percentual de germinação e de emergência de plântula de sementes de pimentão, grão de bico, ervilha e alface em tratamentos com adição de farinha e de *U. lactuca* (g.L^{-1}) após 10 dias de cultivo *in vitro* em substrato de análogo lunar

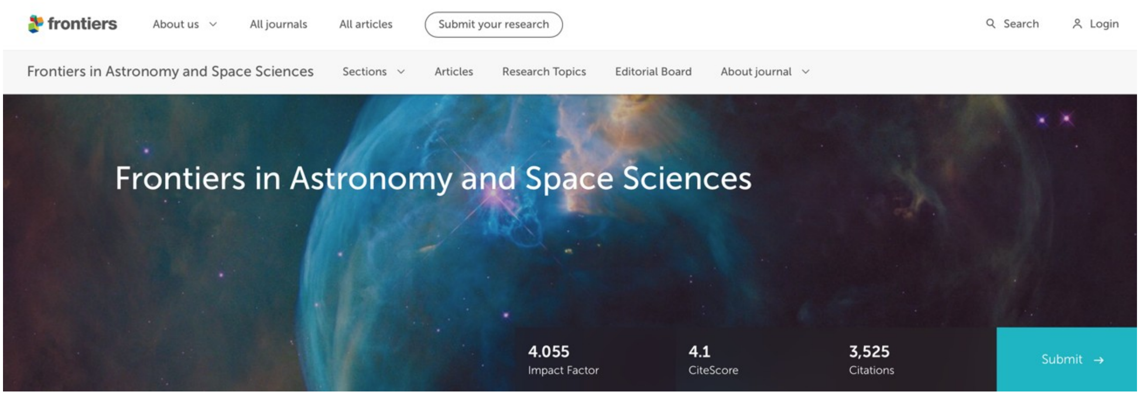
Regolito lunar

Germinação %	**Controle (água destilada)**	***U. lactuca* 0.2 g.L-¹**	***U. lactuca* 0.4 g.L-¹**
Pimentão	90±0,0	87±5,8	83±5,8
Grão de bico	90±0,0	90±10,0	87±11,5

Ervilha	80±0,0	90±10,0	77±11,5
Alface	80±0,0	73±5,8	77±5,8

Emergência de plântula %	**Controle (água destilada)**	***U. lactuca* 0.2 g. L^{-1}**	***U. lactuca* 0.4 g. L^{-1}**
Pimentão	63±5,8	63±15,3	60±10,0
Grão de bico	73±0,8	90±17,3	87±15,3
Ervilha	70±10,0	83±5,8	67±15,3
Alface	67±15,3	77±25,2	83±5,8

9. ARTIGO SUBMETIDO



Seed Priming with *Ulva lactuca L.* in Cultivars Grown in Martian and Lunar Regolith Analogues

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Abstract

Due to the imminent nature of human settlement on lunar and/or Martian bases, there is an urgent need to implement and make compatible food production in these environments by optimizing available lunar and Martian regolith resources. In addition, the Martian and lunar economy will depend on the cultivation and marketing of low-cost food resources obtained through sustainable biotechnologies. Macroalgae extracts are considered promising low-cost resources that can be used in seed priming before sowing to promote faster and more uniform germination. The present work experimentally evaluated the effect of different concentrations of the green macroalgae *Ulva lactuca* L. powder on the germination and growth of the cultivars *Capsicum annuum* L. (pepper), *Lactuca sativa* L. (lettuce), *Cicer arietinum* L. (chickpea) and *Pisum sativum* L. (pea) in Martian and lunar regolith simulators. The germination and emergence of the seeds were evaluated under controlled greenhouse conditions with the addition of two concentrations of *U. lactuca* powder (0.2 and 0.4 $g.L^{-1}$). The qualitative chemical analysis of *U. lactuca* was performed for the purpose of knowledge of its bioactives (alkaloids, terpenoids, phenol, saponins, flavonoids, quinine, and steroids) important as metabolic pathways for the formation of phytohormones. The speed of germination and emergence of the seeds of the cultivars in lunar regolith were higher than those observed in Martian regolith ($p < 0.05$). In Martian regolith, the best treatment for germination of pea and chickpeas seeds was 0.2 $g.L^{-1}$ ($p= < 0.001$; $p= 0.006$), as well as seedling emergence. In lunar regolith, pea seeds germination peaks were obtained with both treatments (0.2 $g.L^{-1}$ $p= < 0.001$ and 0.4 $g.L^{-1}$ $p= 0.001$), as well as beak grain seeds (0.2 $g.L^{-1}$ $p= 0.002$ and 0.4 $g.L^{-1}$; $p= 0.008$). The germination percentage of lettuce seeds grown in lunar regolith was better in 0.2 $g.L^{-1}$ compared to the control ($p= 0.010$). For the other seeds, there was no significant difference. From the results obtained, we propose using *U. lactuca* powder as a biostimulant for the studied cultivars, due to the presence of plant growth regulators (PGRs) that improve germination and seedling emergence under stressful conditions.

Keywords: regolith simulant; plants in space; seed priming; seed vigor; *Ulva* spp biostimulant.

1. Introduction

The establishment of space agriculture will be a decisive event for the survival of human beings outside Earth, mainly because it is a viable food source and income generator, but there are many obstacles to be overcome (Cannon and Britt, 2019). For example, luminosity, crucial for plant development, is considerably lower compared to that of the Earth, making subsidized and fully controlled supplementation necessary (Ming and Brady, 1989; Wamelink et al., 2014; Wheeler, 2017). In addition, the cosmic radiation that reaches the Martian surface affects plant growth since it is 17 times greater than on Earth, and could drastically decrease plant biomass (Tack et al., 2021)

Multidisciplinary studies explored life-support bioregenerative and food production in extreme space conditions (Miller and Ward, 1966; Wheeler et al., 2011; Poulet et al., 2016). For example, the use of marine macroalgae by their ability to convert carbon dioxide and water into oxygen, as well as research with cyanobacteria fed with Martian analogues (MGS-1) and Mars atmosphere as a source of nutrients to be suitable for photobioreactors of life support systems (Miller and Ward, 1966; Verseux et al., 2021). Technologies that aim to measure the gas exchange of the entire plant, under the influence of different air velocities, allow for understanding the photosynthesis measurements performed by the individual (Poulet et al., 2020).

In space agriculture, the challenges to be overcome to ensure efficient growth of cultivars include the production of successful crop cycles or using crops that accumulate a high nutrient content (Wamelink et al., 2019). The improvement in plant development will not only promote increased biomass but will also add phytohormones and bioactives essential to human food to the metabolism of cultivars (Llorente et al., 2018)

For future interplanetary nutrition, it is important to choose crops that do not require processing, requires minimum preparation time, provide essential vitamins (B1, K, C) and do not degrade like most bioactive compounds in stored food systems (Johnson et al., 2021; Poulet et al., 2022). In addition, the production of fresh foods may provide important nutritional and psychological information for long-term missions and/or for future human settlers in the ideal choice of vegetables with organoleptic properties (Massa et al., 2015; Zhang et al., 2022)

Plant germination and performance can be altered or impaired under atypical conditions in soils of absent organic matter, such as those of Mars and moon. To ensure a self-sufficient civilization, it will be necessary to improve the cultivation of food plants through biotechnology, robotics, and agriculture (Wheeler et al., 2011; Wheeler, 2017; Cannon and Britt, 2019) to associate biological systems with local natural resources (Verseux et al., 2016; Cannon and Britt 2019). Thus, these foods' mechanisms and biological processes must be fully understood (Verseux et al., 2016). In the processing of seeds, techniques or specific treatments before sowing provide faster and more uniform germination and the generation of seedlings more tolerant to abiotic stresses, higher productivity, and crop quality (Paparella et al., 2015; Sivritepe and Sivritepe, 2016)

Biostimulants are considered efficient when applying in minimal amounts to promote plant growth and many of these are derived from marine macroalgae (du Jardin, 2015). Species of the cosmopolitan marine green macroalgae *Ulva* spp. are indicated as biostimulants and biorefined products by the presence of bioactive substances (Jaulneau et al., 2011; Hernández-Herrera et al., 2014; Sekhouna et al., 2021; Pappou et al., 2022), as well as macro- and microelements, amino acids, vitamins, phytohormones, betaines and sterols that act in stimulating seed germination (Khan et al., 2009; Hafting et al., 2015; Sekhouna et al., 2021; Pappou et al., 2022). For example, marine macroalgal extracts were used to soak seeds with promising results for organic production in species, such as *Solanum lycopersicum*, *Abelmoschus esculentus*, *Ceratonia siliqua, Triticum aestivum*, *Vigna radiata*, *Vigna sinensis* and *Zea may*s (Ibrahim et al., 2014; Divya et al., 2015; Reis et al., 2020; El Boukhari et al., 2021; Hussein et al., 2021; Zouari et al., 2022)

Seed priming techniques with phytohormones have been reported as an effective complement to minimize the effects of environmental stressful conditions on vegetables (El-Aziz Kasim et al., 2016; García et al., 2020; Hamouda et al., 2022). This technique-type consists of pre-soaking seeds with the help of substances that contain bioactives, such as phytohormones, and that aid in the breaking of dormancy and metabolic stimulation of seeds (Rhaman et al., 2020). There is an improvement in shoot length and fresh root mass of wheat seedlings, as well as a higher percentage of chlorophyll a, b and total, carotenoid and wheat grain carbohydrate (Shahbazi et al. 2015) and, in tomato species, there is an improvement in germination and growth mainly of the stem and it can act as an antibacterial agent (Sekhouna et al., 2021).

The use of *Ulva* spp. extracts as a biostimulant is a promising alternative to improve the metabolic capacity and physiological development of seedlings when they are under stressful conditions (El Boukhari et al., 2021). These macroalgae can be indicated when implemented in seed priming techniques by hydration (Rhaman et al., 2021) because it contains hormones essential for the development and stimulation of germination in its composition and allows the seeds to be prepared to receive this biostimulant before germination and for the improvement of their metabolic processes (El Boukhari et al. 2020; Rhaman et al. 2021).

The use of dry and crushed marine macroalgae in the form of powder may reduce costs due to the facility of production, and low cost of obtaining when compared to the extraction of the polysaccharide called ulvan (Alves et al., 2013; Reis et al., 2020). Verifying the germination and emergence of seedlings of cultivars when submitted to the treatment of *Ulva lactuca* L. is a way of understanding whether there is a crosstalk between the bioactives of marine macroalgae and seed metabolism (Hussein et al., 2021). To this end, it is important to perform the analysis of the presence of bioactive compounds of *U. lactuca* and its phytochemical extracts for compression of its composition, and hormonal analysis of seeds.

All essential macro- and micronutrients for plant growth are present in sufficient amounts, both in lunar and Martian regolith, except the reactive nitrogen (Duri et al., 2022). Nitrogen in absorbable forms (NO_3 and NH_4) is one of the essential minerals necessary for the growth of almost all plants due to the mineralization of organic matter and nitrogen fixation through mutualistic associations between plants and the terrestrial soil microbiome (Stevens et al., 2011). However, organic matter is absent on Mars and the Moon, although both sites contain graphitic carbon (Parnell, 2005) and no microorganism activity has been recorded to date. The absence of sufficient absorbable nitrogen can be resolved using nitrogen-fixing species (Llorente et al., 2018).

Martian soil exhibits high levels of perchlorate ($NaClO_4$) that are toxic to humans, therefore, cultivation in this type of soil will need specific care (Eichler et al., 2021). Studies have been carried out with the aim of reducing the levels of this toxic substance. For example, some organisms have been suggested as potential candidates for Martian regolith, such as plants of woody species, non-woody terrestrial plants, aquatic plants, and potential microbes that carry out phytoremediation through phytoaccumulation, phytodegradation, and rhizodegradation (Misra et al., 2021), but these authors recommend further studies in the area so that agriculture based on ISRU (In-Situ Resource Utilization) become viable.

Therefore, to improve biosynthetic capacity under adverse conditions, as well as biomass production, techniques should be considered that allow for increased vigor and hormonal indices of these plants (Llorente et al., 2018) to play a role in the development of a self-sustaining civilization (Verseux et al., 2016).

Previous studies have demonstrated the success of the cultivation of viable crops in Martian and lunar regolith-based agriculture (RBA) with nutrient supplementation (Wamelink et al., 2014, 2019; Eichler et al., 2021; Kasiviswanathan et al., 2022), but some of these studies did not consider important aspects, such as complete sterilization of the regolith and, in Martian analogues, the addition of perchlorate to the regolith. Although it is considered a viable solution for a continuous and self-sustaining alternative to *in-situ* cultivation, the use of Martian and lunar regolith as the answer to complete ISRU-dependent missions remains uncertain (Eichler et al., 2021).

Here, we propose to experimentally verify the improvement of seed vigor of peppers (*Capsicum annuum* L.), lettuce (*Lactuca sativa* L.), chickpeas (*Cicer arietinum* L.) and peas (*Pisum sativum* L.) in Martian regolith simulators, using different concentrations of *U. lactuca* powder as a seed germinates and develops under greenhouse incubator conditions. In addition, it was also evaluated the phytochemicals of this marine macroalga to understand the possible crosstalk between different phytohormones in the generation of a stress response to each treatment performed.

2. Material and methods

2.1 Collection and obtaining of U. lactuca powder

Ulva lactuca specimens were collected at Arpoador Beach, Rio de Janeiro, RJ (22º 59'S - 43º 11'W) on June 9, 2021. In the laboratory of the Botanical Garden Research Institute of Rio de Janeiro, RJ, Brazil, epiphytes, and associated organisms were removed and this macroalga was identified according to Koeman (1985). The specimens were frozen and later freeze-dried to obtain powder used in in vitro experiments. The *U. lactuca* powder was obtained in a ball mill (SOLAB-SL 38 Solab Científica) for 2 minutes until it reached the granulometry (0.9 mm) (Reis et al. 2020). The pH at a concentration of 0.2 g. L^{-1} was 5.1 (32.5 salinity) and pH at a concentration of 0.4 g. L^{-1} was 5.36 (34.75 salinity).

2.1.1 Regolith

2.1.2. Martian regolith

Experiments with the analog of Martian regolith were carried out with the MGS-1 Mars Global Simulant (University of Central Florida, Exolith Lab). The average particle size of 90 µm. According to CLASS Exolith Lab, the relative abundances of each element, evaluated by the percentage of wet weight (wt%) detected by X-ray fluorescence were: SiO_2 (42.9), TiO_2 (0.6), Al_2O_3 (12.8), FeO (11.2), MnO (0.1), MgO (14.6), CaO (7.4), Na_2O (1.5), K_2O (0.6) and P_2O_5 (0.1).

2.1.3. Lunar regolith

Experiments with the analog of lunar regolith were carried out with the Off Planet Research H3N (Farside Lunar Highlands Simulant) (University of Central Florida, Exolith Lab). The LHS-1 Lunar Highland Simulator is developed by CLASS Exolith Lab (University of Central Florida). Average particle size 50 µm. According to CLASS Exolith Lab, the relative abundances evaluated of each element, detected by the percentage of wet weight (wt%) by X-ray fluorescence, were: SiO_2 (51.2), TiO_2 (0.6), Al_2O_3 (26.6), FeO (2.7), MnO (0.1), MgO (1.6), CaO (12.8), Na_2O (2.9), K_2O (0. 5) and P_2O_5 (0.1).

2.2. Conditions for seed germination and seedling development

Germination tests were performed in greenhouse incubator Conviron (GEN1000 SH) Winston-Salem State University (WSSU), according to the conditions described in Table 1. The illumination was 400 µmol.m^{-2}.s, obtained by fluorescent lamps. The seeds of each cultivar species were previously treated with 5% sodium hypochlorite solution for 5 minutes and sowing was performed in a Petri dish (10 cm in diameter) containing separate lunar and Martian regolith simulants as substrate. Each Petri dish was lined with plastic film to prevent evaporation of the medium.

Table 1. Controlled conditions were maintained in an incubator for seed germination assays in terrestrial soil samples and simulated lunar and Martian regolith.

Conditions	Day	Night
Photoperiod	16:00 h	8:00 h
Temperature	23 °C	18 °C
Relative Humidity	70%	70%
CO_2	400 ppm - 1000 ppm	1000 ppm
Light	~ 400 µmol.m^{-2}. s^{-1} HPLED	N/A

2.3. Experimental design

Seeds (n=10 for each cultivar in each Petri plate) of pepper (*C. annuum*), lettuce (*L. sativa)*, chickpeas (*C. arietinum*), and peas (*P. sativum*) were concentrated in 5mL of the different concentrations (0.2 and 0.4 g.L^{-1}) of *U. lactuca* powder solution, and distilled water, in the control and distributed in Petri plates (n= 4 replicates) containing lunar and Martian soil simulator (treatments). These quantities were adapted from the results obtained by Reis et al. (2020) who also used *U. lactuca* in germination assays. To compare the physiological behavior of the seeds in the control, the ground soil (Elite Soil from The Soil Makers Company [Colchester, Connecticut, United States]) was used. The seeds were obtained from Johnny's selected seeds company (Winslow, ME, USA).

2.4. Evaluation of seed vigor

Seed vigor was determined according to the Seed Analysis Rules (Brasil, 2009). Seed germination performance was evaluated based on radicle elongation (> 1 mm) with positive geotropism, as well as by seedling formation with well-developed essential structures (Brasil, 2009). The evaluations were performed daily until the stabilization of the count. The peak value was derived from the cumulative germination percentages, considering the number of days needed to reach this percentage (Czabator, 1962). The parameters of seed vigor evaluation were germination percentage and seedling emergence percentage, which were calculated with the usual procedure previously described (Labouriau and Pacheco, 1978).

2.5. Analysis of the qualitative chemical profile of U. lactuca

The qualitative analysis of the chemical profile of *U. lactuca* was obtained in the Metabolomics Core Lab, Winston-Salem State University (WSSU). Two types of extracts of *U. lactuca* were prepared, methanol and aqueous. A total of 5 g of *U. lactuca* powder was suspended in water (500 mL) and incubated for 24 h at 40 °C in an orbital shaker (Corning® LSE™ Compact Centrifuge). The resulting solution was then filtered through Whatman number 1 filter paper, and the extracts were stored at 4°C. The qualitative analysis was carried out using the following standard procedure for identifying phytochemical constituents, such as alkaloids, terpenoids, phenol, sugar, saponins, flavonoids, quinines, protein, and steroids (Altemimi et al., 2017). The *U. lactuca* solution was initially characterized using UV-VIS spectroscopy, Fourier Infrared spectroscopy (FTIR), and Gas chromatography-mass spectrometry (GCMS) analysis. The presence of different components was also analyzed using an FT-IR spectrophotometer (Shimadzu: IRAffinity1S), and the IR spectra were recorded in the wavelength range of 400 to 4000 cm^{-1}.

2.6. Hormonal analysis of cultivated seeds

The samples were analyzed at Astrobotany Laboratory, Department of Biological Sciences, Winston-Salem State University (WSS) and aimed to understand the crosstalk between different hormones in generating a stress response under each treatment.

Cytokinin hormones (tZ or cZ), auxins (IAA), ethylene, abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), gibberellins (GA_4 active forms of Gibberellins) of all germinated species were quantified: *C. annuum* (pepper), *L. sativa* (lettuce), *C. arietinum* (chickpeas) and *P. sativum* (pea). For the profile of cytokinins, auxins, and abscisic acid, a fresh weight (FW) of 100 mg was placed in 2mL microcentrifuge tubes along with a 5 mm zirconium oxide grinding ball. The samples were quickly frozen in liquid nitrogen and stored at -80 °C. Smaller samples were collected directly in a microcentrifuge tube with methanol (300 µL).

After the measurement of FW of each sample, homogenization was performed before purification. The samples were placed on a Teflon adapter (15 min) to maintain the temperature of -80°C while they were ground in a MM 301 (Retsch GmbH) mixer mill at 25 Hz frequency for 2 minutes. After the sample was fully homogenized, 0.5 mL of cold extraction solvent (-20 °C) was added to the tubes (for cytokinins and auxin - 75% methanol and formic acid solution and, for abscisic acid, - 75% methanol and methylene chloride). Also, 50 μL of internal standards were added, mixed, and left at - 20 °C for 1 hour.

The samples were centrifuged at 20,000 x g at 4°C for 20 minutes. The overnatant was then transferred to new 2mL tubes. The pellet obtained was extracted again using 0.5 mL of solvent (for each phytohormone) for 30 minutes. This process ensures more than 95% solvent extraction.

During the quantification phase of Liquid Chromatography Coupled to Mass Spectrometry (LC-MS Therm Scientific Orbitrap ID-X Tribrid), the dry samples were dissolved in 30 μL of 15% acetonitrile in water and 30 μL of 5% methanol in water. After being dissolved in the solution, the samples were centrifuged at 20.000 x g at 4°C for 20 minutes. The overnatant was transferred to automatic sampler vials. An aliquot of 1/10 was injected in LC-MS for metabolite analysis. Calibration patterns were also injected to obtain the calibration curve parameters.

2.7. Statistical analysis

To evaluate the effect of seed germination when submitted to treatments with *U. lactuca* (0.2 and 0.4 g.L^{-1}), statistical analysis was used to estimate the degree of significance between these different concentrations using the IBM® SPSS® Statistics software. The tests of homogeneity of the variance of the data were previously tested for parametric analyses of the data by the Shapiro-Wilk test. Unifactorial variance analysis (ANOVA) was performed to evaluate significant differences between each parameter of the different treatments in seed experiments. To evaluate the significant differences between the means of the groups from the ANOVA, the Tukey pos hoc test was used. The data were defined as mean ± standard deviation, and the confidence interval for the difference of tests was 95% ($p = 0.05$).

3. Results

3.1. Effects of U. lactuca powder on germination and seedling emergence

3.1.1. Martian regolith analog MGS-1 Mars Global Simulant

Seed germination of cultivars *C. annum*, *P. sativum*, *C. arietinum,* and *L. sativa* was a total of 100% in terrestrial soil, but variable under the other treatments and in Martian regolith.

The germination percentages of *C. annum* seeds (Figure 1A) were not significantly distinct under the two concentrations: concentrations of 0.2 and 0.4 g. L^{-1} of *U. lactuca* powder (p= 0.135 and p= 0.645, respectively) when compared with those of the control (MGS Control). However, the seedling emergence of *C. annum* (Figure 2A) was significantly different from the control (MGS Control) when applied at 0.4 g. L^{-1} of *U. lactuca* powder (p= 0.001).

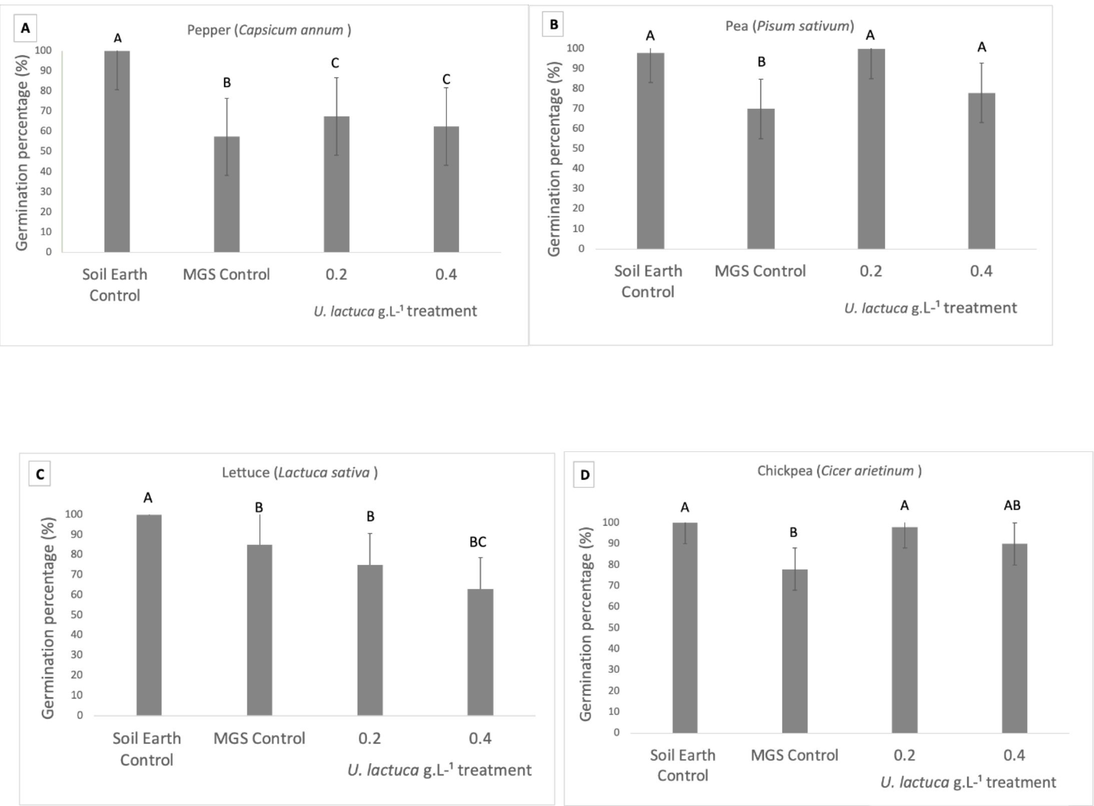

Figure 1. Germination percentage of *C. annum* (A)*, P. sativum* (B)*, L. sativa* (C), and *C. arietinum* (D) in controls (terrestrial soil and Martian regolith MGS-1) and in treatments (0.2 and 0.4 g.L^{-1} of *U. lactuca* powder), n= 10 for each cultivar.

When applied at 0.2 g.L^{-1} in *P. sativum* seeds, germination percentage (Figure 1B) was higher at 97.5 ± 5% and significantly different (p= < 0.001) compared to the MGS Control (70 ± 8.2 %). Treatment subjected to lower concentrations of *U. lactuca* powder on *P. sativum* seeds grown in Martian regolith obtained a germination percentage considerably as good as cultivated in terrestrial soil (97.5 ± 5%). Among the concentrations, 0.2 and 0.4 g.L^{-1} of *U. lactuca* powder, there was a significant difference (p= 0.001) where the concentration in a smaller amount favored the development of germination of *P. sativum* seeds. Regarding seedling emergence (Figure 2B), the best concentration for *P. sativum* seeds was also 0.2 g.L^{-1} compared to the control (MGS Control p= < 0.001) and with significant difference between treatments.

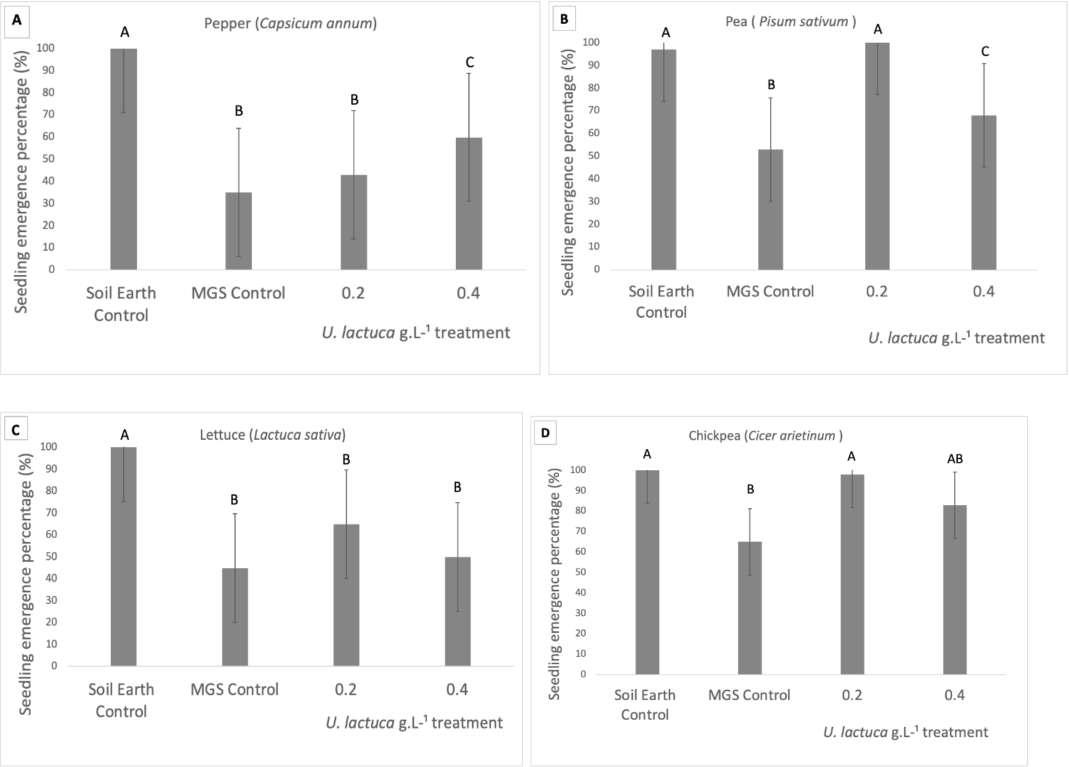

Figure 2. Percentage of the emergence of seedlings of *C. annum* (A), *P. sativum* (B), *L. sativa* (C) e *C. arietinum* (D) in controls (terrestrial soil and Martian regolith MGS-1) and treatments (0.2 and 0.4 g.L^{-1} *U. lactuca* powder), n=10 for each cultivar.

The percentage of germination of *L. sativa* (Figure 1C) submitted to 0.4 g. L^{-1} of *U. lactuca* powder was significantly different (p= 0.025) compared to the control (MGS Control). *Lactuca sativa* seeds germinated well in Martian regolith (85 ± 5.7 MGS Control) without any treatment with *U. lactuca*. When applied 0.2 L^{-1}, there was no significant difference (p= 0.470) in relation to the control, as there was no difference between treatments (p= 0.291). Concentrations of 0.2 L^{-1} (p= 0.064) and 0.4 L^{-1} (p= 0.892) exerted significantly different effects compared to MGS Control on the development of *L. sativa* seedlings (Figure 2C).

Capsicum arietinum seeds (Figure 1D), when cultivated in Martian regolith and submitted to the treatment of 0.2 g.L^{-1}, had a significantly different percentage of germination in relation to the MGS Control (p= 0.006). Furthermore, the percentage of seed germination (97.5 ± 5 %) was different when compared to that in terrestrial soil (100 ± 0 %). The development of *C. arietinum* seedlings (Figure 2D) was significantly different in relation to the MGS Control (p= 0.01) when subjected to lower amounts.

3.1.2. In lunar analog Farside Lunar Highlands Simulant

Seed germination of the cultivars *C. annum*, *P. sativum*, *C. arietinum,* and *L. sativa* was total (100%) in terrestrial soil and very similar in all cultivars, but variable under the other treatments and in lunar regolith, as well as in seedling emergence.

Capsicum annum seeds (Figure 3A) did not germinate with a significant difference between the 0.2 and 0.4 g.L^{-1}treatments and the LHS control (p=1.00 and p= 0.35, respectively). However, when small amounts of *U. lactuca* powder were applied, seed germination was stimulated (90.0 ± 14.1%) when compared to the corresponding LHS control (85.0 ± 5.8%).

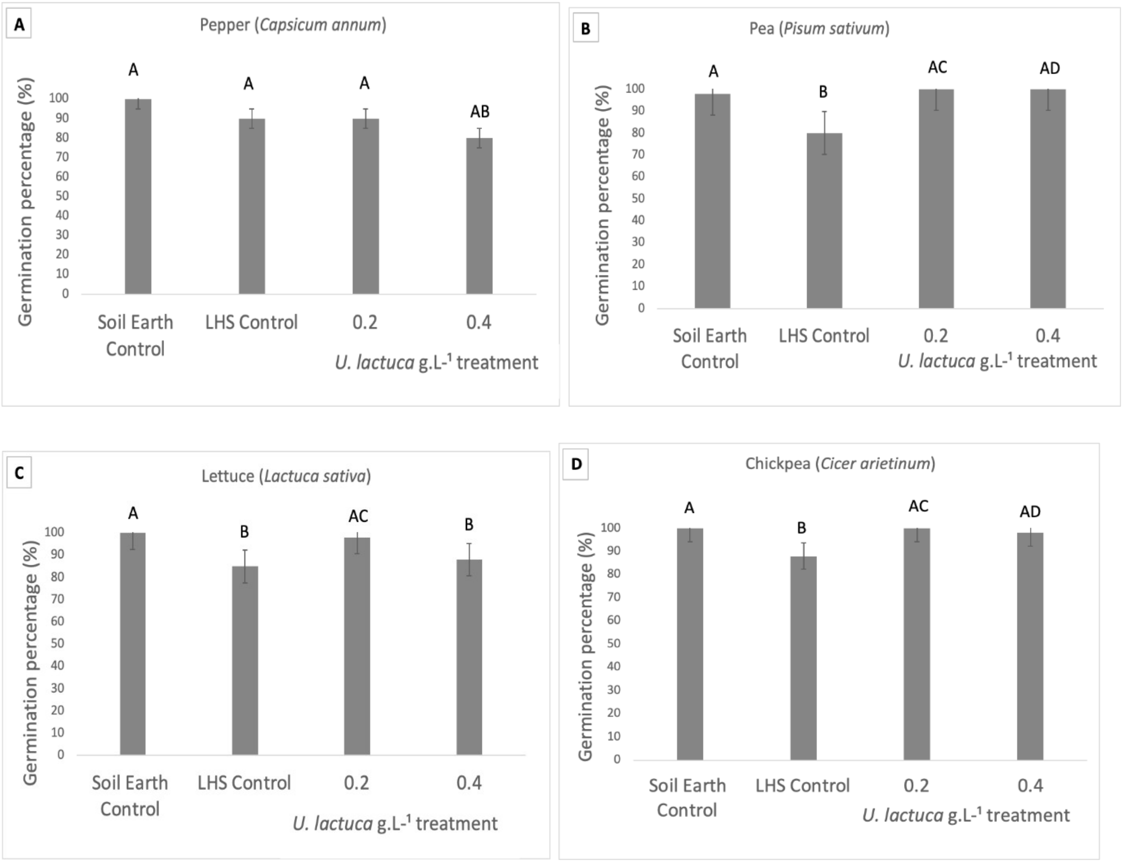

Figure 3. Percentage of germination of *C. annum* (A)*, P. sativum* (B)*, L. sativa* (C), and *C. arietinum* (D) in controls (terrestrial soil and lunar regolith LHS-1) and treatments (0. 2 and 0. 4 g.L^{-1} *U. lactuca* powder), n= 10 for each cultivar.

Regarding seedling emergence (Figure 4A), both treatments (0.2 and 0.4 g. L^{-1}) were not significantly different from the control (LHS control p= 0.224 and p= 0.655m respectively). However, when small amounts of *U. lactuca* powder were applied, the percentage of *C. annum* seedling development was stimulated (80.0 ± 20 %) compared to the corresponding LHS control (63.0 ± 12.5 %).

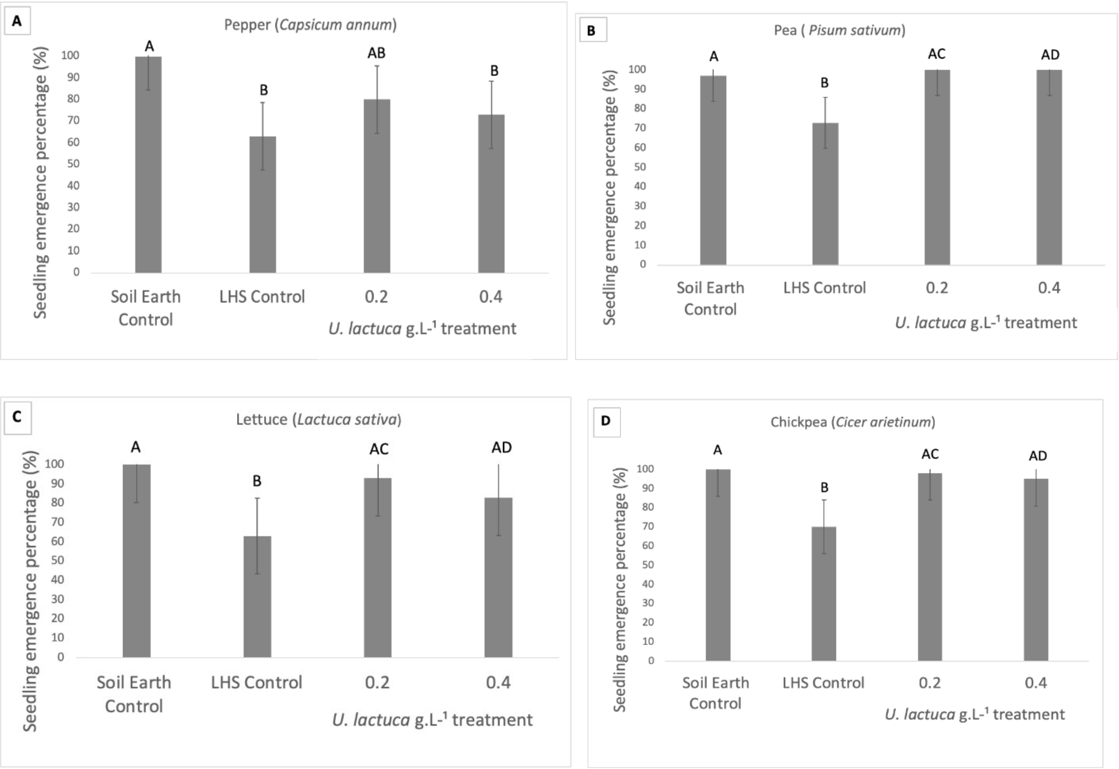

Figure 4. Percentage of the emergence of *C. annum* (A)*, P. sativum* (B)*, L. sativa* (C), and *C. arietinum* (D) in controls (terrestrial soil and lunar regolith LHS-1) and treatments (0. 2 and 0. 4 g. L^{-1} *U. lactuca* powder), n= 10 for each cultivar.

The seeds of *P. sativum* (Figure 3B) had a significant percentage of germination in relation to the control (LHS control) in both concentrations (0.2 p= < 0.001 and 0.4 g.L^{-1}; p= 0.001). The treatment when applied in proportions of 0.2 and 0.4 g. $L^{-1}$of *U. lactuca* powder, seeds grown in lunar regolith resulted in a germination percentage (100 ± 0 %; 100 ± 0 %) considerably as good as seeds grown in terrestrial soil (97.5 ± 5 %).

The results with *P. sativum* seeds were also favorable in relation to seedling emergence (Figure 4B), with a significant difference between the control (LHS Control) and the two concentrations of 0.2 and 0.4 g. L-1 of *U. lactuca* powder (p= < 0.001; p= 0.001).

In *L. sativa*, the percentage of germination (Figure 3C) was significantly different when the seeds were submitted to 0.2 g.L^{-1} of *U. lactuca* powder in relation to the control (LHS Control p= 0.010). Regarding the two treatments, 0.2 and 0.4 g. L^{-1} of *U. lactuca* powder, the one with the lowest concentration, had a difference (p= 0.04), which suggests that for *L. sativa* seeds, the best treatment is 0.2 g.$L^{-1}$of *U. lactuca* powder.

The percentage of the emergence of *L. sativa* seedlings (Figure 4C) had a significant difference in both concentrations in relation to the control (LHS Control p= 0.001 and p= 0.023). However, when subjected to lower concentrations, the percentage of seedling emergence was as good as in terrestrial soil (92.5 ± 5%; 100 ± 0%).

The percentage of germination in *C. arietinum* seeds (Figure 3D) was significantly different when compared to the control (LHS Control) in both concentrations: 0.2 and 0.4 g.L^{-1} of *U. lactuca* powder (p= 0.002; p= 0.008, respectively).

The percentage of the emergence of *C. arietinum* seedlings (Figure 4D), when compared to the control (LHS Control), was also significantly different in both concentrations (both p= < 0.001) and can be considered effective to treat seeds of this cultivar.

3.2. Relationship between germination speed and seedling emergence to lunar and Martian regolith.

The germination speed of seeds of cultivars developed in lunar regolith was higher than in Martian regolith (p= 0.035) (Figure 5). The same pattern was observed in the emergence of seedlings, with the seeds in lunar regolith developing more rapidly (p= 0.023).

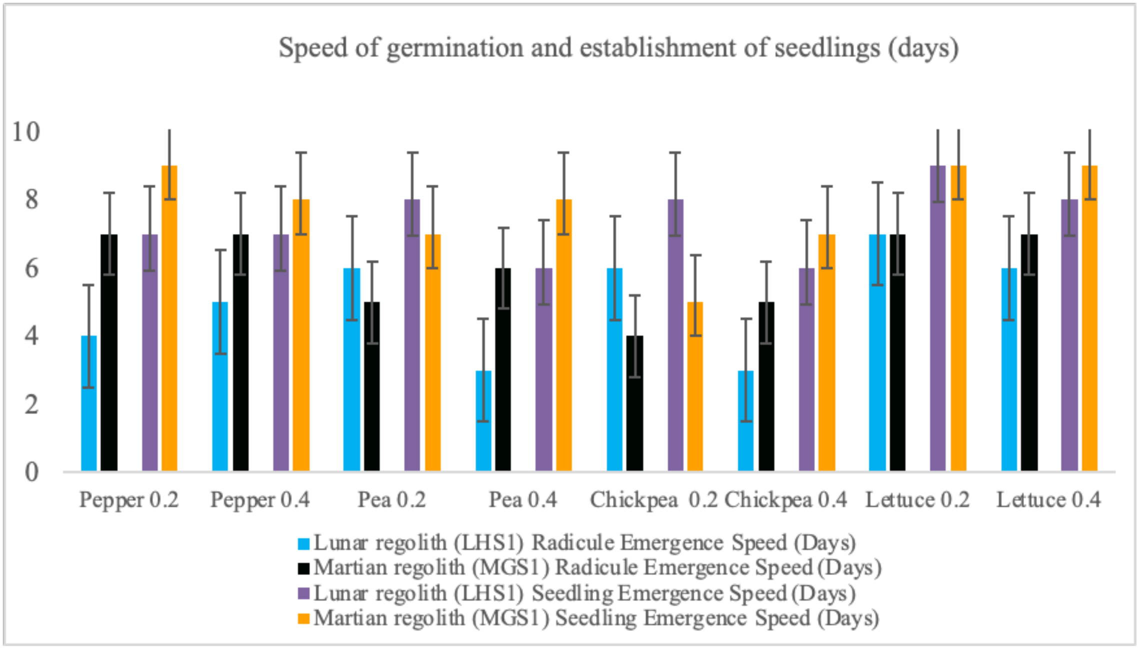

Figure 5. Germination speed and seedling emergence of cultivars developed in lunar and Martian regolith when treatment was applied at concentrations of 0.2 g.L^{-1} and 0.4 g.L^{-1}.

3.3. Indicators of abiotic stresses

In all seeds, the antagonist reaction between ABA and GA occurred to promote dormancy breakage and the beginning of germination development. In addition, all seeds had high peaks of phytohormones when submitted to 0.4 g.L^{-1} treatment compared to the control.

Pisum sativum seeds (Table 2) had lower ABA peaks in Martian regolith (75.8 pmol/FW) and lunar regolith (78.9 pmol/FW). However, when submitted to concentrations of *U. lactuca*, the levels of these phytohormones were high, with peaks of GA_4 in Martian regolith (188.1 pmol/FW) and lunar regolith (223.06 pmol/FW) when applied 0.4 g.L^{-1} compared with control (82.3 pmol/FW; 127.8 pmol/FW).

Table 2. Contents of phytohormones in seeds of *P. sativum*. PeM: Martian regolith pea. PeM0.2: Pea Martian regolith concentration of 0.2 g.L^{-1}. PeM0.4: Pea Martian regolith concentration of 0.4 g.L^{-1}. PeL: Lunar regolith pea. PeL0.2: Pea lunar regolith concentration of 0.2 g.L^{-1}. PeL0.4: Pea lunar regolith concentration 0.4 g.L^{-1}.

Hormones	PeM	PeM0.2	PeM0.4	Pel	PeL0.2	PeL0.4
ABA	75.8	87.9	98.9	78.9	96.5	101.9

IAA	98.9	112.3	119.9	99.2	122.6	129.1
GA_4	82.3	134.7	188.1	127.8	198.9	223.6
tZ or cZ	72.5	81.2	88.7	81.0	87.9	91.0
JA	36.2	38.9	40.1	37.8	40.5	41.0
SA	54.4	65.7	76.9	53.9	68.2	80.2

Cicer anuum seeds (Table 3) had peaks of GA_4 in Martian regolith (96.9 pmol/FW) and lunar regolith (102.9 pmol/FW) when applied 0.4 $g.L^{-1}$, compared with control (70.1 pmol/FW; 88.9 pmol/FW). In addition to IAA peaks in Martian regolith (96.9 pmol/FW) and lunar regolith (98.9 pmol/FW) when subjected to a concentration of 0.4 $g.L^{-1}$ compared to control (79.5 pmol/FW; 80.7 pmol/FW, respectively).

Table 3. Contents of phytohormones in seeds of *C. annum*. Peak hormonal concentration (pmol/FW) for each type of regolith, Martian, and lunar. Legend: PpM: Pepper Martian regolith. PpM0.2: Martian regolith pepper with a concentration of 0.2 $g.L^{-1}$ PpM0.4: Martian regolith pepper with a concentration of 0.4 $g.L^{-1}$. PpL: Lunar regolith pepper. PpL0.2: Lunar regolith pepper with a concentration of 0.2 $g.L^{-1}$. PpL0.4: Lunar regolith pepper with concentration 0.4 g. L^{-1}.

Hormones	**PpM**	**PpM0.2**	**PpM0.4**	**PpL**	**PpL0.2**	**PL0.4**
ABA	55.3	68.9	70.1	63.9	73.7	80.2
IAA	79.5	88.9	90.2	80.7	89.7	98.9
GA_4	70.1	86.9	96.9	88.9	97.8	102.9
tZ or cZ	66.9	78.9	81.0.	68.9	77.9	80.8
JA	29.8	30.9	33.8	30.1	33.9	35.4
SA	43.9	54.8	60.4	42.1	55.8	64.9

Lactuca sativa seeds (Table 4) had IAA peaks in Martian regolith (93.9 pmol/FW) and lunar regolith (97.4 pmol/FW) when 0.4 $g.L^{-1}$ was applied, compared with the control (72.8 pmol/FW; 83.9 pmol/FW). In addition to GA_4 peaks in Martian regolith (89.2 pmol/FW) and lunar regolith (98.9 pmol/FW) when submitted to a concentration of 0.4 $g.L^{-1}$ compared to the control (66.9 pmol/FW; 78 pmol/FW), respectively.

Table 4. Contents of phytohormones in seeds of *L. sativa*. Peak hormonal concentration (pmol/FW) for each type of regolith, Martian, and lunar. Legend: LeM:

Lettuce Martian regolith. LeM0.2: Martian regolith lettuce with a concentration of 0.2 g.L^{-1}. LeM0.4: Martian regolith lettuce with a concentration of 0.4 g.L^{-1}. LeL: Lunar regolith lettuce. LeL0.2: Lunar regolith lettuce with a concentration of 0.2 g.L^{-1}. LeL0.4: Lunar regolith lettuce with a concentration of 0.4 g.L^{-1}.

Hormones	**LeM**	**LeM0.2**	**LeM0.4**	**LeL**	**LeL0.2**	**LeL0.4**
ABA	45.8	52.9	60.1	50.3	58.9	67.0
IAA	72.8	87.9	93.9	83.9	88.8	97.4
GA_4	66.9	78.9	89.2	78.0	86.9	98.9
tZ or cZ	63.9	71.4	77.9	69.2	78.4	82.9
JA	21.9	28.2	31.6	28.3	35.6	38.1
SA	49.0	58.9	67.9	50.1	60.4	68.6

Cicer arietinum seeds (Table 5) had peaks phytohormones in both regolith when submitted to a concentration of 0.4 gL^{-1}, such as ABA peaks (101.6 pmol/FW) in Martian regolith and lunar regolith (104.8 pmol/FW). In addition, there were peaks of IAA (128.2 and 121.1 pmol/FW) and GA_4 (219.7 and 156.9 pmol/FW) in both simulated lunar and Martian regolith, respectively, when subjected to higher amounts of *U. lactuca* powder, compared to the control.

Table 5. Contents of phytohormones in seeds of *C. arietinum*. Peak hormonal concentration (pmol/FW) for each type of regolith, Martian, and lunar. Legend: CpM: Martian regolith chickpea. CpM0.2: Martian chickpea regolith concentration of 0.2 g.L^{-1}. CpM0.4 chickpea Martian regolith concentration of 0.4 g.L^{-1}. CpL: lunar regolith chickpea. CpL0.2: lunar regolith chickpea concentration of 0.2 g.L^{-1}. CpL0.4: chickpea lunar regolith concentration 0.4 g.xL^{-1}.

Hormones	**CpM**	**CpM0.2**	**CpM0.4**	**CpL**	**CpL0.2**	**CpL0.4**
ABA	82.8	94.8	101.6	85.2	98.9	104.8
IAA	100.2	115.9	121.1	105.6	117.7	128.2
GA_4	87.5	122.0	156.9	114.1	189.6	219.7
tZ or cZ	81.8	87.9	93.2	88.1	91.3	96.9
JA	39.8	41.2	44.5	38.1	42.7	45.2
SA	61.1	76.5	81.8	66.2	78.7	89.5

3.4. Qualitative chemical analysis of U. lactuca

In the phytochemical analysis of seaweed constituents, the results of gas chromatography-mass spectrometry (GC-MS) evidenced the presence of alkaloids, terpenoids, flavonoids, fatty acids, and bioactive compounds such as 5-Octadecenal, 1-Trichananol, Neophytadiene, Lactaropallidin, Phytol, Lucenin, Vincadifformine in *U. lactuca*.

Table 6. *U. lactuca* qualitative *analysis.*

Chemical class	Compounds
Alkaloids	Vincadifformin Quinines
Terpenoids	Neophytadiene Phytol Lactaropallidin
Flavonoids	Lucenin 2 Quercetin 7.3′,4′-Trimethoxy
Steroids	Digitoxigenin
Fatty acids	5-Octadecenal Erucic Acid 9-Octadecenoic acid 1-Tricosanol n-Hexadecanoic acid Myristate Isopropyl
Phenols	Uncharacterized

3.5. Proposition of the macroalgae U. lactuca *as a seed priming technique*

The evidence presented here was combined with previous knowledge in the corresponding research area to propose a model regarding the possible interaction of secondary metabolites of *U. lactuca* and its precursors of phytohormones as plant growth regulators (Figure 6) to be used in a seed priming technique.

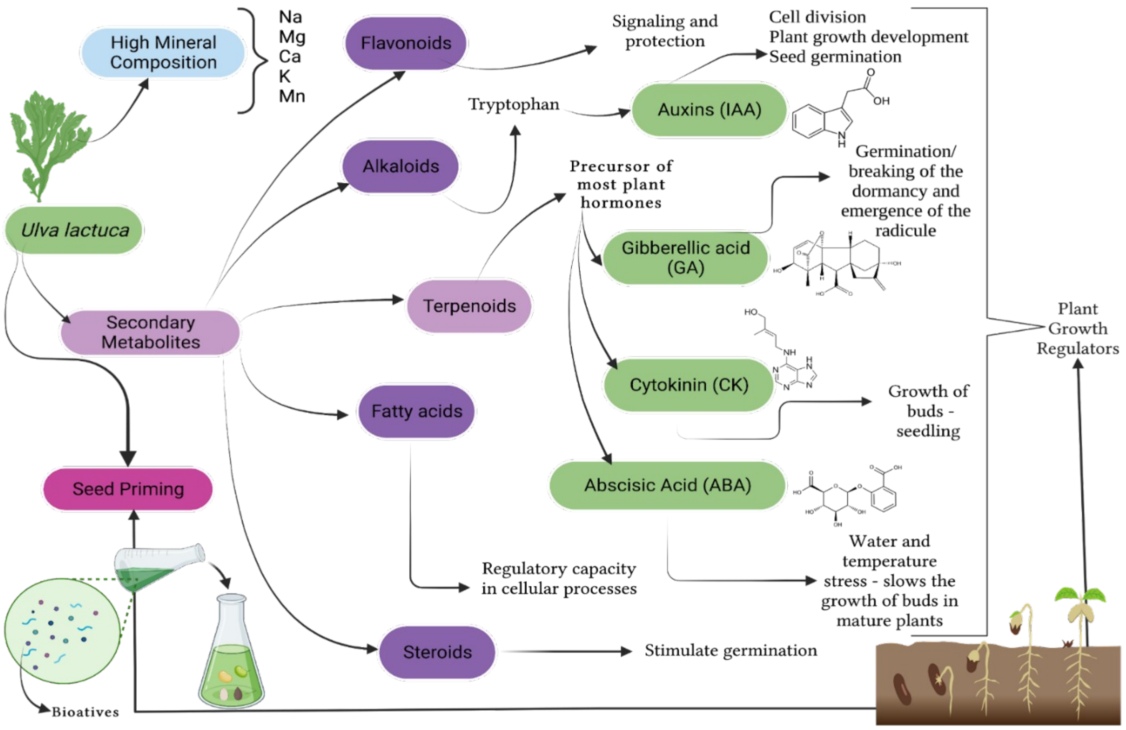

Figure 6. Hypothetical model representing the interaction of secondary metabolites of *U. lactuca* and their phytohormone precursors as plant growth regulators.

4. Discussion

The present work evaluated the effect of different concentrations of *U. lactuca* powder in the germination and growth of the cultivars *C. annuum*, *L. sativa*, *C. arietinum,* and *P. sativum*. Terrestrial soil was expected to perform with better effect than both regoliths, Martian, and lunar. These results obtained indicate that the technique of seed priming using the marine macroalgae *U. lactuca* in small amounts (0.2 g.L^{-1}) is considered a good biostimulant to increase the percentage of seed germination under stressful conditions, mainly on *C. arietinum* and *P. sativum* in both simulated, martian and lunar. Our results are in accordance with the previous study that documented the same patterns of responses using lower *U. lactuca* concentrations in the treatment of tomato seeds (*Solanum lycopersicum* L.) under stressful conditions and which related the invigoration of seeds to the bioactive substances present in this macroalga (Hernández-Herrera et al., 2016).

Lactuca sativa seeds developed well in lunar regolith when 0.2 g.L^{-1} of *U. lactuca* powder was applied, both for germination and seedling emergence. In Martian regolith, without any addition of biostimulant (control), germination rates were satisfactory for the seeds. However, regarding seedling emergence, it was not satisfactory with the probability of loss of this cultivar.

Macroelements are present in marine macroalgae (Hernández-Herrera et al. 2016), as well as in Martian regolith (Caporale et al., 2020; Eichler et al., 2021), may slow or positively affect the osmotic potential of plant seeds (Castellanos-Barriga et al., 2017; Eichler et al 2021). The matric potential of *L. sativa* seeds may have been influenced by the presence of these macroelements of the Martian regolith, reducing the metabolic capacity of seeds and the development as seedlings in the culture medium due to high regolith toxicity (MGS-1) and because their pH is significantly more alkaline (pH > 9) (Eichler et al., 2021). Under these conditions, the addition of the macroalga did not increase the percentage of seed germination; including the addition of *U. lactuca*, to a greater extent, may affect the yield or formation of radicle and plumule (Mzibra et al., 2018; Reis et al 2020).

In Martian regolith, the seeds of *C. annuum* performed well in relation to seedling emergence when higher amounts of biostimulant (0.4 $g.L^{-1}$) were applied. Probably, the more acidic pH of *U. lactuca* in higher concentration (0.4 $g.L^{-1}$, pH= 5.36) promoted *C. annuum* germination in simulated Martian. Most acid extracts of brown seaweed (*Ascophyllum nodosum* and *Fucus vesiculosus*) and acid extracts of green macroalgae *U. lactuca* were also beneficial for *Vigna radiata* and *Vigno mungo* seeds (Sharma et al., 2012; Castellanos-Barriga et al., 2017) and influenced the length of the shoots, root length and fresh weight of seed plants denoting with bioactives of these algae.

Ulva spp. based biostimulants were applied at different concentrations to promote seed germination and root induction in plants that grew under stressful environments or were subjected to stressful conditions (El Boukhari et al. 2021; Zouari et al. 2022). The results were positive for those that were treated at low dosages, while high concentrations inhibited seed performance and seedling development (Hernández-Herrera et al., 2014; Castellanos-Barriga et al., 2017; Reis et al., 2020; Hamouda et al., 2021). In addition, if applied to seeds before stress occurs, the biostimulant helps the plant to become more tolerant (Borges et al., 2014; Kasim et al., 2022)

Macroalgae of the genus *Ulva* contain elements such as Na, K, Ca, Mg, Cl, and NO_3 (Khan et al., 2009; Craigie, 2011; Tabarsa et al., 2012; Hussein et al., 2021), as well as in simulated Martian and lunar contains macroelements such as potassium (K_2O), phosphorus (P_2O_5), magnesium (MgO) and calcium (CaO) (Caporale et al., 2020;

Eichler et al., 2021). Notably, lunar regolith (LHS-1) promoted a faster seed germination rate than Martian regolith (MGS-1). The fact of its best performance can be explained by its high concentrations of calcium in its composition (12.8 wt%), as it is known to stimulate the production of gibberellins (Taiz et al., 2015). Furthermore, most seeds grown in lunar regolith with lower amounts of *U. lactuca* (0.2 $g.L^{-1}$) had a higher germination potential compared to the control.

Radish seeds (*Raphanus sativus*) were pre-soaked with biostimulants based on two macroalgae: *Codium taylorii* and *Pterocladia capillacea* for two hours before sowing. The use of these macroalgae in pre-sowing allowed better metabolic development in normal and stressful situations (Kasim et al., 2022). Similarly, Hamouda et al., (2022) evaluated the effect of seed priming in different concentrations *of Ulva linza* and *Corallina officinalis* extracts on wheat germination (*Triticum aestivum* L.) and provided a good percentage of germination and seedling emergence in relation to control.

Seed preparation increases metabolic activities in pre-germination and antioxidant activities, as well as accelerates metabolic recovery of stress-degraded membranes (El Boukhari et al., 2021). This technique not only increases the biomass of the adult plant but also contributes to a better performance of metabolism and adds bioactive compounds necessary for human food (Hamouda et al., 2022), such as phytohormones that are plant growth regulators (PGRs).

García et al., (2020) identified PGRs in extracts of *U. lactuca* and found higher concentrations of GA_4, which highlights the potential use of this macroalgae as a biostimulant. They were found in species of *Ulva* auxins (indole-3-acetic acid, IAA, and indole-3-butyric acid, IBA), gibberellins (gibberellic acid, GA_3), abscisic acid (ABA), cytokines (ribocytic kinetin, KR) and salicylic acid, which may act in the inhibition or acceleration of seed germination (Crouch and van Staden, 1992; Gupta and Abu-Ghannam, 2011)

Gupta and Abu-Ghannam, (2011)*,* mention that the interactions of the different metabolic pathways of phytohormones are responsible for regulating various physiological processes of the plant. Phytohormones play an important role in the regulatory process of plant growth and development, and are important chemical messengers, allowing plants to develop when subjected to some stress factor (Rhaman et al., 2020) as in the case of regolith. For example, auxins play an important role in plant growth and development, in promoting cell division, maintaining meristem, organogenesis, cell standardization, and root and stem development. Cytokines regulate the development of roots and stems while gibberellins and abscisic acid control germination and act in stimulating germination and dormancy induction (Crouch and van Staden 199; Gupta and Abu-Ghannam, 2011).

For germination to occur, in the first stages of development, it is essential to have an antagonistic reaction between abscisic acid (ABA) and gibberellins (GA) to promote dormancy breakdown (Taiz et al., 2015; Tuan et al., 2018). In addition, it is known that both cytokinin and auxin interact synergistically to control certain important processes, such as the maintenance of the meristem (Su et al., 2011). In the analysis of phytohormones of the seeds (pmol/FW), the expected occurred in relation to this balanced reaction between hormones ABA and GA. There was a decline in ABA and an increased GA sensitivity, which favored germination (Taiz et al., 2015; Tuan et al., 2018). However, when treated at higher concentrations (0.4 $g.L^{-1}$) phytohormones levels increased significantly, which may have caused stress.

Subjecting seeds to high concentrations can extend or delay plant germination time and radicle and plumule formation can degenerate (Selvam and Sivakumar, 2013; Hernandèz-Herrera et al., 2014; 2016; Mzibra et al., 2018). Hormonal peaks had a significant difference in relation to control at ideal concentrations (0.2 $g.L^{-1}$), which probably favored an improvement in hormonal balance and metabolic development of most seeds in this study.

As in our study, previous phytochemical analysis of *U. lactuca* also revealed the presence of alkaloids, terpenoids, flavonoids, and fatty acids (Hernández-Herrera et al., 2014; García et al., 2020). These bioactives are present in the secondary metabolism of macroalgae of the genus *Ulva* (Anjali et al., 2019) and metabolic pathways are essential for the formation of phytohormones (Taiz et al., 2015). However, their performance in the metabolic pathways of plants remains poorly understood (Gupta et al., 2011). We propose a scheme about the possible action and interaction between the bioactive elements of *U. lactuca* and its action as a biostimulant in seeds. Seed germination metabolism is directly induced by the response to seaweed application (El Boukhari et al., 2021) and one can see the crosstalk between the ability of macroalgae secondary metabolite components, such as phenolic compounds, terpenes, and alkaloids to influence the biosynthesis of phytohormones (Lepiniec et al., 2006; Dumas et al., 2010; Gupta et al., 2011; Jaulneau et al., 2011; Taiz et al., 2015). These compounds are of interest due to their antioxidant properties that act as influential protectors against biotic and abiotic stresses (Lepinec et al., 2006; García et al., 2020) and can act on seed germination. For example, terpenes are responsible for the biosynthesis of gibberellins, cytokinin, and abscisic acid (Taiz et al., 2015).

Flavonoids modulate auxin transport through direct and indirect interactions with cell transport and regulatory mechanisms and, in addition, are of current interest due to their antioxidant properties, which accumulate in seed coatings of various plant species, including peas, and which function as protective agents against biotic and abiotic stresses (Lepiniec et al., 2006). Alkaloids are important in tryptophan biosynthesis which is a precursor in auxin synthesis (Taiz et al., 2015; Jiang et al., 2022).

The nutritional deficiencies of lunar and Martian regolith can be overcome by producing nutrient-rich solutions. However, this method of cultivation is not sustainable for space agriculture, as nutrients need to be brought from Earth (Cannon and Britt, 2019). A solution to mitigate this problem would be biostimulants based on marine macroalgal extracts because they are used in small quantities (Castellanos-Barriga et al., 2017; Reis et al., 2020; Hamouda et al., 2021) and mixed in regolith simulators with organic matter from *Ulva* spp. which, as they contain bioactive substances, may directly influence the growth of edible plants and the availability of nutrients in regolith-based agriculture (RBA).

Figuring out how to live on Moon and Mars is the ultimate test of sustainability in agriculture and learning to grow plants in a hostile environment can maximize the use of valuable and harmful resources, such as water and fertilizers (de Micco et al., 2009; Pickett et al., 2020). In addition, space agriculture will be decisive for the survival of humans outside the Earth, mainly because it is viable as a source of income (Cannon and Britt, 2019). Biostimulants offer a potentially new approach to regulating/modifying physiological processes in plants to stimulate growth, reduce stress-induced limitations and increase productivity (Yakhin et al., 2017; Pardilhó et al., 2022). The raw material of *Ulva* spp. may be available in natural banks and periodic blooms, or from integrated multitrophic aquaculture which turns our focus to this promising source of biostimulants as a nutritional additive to Martian and lunar regolith simulators.

5. Conclusion

The metabolism of the studied seeds and the emergence of seedlings were directly stimulated by the response of the application of the macroalgae *U. lactuca* because they contain chemicals that participate in the biosynthesis of phytohormones and prove the efficiency in stimulating germination. In priming seed techniques under stressful conditions, we recommend the use of *U. lactuca* in low concentrations (0.2 g.L^{-1}) for chickpea seeds (*C. arietinum*) and peas (*P. sativum*) in Martian regolith. For lunar regolith, both concentrations were satisfactory for chickpeas (*C. arietinum*) and peas (*P. sativum*). For lettuce (*L. sativa*), the lowest concentrations were satisfactory. We recommend the lowest concentration for the best cost-benefit, in addition, we propose further investigations and protocols for the application of this biostimulant for its proper use. The use of *Ulva* spp. will be important to connect biological systems with local natural resources (lunar and Martian regolith) to improve the feeding of interplanetary civilization and increase the chances of a self-sustaining establishment.

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10. CONSIDERAÇÕES FINAIS

O metabolismo das sementes e estabelecimento de plântulas foi diretamente induzido pela resposta da aplicação de farinha da macroalga *U. lactuca*, por ela conter metabólitos secundários que participam da biossíntese de fitormônios, e comprovam a eficiência no estímulo à germinação.

Em técnicas *de seed priming* sob condições estressantes, recomendamos o uso de *U. lactuca* em baixas concentrações (0.2 g. L^{-1}) para sementes de grão de bico (*C. arietinum*) e ervilha (*P. sativum*) em regolito marciano.

Para o regolito lunar, ambas as concentrações foram satisfatórias para grão de bico (*C. arietinum*) e ervilha (*P. sativum*). Para alface (*L. sativa*), as menores concentrações foram satisfatórias. Recomendamos a menor concentração pelo melhor custo-benefício, além disso, propomos maiores investigações e protocolos de aplicação desse bioestimulante para o seu uso adequado.

A utilização de *Ulva* spp. será importante para conectar os sistemas biológicos com os recursos naturais locais (recursos naturais *in-situ*, regolito lunar e marciano), como uma forma de melhorar a alimentação da civilização interplanetária e aumentar as chances de um estabelecimento autossustentável.

Esses resultados evidenciaram ser possível cultivar plantas em simuladores de regolito marciano, porém, faltam dados moleculares obtidos via ciências ômicas. Essas informações fornecem conhecimentos úteis sobre as principais vias bioquímicas que estão ausentes durante o crescimento das plantas cultivadas em sistema regolítico. Além disso, compreender através das ciências ômicas lacunas do conhecimento na avaliação de como os simuladores afetam o crescimento das plantas, bem como as plantas alteram a composição dos simuladores.

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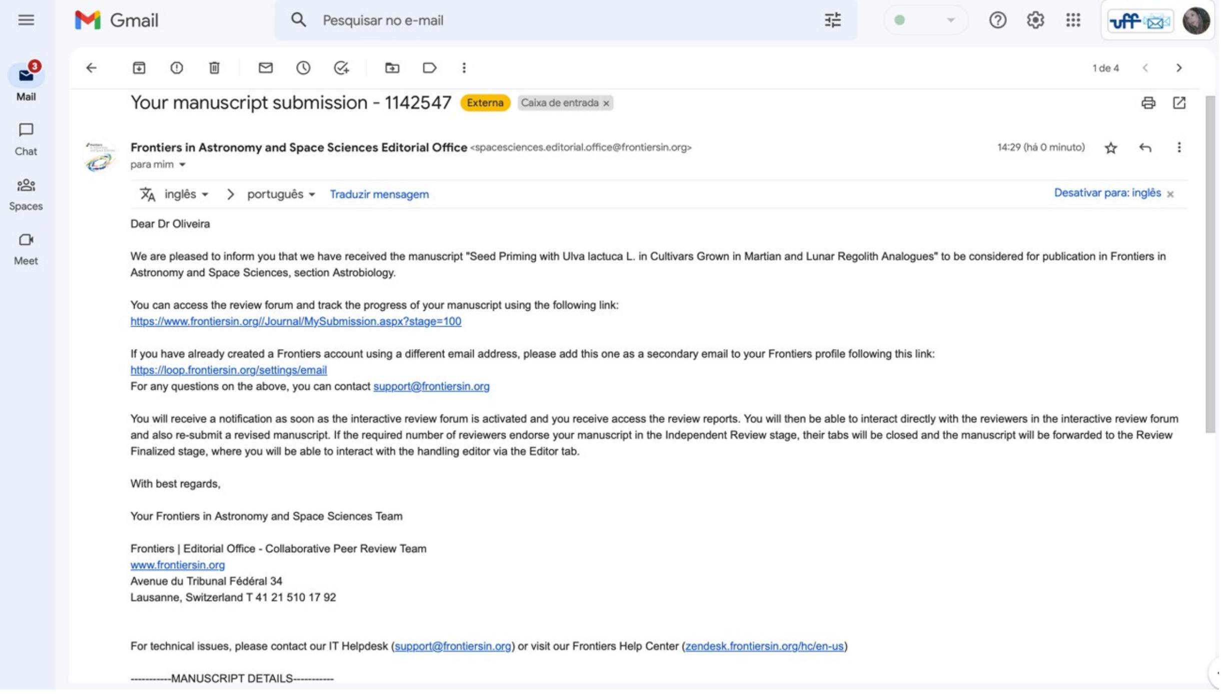

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