UNIVERSIDADE FEDERAL DA FRONTEIRA SUL

CAMPUS ERECHIM

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA AMBIENTAL

Ana Paula Vanin

POTENCIAL NEUROPROTETOR DE ÓLEOS A BASE DE *Cannabis sativa* EM *Caenorhabditis elegans*

Erechim/RS

2022

ANA PAULA VANIN

POTENCIAL NEUROPROTETOR DE ÓLEOS A BASE DE *Cannabis sativa* EM *Caenorhabditis elegans*

Dissertação de mestrado, apresentada para o Programa de Pós-graduação em Ciência e Tecnologia Ambiental da Universidade Federal da Fronteira Sul, como requisito parcial para obtenção do título de Mestre em Ciência e Tecnologia Ambiental.

Orientador: Prof.ª Dra. Rosilene Rodrigues Kaizer Perin (*in memoriam*)

Prof. D. Sc. Leandro Galon

Erechim/RS

2022

Bibliotecas da Universidade Federal da Fronteira Sul - UFFS

, Ana Paula Vanin POTENCIAL NEUROPROTETOR DE ÓLEOS A BASE DE Cannabis sativa EM Caenorhabditis elegans / Ana Paula Vanin . -2022.
43 f.:il.

Orientador: Doutor Leandro Galon

Dissertação (Mestrado) - Universidade Federal da Fronteira Sul, N, N, 2022.

1. Dissertação. I. , Leandro Galon, orient. II. Universidade Federal da Fronteira Sul. III. Título.

Elaborada pelo sistema de Geração Automática de Ficha de Identificação da Obra pela UFFS com os dados fornecidos pelo(a) autor(a).

ANA PAULA VANIN

POTENCIAL NEUROPROTETOR DE ÓLEOS A BASE DE *Cannabis sativa* EM *Caenorhabditis elegans*

Dissertação de mestrado, apresentada para o Programa de Pós-graduação em Ciência e Tecnologia Ambiental da Universidade Federal da Fronteira Sul, como requisito parcial para obtenção do título de Mestre em Ciência e Tecnologia Ambiental.

Este trabalho foi defendido e aprovado pela banca em 16/05/2022.

BANCA EXAMINADORA

_____

Prof. D. Sc Leandro Galon – UFFS
Orientador

_____

Prof. Dr. Paulo Afonso Hartmann – UFFS
Avaliador

_____

Prof.ª Dr.ª Caroline Müller – Bolsista PNPD/CAPES/PPGCTA/UFFS
Avaliadora

Dedico este trabalho ao meu pai/avô João Vanin Filho (in memoriam), minha mãe/avó Ida Algeri Vanin (in memoriam) que sempre acreditaram em mim. Também, a minha eterna orientadora Profª. Dra. Rosilene Rodrigues Kaizer Perin (in memoriam), minha maior incentivadora desde o ínício. Saudades eternas de vocês três.

AGRADECIMENTOS

Primeiramente gostaria de agradecer meu esposo, marido e companheiro Gainete, por ter me incentivado todo esse tempo, principalmente quando perdi minha orientadora, Prof.ª Rosi (foi um dos momentos mais difíceis da minha vida). Sem contar nas outras dificuldades que enfrentamos juntos e só nós dois sabemos muito bem disso, o quão puxado foi. Pode ter certeza de que, sem sua ajuda e companheirismo nesta etapa de minha vida, eu não teria conseguido chegar até aqui. Muito obrigada por tudo.

À toda minha família e amigos pela compreensão e apoio.

Ao Laboratório de Bioquímica Rosilene Rodrigues Kaizer Perin - IFRS Campus Sertão RS, onde realizei meus experimentos.

À Universidade Federal da Fronteira Sul, Campus Erechim, e ao Programa de Pós-Graduação em Ciência e Tecnologia Ambiental pela oportunidade de realização do mestrado.

Ao meu orientador, Prof. D. Sc. Leandro Galon.

A CAPES, pelo auxílio financeiro e concessão da bolsa ofertada.

A APEPI, pela doação dos óleos e parceria firmada conosco para a realização dos experimentos.

As minhas colegas/amigas de mestrado Catarina, Camila e Rubia, que apesar de termos feito um mestrado em tempos de pandemia, dividimos as conquistas e sofrimentos umas com as outras nessa nossa etapa de mestrado, seguindo sempre firmes e fortes. Obrigada meninas.

Aos meus amigos, especialmente a essa guria, Letícia Mesacasa, simplesmente **SENSACIONAL**, uma legítima **NERD**. Meu muito obrigada, você foi fundamental nesta etapa da minha vida. Por isso merece meu eterno agradecimento, tanto que nem sei se um dia vou conseguir te retribuir todo o apoio que me destes, me auxiliando, me incentivando. Muito Obrigada.

Nathiiiiiiiiii, Tafa, Nathália Tafarel Sutorilo, Sutorilo Tarfarel como te agradecer? Obrigada por ter me auxiliando em meus experimentos e também por ter-me ajudado emocionalmente nos momentos mais difíceis, sendo uma verdadeira amiga. Você tornou-se uma mulher incrível, tenho muito orgulho de ti. Se vira sozinha, não pede “bexiga” para ninguém. Tens um futuro brilhante pela frente. Vá, que o teu sucesso está garantido. Obrigada por tudo.

A Profª. Luciani Figueiredo Santin, pelos seus sábios conselhos dados e, por ser uma verdadeira amiga nos momentos em que eu estava totalmente desmotivada e desanimada para continuar seguindo em frente. Com certeza, foram de suma importância para que eu chegasse até aqui.

A Amanda dos Santos (Amandita) e ao Wallace Santin (Remix), que em alguns momentos de dificuldade que passei no lab, pedi auxílio a vocês e, prontamente estavam lá firmes e fortes me auxiliando. Meu muito obrigado.

A Carlotaaaa, a servidora Carla Alves, na verdade, quase uma Dra. Meu muito obrigada pela disponibilidade em vir me ajudar nas atividades laboratoriais e sempre me dando forças nos momentos difíceis, inclusive com a perda da Prof.ª Rosi onde todos nós ficamos desamparados e sem chão, você sempre esteve ali, pronta para ajudar, incentivar e não desistir. Sendo uma verdadeira orientadora quando nós mais precisamos. Obrigada pela parceria, pelas ajudas, pelos desabafos. Ah! E quero te ver como professora, em um futuro bem próximo tá?

Ao Louis, vulgo Wagner Antonio Tamagno. Por onde começar? Vamos começar pelo começo então. Lembro-me quando você chegou no laboratório, um "piá de merda", sem barba e de óculos. Eu disse: pronto, esse é o legítimo NERD. Sempre aprendendo com muita facilidade, ágil e tudo mais. Passamos por "N" perrengues lá no lab. de Bioquímica, principalmente quando sabíamos haver pessoas lá que não gostavam de nós e que não gostam até hoje. As mesmas, mantinham certas posturas nos desmerecendo, te desmerecendo. Tivemos alguns momentos de mágoas um com o outro também, tanto que já discutimos e não foi uma só vez. Mas no fim, até que tudo se ajeita (por enquanto rsrsrs). Enfim, acredito que sempre vou ficar te **devendo obrigação** por tudo que tu fizeste e continua fazendo por mim nos meus experimentos, sempre me auxiliando. Inclusive **é o cara da estatística**, pois foi quem fez ela para mim. Que quando nós não temos capacidade e nem competência para certas coisas, temos que ser humildes e pedir ajuda e saber reconhecer, isso é fundamental. Bom, já falei demais, mas não tudo que temos de história para contar (boas e ruins). Tem os nossos "quebra-paus" entre nós dois, as fofocas, enfim... Valeu Louis pela ajuda de sempre. E, como digo para o meu irmão Guilherme, digo-lhe também: voe, que o mundo é inteiro seu. Como a prof.ª Rosi dizia, meu menino de ouro, meu orgulho. E, pode ter certeza que ela se orgulha muito de ti. **Obs. (parte inserida nestes agradecimentos após dia 5 de abril de 2022): não fique dando "pitis" e "surtando" antes do fim rsrsrsrsr.**

E, UM AGRADECIMENTO EM ESPECIAL a minha eterna orientadora, Prof.ª Dra. Rosilene Rodrigues Kaizer Perin (*in memoriam*), aquela que me chamava de: "Meu malvado favorito", "Ana, eu sei que tu me ama" ou então, "Ana tu és uma vaca mesmo". Lembro-me do ano em que nos conhecemos (2011), quando você ministrava a disciplina de Bioquímica para mim no curso de Gestão ambiental. Jamais imaginaria que você Prof.ª Rosi seria uma das pessoas mais especiais na minha vida. Também, foi você quem me deu a primeira

oportunidade de ser bolsista de iniciação científica. Com o passar dos anos, fomos tendo uma afinidade maior, convivência diária, vi sua batalha para ter seu tão sonhado lab. de Bioquímica (pois quando você começou, tinha apenas uma salinha que nem dava para se mexer dentro e mesmo assim você fazia milagre lá). Enfim, sonho realizado, mudança para o prédio A14. E, em uma brincadeira boba de passatempo de lab. eu disse para você que somente faria mestrado se pudesse fazer com *Cannabis sativa*. Que coisa não?! Então hoje, cheguei até aqui, com um projeto de **MACONHA.**

Passou por muitas dificuldades na vida, tanto pessoal quanto profissional e sempre forte, muito forte. **UMA BAITA MULHER. SIMPLESMENTE INCRÍVEL.** Tenho certeza que estas aí de cima iluminando todos nós aqui, para sermos fortes e continuarmos seguindo. Mais uma vez, muito obrigada por ter me ensinado valores que carrego e sempre carregarei comigo, pelo estímulo, pelas palavras de força ao longo destes anos que convivemos juntas. Sabes que te considero como uma mãe (mas que eu não podia te chamar, pois, você dizia que eu não tinha idade para ser sua filha, mas, quem sabe uma tia minha rsrsrsrs). Obrigada por me olhar de algum lugar. Saudades eternas. Amo-te demais.

E, por fim, gostaria também de agradecer aqueles que não me ajudaram em nada, principalmente aqueles que nos momentos em que mais precisei viraram as costas para mim. Alguns me disseram que após a morte da minha orientadora, estavam me emprestando o laboratório salientando isso, repetidas vezes (Obs: O laboratório é Federal, adquirido com dinheiro público e não particular. Portanto, de LIVRE acesso aos discentes e demais. Entendo que, se estivessem "me emprestando" o ambiente para realização dos meus experimentos, este deveria ser em sua residência, não? Assim, poderia chamar de seu e dizer que estava me emprestando) pra realizar meus experimentos. Aqueles que me perseguiram, tentando me prejudicar de alguma forma, tanto pessoal, quanto profissional. Isso tudo me fez ser mais forte, mais madura, mais cuidadosa, mais confiante e claro, extremamente motivada. Obrigada, vocês foram meu combustível para que eu chegasse até aqui.

RESUMO

As substâncias derivadas de *Cannabis sativa* tem chamado atenção da medicina nos últimos anos, em especial o canabidiol (CBD) e a delta-9-tetrahidrocanabinol (Δ^9-THC). Esses compostos possuem interação farmacocinética e farmacodinâmica opostas, onde o CBD atua como um antagonista ou modulador negativo em relação ao THC, através da ligação aos receptores CB1 e CB2. Dessa forma, as ações destes dois principais canabinóides modulam o sistema nervoso central envolvendo desenvolvimento, plasticidade sináptica e resposta a danos endógenos e ambientais, como característica de muitas doenças neurodegenerativas. Algumas dessas doenças apresentam etiologias ainda desconhecidas, porém todas são mediadas por neurotransmissores específicos que mantêm sistemas em desequilíbrio homeostático, como o Sistema Nervoso Gabaérgico (SNG) e o Sistema Nervoso Colinérgico (SNCol). O nematódeo *Caenorhabditis elegans* é um importante modelo experimental, pois esse organismo possui diferentes sistemas de neurotransmissores que coordenam seu comportamento: dopaminérgico, colinérgico, serotoninérgico, glutamatérgico e gabaérgico. Diante disso, o objetivo geral deste estudo foi avaliar o potencial protetivo e/ou curativo de diferentes concentrações dos canabinóides THC e CBD obtidos de *C. sativa* sobre os parâmetros comportamentais (ciclo de defecação, batimento faríngeo e o parâmetro locomotor de *Body bends*), sistema colinérgico (atividade da enzima acetilcolinesterase; AChE), e peroxidação lipídica em cepa selvagem (N2) e transgênicas (GMC101 e CL2122) no nematódeo *Caenorhabditis elegans.* O composto observado foi eficiente para redução da atividade da enzima AChE na cepa N2 e no aumento na cepa GMC101, destacando-se o óleo rico em CBD. Já os óleos ricos em THC influenciaram os biomarcadores avaliados, tanto na cepa N2 como nas transgênicas GMC101 e CL2122. Portanto, estudos como o apresentado são importantes para esclarecer os verdadeiros mecanismos pelos quais se observam melhorias em doenças crônicas ligadas ao sistema nervoso central. Além disso, são necessários, como uma quebra de tabu quanto ao uso da planta *C. sativa* como alternativa para uso medicinal, principalmente no que se refere a doenças neurodegenerativas, que já demostraram resultados iniciais positivos. Por fim, com base nos resultados obtidos no presente trabalho, observa-se um grande potencial no uso dos óles CBD e THC para o tratamento de doenças neurodegenerativas.

Palavras-chave: Canabidiol, Delta-9-tetrahydrocannabinol, Sistema Nervoso Gabaérgico (SNG), Sistema Nervoso Colinérgico (SNCol).

ABSTRACT

Substances from the *Cannabis sativa* species, especially cannabidiol (CBD) and Delta-9-tetrahydrocannabinol (Δ9-THC), have attracted medical attention in recent years. THC is known for its psychoactive properties, whereas CBD is not a psychostimulant. These compounds have opposite pharmacokinetic and pharmacodynamic interactions, where CBD acts as an antagonist or negative modulator of THC, through binding to CB1 and CB2 receptors. Thus, the actions of these two main cannabinoids modulate the Central Nervous System (CNS) involving development, synaptic plasticity, and response to endogenous and environmental damage, as a characteristic of many neurodegenerative diseases. Some of these diseases still have unknown etiology, but their dynamics are mediated by specific neurotransmitters that maintain systems in homeostatic imbalance, such as the GABAergic Nervous System (GNS) and the Cholinergic Nervous System (SNCol). The nematode *C. elegans* is an important experimental model, has this different neurotransmitter systems that coordinate its behavior: dopaminergic, cholinergic, serotoninergic, glutamatergic, and GABAergic. Therefore, the objective of this study was to evaluate the protective and/or curative potential of terpenoids found in *C. sativa* on the GNS and ChoNS of *C. elegans*. The effect of two *C. sativa* oils with variations in THC and CBD concentrations on the cholinergic system, lipid peroxidation, and behavior of *C. elegans* was evaluated. The compound was efficient in reducing the activity of the enzyme acetylcholinesterase (AChE) in N2 strains and in increasing the GMC101 strain, highlighting the CBD rich-oil. The TCH rich-oils influenced the evaluated biomarkers, both in the N2 strain and in the transgenic GMC101 and CL2122. Consequently, greater investments in scientific research are needed, in addition to breaking the taboo on the use of the *C. sativa* plant as an alternative for medicinal use, especially in neurodegenerative diseases, which have already shown positive initial results. Finally, based on the results obtained in the present work, there is great potential in the use of CBD and THC oils for the treatment of neurodegenerative diseases.

Keywords: Cannabidiol; delta-9-tetrahydrocannabinol; GABAergic Nervous System (GNS); Cholinergic Nervous System (CNS).

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

ACh - Acetilcolina

AChE - Enzima acetilcolinesterase

ANOVA - Análise de variância

APEPI- Apoio à Pesquisa e Pacientes de Cannabis Medicinal

ASChI - Iodeto de acetiltiocolina

CBD - Canabidiol

DA - Doença de Alzheimer

EI - Ionização de elétrons

GABA - Ácido γ-aminobutírico

GFP - green fluorescent protein

KH_2PO_4 - Fosfato de potássio monobásico

HPLC - *High-Performance Liquid Chromatography*

KW - Kruskal-Wallis

mAChR - Receptores muscarínicos

$MgSO_4$ - Sulfato de magnésio

nAChR - Receptores nicotínicos

NaCl - Cloreto de sódio

Na_2HPO_4 – Fosfato de sódio dibásico

NaOH - Hidróxido de sódio

NGM - *Nematode growth medium*

SE - *Standard error*

SNC - Sistema Nervoso Central

SNCol - Sistema Nervoso Colinérgico

SNG - Sistema Nervoso Gabaérgico

TBARS - Substâncias reativas ao ácido tiobarbitúrico

THC – Tetrahidrocanabinol

βA - Beta-amilóide

1. INTRODUÇÃO GERAL

A espécie *Cannabis sativa*, pertencente à família Cannabeaceae, é uma planta com flores, que contém substâncias aromáticas e canabinóides (ARAÚJO, 2020). Essa planta está entre as plantas antigas mais cultivadas pelo homem. Ela tem sido utilizada ao longo dos anos como fornecedora de fibras, como alimento, com propósitos religiosos ou recreativos e como medicamento (ZUARDI, 2008).

A *C. sativa*, possui ainda diversos potenciais ecológicos, principalmente o caule, conhecido como cânhamo. Ao invés da utilização de sacolas plásticas feitas de derivados de petróleo que acabam indo para o mar, as mesmas poderiam ser substituídas pelo celofane a base de cânhamo, que é biodegradável. O isopor também poderia ser substituído por celulose a base de cânhamo compostável (HOLLAND, 2010).

Já para na medicina, a espécie *C. sativa* tem despertado interesse em substâncias derivadas, especialmente o canabidiol (CBD) e a delta-9-tetrahidrocanabinol (Δ^9-THC). Essas substâncias fazem parte do grupo de canabinóides, que possuem mecanismo de ação diferente de muitos fármacos convencionais e apresentam efeitos bem tolerados pelos pacientes (FARIAS, 2020). O CBD apresenta propriedades anticonvulsivantes, ausência de efeitos psicomiméticos (que causam alterações mentais e psíquicas) e não causa dependência. O THC contém propriedades psicoativas e pode causar dependência, apesar de possuir propriedades analgésicas e ser relaxante muscular (FARIAS, 2020). Entretanto, o uso de CBD tem o potencial de antagonizar os efeitos do THC, quando utilizados concomitantemente.

No cérebro humano existem cerca de 100 bilhões de neurônios, onde cerca de 20-30% são neurônios do sistema nervoso central (SNC) e são gabaérgicos (CARVER; REDDY, 2013). O neurotransmissor ácido γ-aminobutírico (GABA) é considerado o maior neurotransmissor inibitório do SNC, além disso esse tem importante papel na regulação da excitabilidade e na plasticidade neuronal durante as fases de maturação do cérebro (LE MANGUERESSE; MONYER, 2013). Já o sistema nervoso colinérgico (SNCol) é o responsável pela cognição, memória e sentidos, e tem como neurotransmissor a acetilcolina (ACh).

A ACh por sua vez é um mediador químico de sinapses no SNC, no sistema nervoso periférico e na junção neuromuscular (BRUNEAU; AKAABOUNE, 2006). A ACh foi classificada por ações muscarínicas e nicotínicas, onde os receptores colinérgicos são capazes de se ligar à muscarina e a nicotina, respondendo assim a ativação colinérgica com alta afinidade. Pacientes com doença de Alzheimer apresentaram melhoras quando tratados com inibidores de AChE. (OH et al., 2004).

Estudos relatam que alterações nos sistemas nervosos gabaérgico e colinérgico podem ser responsáveis por distúrbios comportamentais em pacientes com doença de Alzheimer (TERRY *et al*., 2008; NAVA-MESA *et al*., 2014; LI *et al*., 2016), uma vez que tais alterações comportamentais podem prejudicar a qualidade de vida das pessoas com essa doença. Na busca por uma melhor compreensão desses processos, a utilização de organismos modelos como o *Caenorhabditis elegans* tem se destacado.

O nematódeo *C. elegans* é membro da família Rhabditidae, sendo importante modelo experimental em áreas de pesquisa como biologia molecular, toxicologia e farmacologia (COX *et al*., 2012). Esses organismos cumprem um importante papel ecológico, com diversas vantagens de usos em testes biológicos, principalmente sobre toxicidade de metais (JIANG et al, 2016; MOYSON *et al.*, 2019), compostos orgânicos (BIAN *et al.*, 2018) e nanopartículas (SCHARF *et al.*, 2013; WU *et al.*, 2019). O *C. elegans* possui um ciclo de vida curto, tendo em 3 a 4 dias as quatro fases larvais (L1-L4) e, posteriormente ocorre a produção de oócitos pelo hermafrodita adulto durante um período fértil de 4 dias. Os adultos maduros vivem por mais um período de 10 a 15 dias. O comportamento e sistema fisiológico é coordenado por diferentes sistemas de neurotransmissores, os quais incluem os sistemas dopaminérgico, colinérgico, serotoninérgico, glutamatérgico e gabaérgico (HARVEY *et al*., 2009). No entanto, as respostas comportamentais, a duração da vida adulta, a fecundidade e o comprimento do corpo do nematoide podem variar de acordo com a qualidade ambiental em que esses organismos se encontram (MOYSON *et al.*, 2019).

Assim, considerando os diferentes sistemas de neurotransmissores presentes no nematódeo *C. elegans*, e o seu curto ciclo de vida, este organismo torna-se um importante aliado em estudos para avaliar a atividade de canabinóides e a possível eficácia na amenização dos sintomas de doenças neurodegenerativas.

Diante do exposto, o objetivo geral do presente estudo foi avaliar o potencial protetivo e/ou curativo de diferentes concentrações dos canabinóides THC e CBD obtidos de *C. sativa* sobre os parâmetros comportamentais (ciclo de defecação, batimento faríngeo e parâmetro locomotor de *Body bends*), sistema colinérgico (atividade da enzima acetilcolinesterase; AChE), e peroxidação lipídica em cepa selvagem e duas cepas transgênicas (GMC101 cepa que possui mutação e expressa a proteína Beta-amilóide e CL2122 que é a cepa controle da GMC101) no nematódeo *Caenorhabditis elegans*.

2. REFERÊNCIAS

ARAÚJO, R. ***Cannabis*** **medicinal no combate à dor crônica**. São Paulo: Editora Europa, 2020.

BIAN, J.; ZHANG, H.; MENG, S.; LIU, Y. Chemotaxis of *Caenorhabditis elegans* toward volatile organic compounds from *Stropharia rugosoannulata* induced by amino acids. **Journal of Nematology**, 50:3-8, 2018.

BRUNEAU, E. G.; AKAABOUNE, M. Running to stand still: ionotropic receptor dynamics at central and peripheral synapses. **Molecular Neurobiology**, 34:137-51, 2006.

CARVER, C. M.; REDDY, D. S. Neurosteroid interactions with synaptic and extrasynaptic GABAA receptors: regulation of subunit plasticity, phasic and tonic inhibition, and neuronal network excitability. **Psychopharmacology**, 1-38, 2013.

COX, M. M.; DOUDNA, J. A.; O'DONNELL, M. **Biologia Molecular: Princípios e Técnicas.** Porto Alegre: Artmed, 2012.

FARIAS, A. ***Cannabis*** **medicinal no combate à doença de Parkison**. São Paulo: Editora Europa, 2020.

HARVEY, S. C.; SHORTO, A.; VINEY, M. E. Erratum to: Quantitative genetic analysis of life-history traits of *Caenorhabditis elegans* in stressful environments. **BMC Ecology and Evolution,** 9, 96, 2009.

HOLLAND, J. **O livro da maconha: o guia complete sobre a *Cannabis*, seu papel na medicina, política, ciência e cultura.** Editora Vista Chinesa, 2010.

JIANG, Y.; CHEN, J.; WU, Y.; WANG, Q.; LI, H. Sublethal Toxicity Endpoints of Heavy Metals to the Nematode *Caenorhabditis elegans*. **PLoS One,** 11: e0148014, 2016.

LE MAGUERESSE, C.; MONYER, H. GABAergic interneurons shape the Functional Maturation of the Cortex. **Neuron**, 77: 388–405, 2013.

LI, Y.; SUN, H.; CHEN, Z.; XU H.; BU, G.; ZHENG H. Implications of GABAergic Neurotransmission in Alzheimer's Disease. **Frontiers in Aging Neuroscience**, 8: 31, 2016.

MOYSON, S.; TOWN, R.M.; VISSENBERG, K; BLUST, R. The effect of metal mixture compositionon toxicity to *C. elegans* at individual and population levels. **PLoS ONE** 14: e0218929, 2019.

NAVA-MESA, M. O.; JIMÉNEZ-DÍAZ, L.; YAJEYA, J.; NAVARRO-LOPEZ, J. D. GABAergic neurotransmission and new strategies of neuromodulation to compensate synaptic

dysfunction in early stages of Alzheimer's disease. **Frontiers in Cellular Neuroscience**, 8, 2014.

SCHARF, A.; PIECHULEK, A.; MIKECZ, A. V. Effect of Nanoparticles on the Biochemical and Behavioral Aging Phenotype of the Nematode *Caenorhabditis elegans*. **ACS Nano**, 7:10695-703, 2013.

TERRY, A. V.; BUCCAFUSCO, J. J.; WILSON, C. Cognitive dysfunction in neuropsychiatric disorders: selected serotonin receptor subtypes as therapeutic targets, **Behavior Brain Research**, 195: 30-38, 2008.

WU, T.; XU, H.; LIANG, X.; TANG, M. *Caenorhabditis elegans* as a complete model organism for biosafety assessments of nanoparticles. **Chemosphere**, 221: 708-726, 2019.

ZUARDI, A. W. ***Cannabis* e saúde mental**. Editora: FUNDEC, 2008.

3. Artigo - Neuroprotective potential of *Cannabis sativa*-based oils in *Caenorhabditis elegans*

Artigo submetido para a revista Scientific Reports

ABSTRACT

Cannabinoids from *Cannabis sativa* that have therapeutic functions have been known for a long time, however, studies elucidating the compounds cannabidiol (CBD) and delta-9-tetrahydrocannabinol (Δ9-THC) are very recent. Model organisms, such as *C. elegans*, have a complex neurotransmitter system and are important in studies related to neurological diseases. Therefore, the objective of this work was to evaluate the protective and/or curative potential of different concentrations of THC and CBD cannabinoids obtained from C. sativa on behavioral parameters (defecation cycle and pharyngeal beating and locomotor parameter of body curves), cholinergic (acetylcholinesterase enzyme activity; AChE) and lipid peroxidation in wild type and two transgenic strains (GMC101 and CL2122) in the nematode *C. elegans*. For this, different concentrations of tetrahydrocannabinol (THC; 1.25% and 2.5%) and cannabidiol (CBD; 1.25% and 5%) were used on behavioral parameters, cholinergic system and lipid peroxidation. As a result, the observed compound was efficient in reducing AChE activity in N2 strains and in increasing the GMC101 strain, with emphasis on the oil rich in CBD. On the other hand, THC-based oils influenced the evaluated biomarkers, both in the N2 strain and in the transgenic GMC101 and CL2122. These results improve the potential to use medicinal oils to treatment in neurodegenerative disorders.

Keywords: Cannabidiol, THC, Acetylcholinsterase, GABA, Alzheimer's Disease.

1. INTRODUCTION

The *Cannabis sativa* plant has hundreds of chemical compounds produced by secondary metabolism, including cannabinoids, terpenes, and phenolic compounds, each with potential biological properties[1]. Among the cannabinoids, delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) stand out as the two main ones[2].

THC is well known for its psychoactive properties, while CBD is a non-psychostimulant cannabinoid[3]. These compounds have opposing pharmacokinetic and pharmacodynamic interactions, where CBD acts as an antagonist or negative modulator towards THC, through binding to CB1 and CB2 receptors[4]. Thus, the actions of these cannabinoids modulate the central nervous system (CNS) involving development, synaptic plasticity, response to endogenous and environmental damage, as a characteristic of many neurodegenerative diseases[5]. Through the interaction between cannabinoids and their receptors, an anti-inflammatory response is generated[6], which is an important strategy for maintaining physiological homeostasis and acting on the central nervous system, including pain reduction[7].

Some neurodegenerative diseases have an unknown etiology, but the dynamics of these diseases are mediated by specific neurotransmitters that maintain systems in homeostatic imbalance. When it comes to diseases related to nerve synapses, the most studied systems include the GABAergic nervous system (SNG) and the cholinergic nervous system (SNCol)[8], as they are directly linked to neurodegenerative diseases.

The SNG comprises the most abundant synapses in the central region of the CNS, of the inhibitory type, mediated by the action of the neurotransmitter γ-aminobutyric acid (GABA), which plays a dominant role in inhibitory processes[9]. The inhibitory action of GABA occurs through interaction with the GABAergic receptors, GABA A, GABA B and GABA C[10]. It is known that patients with depression have reduced levels of the neurotransmitter GABA in the CNS[11]. GAbaergic neurotransmission occurs in interneurons that modulate local neurotransmission including noradrenergic, dopaminergic and serotonergic neurons[12]. Thus, it is believed that the reduction of GABAergic transmission over a long period is related to the characteristics associated with Alzheimer's disease[12,13], which demonstrates that the serotonergic interaction with the receptors GABA B and GABAergic neurotransmission are important modulators of the effects of Alzheimer's disease and, therefore, this is related to the etiology of this disease[14,15].

SNCol is responsible for cognition, memory and senses, and its neurotransmitter is acetylcholine (ACh). ACh is an excitatory neurotransmitter formed from acetyl coenzyme-A and choline, which acts at neuromuscular junctions, memory, and areas of the brain related to

learning[16]. ACh develops its role through binding to nicotinic (nAChR) and muscarinic (mAChR) receptors. Muscarinic receptors are divided into 5 types (M1-M5), and they belong to a superfamily of G-protein-coupled receptors, which have seven transmembrane hydrophobic domains[12]. Nicotine receptors, on the other hand, belong to a family of ligand-dependent ion channel receptors, divided into three general classes considering their pharmacological and physiological characteristics, which distribute them at the muscular and neuronal level, with the third type being present in the human CNS[17,18]. In this way, SNCol acts on the peripheral nervous system along with the neuromuscular connections, being, therefore, affected in neurodegenerative diseases such as Parkinson's and Alzheimer's[19].

Changes that occur in the GABAergic and cholinergic nervous systems are also responsible for behavioral disturbances in AD patients[20,21,22], since the enzyme acetylcholinesterase (AChE) develops the nerve impulse through the hydrolysis of ACh into acetate and choline. This neurotransmitter is synthesized and stored in vesicles in the presynaptic neuron. The release of acetylcholine occurs in the synaptic cleft and propagates to the receptor located on the postsynaptic neuron. At the same time that the new release of acetylcholine occurs, the previously released molecule must be hydrolyzed by the enzyme AChE[23]. Thus, a study by OH *et al.*[24] showed that patients with Alzheimer's disease improved when they were treated with AChE inhibitors.

For a better understanding of neurological disorders and their effects on physiological processes, model organisms have been used. Among these, the free-living nematode *C. elegans* is consolidated in studies in the areas of biochemistry, toxicology and genetics. The nematode *C. elegans* has different neurotransmitter systems that coordinate its behavior, including dopaminergic, cholinergic, serotonergic, glutamatergic and GABAergic systems[25]. Furthermore, they are transparent, which allows the visualization of gene or protein expression patterns using the reporter genes, such as fluorescence marker proteins[26,27]. These characteristics make it possible to detect neuronal cell death and protein inclusions through optical and fluorescence microscopy techniques, facilitated by the simple organization of its nervous system, composed of 302 neurons in an adult hermaphrodite[28].

C. elegans presents behaviors that can be evaluated under controlled conditions, such as swimming, locomotion, pharyngeal beating (which controls food consumption), contraction of the gonadal sheath (involved in the process of ovulation and egg production), and defecation. Both GABA and ACh neurotransmitters are present in *C. elegans*. However, in contrast to what occurs in vertebrates, where GABA acts on CNS synapses, in nematodes, the action of GABA, as well as ACh, is primarily on neuromuscular synapses[29].

Based on this, it is hypothesized that *C. sativa*-based THC and CBD oil blends are effective in decreasing or delaying the symptoms caused by Alzheimer's disease. This study aimed to evaluate the protective and/or curative potential of different concentrations of THC and CBD cannabinoids obtained from *C. sativa* on behavioral parameters (defecation cycle, pharyngeal beating, and the locomotor parameter of body bends), cholinergic system (activity of the enzyme acetylcholinesterase; AChE), and lipid peroxidation in wild type and two transgenic strains, GMC101 strain that has a mutation and expresses amyloid B protein, and CL2122 which is the control strain of GMC101, in the nematode *C. elegans*.

2. MATERIAL AND METHODS

2.1. Obtaining the compounds

The oils were donated by APEPI - RJ (Support for Medicinal *Cannabis* Research and Patients, Rio de Janeiro State), a non-profit initiative that aims to support research and also the individual cultivation of *Cannabis* for medicinal purposes. As the oils obtained were donated and the extraction protocols were not provided by APEPI, they were used for analysis on nematodes in the same way that they are marketed for medicinal purposes for patients with various diseases, including AD.

2.2 Analysis of Major Compounds from the mix of THC and CBD oils

For sample preparation, aliquots (100 mg) of THC and CBD-based oils were added to 10 mL of methanol:n-hexane solvent (9:1 v/v), homogenized in a vortex mixer for 1 minute and kept in an ultrasound bath for 30 minutes.

The samples were then refrigerated at -20ºC for 30 minutes, centrifuged at 4000 rpm for 20 minutes, and the supernatant filtered through PTFE polytetrafluoroethylene membranes (0.22µm) and stored in vials (in a refrigerator) for further analysis. For the analyses, a gas chromatograph coupled to the detector by mass spectrometry (CGMS 7890) was used with the following chromatographic conditions: column, DB5-HT (30mx 250µm x 0.1µm); oven, 150°C (1 minute); 15°C/minute; 270°C (6 minutes); 20°C/minute; 300ºC (2 minutes); Running time: 18.5 minutes; Flow: 0.6 mL/minute; 1µL Injection, Split: 50:1; injector and transfer line temperatures, 250ºC; Mass spectrometer programmed for acquisition in Full Scan mode 50-500 m/z, electron ionization source (EI) 70eV. Mass spectra were compared to those from the Nist library.

2.3 *Caenorhabditis elegans*

The strains used in the study, both the N2 type and mutants (GMC101, CF1553, CL2166, CL2070, GA800, and CL2122) were obtained from the *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota (Minneapolis, MN, USA) which were placed in NGM (nematode growth medium) with *Escherichia coli* OP50 at 20°C, as described by Brenner[53]. The study was carried out in the biochemistry laboratory of the Federal Institute of Rio Grande do Sul- Campus Sertão.

Different strains of *C. elegans* were used for the assays: wild type (N2), and the transgenic strains GMC101 and CL2122, and the strains that express GFP CF1553, CL2166, CL2070, and GA800. The transgenic strain GMC101 is widely used in Alzheimer's disease studies because it expresses constructs that contain the coding region of the human β-amyloid peptide[54]. The strain GMC101 had the induced expression of the protein Beta-amyloid (βA) through heat stress (25°C), in larval stage L1. Strain CL2122 is a negative control of strain GMC101.

Nematodes of the CF1553 strain were used as markers of response to oxidative stress, since this strain induces the sod-3 gene in response to oxidative stress. CL2166 was used to express gst-4, which acts on endo and xenobiotic substance's excretion pathways, protecting cells against chemical toxicity and stress. The CL2070 strain was used to determine the hsp-16.2 known as heat shock proteins, which are known to be regulated by the transcription factors SKN-1, DAF-16, and HSF. The GA800 was used to analyze the catalase green fluorescent expression (ctl-1, 2, 3 and 4).

2.4 Experimental conditions

For the stress-free acute treatment, wild-type N2 strains, GMC101, CL2122, CF1553, CL2166, CL2070 and GA800 were maintained at 20°C. In these trials of acute exposure without the stressor, after synchronization, both seven strains returned for another 48 hours at 20°C. In this case, the GMC101 strain does not express the amyloid beta protein.

For the acute treatment with the stressor, the wild N2 strain was kept at a temperature of 20°C, whereas the strains GMC101 and CL2122, after their synchronization, were raised to a temperature of 25°C so that the strain GMC101 expressed the Beta protein – amyloid. After 48 hours in the bacteriological oven, the nematodes were exposed to the oils, containing a total of six groups for exposure: water control / olive oil control (together with the oil mixers that

APEPI sends, 20 mL of what is in the bottle is olive oil, the rest is *C. sativa* extract) CBD 1.25% and 5% and THC; 1.25% and 2.5% totaling the 6 groups.

For both treatments, the nematodes were transplanted and sown in NGM) with *Escherichia coli* as food, and kept growth chambers at 20ºC, until they reached the L4 larval stage and after that they were synchronized (pregnant or with eggs). The nematodes were washed 3 times, where the supernatant was discarded, centrifuged for 2 minutes at 3000 rpm. The nematodes were left, and they were exposed to a synchronization solution for 5 ml (hypochlorite: 1.5 ml, NaOH: 0.250 ml, water: 3.250 ml). The hypochlorite and NaOH in the synchronization solution are toxic to the nematodes, causing them to break the cuticle and release their eggs. Then the eggs were placed on plates with the M9 solution, and the eggs were allowed to hatch for 24 hours in at 20°C and reach the L1 larval stage. After that, they are transferred to NGM and feed with E. coli. Next day when they grow to L4 stage they can be exposed.

Then, nematodes from both treatments were exposed to acute exposure for 1 hour. The treatments consisted of exposure to THC-based oils at concentrations of 1.25% and 2.5%, and CBD-based oils at concentrations of 1.25% and 5%. Two control treatments were also carried out: one using only water and the other using only olive oil. After 1 hour of exposure to the treatments, the organisms were washed 3 times in M9 buffer solution, transferred to Petry dishes containing *E. coli* and acclimated for another 30 minutes for further behavioral evaluation.

2.5 Behavioral assessment

The following behavioral parameters were evaluated: defecation cycle, pharyngeal pumping and body bends. To measure the duration of the defecation cycle, defecation frequency was counted under a microscope (100×) among synchronously grown young adults. Six nematodes were examined per strain, per treatment. Each nematode was observed for three consecutive defecation cycles and the time between the two consecutive cycles was calculated[55]. To measure the pharyngeal pumping, six nematodes were also examined, where each one was observed three times for 10 seconds each, closing a total of 30 seconds (10 s-10 s-10 s)[55]. They are fed by sucking bacteria and grinding them in their terminal bulbs. As a complete cycle of synchronous contraction and relaxation of the terminal bulb is called a "pump", the pharyngeal beat rate (pumps per minute) was measured by visually observing the nematodes under a microscope (100×).

Still, the locomotor parameter of body bends was evaluated, where eight nematodes (performed in the 3 strains) were exposed to each treatment in the larval stage L4, where they were evaluated individually 2 times for 30 seconds with a total time of 60 seconds for each nematode. These were collected and placed in a Petry dish (5 cm in diameter) containing only agar and allowed to acclimate for 1 minute. Each of the nematodes was kept in a horizontal position (X axis) and the number of body curves generated in 30 seconds was counted. Body curvature was defined as a change in the direction of locomotion of the nematode's anterior part, ie, thepharynx, along the Y axis. The number of curvatures per minute was calculated according to the methodology of Tsalik & Hobert[56].

2.6 Acetylcholinesterase enzyme activity

To determine the activity of the enzyme acetylcholinesterase (AChE) it was analyzed in L4 stage nematodes, using a colorimetric assay[57] with minor modifications. After the exposure period, 10.000 nematodes were washed three times with M9 buffer and transferred to microcentrifuge tubes. Samples were frozen and thawed 3 times in liquid nitrogen, followed by 5 rounds of sonication, each of 15 seconds in the ice at 30% amplitude, with 10-second intervals in between. These were centrifuged for 30 minutes at 15.000 rpm and the supernatants were collected. A 160 µL aliquot of the supernatant was mixed with 1200 µL of a solution containing 0.25 mM DTNB and 40 µL of 156 mM acetylthiocholine iodide (ASChI) and incubated at 30 °C for 5 minutes. The rate of change in absorbance was measured at 405 nm at 30 second intervals for 4 minutes. Kinetic measurements were recorded and converted to total cholinesterase activity using the extinction coefficient for the yellow-colored product, 5-thio-2-nitro-benzoic acid (II). AChE activity was expressed as µmol of ASChI per minute per mg of protein[58].

2.7 Antioxidant enzymes

Determination of the green fluorescent expression of Superoxide dismutase (SOD), Catalase (CAT), Glutathione-S-transferase (GST), and Heat shock protein (HSP) was used the strain with the expression GFP (CF1553 [muls84 (pAD76) *sod-3p::*GFP + *rol-6(su1006)*] and GA800 [wuls151 *ctl-1* + *ctl-2* + *ctl-3* + *myo-2*::GFP], CL2166 [dvIs19 (pAF15) *gst-4p*::GFP::nls], CL2070 (dvIs70 [hsp-16.2p::GFP + rol-6(su1006)]), were exposed as described in the methodology, after exposed, they were washed and transferred to 96-well microplates with 200 µL of buffer M9, using excitation: 485nm and emission: 530nm to measure total fluorescence. Assays that were fluorescence-based were conducted on a microplate reader[47,59].

2.8 ROS levels

To evaluate ROS levels 1500 nematodes of N2 strain were separated in L4 stage, which were pre-exposed to different concentrations of the oil extracts as described above, were later washed out of the treatment, and then exposed to 0.5 mM of H2DCF-DA (2.7-dichlorofluorescein-diacetate) for one hour, then the nematodes were washed again, containing the fluorescence dye and then exposed to hydrogen peroxide (0.4 mM). ROS was analyzed inside the nematodes by measuring fluorescence (Exc: 485 nm; Em: 535 nm) for 1 h in the presence of hydrogen peroxide and the results were expressed as function-time curves per mg of protein for normalization.

2.9 Determination of lipid peroxidation

For the determination of the lipid peroxidation biomarker (TBARS) the preparation of extracts was performed in the same way as the extracts for AChE. The extracts were incubated with 60 µL of sample + 60 µL of phosphoric acid (0.1%) + 60 µL of TBA (0.6%) + 60 µL of SDS (0.6%) and placed in a water bath in tubes of test at 100 °C for 1.5 hours. The samples were read in a microplate reader with absorbance of 532 nm[60].

2.10 Statistical analyze

Statistical analysis was performed using ANOVA – one way (one-way analysis of variance), followed by Tukey's post hoc multicomparative test or Kruskal-Wallis (KW) test with post hoc test of Dunn's. The choice of the post hoc test was defined based on the homoscedasticity and homogeneity of variance, defined by the Kolmogorov-Smirnov and Bartlett tests. Means were considered different when $p<0.05$. The results of the parametric data were expressed in the graphs by means ± standard error (SE). For data considered non-parametric were expressed by the medians ± interquartile ranges. The representation of the statistical differences was given by the representation of different lowercase letters on the axes of the bars. Statistical analyzes were performed using Graph Pad Prism version 8.0.1 (GraphPad Software).

3. RESULTS

3.1 Effect of *Cannabis* oils on *per se* exposure

3.1.1 Behavioral biomarkers on *per se* exposure (N2, GMC101, and CL2122 strains)

Body bends rate was decreased in the group treated with 1.25% of THC (P=0.01) in comparison to CBD 1.25% in the GMC101 transgenic strain (Fig. 1A). The body bends rate on CL2122 transgenic strain was increased in the group treated with CBD 5% in comparison to both olive (P=0.05) and water (P=0.001) controls, as well as the group treated with CBD 1.25% (Fig. 1B). In the wild-type (N2) strain, the body bends rate was decreased in the 1.25% THC-treated group in comparison to the water control group (Fig. 1C).

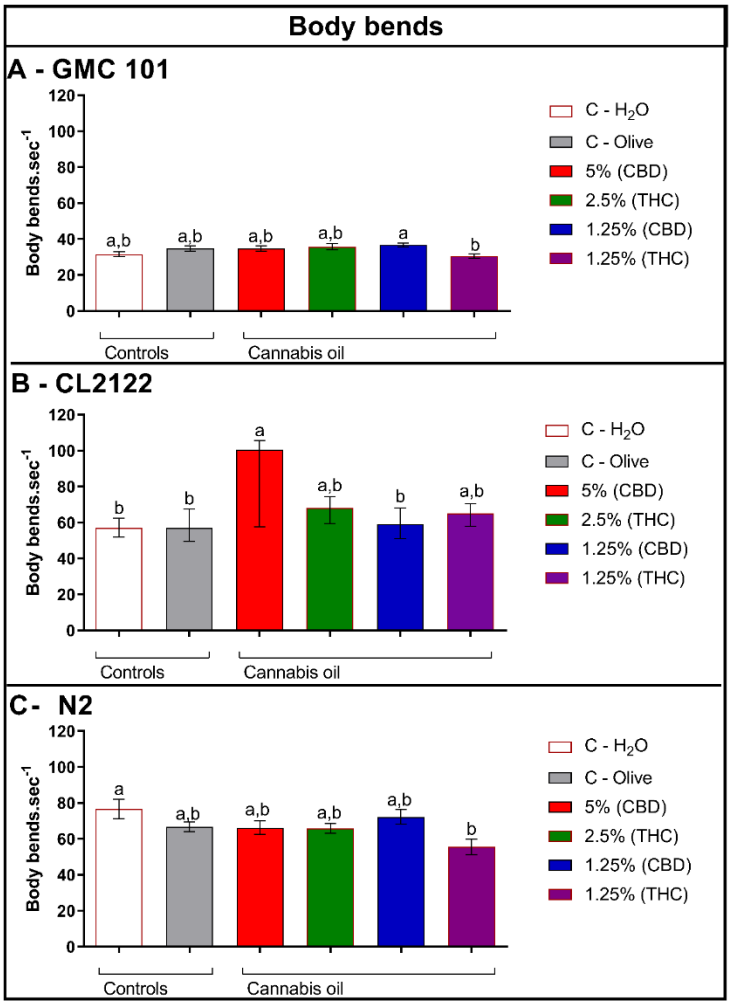

Fig. 1 – *Per se* effect of different doses of *Cannabis* oils on Body bends rate in GMC101 (A), CL2122 (B), and N2 (C). Data in panels A and C are expressed by the mean ± SEM analyzed by ANOVA one-way with Tukey's post hoc test. Data in panel B is expressed by the median ± interquartile range analyzed by Kruskal-wallis non-parametric test with post hoc test of Dunn's. Different letters mean statistical difference.

Defecation cycle in GMC101 strain was increased in the group treated with 5% of CBD in comparison to the water (P=0.001) and olive (P=0.0001) controls, and in comparison to the 1.25% CBD (P=0.05) and 1.25 % THC (P=0.0001) (Fig. 2A). In addition, the defecation cycle was increased in the group treated with 2.5% THC in comparison with all the other groups (p=0.0001). Defecation cycle on CL2122 strain was decreased in the groups treated with 2.5% THC, 1.25% CBD, and 1.25% THC in comparison to water and olive controls (p=0.0001) (Fig. 2B). Defecation cycle on N2 strain was decreased in the groups treated with 5% CBD and 1.25% THC in comparison to the controls water (p=0.0001) and olive (p=0.0001) (Fig. 2C).

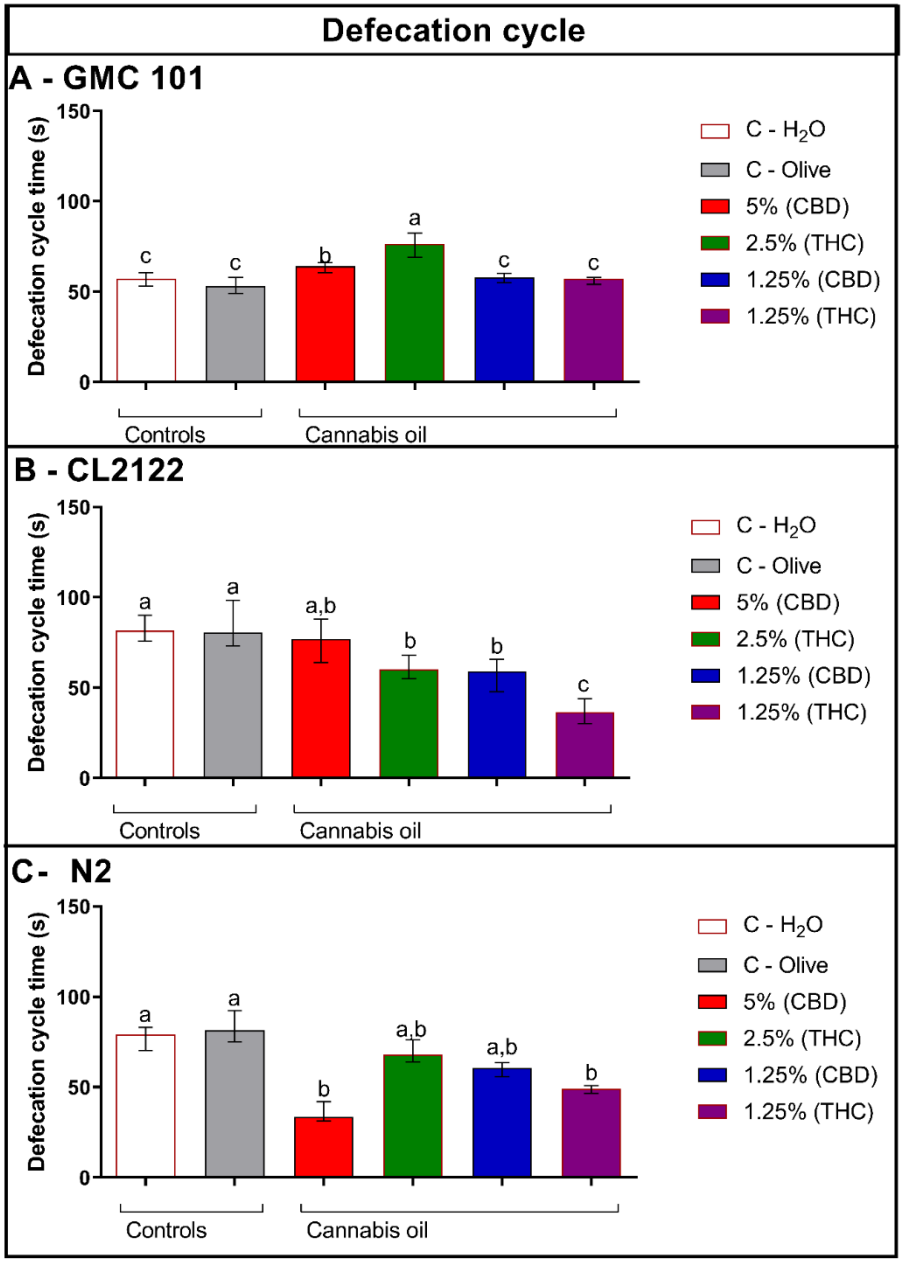

Fig. 2 - *Per se* effect of different doses of *Cannabis* oils on defecation cycle in GMC101 (A), CL2122 (B), and N2 (C). Data in panels A, B, and C are expressed by the median ± interquartile range analyzed

by Kruskal-wallis non-parametric test with post hoc test of Dunn's. Different letters mean statistical difference.

Pharyngeal pumping rate was increased in the GMC101 strain in the group treated with 1.25% CBD in comparison to the groups treated with water (p=0.01), olive (p=0.001), 5% CBD (p=0.01, 2.5% THC (p=0.01), and 1.25% THC (p=0.01) (Fig. 3A). In the CL2122 transgenic strain the pharyngeal pumping was increased in the group treated with 1.25% CBD in comparison to water (p=0.001), olive (p=0.001), 5% CBD (p=0.001), 2.5% THC (p=0.001), and 1.25% THC (p=0.001) (Fig. 3B). The pharyngeal pumping was decreased in the group treated with 5% CBD in comparison to water, olive, and 1.25% CBD. The pharyngeal pumping on N2 strain was increased in the groups treated with 1.25% CBD and 5% CBD in comparison to water and olive controls (Fig. 3C).

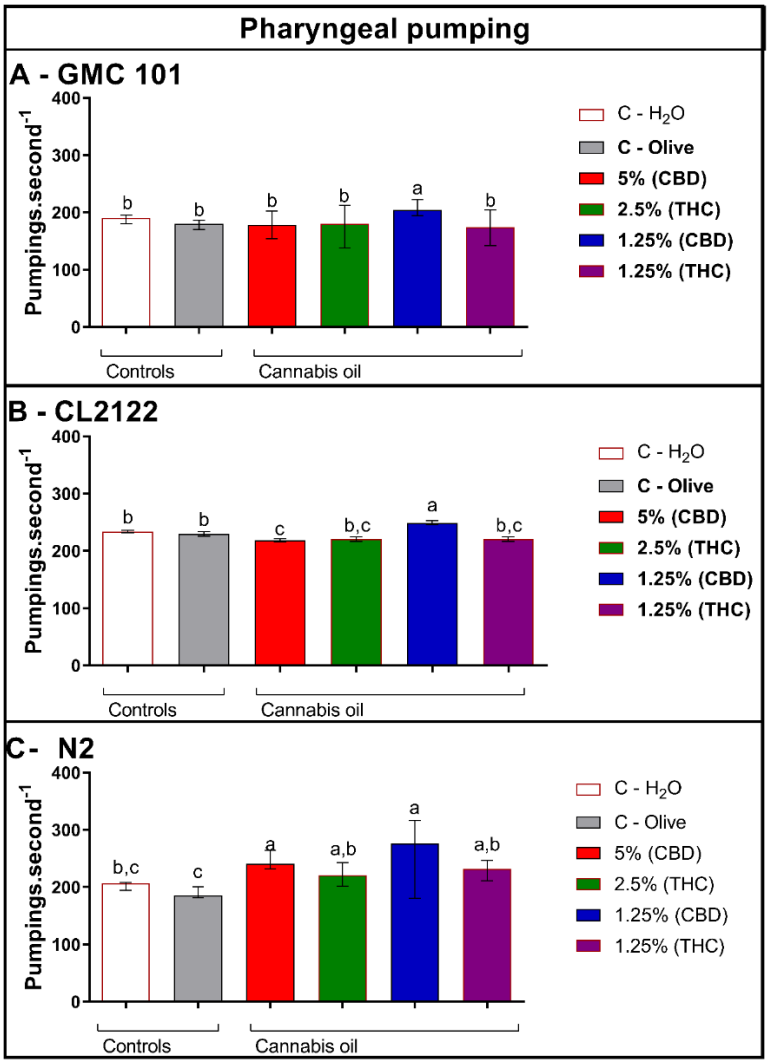

Fig. 3 - *Per se* effect of different doses of *Cannabis* oils on Pharyngeal pumping rate in GMC101 (A), CL2122 (B), and N2 (C). Data in panels A and C are expressed by the median ± interquartile range analyzed by Kruskal-wallis non-parametric test with post-hoc test of Dunn's. Data in panel B is

expressed by the mean ± SEM analyzed by ANOVA one-way with Tukey's post hoc test. Different letters mean statistical difference.

3.1.2 Biochemical biomarkers on *per se* exposure

Acetylcholinesterase activity on N2 was decreased in both CDB-treated groups (1.25% and 5%) in comparison to the groups treated with water, olive, 2.5% THC, and 1.25% THC (Fig. 4) with p=0.001 for all comparisons.

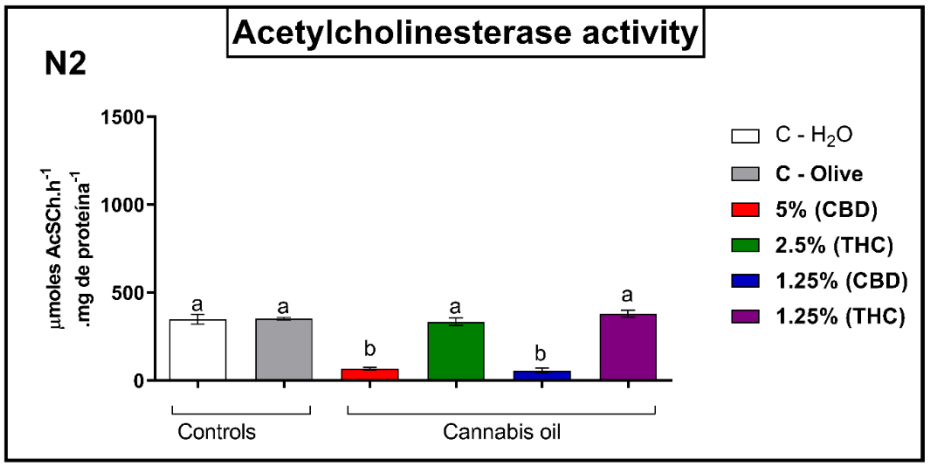

Fig. 4 - *Per se* effect of different doses of *Cannabis* oils on Acetylcholinesterase (AChE) activity N2 (wild-type) strain. Data is expressed by the mean ± SEM analyzed by ANOVA one-way with Tukey's post hoc test. Different letters mean statistical difference.

AChE activity on *per se* exposure in GMC101 strain was increased in the groups treated with 5% CBD and 2.5% THC in comparison to both water and olive (p=0.05) controls (Fig. 5A). AChE activity on *per se* exposure in CL2122 strain was increased in the group treated with 2.5% THC in comparison to both water and olive controls (p=0.01) as well as in the 5% CBD (p=0.001), and 1.25% THC (p=0.05) (Fig. 5B).

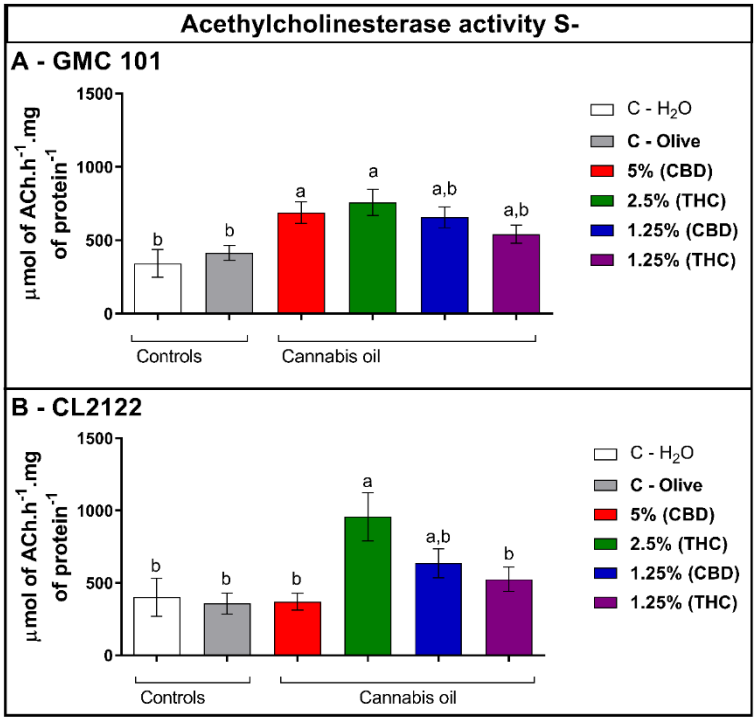

Fig. 5 - *Per se* effect of different doses of *Cannabis* oils on Acetylcholinesterase (AChE) activity in GMC101 (A) and CL2122 (B) transgenic strains. Data are expressed by the mean ± SEM analyzed by ANOVA one-way with Tukey's post hoc test. Different letters mean statistical difference.

3.1.3 Redox status on *per se* exposure (N2, CL2070, CF1553, GA800, and CL2166 strains)

The ROS level on wild-type was decreased in the groups treated with 5% CBD and 2.5% THC in comparison with both water (p=0.05 and p=0.01) and olive (p=0.05 and p=0.05) (Fig. 6).

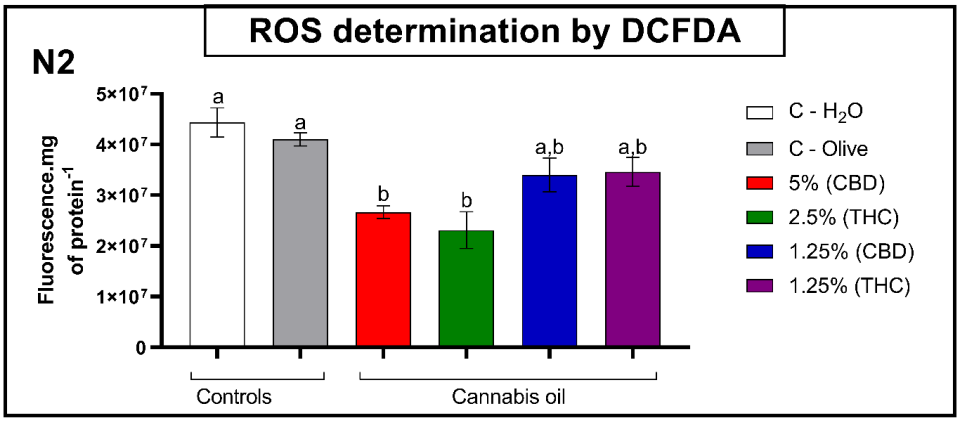

Fig. 6 - *Per se* effect of different doses of *Cannabis* oils on ROS identification (DCFDA) levels in N2 (wild-type) strain. Data is expressed by the mean ± SEM analyzed by ANOVA one-way with Tukey's post hoc test. Different letters mean statistical difference.

The SOD fluorescent expression on CF1553 strain was increased in the group treated with 2.5% THC in comparison to both water (p=0.001) and olive (p=0.05) controls, as well as 5% CBD (p=0.05), and 1.25% THC (p=0.01) (Fig. 7A). The CAT fluorescent expression on GA800 strain was increased in the group treated with 1.25% THC in comparison to water and olive controls, 5% CBD, 2.5% THC, 1.25% CBD (p=0.01 for all) (Fig. 7B). GST fluorescent expression on CL2166 strain was decreased in comparison to all the treatment groups (Fig. 7C). The groups treated with 1.25% CBD and THC were increased in comparison to water and olive controls, as well as 5% CBD and 2.5% THC (Fig. 7D).

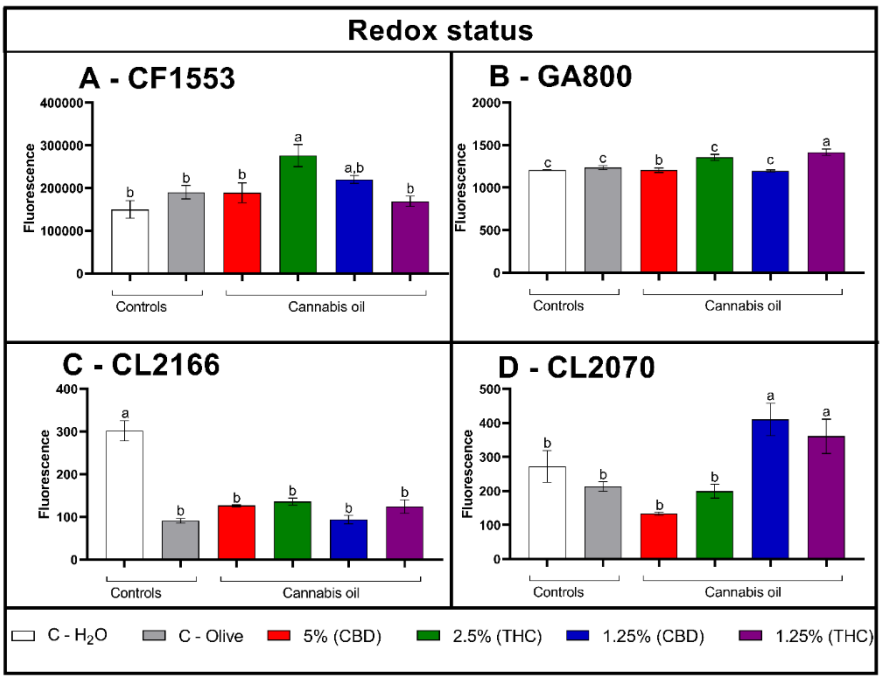

Fig. 7 - *Per se* effect of different doses of *Cannabis* oils on antioxidant enzymes expression by GFP in CF1553 (A), GA800 (B), CL2166 (C), and CL2070 (D) transgenic strains. Fluorescent expression of Superoxide dismutase (A), Catalase (B), Glutathione-S-transferase (C), and Heat shock protein (D). Data are expressed by the mean ± SEM analyzed by ANOVA one-way with Tukey's post hoc test. Different letters mean statistical difference.

Lipid peroxidation in wild-type was decreased in the groups treated with 5% CBD in comparison to water, olive controls (Fig. 8). In addition, the group treated with 1.25% CBD was decreased in comparison to the water and olive controls (p=0.001 for all comparisons) (Fig. 8).

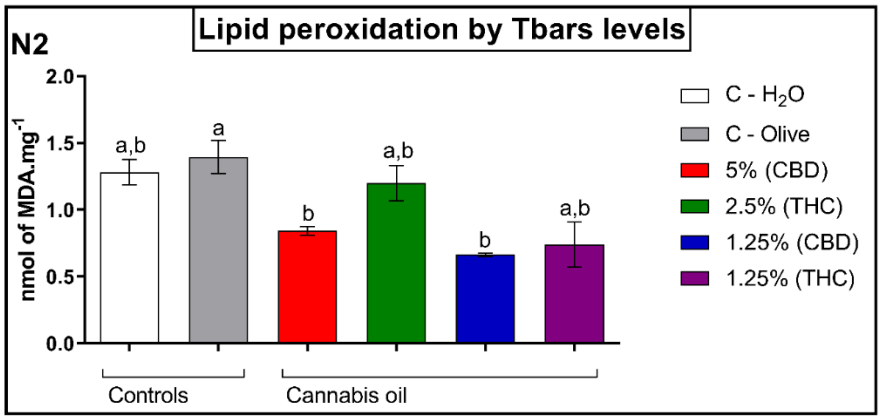

Fig. 8 - *Per se* effect of different doses of *Cannabis* oils on lipid peroxidation (Tbars) levels in N2 (wild-type) strain. Data is expressed by the mean ± SEM analyzed by ANOVA one-way with Tukey's post hoc test. Different letters mean statistical difference.

3.2 Effect of Cannabis oils on heat stressed transgenic strains

3.2.1 Behavioral biomarkers on heat stressed nematode (GMC101 and CL2122)

Body bends on heat-stressed GMC101 was increased in 5% CBD, 1.25% CBD, and 1.25% THC in comparison to both water (p=0.01) and olive control treatments (Fig 9A). In the heat-stressed CL2122, the body bends rate was increased in the groups treated with 5% CBD, and 2.5% THC in comparison to water, olive, 1.25% CBD and 1.25 THC (p=0.001 for all comparisons) (Fig. 9B).

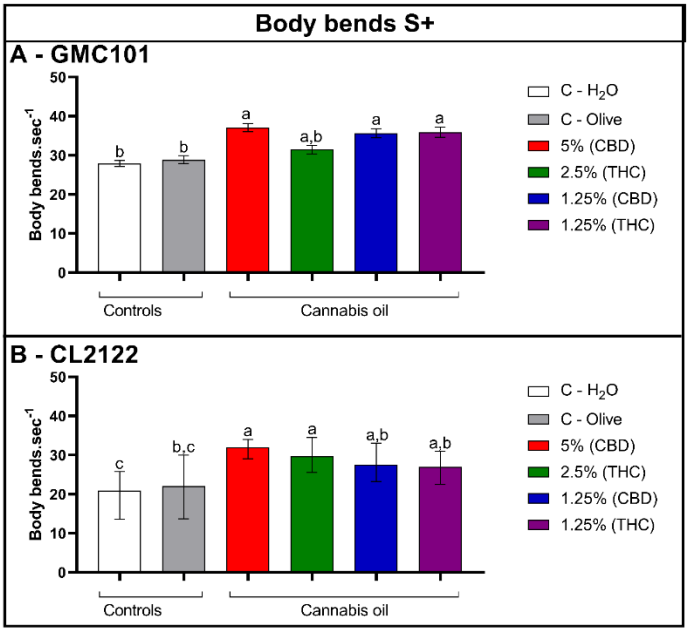

Fig. 9 - Effect of different doses of *Cannabis* oils on Body bends rate in heat stressed nematode from GMC101 (A) and CL2122 (B) transgenic strains. Data in panels A is expressed by the mean ± SEM analyzed by ANOVA one-way with Tukey's post hoc test. Data in panel B is expressed by the median

± interquartile range analyzed by Kruskal-wallis non-parametric test with post hoc test of Dunn's. Different letters mean statistical difference.

Defecation cycle on heat-stressed GMC101 was decreased in the group treated with 2.5% THC in comparison to water, olive, 5% CBD, 1.25% CBD, and 1.25% THC (Fig. 10A). The heat-stressed CL2122 defecation cycle was not significant among the treatment groups (Fig. 10B) (p=0.001 for all comparisons).

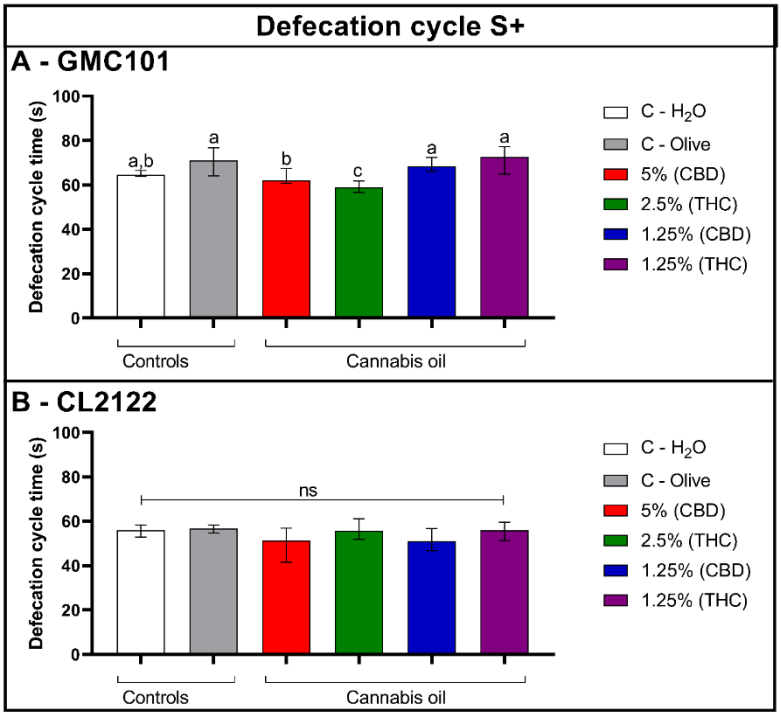

Fig. 10 - Effect of different doses of *Cannabis* oils on defecation cycle rate in heat stressed nematode from GMC101 (A) and CL2122 (B) transgenic strains. Data are expressed by the median ± interquartile range analyzed by Kruskal-Wallis non-parametric test with post hoc test of Dunn's. Different letters mean statistical difference.

Pharyngeal pumping on heat-stressed GMC101 was increased in the group treated with 5% CBD in comparison to the controls water (p=0.01), olive (p=0.001), 2.5% THC (p=0.001), 1.25% CBD (p=0.001), and 1.25% THC (p=0.001) (Fig. 11A). On the heat-stressed CL2122, the pharyngeal pumping was decreased in the groups treated with 1.25% CBD and THC in comparison to the water, olive, 5% CBD, and THC 2.5% (Fig. 11B) (p=0001 for all comparisons).

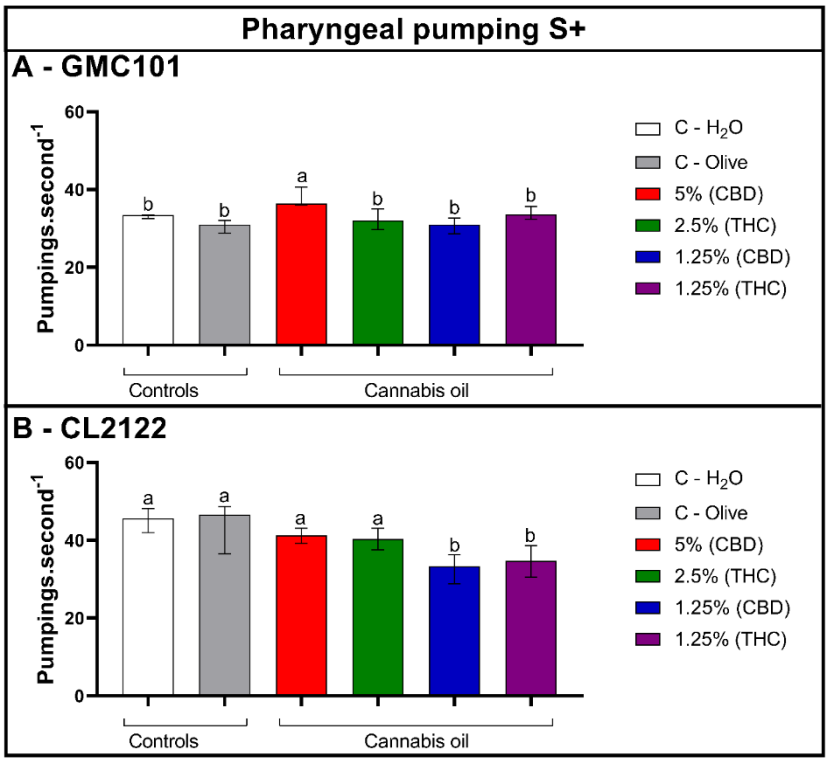

Fig. 11 - Effect of different doses of *Cannabis* oils on pharyngeal pumping rate in heat stressed nematode from GMC101 (A) and CL2122 (B) transgenic strains. Data are expressed by the median ± interquartile range analyzed by Kruskal-wallis non-parametric test with post hoc test of Dunn's. Different letters mean statistical difference.

3.2.2 Biochemical biomarkers on heat stressed nematode (GMC101 and CL2122)

AChE activity on heat-stressed GMC101 was decreased in the group treated with 1.25% THC in comparison to water (p=0.001) and olive (p=0.01) controls (Fig. 12A). In addition, the groups treated with 5% CBD and 2.5% THC were decreased in comparison to water and olive controls as well as with the group 1.25% CBD (Fig. 12A) (p=0.001 for all comparisons). On heat-stressed CL2122 strain, the AChE activity was increased in the group treated with 2.5% THC in comparison to 1.25% CBD and 1.25% THC (Fig. 12B). In addition, the groups treated with 1.25% of CBD and THC were decreased in comparison to the groups treated as water, olive, 5% CBD, and 2.5% THC respectively (Fig. 12B) (p=0.001 for all comparisons).

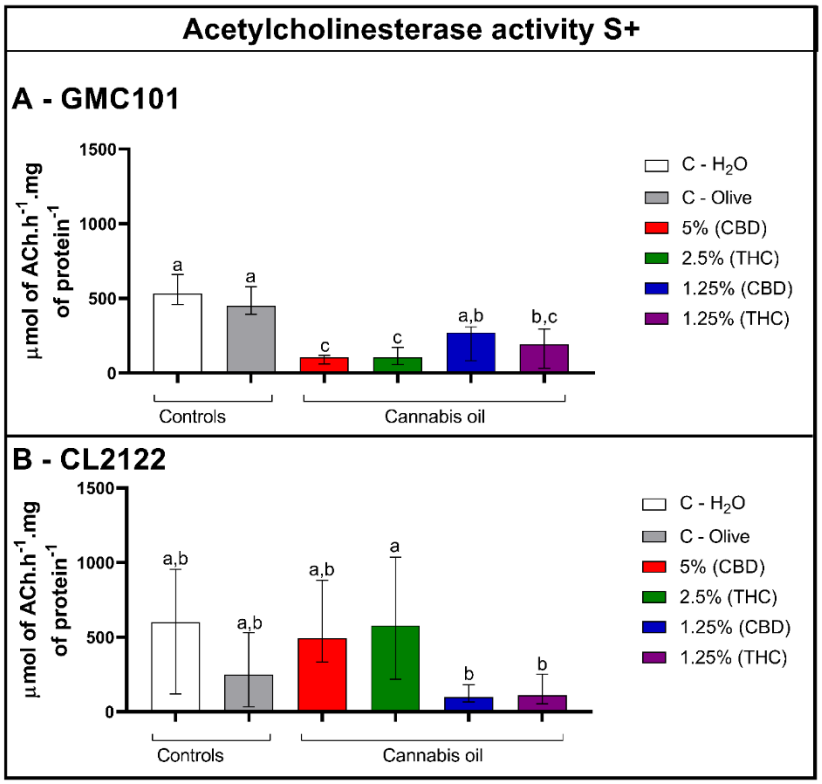

Fig. 12 - Effect of different doses of *Cannabis* oils on acetylcholinesterase (AChE) activity in heat stressed nematode from GMC101 (A) and CL2122 (B) transgenic strains. Data are expressed by the median ± interquartile range analyzed by Kruskal-wallis non-parametric test with post hoc test of Dunn's. Different letters mean statistical difference.

4. DISCUSSION

Different strains of *C. elegans* nematodes showed differential responses to treatments based on tetrahydrocannabinol (THC) and cannabidiol (CBD), when behavioral activity, AChE enzyme activity, and lipid peroxidation were evaluated. In this study, we observed an increase in the neurodegenerative conditions of the nematode, with increased behavior, pharyngeal pumping and body bends. Higher concentrations of the CBD and THC oils showed a decrease in the reactive oxygen species (ROS) production, which could act as an antioxidant effect, with the enzyme results showing the potential for the medicinal use of the oils to treat neurodegenerative conditions.

Among the various diseases to which the oils are applied, there are seizures, epilepsy, multiple sclerosis, anxiety, depression, migraine, Alzheimer's, and Parkinson's disease, among others that affect the nervous system[30,31]. As many of these treatments have no scientific basis, it is essential to demonstrate the real effects at a biochemical level, especially the pharmacodynamics and pharmacokinetics of these compounds.

The species used in the manufacture of oils is *Cannabis sativa*, and different concentrations of THC and CBD are produced by the plant due to the effects of climate, soil, management, among others adopted in the cultivation of the species[32]. In the present study, both terpenes were relativized in terms of their contents by HPLC (High-Performance Liquid Chromatography). High concentrations of THC were observed in both oils, but the same was not observed with CBD. As for CBD, it is present in higher concentrations in the so-called CBD rich-oil and its concentrations are almost undetectable in the so-called THC-rich oil. In addition to these two terpenes, plants of the *Cannabis* genus are known to produce several other terpenes, which have a high influence on the nervous system, mainly on the CB1 and CB2 endocannabinoid receptors[33].

The different concentrations of THC and CBD are the result of genetic improvement processes, where strains with high levels of specific desired compounds are prepared. Thus, elucidating the efficiency of the breeding to ensure the presence of a particular compound in the oil is essential, as well as paying attention to pollination between different strains, which culminates in genetic variability[34]. In addition, another factor that influences and guarantees the desired concentration of these terpenes is to certify that the extraction was efficient for each of these compounds[35]. In the present study, the extraction methods were not compared, since the objective was only to elucidate the biochemical effect of the oils already produced.

In the *in vivo* use of these oils, it was observed that several biomarkers evaluated were altered. In the wild-type N2 strain, an increase in pharyngeal pumping was observed when CBD-rich oil was added at both concentrations. Furthermore, the duration of the defecation cycle was decreased at the highest dose of CBD and the lowest dose of THC. The effect observed in the increase in pharyngeal pumping and decrease in the defecation cycle in the group treated with 5% CBD is related to the decrease in acetylcholinesterase (AChE) enzyme activity, which was also evidenced.

In the transgenic GMC strains that express β-amyloid and the CL2122 strain, our control, we could observe that the higher concentration of CBD increased the movement of the nematode. The same occurred in the increase in pharyngeal beat. Taken together, also with the decrease in AChE, this confirms the results for the strain that expresses β-amyloid, the smaller the synaptic cleft, the greater the release of acetylcholine.

AChE is a central nervous system enzyme that acts in the synaptic cleft and neuromuscular junctions and is responsible for the hydrolysis of acetylcholine (Ach) into acetic acid and choline[36]. ACh is one of the main neurotransmitters that make up the nervous system, being responsible for behavior, memory, movement, and reasoning[37].

Inhibition of AChE enzyme activity is responsible for causing high excitability in cholinergic neurons, and it forces ACh to remain longer in the synaptic cleft, inducing an excitatory effect in the system. The reflexes of this excitatory effect are mostly evidenced in high muscle contraction; thus, the increased pharyngeal beat rate characterizes the reduced effect of AChE[38,39]. The pharynx is a bilobed neuromuscular organ and in the nematode phylum it plays an important role, as it is where the pharyngeal ring is located, that is, the highest concentration of neurons is present in this space[40]. Thus, the pharynx is mainly regulated by the action of the ACh neurotransmitter and the increase in pharyngeal rhythm is related to improvement in neurodegenerative diseases[41].

However, the defecation cycle is also regulated by the ACh levels in the synaptic clefts, but it is strongly influenced by two other factors, namely calcium (Ca^{+}) levels and the neurotransmitter γ-aminobutyric acid (GABA). It is known that muscle contractions are linked with the ACh transmitter, but the frequency in which they occur mainly concerns the levels of GABA and Ca^{+} [38]. Thus, it can be predicted that the altered effect observed in the cholinergic system will also be reported at the GABAergic level, which is the main candidate for the defecation cycle time-reducing effect. In addition, the accentuation of the reduction of the defecation interval is closely linked to the endocannabinoid system CB1 receptors, which are found throughout the central nervous system, peripheral, synaptic endings, intestine, liver, adipose tissue, and immune cells, being that in the intestine the endocannabinoid system plays an important role in its motility[42,43]. CB1 receptors act more actively in the presence of CBD, thus increasing the number of defective contractions and reducing the interval between them in *C. elegans*.

The observed effect in the mutant strains CL2122 and GMC101 can predict how the oil would behave in an organism predisposed to manifest diseases related to the central nervous system[44]. The GMC101 strain has the addition of the gene encoding the human β-amyloid protein, and this protein is expressed at a low rate in the strain and can be transcriptionally activated through heat stress (25 °C)[45]. The β-amyloid protein is one of the main proteins involved in the onset and progression of Alzheimer's disease. Patients affected by Alzheimer's have aggregations of different isoforms of this protein in the synaptic clefts, which under normal conditions would be precipitated and eliminated periodically, which does not occur in patients predisposed to neurodegeneration[46]. These aggregations, in addition to hindering communication between neurons, causing neurotransmitters to lose efficiency, are also responsible for the death of cholinergic neurons and thus leading to the progression of neurodegenerative diseases.

On the other hand, strain CL2122 is considered a reference for GMC101, because at high temperatures (25 °C) it does not transcribe β-amyloid protein. In this way, observing the result of both strains is important. In this study, there was no thermal stress (temperature rise to 25 °C after hatching), that is, both strains were treated as N2. The purpose of this treatment (without stress) is to observe and establish the effects of exposure *per se* of the cannabis compound in organisms only predisposed to the involvement of neurodegenerative diseases[47].

As in the N2 strain, an increase in the pharyngeal beating rate was observed in the GMC101 and CL2122 strains at 1.25% CDB concentration. In aging tests, the decrease in the pharyngeal beat rate is gradual and can be accelerated by neurodegenerative diseases[48]. In this way, observing an increase in the pharyngeal beat, mainly in the GMC101 strain, indicates a possible improvement in the framework of neurodegenerative diseases when oil is applied. Furthermore, changes in defecation cycle intervals, as mentioned above, will be linked to GABAergic signaling and Ca^{+} concentrations. Taking into account that the neurofibrillary aggregates influence the permeability and communication of nerve cells and that even at low concentrations they influence basic behaviors, thus clarifying the different results presented by the defecation cycle. In strain GMC101, an increase in the time between defecation cycles was observed, whic is related to the increase in AChE activity with the inhibition of the excitability of the cholinergic system, reducing the defecation cycle[45].

Naturally, in *C. elegans* mutant strains for neurodegenerative diseases, as well as in patients affected by neurodegenerative diseases, there is a harmful decrease in the levels of neurotransmitters, as well as in the activity of enzymes that act in the synapse process. To reverse this effect, defining compounds capable of activating and stabilizing these enzymes at normal levels is an important process to help reduce the progression of neurodegenerative diseases. It was observed, mainly in the activity of the AChE enzyme in the strain GMC101, which in the groups treated with the oil had its activity increased, especially at the highest doses of CBD (5%) and THC (2.5%). This increase can positively influence neurotransmission in neurodegenerative patients, since in many diseases such as Alzheimer's and Parkinson's, intense and dense protein neurofibrillary tangles are formed in the synaptic clefts. Therefore, the activation of nervous system enzymes intensifies the processes, equalizing the synapses[43].

In addition to the aforementioned, the endocannabinoid system receptors, mainly CB1, are an important biomarker of body weight gain. Traditionally known, *Cannabis* increases appetite and cravings for particularly high-fat foods. One explanation for this phenomenon is that the endocannabinoid system regulates energy balance and food intake at various functional levels, both in the brain and gastrointestinal tract[49]. As in humans, *C. elegans*, and many other

species, agonists of endocannabinoid receptors, such as THC, cause an increase in food intake, promoting body weight gain through the activation of the CB1 receptor[50,51]. On the other hand, selective CB1 receptor antagonists reduce food intake and body weight in animals and humans[52]. These observations highlight the increase in pharyngeal rate that is linked with feeding in the mutant strains. In this sense, another use for the oils is related to the food-inducing effect, that is, in patients affected by various diseases, there is a loss of appetite. In this way, an increase in intake can be induced via the endocannabinoid system (CB1) of food.

As pointed out, THC is one of the main agonists of the endocannabinoid system, acting as an inducer of this system. Thus, its administration leads to large changes observed in almost all parameters evaluated when oils with high THC contents are administered[42].

Studies such as the one presented are important to clarify the true mechanisms by which fragmented or small improvements are observed in chronic diseases linked to the central nervous system. However, even more importantly, it is necessary to evidence with science the need to discriminate these therapies, considering that in many cases it is the most effective and perhaps the only alternative. In this way, research that proves the destruction of some beliefs and taboos is essential, because far from recreational use, there are patients, family members, and also medical teams that struggle to alleviate symptoms of diseases that only cause prolonged suffering.

6. REFERÊNCIAS BIBLIOGRÁFICAS

1. Andre, C. M., Hausman, J. F., & Guerriero, G. *Cannabis sativa*: The plant of the thousand and one molecules, *Front. Plant Sci.* **7**, 19 (2016).
2. Crippa, J. A., Guimarães, F. S., Campos, A. C., & Zuardi, A. W. Translational investigation of the therapeutic potential of cannabidiol (CBD): toward a new age. *Front. Immunol.* **9**, 1256-1265 (2018).
3. Martínez, V., *et al.* Cannabidiol and Other Non-Psychoactive Cannabinoids for Prevention and Treatment of Gastrointestinal Disorders: Useful Nutraceuticals? *Int. J. Mol. Sci.* **21**, 3067 (2020).
4. Ligresti, A., De Petrocellis, L., & Di Marzo, V. From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. *Physiol. Rev.* **96**, 1593-1659 (2016).
5. Lu, H.C., & Mackie, K. An introduction to the endogenous cannabidiol system. *Biol. Psychiatry* **79**, 516-525 (2016).
6. Thomas, A., Baillie, G. L., Phillips, A. M., Razdan, R. K., Ross, R. A., & Pertwee, R. G. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br. J. Pharmacol.* **150**, 613-623 (2007).
7. Peterwee, R. G. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Delta-9-tetrahydrocannabinol, cannabidiol and delta-9-tetrahydrocannabivarin. *Br. J. Pharmacol.* **153**, 199-215 (2008).
8. Giacomello, M., Hudec, R., & Lopreiato, R. Huntington's disease, calcium, and mitochondria. *Biofactors* **37**, 206-218 (2011).
9. Salari, A.-A., Bakhtiari, A., & Homberg, J. R. Activation of GABA-A receptors during postnatal brain development increases anxiety- and depression-related behaviors in a time- and dose-dependent manner in adult mice. *Eur. Neuropsychopharmacol.* **25**, 1260-1274 (2015).
10. Barnard, E. A. *et al*. International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. *Pharmacol. Rev.* **50**, 291–313 (1998).
11. Pehrson A. L., & Sanchez C. Altered γ-aminobutyric acid neurotransmission in major depressive disorder: a critical review of the supporting evidence and the influence of serotonergic antidepressants. *Drug Des. Devel. Ther.* **9**, 603–624 (2015).
12. Pytka, K. *et al*. The role of glutamatergic, GABA-ergic, and cholinergic receptors in depression and antidepressant-like effect. *Pharmacol. Rep.* **68**, 443-450 (2016).
13. Jiménez-Balado, J., & Eich, T. S. GABAergic dysfunction, neural network hyperactivity and memory impairments in human aging and Alzheimer's disease. *Semin. Cell. Dev. Biol.* **116**, 146-159 (2021).
14. Cryan, J. F., Markou, A., & Lucki, I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol. Sci.* **23**, 238–45 (2002).
15. Millan, M. J. *et al*. Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. *Nat. Rev. Drug Discov.* **11**, 141-168 (2012).
16. Baynes, J. W., & Dominiczak, M. K. *Bioquímica Médica*. 4th ed. GEN Guanabara Koogan. 664p. (2015).

17. Bertrand, D. The possible contribution of neuronal nicotinic acetylcholine receptors in depression. *Dialogues Clin. Neurosci.* **7**, 207-216 (2005).
18. Gotti, C., Zoli, M., & Clementi, F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol. Sci.* **27**, 482–91 (2006).
19. Craig, L. A., Hong, N. S., & McDonald, R. J. Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. *Neurosci. Behav. Rev.* **35**, 1397-1409 (2011).
20. Terry, A. V., Buccafusco, J. J., & Wilson, C. Cognitive dysfunction in neuropsychiatric disorders: selected serotonin receptor subtypes as therapeutic targets, *Behav. Brain Res.* **195**, 30-38 (2008).
21. Nava-Mesa, M. O., Jiménez-Díaz, L., Yajeya, J., & Navarro-Lopez, J. D. GABAergic neurotransmission and new strategies of neuromodulation to compensate synaptic dysfunction in early stages of Alzheimer's disease. *Front. Cell. Neurosci.* **8**, 167 (2014).
22. LI, Y. *et al.* Implications of GABAergic Neurotransmission in Alzheimer's Disease. *Front. Aging Neurosci.* **8**, 31 (2016).
23. Mota, W. M. *et al*. Avaliação da inibição da acetilcolinesterase por extratos de plantas medicinais. *Rev. Bras. Plantas Med.* **14**, 624-628 (2012).
24. Oh, M. H., Houghton, P. J., Whang, W. K., & Cho, J. H. Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. *Phytomedicine* **11**, 544-548 (2004).
25. Riddle, D. L., Blumenthal, T., Meyer, B. J., & Priess, J. R. *C. elegans* II. Cold Spring Harbor Monograph Series 33. Cold Spring Harbor Laboratory Press, New York, 1222p. (1997).
26. Fire, A. *et al*. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806-811 (1998).
27. Wicks, S. R., Yeh, R. T., Gish, W. R., Waterston, R. H., & Plasterk, R. H. A. Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. *Nat. Genet.* **28**, 160-164 (2001).
28. White, J. G., Southgate, E., Thomson, J. N., & Brenner, S. The structure of the nervous system of the nematode *C. elegans*. *Philos. Trans. R. Soc. Lon. B Biol. Sci.* **314**, 1–340 (1986).
29. Shan, G., Kim, K., Li, C., & Walthall, W. W. Convergent genetic programs regulate similarities and differences between related motor neuron classes in *Caenorhabditis elegans*. *Dev. Biol.* **280**, 494-503 (2005).
30. Coatti Neto, T. A luta pelo acesso ao direito à saúde por meio do uso terapêutico da Cannabis Sativa no Estado da Paraíba. (Dissertation in Human Rights) Federal University of Campina Grande. https://repositorio.ufpb.br/jspui/handle/123456789/18535 (2020).
31. Mano-Souza, B. J., Lima, P. L., Chequer, F. M. D., & Duarte-Almeida, J. M. Os derivados da *Cannabis sativa* têm potencial para limitar a severidade e a progressão da COVID-19? Uma revisão da literatura. *Braz. J. Health Pharm.* **2**, 83-96 (2020).
32. Morais, M. E. F. *Cannabis sativa* L. (Cannbaceae): uma abordagem morfológica e medicinal. (Monography in Biological Sciences) Federal University of Paraiba. http://dspace.sti.ufcg.edu.br:8080/jspui/handle/riufcg/8121 (2018).
33. Vargas, B. M. Comparação dos processos de extração dos canabinoides da cannabis Medicinal-revisão literária. (Monography in Chemical Engineering). Federal University of Pampa. http://dspace.unipampa.edu.br:8080/jspui/handle/riu/5773 (2021).
34. Cordeiro, M. H. M. *et al*. Contribution of male genitors in open-pollination progenies of sour passion fruit. *Euphytica* **216**, 147 (2020).

35. Nuapia, Y., Tutu, H., Chimuka, L., & Cukrowska, E. Selective extraction of cannabinoid compounds from cannabis seed using pressurized hot water extraction. *Molecules* **25**, 1335 (2020).
36. Pundir, C. S., & Chauhan, N. Acetylcholinesterase inhibition-based biosensors for pesticide determination: A review. *Anal. Biochem.* **429**, 19–31 (2012).
37. Kaizer, R. R., Silva, A. C., Morsch, V. M., Corrêa, M. C., & Schetinger, M. R. C. Diet-induced changes in AChE activity after long-term exposure. *Neurochem. Res.* **29**, 2251–2255 (2004).
38. Avery, L., & You, Y.-J. *C. elegans* feeding. ed. The *C. elegans* Research Community, WormBook, https://doi.org/10.1895/wormbook.1.150.1 (2012).
39. Philippsen, D. F. *et al.* Copper uses in organic production are safe to the nervous system of *Caenorhabditis elegans*? *Environ. Qual. Manag.* **30**, 61-70 (2021).
40. Rand, J. Acetylcholine. ed. The *C. elegans* Research Community, WormBook, https://doi.org/ 10.1895/wormbook.1.131.1 (2007).
41. Zamberlan, D. C., Halmenschelager, P. T., Silva, L. F.O., & Rocha, J. B. T. Copper decreases associative learning and memory in *Drosophila melanogaster*. *Sci. Tot. Environ.* **710**, 135306 (2020).
42. Izzo, A. A., & Sharkey, K. A. Cannabinoids and the gut: new developments and emerging concepts. *Pharmacology & Therapeutics* **126**, 21–38 (2010).
43. Savonenko, A. V. *et al.* Cannabinoid CB2 receptors in a mouse model of Aβ amyloidosis: immunohistochemical analysis and suitability as a PET biomarker of neuroinflammation. *PLoS One* **10**, e0129618 (2015).
44. Bortoli, P. M. *et al*. *Ilex paraguariensis*: Potential antioxidant on aluminium toxicity, in an experimental model of Alzheimer's disease. *J. Inorg. Biochem.* **181**, 104–110 (2018).
45. Yang, T., Zhao, X., Zhang, Y., Xie, J., & Zhou, A. 6‴–Feruloylspinosin alleviated beta-amyloid induced toxicity by promoting mitophagy in *Caenorhabditis elegans* (GMC101) and PC12 cells. *Sci. Tot. Environ.* **715**, 136953 (2020).
46. Bal, S., Ghosh, C., Ghosh, T., Vijayaraghavan, R. K., & Das, D. Non-equilibrium polymerization of cross-β amyloid peptides for temporal control of electronic properties. *Angew. Chem.* **132**, 13608–13612 (2020).
47. Tambara, A. L. *et al.* Purple pitanga fruit (*Eugenia uniflora* L.) protects against oxidative stress and increase the lifespan in *Caenorhabditis elegans* via the DAF-16/FOXO pathway. *Food Chem. Toxicol.* **120**, 639–650 (2018).
48. Rodriguez, M., Snoek, L. B., Bono, M., & Kammenga, J. E. Worms under stress: *C. elegans* stress response and its relevance to complex human disease and aging. *Trends Genet.* **29**, 367–374 (2013).
49. Bellocchio, L., Cervino, C., Pasquali, R., & Pagotto, U. The endocannabinoid system and energy metabolism. *J. Neuroendocrinol.* **20**, 850–857 (2008).
50. Kunos, G. Understanding metabolic homeostasis and imbalance: what is the role of the endocannabinoid system? *Am. J. Med.* **120**, S18–S24 (2007).
51. Matias, I., & Di Marzo, V. Endocannabinoids and the control of energy balance. *Trends Endocrinol. Metab.* **18**, 27–37 (2007).
52. Di Marzo, V. CB1 receptor antagonism: biological basis for metabolic effects. *Drug Discov. Today* **13**, 1026–1041 (2008).
53. Benner, S. The genetics of *Caenorhabditis elegans. Genetics* **77**, 71-94 (1974)
54. Dimitriadi, M., & Hart, A.C. Neurodegenerative disorders: Insights from the nematode *Caenorhabditis elegans*. *Neurobiol. Dis.* **40**, 4-11 (2010).

55. Wang, M. C., O'rourke, E. J., & Ruvkun, G. Fat metabolism links germline stem cells and longevity in *C. elegans*. *Science*, **322**, 957-960 (2008).

56. Tsalik, E. L. & Hobert, O. Functional mapping of neurons that control locomotory behavior in *Caenorhabditis elegans*. *J. Neurobiol.* **56**, 178-197 (2003).

57. Cole, R. D., Anderson, G. L., & Williams, P. L. The nematode *Caenorhabditis elegans* as a model of organophosphate-induced mammalian neurotoxicity. *Toxicol. Appl. Pharmacol.* **194**, 248-256 (2004).

58. Ellman, J., Mendel, D., Anthony-Cahill, S., Noren, C. J., & Schultz, P. G. Biosynthetic method for introducing unnatural amino acids site-specifically into proteins. *Methods Enzymol.* **202**, 301-336 (1991).

59. Tamagno, W. A. *et al.* Pitaya fruit extract ameliorates the healthspan on copper-induced toxicity of *Caenorhabditis elegans*. *J. Food Biochem.* **46**, e14050 (2022).

60. Gavino, V. C., Miller, J. S., Ikharebha, S. O., Milo, G. E. & Cornwell, D. G. Effect of polyunsaturated fatty acids and antioxidants on lipid peroxidation in tissue cultures. *J. Lipid Res.* **22**, 763-769 (1981).

5. CONSIDERAÇÕES FINAIS

No presente trabalho, observou-se o potencial no uso dos óleos CBD e THC para o tratamento de doenças neurodegenerativas, já que o composto foi eficiente na redução da atividade da AchE nas cepas N2 e no aumento da cepa GMC101, com destaque para o óleo rico em CBD.

O óleo a base de THC influenciou os biomarcadores avaliados, tanto na linhagem N2 como nos transgênicos GMC101 e CL2122.

Ressalta-se também que estudos como este são primordiais, uma vez que buscam esclarecer os verdadeiros mecanismos pelos quais se observam melhorias fragmentadas ou pequenas em doenças crônicas ligadas ao sistema nervoso central. No entanto, ainda mais importante, é necessário evidenciar com a ciência a necessidade de discriminar essas terapias, considerando que em muitos casos é a mais eficaz e talvez a única alternativa. Dessa forma, pesquisas que comprovem a destruição de algumas crenças e tabus são essenciais, pois longe do uso recreativo, há pacientes, familiares e também equipes médicas que lutam para aliviar sintomas de doenças que só causam sofrimento prolongado.