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HELICONIA PSITTACORUM: INFLUÊNCIA DE FITOESTIMULANTES NO CONTROLE DE MANCHAS FOLIARES E NA QUALIDADE DE FLORES PÓS-COLHEITA

São Luís
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Engenheiro Agrônomo

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Tese apresentada ao Programa de Pós-Graduação em Agroecologia da Universidade Estadual do Maranhão, para obtenção do título de Doutor em Agroecologia.

Orientadora: Dra. Antonia Alice Costa Rodrigues

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SUMÁRIO

RESUMO GERAL

Um dos entraves à produção de helicônias são as doenças foliares que podem reduzir a fotossíntese, danificar as brácteas e inviabilizar as flores para comercialização. A utilização de fitoestimulantes pode reduzir a incidência das manchas foliares e influenciar a qualidade das flores pós-colheita. O objetivo deste trabalho foi identificar fitoestimulantes capazes de induzir resistência em *H. psittacorum* cv. Golden Torch e influenciar positivamente a pós-colheita desta espécie. Os fitoestimulantes Agro-Mos® (10 ml L^{-1}), Bion® (0,5 g L^{-1}), Quartz® (40 ml L^{-1}), K - Fosfitotal® (3 g L^{-1}) e Ca - Fosfitotal® (3 g L^{-1}) foram aplicados com auxílio de pulverizador costal e cada parcela foi protegida durante a aplicação eliminando a deriva dos produtos. A indução foi avaliada durante o cultivo experimental com a quantificação da severidade causada pelo complexo fúngico, da taxa fotossintética das plantas e da coleta de material vegetal para análise da atividade enzimática de peroxidase, polifenoloxidase, β-1,3 gluconase. A influência pós-colheita foi mensurada em flores de *H. psittacorum* cv. Golden Torch colhidas no período da manhã considerando os parâmetros: aspecto visual, perda de massa fresca, extravasamento de eletrólitos, peroxidação lipídica, além da quantificação de carboidratos solúveis e das enzimas peroxidase, polifenoloxidase, superóxido dismutase e compostos fenólicos solúveis totais. Os resultados indicaram que os fitoestimulantes Bion® e os fosfitos aplicados, reduziram a severidade do complexo fúngico, aumentaram a atividade de peroxidase, polifenoloxidase, β-1,3 gluconase e não promoveram alterações na taxa de fotossíntese liquida das plantas. O valor encontrado para $\beta > 1$, sugere que a estimativa visual da severidade do complexo fúngico é um bom indicador visual do efeito dos fitopatógenos na taxa fotossintética do hospedeiro. Já a influência dos fitoestimulantes na qualidade das hastes demonstraram ser positivas, preservando a qualidade das hastes florais colhidas. Nos parâmetros avaliados os fosfitos, sobretudo o K - Fosfitotal®, obtiveram as melhores notas para o aspecto visual, apresentando reduzido extravasamento de eletrólitos e menor peroxidação lipídica. Os resultados deste trabalho, são respostas concretas a produtores do setor, sobre alternativas de manejo das doenças associadas ao cultivo de helicônias.

Palavras-chave: Indução de resistência, espécies reativas de oxigênio, flores tropicais, vida de vaso, complexo fúngico.

GENERAL ABSTRACT

Obstructions to the production of heliconia are foliar diseases that can reduce photosynthesis, damage as broccoli and become unfeasible as flowers for commercialization. The use of phytostimulants can reduce leaf spot and influence the quality of post-harvest flowers. The objective of this work was to identify resistance forms in H. psittacorum cv. Golden Torch and positively influence the post-harvest of this species. Agro-Mos® phytostimulants (10 ml L-1), Bion® (0.5 g L-1), Quartz® (40 ml L-1), K - Fosfitotal® (3 g L-1) and Ca - Fosfitotal® (3 g L-1) was the auxiliary commissioner of costal spraying and each plot was protected during an application that eliminated the drift of the products. The induction was evaluated during the experimental cultivation with the quantification of the growth rate of the fungal complex, the photosynthetic of the plants and the collection of vegetal material for the enzymatic analysis of the enzyme peroxidase, polyphenoloxidase, β-1,3 gluconase. Post-harvest color was measured on flowers of H. psittacorum cv. Golden Torch. At the moment of the analysis, the visual effects, fresh mass loss, electrolyte extravasation, lipid peroxidation, besides the quantification of soluble carbohydrates and enzymes peroxidase, polyphenoloxidase, superoxide dismutase and total soluble phenolic compounds. The results indicate that Bion® phytostimulants and applied phosphites reduced fungal complex severity, increased peroxidase, polyphenoloxidase, β-1,3 gluconase activity, and did not promote changes in the net photosynthesis rate of plants. The value found for β> 1, which is visual visual of the severity of the fungal complex, is a good visual indicator of the effect of phytopathogens on the photosynthetic rate of the host. The quality of the vitamins demonstrated is positive, preserving the quality of the plants harvested. The parameters were the phosphites, especially K - Fosfitotal ®, obtained the best notes for the visual aspect, revealed the extravasation of electrolytes and lower lipid peroxidation. The results of this work are concrete answers to the producers of the sector, on the alternatives of management of the diseases associated with the cultivation of heliconia.

Keywords: Induction resistance, reactive oxygen species, tropical flowers, pot life, fungal complex.

Capitulo I - Referencial Teórico

1 INTRODUÇÃO

1.1 Mercado de flores e plantas ornamentais no Brasil e no mundo

A comercialização mundial de flores e plantas ornamentais movimentou em 2013 cerca de 21 bilhões de dólares. Historicamente, a Holanda é o principal país produtor e comercializador de flores, acompanhado da China, Estados Unidos e Japão (NEVES; PINTO, 2015). Nos anos de 2014 a 2015 o volume das exportações brasileiras chegou a apenas 21,9 mil dólares segundo o Ministério do Desenvolvimento Indústria e Comércio Exterior, valores muito abaixo dos apresentados entre os anos de 2004 a 2005, que somaram em torno 12,7 milhões de dólares (BRASIL, 2017), no entanto vale destacar que o principal consumidor das flores e plantas ornamentais produzidas no Brasil é o próprio mercado interno brasileiro com mais de 96 % do total (SEBRAE, 2015).

No Brasil, todo o setor de flores e plantas ornamentais obteve faturamento, no ano de 2015, de mais de R$ 6 bilhões (ALENCAR; GALERA, 2016), o que mostra o seu tamanho e importância na economia nacional. Apresentando no mesmo período uma área plantada de aproximadamente 15.000 hectares. Esse número é resultado de um aumento recorrente da área destinada a essa atividade no país, já que em 2012 a área foi estimada em torno de 11.800 hectares e em 2013 de aproximadamente 14.000 hectares (NEVES; PINTO, 2015).

Em nível nacional, o estado de São Paulo é o maior produtor de flores e plantas ornamentais do Brasil, concentrando 45 % da área de produção, e quase 30 % dos produtores que se dedicam à atividade e o varejo tem tido uma atuação relevante, em termos de volume comercializado, com uma movimentação que atingiu em 2014 cerca de R$ 1,98 bilhão (NEVES; PINTO, 2015). No entanto, essa produção vem se expandindo para outras áreas ou regiões do país, de acordo com o Instituto Brasileiro de Floricultura – IBRAFLOR (2014), a expansão do cultivo de flores e plantas ornamentais no Brasil deriva, dentre outros fatores, da própria biodiversidade e a amplitude de climas e solos do País.

1.2. O Segmento das Flores Tropicais

Entre os vários segmentos do mercado e dentre as diversas espécies de flores, destaca-se a floricultura tropical, que há algum tempo é uma atividade em ascensão no Brasil e segundo Loges et al., (2005), deve-se a características peculiares, tais como durabilidade, beleza e diversidade de cores e formatos, que propiciam grande aceitação pelo mercado consumidor e elevado potencial de crescimento no mercado nacional e internacional.

No Brasil o cultivo de flores tropicais, propriamente dito, é realizado há vários anos e principalmente nos estados de Pernambuco, Alagoas, Ceará, Bahia, Sergipe, Pará, Amazonas, Rio de Janeiro, São Paulo e no Distrito Federal (JUNQUEIRA; PEETZ, 2007), não existindo dados de produção oficiais para o seguimento. Para Villela (1999) o Brasil possui uma ampla variedade de solos e condições climáticas que favorecem o cultivo de uma grande diversidade de flores ornamentais tropicais, sendo que o Nordeste desponta como grande produtor, destacando-se os Estados de Pernambuco, Alagoas e Ceará, onde as condições de clima permitem o cultivo durante todo o ano, sem a necessidade de investimentos em insumos mais caros, resultando em custos que possibilitem a região competir com vantagem no mercado mundial.

Não existem estudos recentes sobre a cadeia produtiva no estado do Maranhão, sendo a produção local, de caráter não empresarial e focada essencialmente no abastecimento de São Luís, é concentrada na exploração de flores e folhagens tropicais de corte, palmeiras, bromélias, samambaias, mini rosas e crótons, entre outros produtos. (SEBRAE, 2015).

O último estudo sobre a cadeia produtiva de flores ornamentais tropicais no Maranhão foi realizado a mais de uma década (SEBRAE, 2003) e quantificou 41 produtores, sendo 14 em Paço do Lumiar, 14 em São José de Ribamar e 12 em São Luís, ocupando, ao todo, aproximadamente 35 ha. Na sua maioria, produtores de pequeno porte, que utilizavam mão-de-obra familiar, empregando, em média, de três a quatro pessoas por unidade de produção, estas quase sempre inferiores a 01 ha (um hectare) (SEBRAE, 2015).

Segundo estudos conduzidos pela Universidade Estadual do Maranhão, apenas nove dos produtores que participam da produção de flores e plantas ornamentais, tem como produto principal de suas atividades, o cultivo de espécies consideradas tropicais (SARDINHA, 2008). Não existem dados oficiais publicados recentemente, porém informações coletadas durante está pesquisa sugerem manutenção do número de produtores. As espécies tradicionalmente cultivadas no Estado do Maranhão são das famílias Musaceae, Heliconiaceae, Zingiberaceae, Marantaceae (SARDINHA et al., 2012), dentre estas famílias, as espécies de maior aceitação no mercado são as helicônias (*Heliconia* spp., bastão do imperador (*Etlingera elatior* (Jack) R. M. Smith) e alpínias (*Alpinia purpurata* (Vieill.) K. Schum).

1.3 Família Heliconiaceae

Originalmente as helicônias pertenciam à família Musaceae, o gênero Heliconia, em função de suas características próprias de individualização, passou a constituir a família Heliconiaceae como único representante (CASTRO et al., 2011). São plantas de origem neotropical que aparecem naturalmente em clareiras, bordas de florestas e matas ciliares, na América Central e América do Sul.

Existem aproximadamente 250 espécies de helicônias e algumas dessas espécies são muito utilizadas como plantas de jardim e flores de corte (MOSCA et al., 2004). No Brasil ainda não há um consenso, mas devem existir entre 40 e 65 espécies nativas de helicônias, dentre as quais: *H. episcopalis, H. bihai, H. stricta, H. spathocircinata, H. lourteigiae, H. farinosa, H. kautzkiana, H. rivularis, H. sampaioana, H. velloziana, H. chartaceae, H. juruana, H. pendula, H. acuminata, H. angusta, H. psittacorum, H. richardiana, H. aemygdiana, H. pseudoaemygdiana, H. densiflora, H. lasiorachis, H. metallica, H. subulata, H. apparicioi, H. hirsuta, H. marginata, H. latispatha, H. x rauliniana, H. julianii, H. rostrata, H. standley, H. tenebrosa, H. timothei e H. velutina* (CASTRO et al., 2011).

As helicônias são plantas de porte herbáceo, com diferentes tamanhos, podendo chegar até 12 m de altura. Propagam-se por meio de rizomas subterrâneos, que emitem brotações à superfície, podendo ser solitários ou agregados. Cada planta é composta por pseudocaule, folhas e uma única inflorescência (MOSCA et al., 2004).

1.4 Aspectos Fitossanitários de Plantas Ornamentais Tropicais

Os prejuízos relacionados com a produção e qualidade de flores tropicais podem ser significativos caso não exista um controle eficiente sobre fitopatógenos presentes nas áreas de cultivo. Os agentes causais de doenças em flores tropicais podem estar associados ao rizoma, às raízes das plantas e às folhas, o que pode ser agravado pela importação de mudas não certificadas, em decorrência da expansão do cultivo, contribuindo para o aumento da incidência e severidade dos problemas fitossanitários. Em alguns casos, as espécies tropicais se tornam veículo de disseminação de doenças para outras espécies cultivadas. Gasparotto et al. (2005) relata a sigatoka-negra, causada pelo fungo *Mycosphaerella fijiensis* Morelet patogênica a *Heliconia psittacorum*, e afirma que *H. psittacorum* pode atuar como veículo de disseminação desse fungo a longas distâncias, principalmente quando suas flores são exportadas para regiões do País onde não ocorre a doença.

Há algum tempo estudos avaliam a presença de fitopatógenos associados a espécies de flores tropicais, dentre os quais Lins; Coelho (2004) que em levantamento na zona da mata pernambucana relataram doenças causadas por fungos, sendo assinaladas a antracnose (*Colletotrichum gloeosporioides* Penz) em *Heliconia* spp., *Etlingera elatior*, *Tapeinochilos ananassae*, causando lesões em folhas e inflorescências; manchas foliares (*Bipolaris* spp., *Cercospora* sp., *Curvularia lunata* (Walker) Boedijn, *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, *Guignardia* sp. e *Deightoniella torulosa* (Syd.) M.B. Ellis em *Heliconia* spp., *Calathea burle* marx e *Musa coccinea*; podridão de rizomas e raízes (*Rhizoctonia solani* Kuhn e *Fusarium oxysporum* f. sp. *cubense* (E.F. Sm.) W.C. Snyder & H.N. Hansen em *E. elatior* e *Heliconia chartacea* cv. Sex Pink.

Coutinho (2006) abordou uma série de doenças fúngicas que atacam plantas ornamentais e seu controle. Em helicônias, de acordo com o processo fisiológico afetado as doenças foram classificadas em: doenças do rizoma e raiz (*Calonectria spathiphylli* El-Gholl, J.Y. Uchida, Alfenas, T.S. Schub., Alfieri & A.R. Chase, *Phytophthora nicotianae* Breda de Haan e *Pythium* sp.) causando podridão em raízes e rizomas de plantas suscetíveis; e, doenças foliares: *C. spathiphylli* causando amarelecimento e secamento das margens da folha, bainha e queimadura dos pecíolos, *Bipolaris* spp. e *B. incurvata*, cujos sintomas nas folhas iniciam-se com pequenas pontuações, que aumentam de tamanho e número, passando a manchas ovais ou irregulares de coloração marrom

claro com bordos escuros e halo amarelado ao redor, atacando também pecíolo, bainha, brácteas e flores, e *Exserohilum rostratum* (Drechsler) Leonard; Suggs, que causa lesões semelhantes ao *Bipolaris* spp.. E como manejo dessas doenças cita a qualidade sanitária do material de propagação, o controle da umidade, a remoção de plantas velhas e sem função, o controle de plantas daninhas, retirada e queima de folhas ou partes da planta atacadas e restos de cultura.

Em outro estudo Coelho; Lins (2002) e Coelho; Warumby (2002), relatam que no Estado de Pernambuco, as doenças em plantas tropicais comumente encontradas são: manchas foliares causadas por *Bipolaris* sp. *Cercospora* sp. e *Curvularia lunata* em helicônia, podridão de rizomas e raízes, associadas aos fungos *Rhizoctonia solani* e *Fusarium oxysporum* (S.F.Smith) em bastão do imperador, murcha bacteriana (*Ralstonia solanacearum* (Smith) Yabuuchi, Kosako, Yano, Hotta & Nishiuchi em helicônias, fitonematoses causadas por *Meloidogyne* sp., *Radopholus* sp. *Helicotylenchus* sp. e *Pratylenchus*, principalmente em alpínias, bastão do imperador, musas e helicônias. A única virose detectada foi observada em *Tapeinochilo ananassae* causando nanismo, clorose das folhas e variegação das inflorescências.

Em estudos detalhados sobre os patógenos causadores de doenças em helicônias no Havaí, Sewake; Uchida (1995) observaram que os fungos encontram-se em maior diversidade, causando lesões nas folhas, flores, podridão de rizoma e raízes, destacando-se *C. spathiphylli*, *Bipolaris* sp., *Exserohilum rostratum*, *Pyriculariopsis* sp., *Cercospora* sp., *Colletotrichum* spp., *Pestalotiopsis* sp., *Phomopsis* sp., *Fusarium* spp., *Pythium* sp. e *P. nicotianae*. Entre as bactérias, apenas *R. solanacearum* foi detectada causando murcha, porém, os fitonematóides associados ao sistema radicular foram mais diversificados, destacando-se *Radopholus* sp. *Meloidogyne* sp., *Rotylenchus reniformis* e *Helicotylenchus* sp.. No bastão do imperador, um dos problemas mais sérios está relacionado com a podridão de rizoma e raízes, provocada por *Rhizoctonia solani*, associadas em cultivos instalados em locais de drenagem inadequada (LAMAS, 1999; COELHO; LINS, 2002).

No Maranhão são escassos os diagnósticos, entretanto, Sardinha et al. (2012) verificou a ocorrência de 16 agentes causais de doenças em flores tropicais na Ilha de São Luís, destacando-se as doenças causadas por fungos. Os agentes causais de doenças fúngicas com maior frequência em espécies da família Heliconiaceae foram *Curvularia*

eragrostides (Henn.), *Pestalotiopsis* sp., *Colletotrichum gloeosporioides* (Penz) e *Curvularia lunata* (Wakker) Boedijn.

1.5 Ativação de defesa a doenças em plantas

Os vegetais produzem grande variedade de compostos orgânicos, conhecidos como metabólitos secundários. Atualmente sabe-se que muitos desses produtos do metabolismo secundário têm funções ecológicas importantes, como atuar na competição planta-planta e na simbiose plantas-microrganismos. Também podem agir como atrativos (odor, cor, sabor) para animais polinizadores e dispersores de sementes. Ou ainda, protegem as plantas contra herbívoros e contra infecção por microrganismos patogênicos (TAIZ; ZEIGER, 2013).

Atualmente, não existe uma solução única para o controle de pragas e doenças na floricultura, o melhor enfoque baseia-se na integração de diferentes estratégias de manejo, incluindo medidas de controle químico, cultural, físico e biológico. Destes, o controle químico ainda é o mais utilizado e eficiente, entretanto é o mais danoso ao meio ambiente. Entre as alternativas de controle atualmente estudadas estão os métodos físicos como a solarização, inseticidas naturais extraídos de plantas, utilização de indutores de resistência e o controle biológico propriamente dito.

A resistência sistêmica adquirida (RSA) ou resistência sistêmica induzida (RSI) são as duas formas conhecidas para explicar o fenômeno da indução de resistência, sendo o processo de indução efetivo, contra amplo espectro de patógenos, associado à produção de proteínas relacionadas à patogênese (PRPs), neste caso desencadeando a RSA (PIETERSE et al., 2014). As várias PRPs produzidas são reconhecidas há algum tempo, muitas possuem atividade microbiana e funcionam como sinalizadoras de indução de resistência (HAMMERSCHMIDT; DANN, 1997). Já a RSI tem como mediadores na sinalização, o ácido jasmônico e o etileno e não ocorre o acumulo de PRPs (VAN LOON, 2006; PIETERSE, et al. 2014). Entre as várias enzimas envolvidas na indução de resistência destacam-se as β-1,3- glucanases, peroxidades, polifenoloxidases e a fenilalanina amônia-liase, que quando ocorre à indução, após contato com agente indutor, aumentam sua atividade na planta (LEON-KLOOSTERZIEL et al., 2005).

Podemos citar como produtos utilizados com indutores o Bion® ou Acibenzolar-S-Methyl (ASM) que é um análogo de ácido salicílico e atua no metabolismo vegetal induzindo processos fisiológicos e bioquímicos (LIMA et al., 2017), regulando rotas metabólicas secundárias ou síntese de compostos de defesa estruturais (GLAZEBROOK, 2005), o que é favorecido pela sua rápida absorção pelos tecidos foliares (FURTADO et al., 2010) e consequente atuação na ativação e acumulo das PR proteínas (LOON; REP; PIETERSE, 2006). O Bion® já vem demonstrando resultados interessantes em diferentes patosistema controlando fitopatógenos, podemos citar o controle da ferrugem e cercosporiose em cafeeiro (FERNANDES et al., 2013), controle de Sigatoka negra em banana (UCHÔA et al., 2014), além de induzir resistência a *Xanthomonas axonopodis* pv. *passiflorae* em maracujazeiro (BORO, 2011). O acibenzolar-S-metil também é utilizado em associação com fungicidas já tendo efeito positivo no combate a ferrugem da soja (CARVALHO, 2013)

O Agro-Mos®, constituído à base de um mananoligossacarídeo fosforilado proveniente da parede celular de *Saccharomyces cerevisiae* Meyen e tem sido utilizado objetivando o controle de doenças pós-colheita (COSTA et.al., 2010; MELO et al., 2016). Resultados de pesquisas ao longo dos anos demonstram que o Agro-Mos® pode controlar fitopatógenos dentre os quais podemos citar *Colletotrichum gloeosporioides* e *Uncinula necator* em videira (GOMES et al., 2007), *Colletotrichum gloeosporioides* e *Fusarium* spp. em mamão (DANTAS et al., 2004), *Fusarium guttiforme* em abacaxi (MELO et al., 2016).

O Quartz® um produto biológico a base de *Bacillus methylotrophicus*, o gênero Bacillus tem sido estudado visando à qualidade nutricional de plantas, promoção de crescimento e controle de doenças (MOREIRA et al, 2013; HARSHAVADHAN et al, 2016). Yánez-Mendizábal; Falconí (2018) relatam que o gênero Bacillus é eficaz para controlar a infecção por antracnose em sementes de tremoço, além de induzir a ativação de enzimas de defesa peroxidase e catalase.

Os fosfitos são uma forma reduzida de fosfatos, estão surgindo como bioestimulador na horticultura e podem atuar no metabolismo vegetal (GOMES-MERINO, 2015). A ação dos fosfitos sobre fitopatógenos já é estudada a algum tempo e algumas publicações evidenciam sua eficácia em reduzir a severidade de doenças em

plantas (MITCHELL; WALTERS, 2004; MANN; KETTLEWELL, 2004; DIANESE et al., 2008; BUFFARA et al., 2013).

Os produtos citados podem ser classificados como fitoestimulantes ou elicitores de natureza biótica ou abiótica, estes podem eficientemente controlar doenças em plantas e frutos de forma direta ou indireta, através da indução de resistência. A eficiência destes elicitores no controle de doenças em plantas tem sido reafirmada em diversos trabalhos (DANTAS; COELHO, 2006; GOMES et al., 2007; GOMES et al., 2011).

1.6 Aspectos pós-colheita de flores tropicais

As flores de corte são um produto extremamente perecível e perdem suas características ótimas para a comercialização de forma muito rápida, principalmente pelo aumento da atividade respiratória, resultando em um maior consumo das reservas energéticas (PROMYOU et al., 2012) consequentemente acelerando os efeitos da senescência. Outra consequência do efeito da senescência, é o aumento das espécies reativas de oxigênio (ERO), que são moléculas danosas a membrana celular (MILLER et al., 2008), entretanto, são produzidas normalmente, mas, quando submetidas a algum tipo de estresse tem seus níveis aumentados, resultando em extravasamento de conteúdo celular e morte (CAO et al., 2010).

As ERO`s não são específicas e reagem com muitas moléculas biológicas como os lipídeos de lipoproteínas e membranas celulares iniciando o que denominamos de peroxidação lipídica. A peroxidação lipídica se inicia quando os lipídeos poli-insaturados das membranas biológicas são atacados por radicais livres e sofrem uma série de reações autocatalíticas, produzindo uma ampla variedade de intermediários e outros subprodutos altamente tóxicos (SINGH et al., 2015). O malondialdeído é um dos subprodutos mais abundantes, servindo como um sinalizador da peroxidação lipídica, a qual implica na perda da integridade da membrana celular, acarretando danos graves, como redução da fluidez e perda da seletividade.

As plantas desenvolveram mecanismos antioxidantes, enzimáticos e não enzimáticos, com o objetivo de combater os efeitos maléficos das ERO’s. O mecanismo antioxidande enzimático é constituído por proteínas que catalisam reações químicas e mediam grande variedade de reações bioquímicas que constituem a vida, portanto, são

essenciais para a manutenção adequada de qualquer organismo. As enzimas antioxidantes estão presentes em diferentes compartimentos celulares e contribuem para o controle das ROS em plantas, o que confere um estádio de homeostase redox no sistema. Destacam-se entre as enzimas antioxidantes a superóxido dismutase (SOD, EC: 1.15.1.1), peroxidases (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6) e polifenoloxidase (PPO, EC 1.14.18.1) (BARBOSA et al., 2014).

Cada uma destas enzimas possui um papel específico dentro do sistema antioxidante. Após a colheita das flores, alterações na atividade destas enzimas, podem interferir nos processos fisiológicos, por exemplo a reação responsável pelo escurecimento do tecido vegetal ocorre por meio da ação da polifenoloxidase (PPO) e da peroxidase (POD) na presença de oxigênio. O rompimento das membranas celulares, devido ao envelhecimento ou qualquer tipo de injuria, pode causar uma desestruturação das células unindo essas enzimas e seus substratos fenólicos que se acumulam em diferentes compartimentos celulares (AZEVEDO, 2004). Em tecidos intactos, os compostos fenólicos e as enzimas são separados em diferentes organelas, limitando o escurecimento enzimático. Enquanto os compostos fenólicos estão presentes no vacúolo, a PPO é encontrada nos plastídeos (KOU et al.2015).

A SOD é a primeira enzima na linha de defesa contra EROs e catalisa a dismutação de radicais O_2^- a H_2O_2 e O_2. Em vegetais, três maiores categorias de SOD são descritas e cada uma definida pela utilização de um metal particular como grupo prostético delas (Mn, Fe e Cu/Zn) (VAN CAMP et al., 1996). As enzimas SOD são localizadas em diferentes compartimentos celulares: a isoforma Cu/Zn-SOD é encontrada no cloroplasto, no citosol e nos peroxissomos; a isoforma Fe-SOD, no cloroplasto; enquanto a Mn-SOD é localizada primariamente na mitocôndria (HALLIWELL, 1987; BARBOSA et al., 2014; BROETTO et al., 2014).

Acredita-se que os diferentes produtos avaliados neste trabalho possam interferir nos processos fisiológicos de *Heliconia psittacorum* proporcionando efeitos positivos no controle de manchas foliares, favorecendo a tolerância da planta ao ataque de fitopatógenos. Além disso, os diferentes produtos poderiam reduzir os efeitos negativos das espécies reativas de oxigênio em pós-colheita, aumentando assim a vida útil das hastes florais *Heliconia psittacorum.*

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Capitulo II - Inducing Resistance in Heliconia psittacorum cv. Golden Torch to Naturally Occurring Leaf Diseases

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Inducing Resistance in *Heliconia psittacorum* cv. Golden Torch to Naturally Occurring Leaf Diseases

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Abstract

One of the bottlenecks to heliconia production is leaf diseases, which have the main characteristic of forming necrotic spots, that can reduce photosynthesis, damage the heliconia bracts and make the flowers unsuitable for commercialisation. The objective of the present study was to identify inducers capable of inducing resistance in *H. psittacorum* cv. Golden Torch, assessing by enzymatic action the reduction in the severity of the fungus complex associated to the cultivation of this species and verifying the action of the severity in relation to the net photosynthesis rate of the plants. The inducers were applied to the plants using a back spray and during application the plots were protected to prevent the products from drifting. The concentrations applied were Agro-Mos® (10 ml L^{-1}), Bion® (0.5 g L^{-1}), Quartz® (40 ml L^{-1}), potassium phosphite (3 g L^{-1}) and calcium phosphite (3 g L^{-1}). During the experimental cultivation, the severity caused by the fungus complex and the photosynthetic rate of the plants were assessed and plant material was collected to analyse the enzymatic activity. The results indicated that the inducers Bion® and the phosphites applied reduced the fungus complex severity, increased the activity of peroxidase, polifenoloxidase and β-1.3 gluconase but did not cause alterations in the net photosynthesis rate of the plants. The value found for $\beta > 1$ suggested that the visual estimation of the fungus complex severity is a good visual indicator of the effect of the plant pathogens on the host photosynthetic rate. The results are concrete responses to producers in the sector on management alternatives for diseases associated to heliconia cultivation.

Keywords: tropical flowers, disease management, enzymatic action, photosynthetic rate

1. Introduction

Heliconia are tropical herbaceous plants, belonging to the family Heliconiaceae. They are popularly known and appreciated because the blossoms have a wide range of colours and shapes (Taniguchi, Castro, T. F. Silva, E. B. da Silva, & Martins, 2016). One of the bottlenecks to heliconia production is the occurrence of leaf diseases, which have the main characteristic of forming dark necrotic spots but can also damage the heliconia bracts and prevent their commercialisation. The leaf spots can also reduce photosynthesis because, according to Xavier et al. (2015), the main visual effect of leaf spot causing pathogens is reduced healthy, photosynthesising leaf area.

In the state of Maranhão, Brazil, diagnosis by Sardinha et al. (2012) indicated the occurrence of a fungus complex, causer of leaf spots, associated to species of Heliconia, including the fungal diseases caused by *Curvularia eragrostides* (Henn.), *Curvularia lunata* (Wakker) Boediin, *Pestalotiopsis* sp. and *Colletotrichum gloeosporioides* (Penz) frequently found in species of the family Heliconiaceae. Currently there is no single solution to control pests and diseases in floriculture and the best approach is based on integrating different management strategies, including chemical, crop, physical and biological control measures.

Management alternatives include the use of inducers that can induce plant resistance to plant pathogens. These inducers include Bion® or Acibenzolar-S-Methyl (ASM) that is a salicylic acid analog and acts on the plant metabolism inducing physiological and biochemical processes (Lima et al., 2017). Agro-Mos®, constituted on the base of a phosphorylated mannanoligosaccaride from the cell wall of *Saccharomyces cerevisiae* Meyen, has

been used to control post harvest diseases (Costa et al., 2010; Melo et al., 2016). Quartz® is a biological product based on *Bacillus methylotrophicus*, the genus Bacillus has been studied for plant nutritional quality, growth promotion and disease control (Harshavadhan, Kumar, Yathindra, Rajesh, & Hongal, 2016; Moreira & Araújo, 2013). Lastly the phosphites are a reduced form of phosphates and are suggested as biostimulators in horticulture that act on the plant metabolism (Gómez-Merino & Trejo-Téllez, 2015). These products may influence the activity of some enzymes that are in directly associated to plant defence processes, including the enzymes β-1.3-glucanases, peroxidases and polyphenol oxidases.

In this context the objective of the present study was to identify inducers capable of inducing resistance in *H. psittacorum* cv. Golden Torch, assessed by enzymatic action and reduced severity of the fungus complex associated to the cultivation of this species, and to verify the action of the severity on the net photosynthesis rate of the plant.

2. **Material and Methods**

2.1 Experimental Field

The experiments were carried out at the Nucleus of Agronomic Biotechnology at the State University of Maranhão, Brazil (2°30′ S and 44°18′ W), where *H. psittacorum* cv. Golden Torch plants were cultivated in soil classified as dystrophic sandy red yellow Argissolo (EMBRAPA, 2013), corrected to pH 6 by applying limestone and fertilized monthly with cattle manure (0.2 kg/m^2). The cultivation was standardized by drastic pruning in all the area planted with *H. psittacorum* cv. Golden Torch and 30 days later the experiment was started by applying inducers to control the naturally occurring leaf plant pathogens associated to the culture. The inducer applications were repeated every 20 days, totaling five applications during the experiment. The inducers were applied using a back spray and during application the treatment plots were protected to prevent the products from drifting. The concentrations applied were: Agro-Mos® (10 ml L^{-1}), Bion® (0.5 g L^{-1}), Quartz® (40 ml L^{-1}), potassium phosphite (3 g L^{-1}) and calcium phosphite (3 g L^{-1}).

During the experiment, the severity of the leaf spots nd the net photosynthetic rate of the plants were assessed and plant material was collected to analyse the enzymatic activity.

2.2 Identification of Naturally Occurring Plant Pathogens in the Experimental Area

Leaves with disease symptoms were collected in the experimental area throughout assessment period. The collected material was isolated and identified according to Sardinha et al. (2012). Due to the similar symptomology of the various diseases associated to *H. psittacorum* cv. Golden Torch cultivation the term fungus complex was used for the group of plant pathogens identified in experimental area.

2.3 Determining Leaf Spot Severity and Net Photosynthesis

Leaf spot severity and net photosynthesis were assessed at 30, 60 and 120 days after the start of the experiment. All treatments were compared to each other to demonstrate the results. To quantify the leaf spot, four leaves with similar physiological age were randomly collected with various infection levels. All the material was scanned and the lesions quantified using the software WinDias-Image Analysis System.

The gas exchanges of the *H. pisitacorum* cv. Golden Torch leaves were measured using the Li-6400 XT infrared gas analyser (LI-Cor, Lincoln, NE, USA), in response to irradiance of 2000 μmol photons m^{-2} s^{-1} and 400 μmol mol^{-1} CO_2. Measurements were only taken between 8 a.m. and 10:30 a.m. and on uniformly sunny days to minimize the sources of diurnal heterogeneity.

2.4 Severity X Leaf Photosynthesis

The relation between the fungus complex severity and the relative net photosynthetic rates (ratio between the net photosynthesis of diseased leaves and the mean net photosynthetic rate of healthy leaves, P_x/P_o) was determined by the model by Bastiaans (1991). The β values were estimated by the equation $P_x/P_o = (1 - x)^{\beta}$. The T test was carried out to verify whether the β values differed from the unit.

2.5 Enzymatic Analyses

Plant material for the enzymatic analysis was collected 0, 24, 48, 96 and 120 hours after applying the products, 50 days after the drastic pruning. Immediately after collection, the samples were frozen using liquid nitrogen, identified and kept in an ultrafreezer at -80 °C until the assessments.

2.5.1 Obtaining the Extract for Enzyme Quantification

The extraction was made according to the methodology adapted by Simões et al. (2015). Liquid nitrogen was used to homogenize 1 g fresh leaf tissue in 13 mL potassium phosphate buffer 0.2 mol (pH 6.0) previously kept

at 4 ºC. The extract was centrifuged at 13.000 × *g* for 21 minutes at 4 ºC. The extract was stored in an ultrafreezer at -80 °C until the analyses were made.

2.5.2 Peroxidase (POD, EC: 1.11.1.7) and Polyphenol Oxidase (PPO, EC: 1.10.3.1) Activity

The POD trial was determined by adding 300 µL of the supernatant to the reaction medium containing 1000 µL phosphate buffer 0.2 mol (pH 6.0), 100 µL guaiacol (0.5%) and 100 µL hydrogen peroxide (0.08%). The readings were made on a spectrophotometer at 470 nm and 25 ºC, for three minutes. The peroxidase activity was calculated based on the molar extinction coefficient of 26.6 mM cm^{-1} for guaicol, and expressed in µmol g^{-1} min^{-1}. (MF)

The PPO trial was determined by adding 50 µL of the supernatant to reaction medium containing 1650 µL phosphate buffer 0.2 mol (pH 6.0) and 1300 µL catechol (0.2 mol). The readings were made in a spectrophotometer at 425 nm and 25 ºC, for two minutes. The PPO activity was calculated based on the molecular extinction coefficient of 34 mmol cm^{-1} for catechol and expressed in µmol g^{-1} min^{-1} (MF).

2.5.3 β-1.3-Gluconase (EC: 3.2.1.39) Activity

β-1.3-gluconase was determined by the dosage of glucose released with lamarine hydrolysis (Tuzun, Rao, Vogeli, Schardl, & Kuc, n.d.). The following were transferred to two test tubes: 25 µL enzymatic extract, 200 µL potassium phosphate buffer (0.2 mol and pH 6.0) and 200 µL laminarine (5 mg mL^{-1}). This material was incubated at 37 ºC for 30 minutes and then 1 mL Somogyi reagent (Smogyi, 1952) and 5 ml deionised distilled water were added and shaken for 10 minutes. After shaking, the material was heated to 100 ºC for 15 minutes and chilled in a ice bath. Then 1 mL Nelson reagent (Smogyi, 1952) and 15 ml deionised distilled water were added and shaken for 15 minutes. The spectrophotometric readings at 760 nm of the samples were compared with glucose standards. The standard glucose curve was prepared by adding the standard, in the same way as the samples, substituting the laminarine with glucose solutions ranging from 0 to 800 mg L^{-1}.

2.6 Statistical Analysis

The experimental field measured 22 × 14 meters and the experimental plots 2 × 2 m, totaling 4 m² and 1 m² central useful area per plot. A completely randomised design was used, placed in random blocks, with four replications. The data found for severity and relative net photosynthesis were correlated and submitted individually to analysis of variance in a factorial scheme (6 products × 3 periods), while the enzymatic activity was also submitted to analysis of variance, but analyzed separately in each period. The means of the parameters, when significant, were compared by the Tukey test ($p < 0.05$), using the software STATISTICA (Stat-Soft, Tulsa, EUA).

3. Results

3.1 Identification of Naturally Occurring Plant Pathogens in the Experimental Area

The following naturally occurring plant pathogens were identified in the experiment: *Curvularia eragrostides* (Henn.) J. A. Mey, *Pestalotiopsis* sp., *Colletotrichum gloeosporioides* (Penz), *Curvularia lunata* (Wakker) Boedijn and *Alternaria* sp. The plant pathogens cited formed the fungus complex associated with *H. psittacorum* cv. Golden Torch cultivation, present in the lesions, that tended to coalesce causing complete drying of the leaves and served as inoculum source for the bract infection.

3.2 Severity

The statistical analysis was significant for the measured factors. Generally the fungus complex severity, associated with *H. psittacorum* cv. Golden Torch cultivation, increased during the three assessment periods. However, the fungus complex was shown to expand its colonisation differentially when plants treated with inducers were compared with the control treatment (Figure 1).

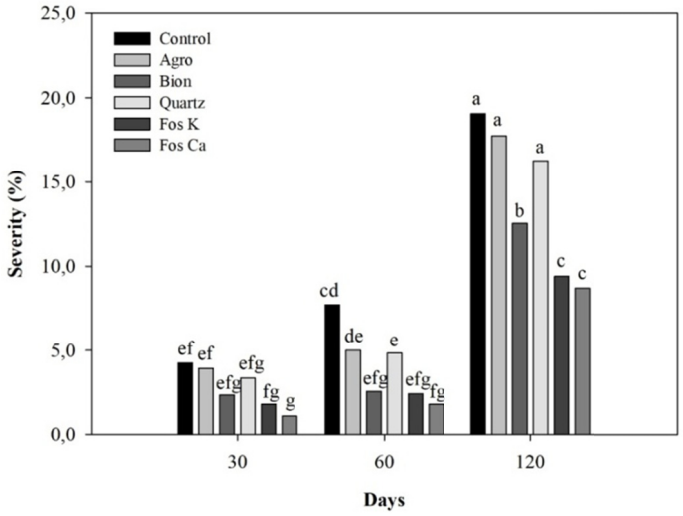

Figure 1. Severity of leaf diseases associated with *Heliconia psittacorum* cv. Golden Torch treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca). The same letters indicate equality among the treatments by the Tukey test (5%)

Among the inducers used, calcium phosphite differed statistically from the control treatment throughout the assessment period. Starting at the second assessment, potassium phosphite and Bion® were also different from the control. Notably, the plants treated with calcium phosphite, potassium phosphite and Bion® presented reduced severity at the end of the assessments, compared to the other treatments.

3.3 Leaf Photosynthesis

The statistical analysis was significant only for the assessment period. Generally a decrease was observed in the photosynthetic rate, especially in the 60-120 day interval in the assessments. However, the inducers assessed did not cause significant alterations in the photosynthetic rate compared to the control (Figure 2).

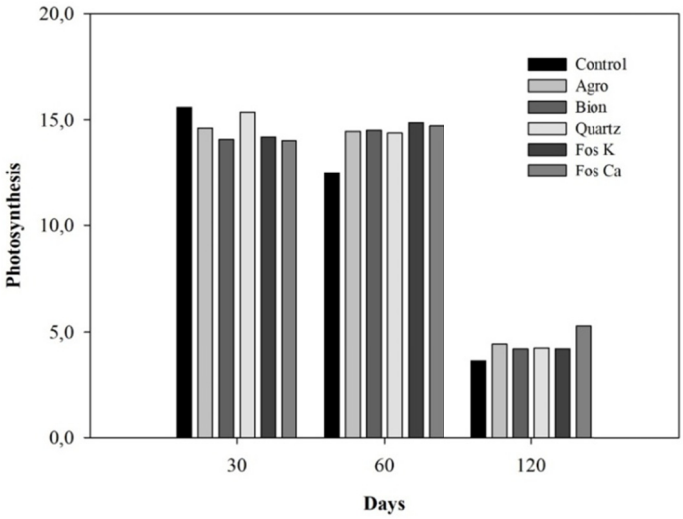

Figure 2. Photosynthesis measured in *Heliconia psittacorum* cv. Golden Torch plants treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca)

3.4 Severity X Leaf Photosynthesis

Photosynthesis and severity were strongly and negatively correlated (r = -0.8527) and generally leaves with fungus lesions had a lower photosynthetic rate compared to healthy leaves (Figure 3).

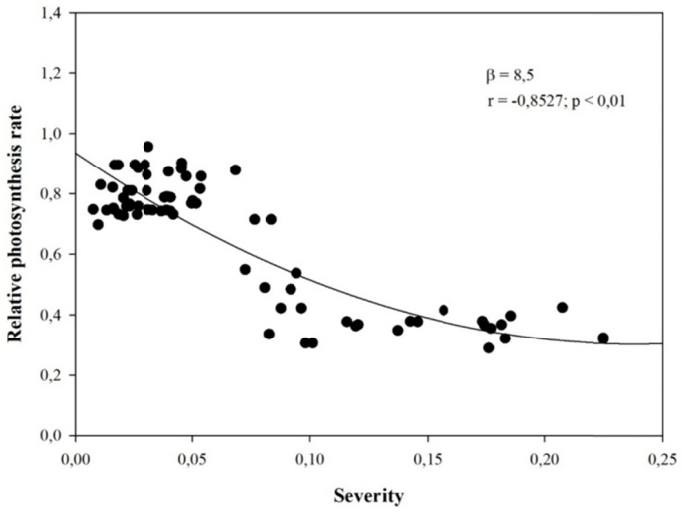

Figure 3. Relative net photosynthetic rate (Px/Po) of *Heliconia psittacorum* Golden Torch leaves infected naturally, in function of the magnitude of the fungus complex severity

The maximum severity observed in the *H. psittacorum* cv. Golden Torch leaves was 19.05 %, the mean value observed in the plants of the control treatment (Table 1).

Table 1. Observations, maximum severity, β coefficients and confidence interval, for the fungus complex associated with *Heliconia psittacorum* cv. Golden Torch

Observations	Maximum severity (%)	β Coefficients	Confidence interval of the β coefficient	
			Inferior	Superior
72	19.05	8.5	5.93	11.06

The estimated β (±standard error) parameter was 8.5 (±0.78) ($p < 0.01$) for leaves attacked by the fungus complex. The β value obtained for the fungus complex associated to *H. psittacorum* cv. Golden Torch was statistically bigger than 1 ($p < 0.01$).

3.5 Peroxidase (POD, EC: 1.11.1.7) and Polyphenol Oxidase (PPO, EC: 1.10.3.1) Activity

It was usually possible to characterize increase in enzymatic activity for the enzymes peroxidase (POD) and polyphenol oxidase (PPO) in inducer treated plants. The POD enzyme performance was characterised by increase in activity after 24 hours, when the potassium phosphite stood out, but the activity peak of this enzyme in plants treated with inducers occurred 48 hours after the applications, for most of the treatments, highlighting Bion®, calcium phosphite and potassium phosphite, in this order, that presented bigger enzymatic activity, differing statistically from the control treatment and other inducers (Figure 4). The other inducers, Quartz® and Agro-Mos®, presented POD activity peak 96 hours after application, and differed statistically from the control treatment, but were not different compared to Bion®, calcium phosphite and potassium phosphite.

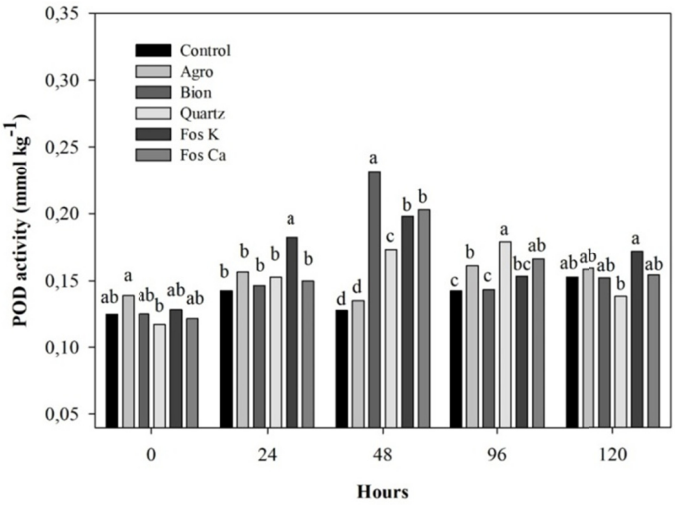

Figure 4. Peroxidase activity associated to *Heliconia psittacorum* cv. Golden Torch treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca)

The PPO enzyme presented pronounced activity peak 48 hours after applying inducers. Plants treated with calcium phosphite, Bion® and potassium phosphite, in this order, differed from the other treatments, including the control. The inducers Quartz® and Agro-Mos® presented PPO activity peak 24 hours after application and were different statistically from the control treatment, but did not differ compared to the other inducers, calcium phosphite, Bion® and potassium phosphite (Figure 5).

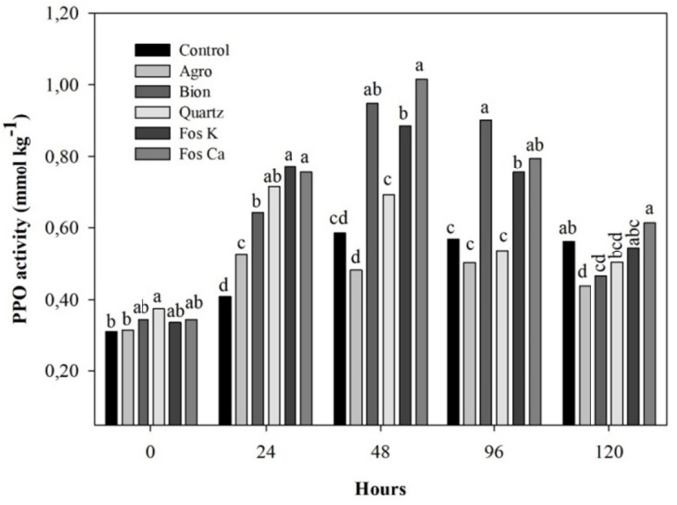

Figure 5. Polyphenol oxidase activity associated with *Heliconia psittacorum* cv. Golden Torch treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca)

3.6 β-1.3-Glucanase Activity

Generally, β-1,3-glucanase activity increased during the period assessed, but an activity peak could not be characterized for all the inducers tested. Only Bion® presented an activity peak statistically bigger than the control treatment and only 96 hours after application (Figure 6).

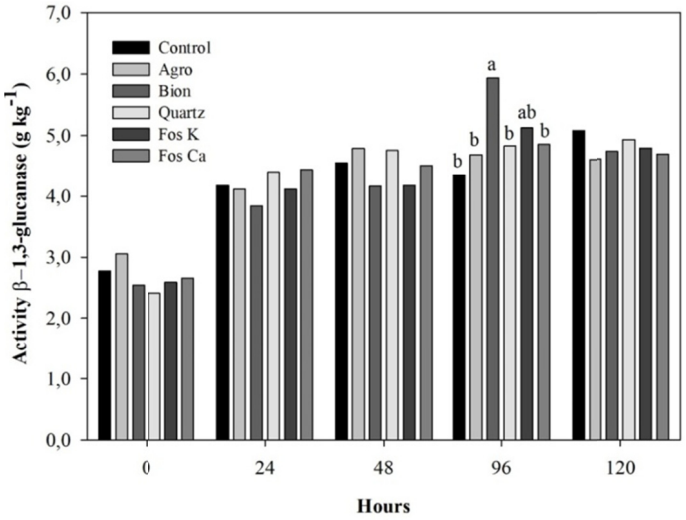

Figure 6. β-1,3-glucanase activity associated to *Heliconia psittacorum* cv. Golden Torch treated with Agro-Mos® (Agro), Bion®, Quartz®, potassium phosphite (Fos K) and calcium phosphite (Fos Ca)

4. Discussion

The naturally occurring plant pathogens identified during the experiment had already been reported in tropical flower cultivation on the island of São Luís, Brazil, as causal agents of leaf diseases in heliconia species (Sardinha et al., 2012) and can be considered a fungus complex causer of diseases in *H. psittacorum* cv. Golden Torch.

According to the results found, the inducers calcium phosphite, potassium phosphite and Bion® were able to reduce damage caused by the fungus complex, possibly ensuring better conditions for the plant to produce higher quality flowers. The reduction in severity resulting from the phosphites probably involved direct control of the fungus complex because the fungitoxic action of phosphite was studied previously in several pathogen systems over the years (Araújo, Valdebenito-Sanhueza, & Stadnik, 2010; Dianese et al., 2008; Sobrinho et al., 2016; Spolti, Valdebenito-Sanhueza, Campos, & Del Ponte, 2015), including the genus Colletotrichum (Oliveira, Pinto Viana, & Valentim Martins, 2016) that is frequently found in the fungus complex associated to *H. psittacorum* cv. Golden Torch cultivation.

In addition to direct control, it can be suggested that the phosphites induced resistance in *H. psittacorum* cv. Golden Torch plants because peroxidase and polyphenol oxidase activity increased compared to the control treatment. The use of inducers and plant stress can cause increase peroxidase activity and consequently cell protection against oxidative reactions (Anterola & Lewis, 2002). Increases in the percentages of peroxidase activity were also been detected in peachtree plants with early death symptoms, consequence of the situation of physiological stress caused by the disease (Marafon, Herter, Bacarin, & Hawerroth, 2009), since the infectious action caused by the pathogen can result in hydrogen peroxide synthesis (Furstenberg-Hagg, Zagrobelny, & Bak, 2013). Peroxidase and phenol oxidase are integrated in various physiological processes in the plant, such as lignification, suberization, cell wall component formation and reticulation and senescence (Nascimento & Barrigossi, 2014).

According to Lobato et al. (2011) potassium phosphite leaf applications induced increase in peroxidase and polyphenol oxidase activity in potato tubers, and these alterations were part of the defence mechanism induced by phosphites. Ramezani et al. (2017) suggested that potassium phosphite prepared plants for a rapid, intense response to infection, involving the activation of various defence responses, including the expression of defence genes via the phenylpropanoid route.

The reduction in severity caused by Bion® was not associated to direct control because it showed no or low fungitoxic activity (Barros, Fonseca, Balbi-Peña, Pascholati, & Peitl, 2015; Oliveira et al., 2016), but its rapid absorption by the plant stimulated resistance by interfering in physiological and biochemical processes (Debona et al., 2009). It is also frequently associated to the salicylic acid metabolic paths because it activates genes referent to resistance signalling (Vitti, 2009) and consequently the action of proteins relative to the pathogenesis (PRP`s) (Felipini & Piero, 2013). In the present study, the enzymes assessed, peroxidase, polyphenol oxidase and

β-1,3-gluconase, increased their activity and the plants treated with this inducer characterized the process of resistance induction.

The other inducers, Agro-Mos® and Quartz®, although they acteds efficaciously in other pathogen systems as resistance inducers or even plant pathogen antagonists (Melo et al., 2016; Yánez-Mendizábal & Falconí, 2018), did not reduce the severity of the fungus complex in *H. psittacorum* cv. Golden Torch cultivation.

None of the inducers assessed interfered directly in the net photosynthesis rate of *H. psittacorum* cv. Golden Torch, but the severity and net photosynthesis rate were strongly and negatively correlated, suggesting that fungus infection, if it is not controlled, may reduce net photosynthesis in the plant.

The β value estimated at 8.5 (±0.78) indicated that the plants infected by the fungus complex had photosynthesis damage not only in the lesion area, but also in the apparently healthy region of the leaf. Consequently, the calculated severity is a good indicative of the effect of the fungus complex on photosynthesis in *H. psittacorum* cv. Golden Torch plants. Although the net photosynthetic rate measurements demonstrated that the fungus complex reduced photosynthesis in the remaining green tissue, these measurements did not elucidate the mechanism responsible for the reduction or indicate the localization of this effect. Johnson (1987) considered that pathogen presence in diseased tissue may influence the crop development by reducing the solar radiation interception (RI) by the green matter or by interference in the radiation use efficiency (RUE). This experiment demonstrated that the spots caused by the fungus complex is an example where both effects occur. References were not found for this parameter in this pathogen system. However, this effect has been observed in pathogen systems such as Phaeosphaeria maydis in corn (Godoy, Amorim, & Bergamin Filho, 2001) and Corynespora cassiicola in soybean (Xavier et al., 2015).

These results suggested that when promoting reduction in the fungus complex severity associated to *H. psittacorum* cv. Golden Torch the phosphites, indirectly, permitted the plant to maintain its net photosynthesis rate close to that found in healthy plants, that would probably permit better quality flower production. These results have been confirmed in research already carried out by the authors (data in publication) when the effects were observed of the inducers, potassium phosphite and calcium phosphite, applied in the field, on the quality of *H. psittacorum* cv. Golden Torch flowers post harvest.

5. Considerations

The inducer Bion® and the phosphites applied in the field were efficacious in reducing the fungus complex severity associated to *H. psittacorum* cv. Golden Torch

The net photosynthesis rate was not affected by the inducers assessed, but the fungus complex can reduce the photosynthesis rate as severity increases.

The value found for β bigger than 1, suggests that the visual estimate of the fungus complex severity is a good in visual indicator of the effect of the plant pathogens on the photosynthetic rate of the host.

The results presented here contribute to the understanding of a little studied pathogen system and give concrete responses to tropical flower producers on management alternatives for the diseases associated to heliconia cultivation.

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Capitulo III - Phytostimulants Influence the Vase Life of *Heliconia psittacorum* cv. Golden Torch

PHYTOSTIMULANTS INFLUENCE THE VASE LIFE OF *HELICONIA PSITTACORUM* CV. GOLDEN TORCH

ABSTRACT

Many factors influence post-harvesting of tropical flowers, including management of the plant still in the field and oxidative stress, which can be considered the main factor for determining quality during vase life, as it involves complex chemical and physiological changes in the plant. In this context the present study aimed to assess the influence of phytostimulants applied to *Heliconia psittacorum* cv. Golden Torch in quality maintenance during the vase life of this species. The products applied in the field were Bion®, Agro-Mos®, Quartz®, Ca - Fosfitotal® (calcium phosphite) and K - Fosfitotal® (potassium phosphite). The flower stems used during the experiment were harvested in the morning to assess the visual aspect, fresh matter loss, electrolyte extravasation, lipid peroxidation and the soluble carbohydrates and the enzymes peroxidase, polyphenoloxidase, superoxide dismutase and total soluble phenolic compounds were quantified. The results showed a positive influence of the Fosfitotal® in the maintenance of stem quality of *Heliconia psittacorum* cv. Golden Torch during vase life. In the parameters assessed, the Fosfitotal®, especially K - Fosfitotal®, obtained the best scores for the visual aspect, presenting reduced electrolyte extravasation and less lipid peroxidation. These results were confirmed with the quantifications of the enzymes peroxidase, polyphenoloxidase, superoxide dismutase and total soluble phenolic compounds. Treatments in the field, with phytostimulants, are interesting to maintain the stem quality *Heliconia psittacorum* cv. Golden Torch during vase life.

Keywords: Tropical flowers, Oxidative stress, Electrolyte extravasation, Lipid peroxidation, Enzymatic defense.

1. INTRODUCTION

Heliconias are tropical herbaceous plants, belonging to the family Heliconiaceae and are popularly known due to their flowers in a wide range of colors and shapes (Taniguchi et al., 2016). Inflorescences are perishable products and after harvest can have their quality reduced quickly, making it impossible to commercialize them. Quality can be lost naturally through senescence, chlorosis, stem bending, excessive desiccation and transpiration (Folha et al., 2016). Furthermore, plant species interact constantly with external factors, that constantly alter and are potentially harmful (Ncube et al., 2012).

Reactive oxygen species (ROS) are inevitably formed by aerobic organisms (Sáenz et al. 2015). ROS are toxic, but to prevent ROS accumulation in the cells, plants have developed enzymatic and non-enzymatic antioxidant defense systems. The enzymatic defense system consists of several enzymes including superoxide dismutase (SOD), peroxidase (POD) and polyphenoloxidase (PPO) (Barbosa et al., 2014). The first plays a key role in the antioxidant defense system by O_2^- dismutation in H_2O_2 and O_2 and the other to catalyze reactions using H_2O_2 as oxidant and phenolic compounds as electron donors (Barbosa et al., 2014; Blokhina et al., 2003; Scandalios, 2005). The imbalance between ROS production and elimination results in oxidative stress which is partly responsible for the loss of quality during vase life and involves complex chemical and physiological changes, resulting in overproduction and accumulation of reactive oxygen species and consequently, activation of stress or cell death (Sáenz et al. 2015).

Applying phytostimulants to heliconia flowers may influence enzymatic activity in the bract tissue, the percentage of absolute cell membrane integrity and stem weight maintenance in the shelf period (Mangave et al., 2013). Products such as Bion®, Agro-Mos®, Quartz®, Ca - Fosfitotal® (calcium phosphite) and K - Fosfitotal® (potassium phosphite) are used in health management in several plant species and their use while still in the field is a factor that may

influence cut flower post-harvest quality. Bion® or Acibenzolar-S-Methyl (ASM) is an analogue of salicylic acid and acts on the plant metabolism inducing physiological and biochemical processes (Lima et al., 2017). Agro-Mos® is constituted on the base of phosphoryl Mannan oligossacaride derived from the *Saccharomyces cerevisiae* Meyen cell wall and has been used to control postharvest diseases (Costa et al., 2010; Melo et al., 2016). Quartz® is a biological product based on *Bacillus methylotrophicus*, the genus *Bacillus* has been studied for plant nutritional quality, growth promotion and disease control (Harshavadhan et al., 2016; Moreira and Araújo, 2013). The phosphites are a reduced form of phosphates and are used as bioestimulants in horticulture and may act on the plant metabolism (Gómez-Merino and Trejo-Téllez, 2015).

Thus, the present study aimed to assess the influence of the products Agro-Mos®, Bion®, Quartz®, Ca - Fosfitotal® (calcium phosphite) and K - Fosfitotal® (potassium phosphite), applied in the field, on the postharvest quality of *Heliconia psittacorum* cv. Golden Torch flowers during vase life.

2. MATERIAL AND METHODS

Heliconia psittacorum plants were grown in an experimental field at the State University of Maranhão, Brazil, (2°30' S and 44°18' W) in soil classified as Red-yellow dystrophic argissol, sandy texture (EMBRAPA, 2013), corrected to pH 6 by applying lime, fertilized with cattle manure (0.2 kg $m^{2\,-1}$) and sprinkler irrigation daily. During cultivation six groups of plants were sprayed every 20 days for six months with sterile distilled water, Agro-Mos® (10 mL L^{-1}), Bion® (0.5 g L^{-1}), Quartz® (40 mL L^{-1}), Ca - Fosfitotal® (3 g L^{-1}) and K - Fosfitotal® (3 g L^{-1}). Phytostimulants were applied with a backpack sprayer and 2 L syrup volume in all treatments. Concentrations were used according to the manufacturers' recommendations.

The flower stems were harvested at approximately 07:30 AM, 48 h after the last spraying and hydrated for 12 h, when they were standardized to 40 cm length and kept in pots with sucrose *pulsing* at 20 % for 48 h. After this period the solution was replaced with 0,5 L distilled water, renewed every three days. During the experiment the temperature, humidity and photoperiod were maintained constant at 22 ± 2 ºC, RH 70 ± 5 % and 12 h, respectively.

The parameters assessed were the visual aspect, fresh matter loss, electrolyte extravasation and the enzymes peroxidase, polyphenoloxidase, total soluble phenols, superoxide dismutase, soluble carbohydrate content and associated lipid peroxidation. Samples of the bracts for enzyme quantification were collected in five periods throughout the storage period and kept in ultra-freezer at -80 °C. All the data were expressed on a fresh weight basis.

2.1.Visual Aspect

The visual aspect was quantified until the twelfth day of vase life. A subjective grading scale ranged from 1 to 5 (Figure 1). Each grade represented: score 1 - necrosis and a marked darkening on the stem and/or bract; improper for commercialization; score 2 - flower with dark marks on at least two bracts, marked darkening and turgidity loss, improper for commercialization; score 3 - turgidity loss, dryness at the bract tips and coloring loss, minimum quality threshold for commercialization; score 4 - flower with no darkening, start of coloring loss, still improper commercialization; score 5 - up to 3 bracts open and without apparent damage; turgid stem and bracts, bright coloring and perfect flower for commercialization (adapted from de Souza et al., 2008). The final grade represented the average scores assigned by five independent evaluators.

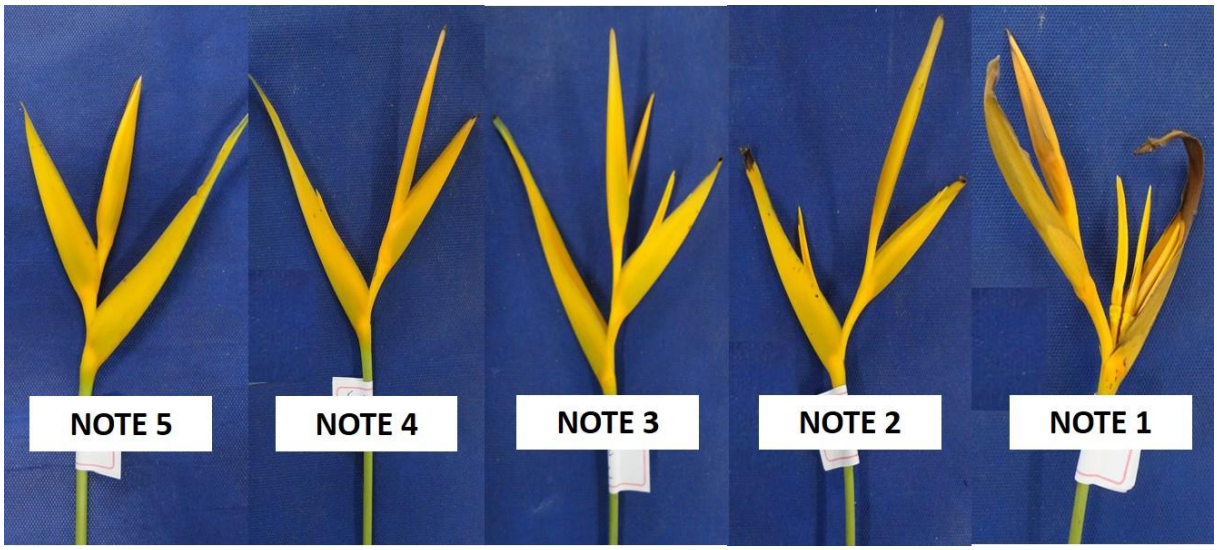

Figure 1. Visual aspect for score assignment.

2.2. Fresh matter loss

The stems were weighed on semi-analytical scales. The dry matter values of the stems were obtained by the percentage difference from the initial dry matter and the dry matter on the day of analysis. The percentage loss of dry matter was determined by the following formula, where: DML: dry matter loss (%), Idm: initial dry matter, Fdm: final dry matter, on the day of analysis.

$$DML: [(Idm – Fdm)/Idma] \times 100$$

2.3.Electrolyte extravasation

Electrolyte extravasation was determined, as described by Shanahan et al. (1990), by weighing 0.3 g of 4 cm discs from the bracts that were immersed in 10 mL deionized distilled water in closed test tubes and incubated for 24 h (Mangave et al., 2013), obtaining the extract called C1 at 25 ºC. Next, the electric conductivity was measured using a conductivity meter. The same tubes were incubated at 100 °C for one hour in a water bath and then kept at ambient temperature until they reached 25 ºC and the C2 extract was obtained, in which the electric

conductivity was again that measured. The electrolyte extravasation was estimated by the ratio below and the result was expressed in percentage.

$$EE = (C1/C2) \times 100$$

2.4.Peroxidase (POD, EC:1.11.1.7) and Polyphenoloxidase (PPO, EC:1.10.3.1) extraction and activity trial

Extraction was made following the methodology adopted by Simões et al. (2015). Homogenization in liquid nitrogen was performed on 0.1 g fresh tissue in 1.3 mL phosphate buffer 0.2 M (pH 6.0) kept previously at 4 ºC. The extract was centrifuged for 21 min at 13.000 x *g* and 4 ºC.

The POD trial was determined by adding 0.3 mL supernatant to the reaction medium containing 1 mL phosphate buffer 0.2 M (pH 6.0), 0.1 mL guaiacol (0.5 %) and 0.1 mL hydrogen peroxide (0.08 %). The readings were made in a spectrophotometer at 470 nm, at 25 ºC, for three minutes. The POD activity was calculated based on the coefficient of molar extinction of 26.6 $mmol^{-1}$ cm^{-1} for guaiacol and expressed in mmol kg^{-1}.

The PPO trial was determined by adding 0.05 mL supernatant to the reaction medium containing 1.65 mL phosphate buffer 0.2 M (pH 6.0) and 1.3 mL catechol (0.2 M). The readings were made in a spectrophotometer at 425 nm, at 25 ºC, for two minutes. The PPO activity was calculated based on the coefficient of molar extinction of 2.47 $mmol^{-1}$ cm^{-1} for catechol and expressed in mmol kg^{-1}.

2.5.Total soluble phenols

The total soluble phenols were quantified following the method by Folin and Ciocalteu (1927). The extraction was made from 0.1 g tissue squashed in a pestle and mortar containing

1.3 mL methanol. The samples then rested for 20 h in the dark at 4 °C. After this period the methanol extract was centrifuged for 21 min at 13.000 x g and 4 ºC. The trial was carried out using 0.15 mL supernatant, 2.4 mL distilled water, 0.15 mL Folin Cioucauteu reagent. The mixture was shaken for three minutes and 0.3 mL sodium carbonate were added. The tubes were kept in the dark at 25 ºC for two hours. The readings were made in a spectrophotometer at 725 nm and the results expressed in g kg^{-1}.

2.6.Superoxide dismutase activity extraction and trial (SOD, EC:1.15.1.1).

Extraction was made using liquid nitrogen and homogenizing 0.15 g tissue in 2 mL phosphate buffer 0.1 M (pH 7.0). The extract was centrifuged for 21 min at 13.000 x g and 4 ºC. The SOD was determined as described by Giannopolitis and Ries (1977). Alequots of 0.1 mL supernatant were added to 1.66 mL phosphate buffer 0.05 M (pH 7.8) containing (0.01 M EDTA and 0.013 M methionine), 0.04 mL riboflavin 0.002 M and 0.2 mL Nitro Blue Tetrazolium chloride (NBT) at 0.75 M.

The reaction medium was kept under light incidence (25 W fluorescent lamp) for six minutes and later read in a spectrophotometric tower at 560 nm. The activity was determined based on the inhibition of NBT reduction, defined as unit of the activity, the positive enzyme necessary to inhibit 50 % photoreduction (Beauchamp and Fridovich, 1971). The activity was expressed in U kg^{-1}.

2.7.Lipid peroxidation – TBARS

Extraction was made using liquid nitrogen and homogenizing 0.5 g tissue in 2 mL trichloroacetic acid - TCA (1 %). The extract was centrifuged for 21 min at 13.000 x g and 4 ºC. To determine the lipid peroxidation, 0.5 mL aliquots of the sample and 2 mL thiobarbituric acid – TBA (20 %) were added to test tubes with buffer. The tubes were kept in a water bath at

100 °C for one hour and were then placed in an ice bath for five minutes. Supernatant absorbance was measured at 532 nm. The ideal for the non-specific absorption at 600 nm was subtracted. The TBARS concentration was calculated using the absorption coefficient of 155 $mmol^{-1}$ cm^{-1} and the results was expressed in mmol kg^{-1}.

2.8.Soluble carbohydrate extraction and determination

The soluble carbohydrates were extracted and determined according to Dubois et al. (1956), using liquid nitrogen and homogenizing 0.05 g of the tissue in 1.3 mL distilled water. The extract was centrifuged for 21 min at 13.000 x g and 4 ºC.

After separating 0.025 mL supernatant, 0.475 mL distilled water were added and placed in test tubes, together with 0.5 mL phenol (5 %) and 2.5 mL sulfuric acid PA. The extract rested for 10 min, and then the test tubes were shaken and kept on a tray containing water at 25 °C for 20 min. The readings were made in a spectrophotometer at 490 nm and the results was expressed in g kg^{-1}.

2.9.Statistical analysis

A randomized complete block design with treatments arranged in split plots was used, where products were allocated to plots and time to sub-plots. The plot useful area comprised twenty-five flower stems and the subplot five flower stems, stored in vases filled with distilled water. The results were submitted to analysis of variance and the means compared by the Tukey test at the 5 % level of probability using the STATISTICA software (Stat-Soft, Tulsa, USA).

3. RESULTS

3.1.Visual Aspects

The scores attributed to the *Heliconia psittacorum* flower stems decreased during the vase life. The flower stems markedly lost points, considering the score scale, accumulating

undesirable characteristics over time. However, in general, the stems treated with phosphite obtained higher visual scores, especially when compared to the control treatment. At the start of vase life, all the treatments received score 5, maximum for quality but starting on the third day the flower stems treated with the K - Fosfitotal® and Ca - Fosfitotal® phosphites already stood out by obtaining higher scores compared to the control, Agro-Mos®, Bion® and Quartz® treatments, that continued throughout the vase life (Figure 2). The other stems treated with Agro-Mos®, Bion® and Quartz® obtained intermediate scores during the assessment, compared to the control and the phosphites, especially the treatments with Bion® and Quartz®, that scored higher than the control, starting on the sixth and third day, respectively. On the 12th day, only the flower stems treated with K - Fosfitotal® obtained score 3, the minimum necessary for commercialization. The scores attributed to the other treatments on the 12th day, considered the stems with dark marks on at least two bracts, darkening and accentuated turgidity loss, making them improper for commercialization according to the cut grade, below 3 used for this in the experiment.

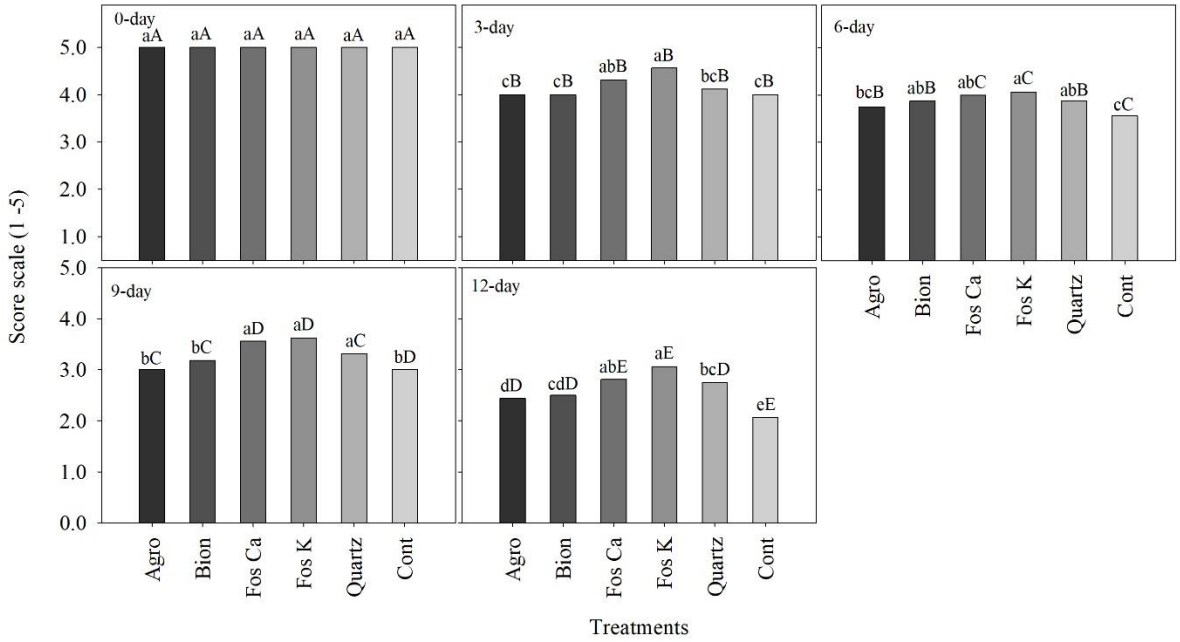

Figure 2. Score scale (scores from 1 to 5) for *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.2.Electrolyte extravasation

Statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. The results showed increased electrolyte extravasation over the assessment period, that indicated bigger deterioration of the bract tissue. In each evaluated period, the stems treated with potassium and calcium Fosfitotal® maintained low electrolyte extravasation levels compared to the control (Figure 3). At the start of vase life Fosfitotal®- treated stems presented reduced electrolyte extravasation and among these, the Ca - Fosfitotal® was best. On the third and sixth vase life days, stems treated with Quartz® and Bion® also maintained electrolyte extravasation levels compared to the control and up to the

12th vase life day it was possible to characterize the Fosfitotal® as the best treatments for this parameter. The stems treated with Agro-Mos® did not show reduced electrolyte extravasation compared to the control except in an isolated form on the 6th vase life day.

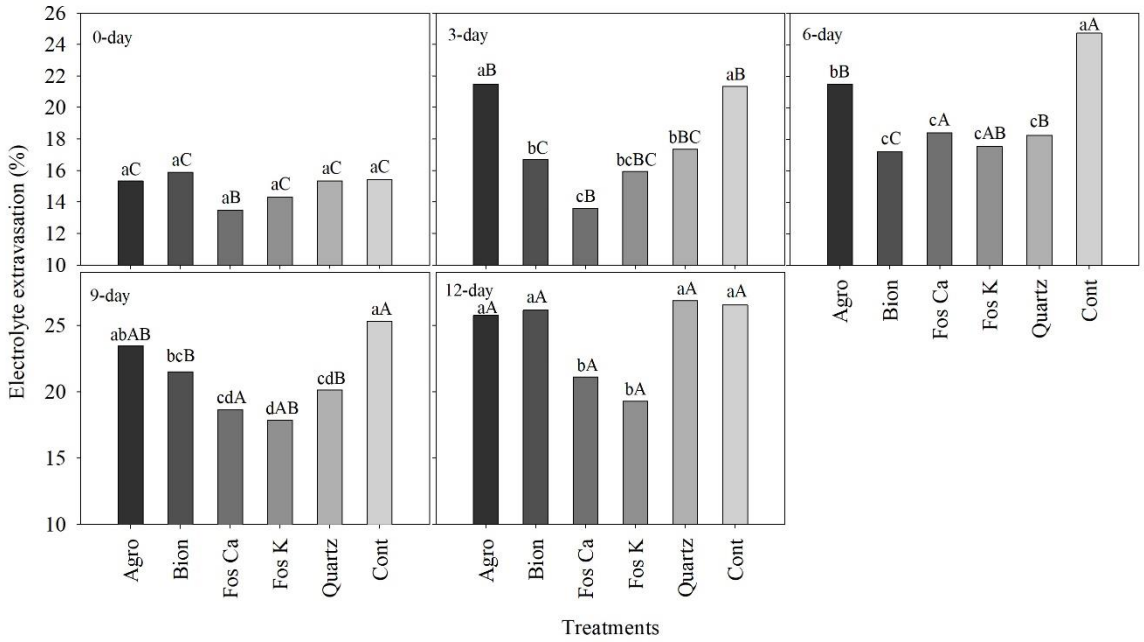

Figure 3. Electrolyte extravasation (%) in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.3.Fresh matter loss

Statistical analysis did not identify significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. Only time significantly influenced the treatments. Fresh matter loss in the *H. psittacorum* stems increased throughout the assessment period, but no statistical difference was observed between the treatments for this parameter (Figure 4).

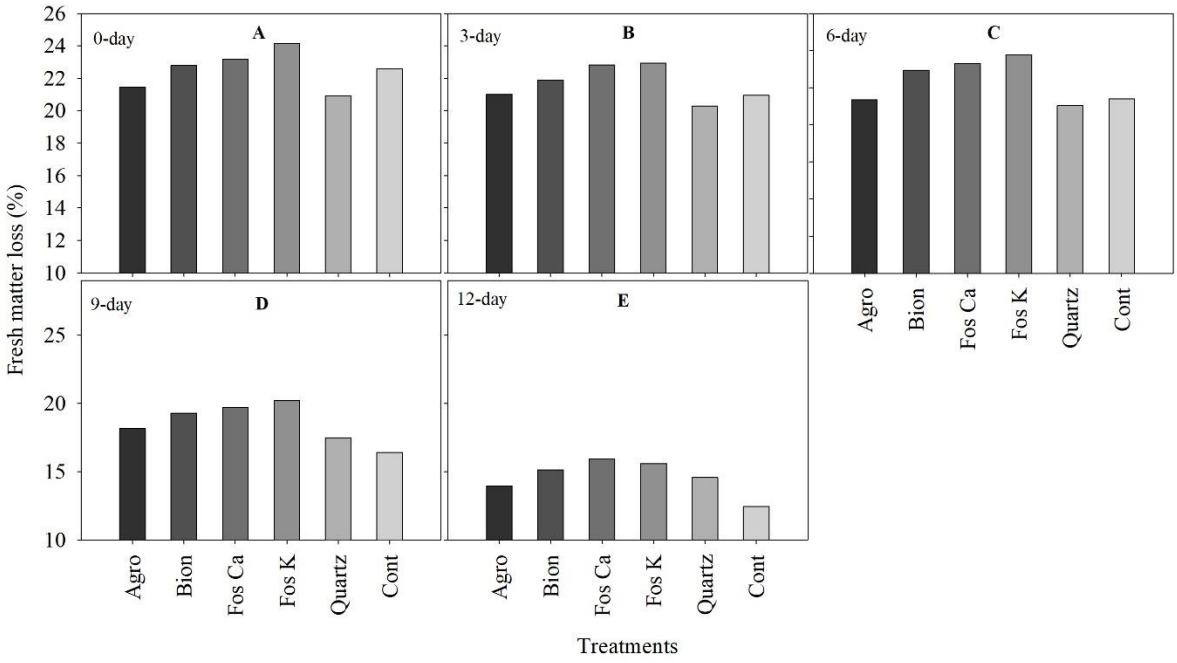

Figure 4. Fresh matter loss in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). The letters compare the different periods of evaluation throughout the vase life.

3.4.Determining soluble carbohydrates

Statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. The soluble carbohydrate content in the *H. psittacorum* flower stems decreased throughout the vase life, that indicated reserve consumption, to maintain the physiological functions.

Figure 5 shows that at the start of the experiments the Fosfitotal® - treated stems already presented a higher carbohydrate concentration than the other treatments, including the control. In the following periods, the treatments with Fosfitotal® continued to present higher levels of

carbohydrates in comparison with the other treatments but were equal to them only in the last evaluated period.

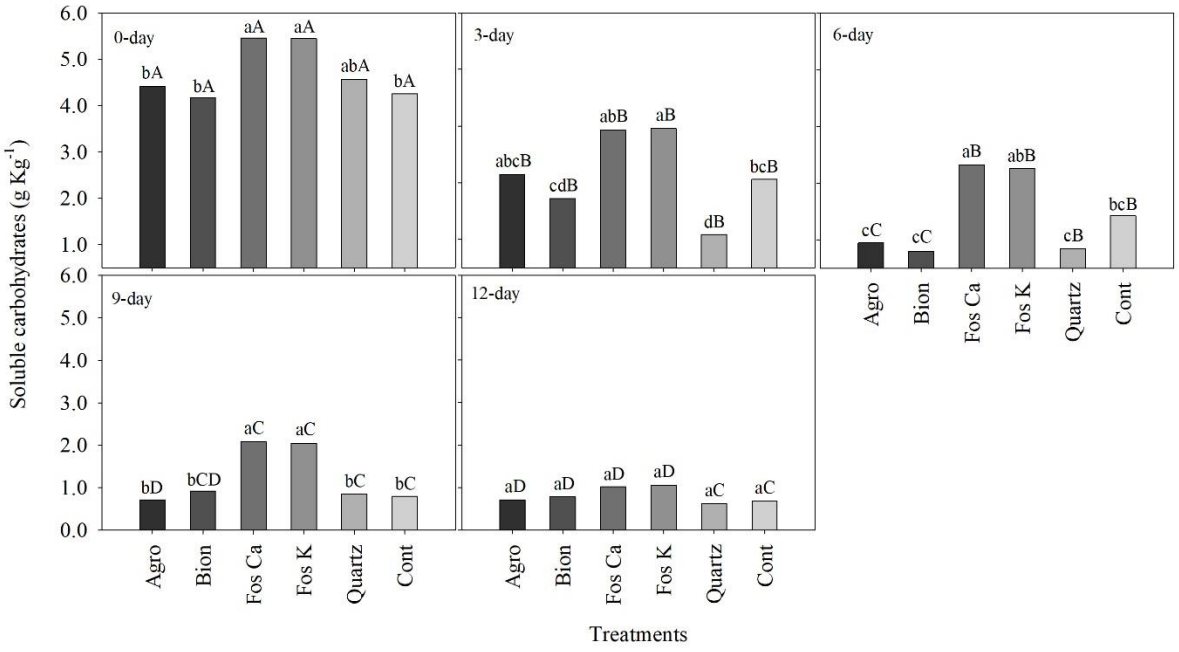

Figure 5. Soluble carbohydrate in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.5.Peroxidase (POD, EC:1.11.1.7) and Polyphenoloxidase (PPO, EC:1.10.3.1) activity

The statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for POD and PPO enzymes. POD activity in *H. psittacorum* flower stems increased during the period assessed and peaked between the sixth and ninth days but decreased on the 12th day in the vase for treatments with Fosfitotal® and Quartz® (Figure 6). The treatments evaluated differed only on the 12th day in the vase, when Ca - Fosfitotal® and K - Fosfitotal® presented lower activity of this enzyme in relation to the control treatments.

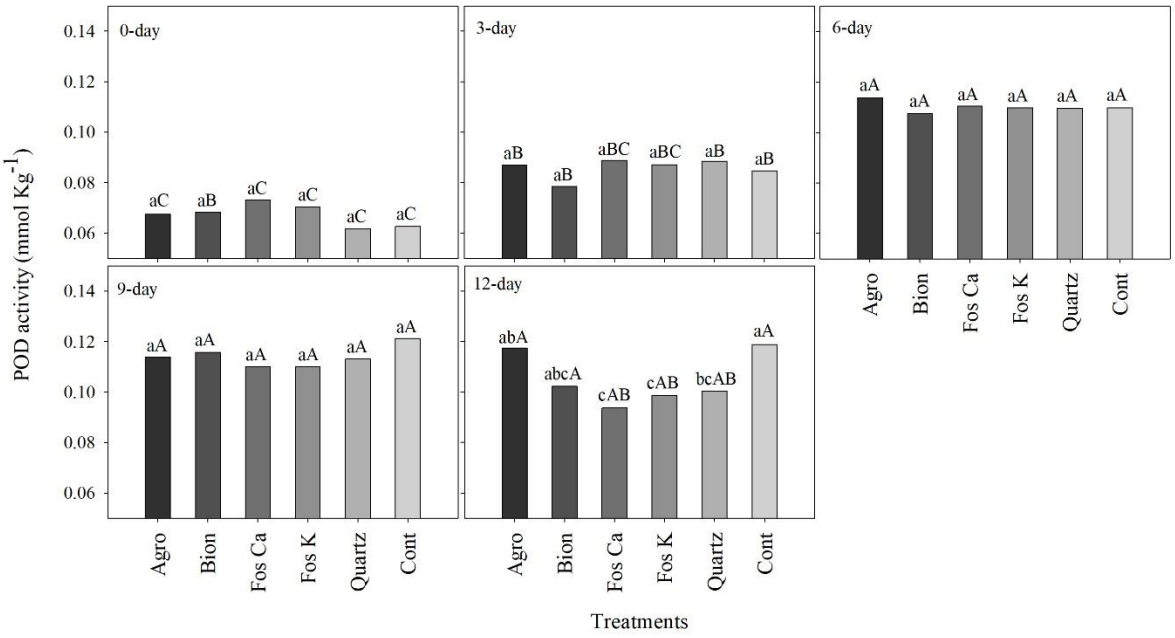

Figure 6. Peroxidase (POD) activity in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

Activity peaked between the sixth and ninth days and decreased on the last day in the vase, similar to the POD enzyme performance. On the first vase life day there was no significant difference between the treatments assessed but during the assessments the stems treated with potassium and calcium Fosfitotal® maintained PPO levels lower than the control treatment. Only on the sixth day of vase life the stems treated with Agro-Mos® and Bion® presented lower PPO levels compared to the control (Figure 7).

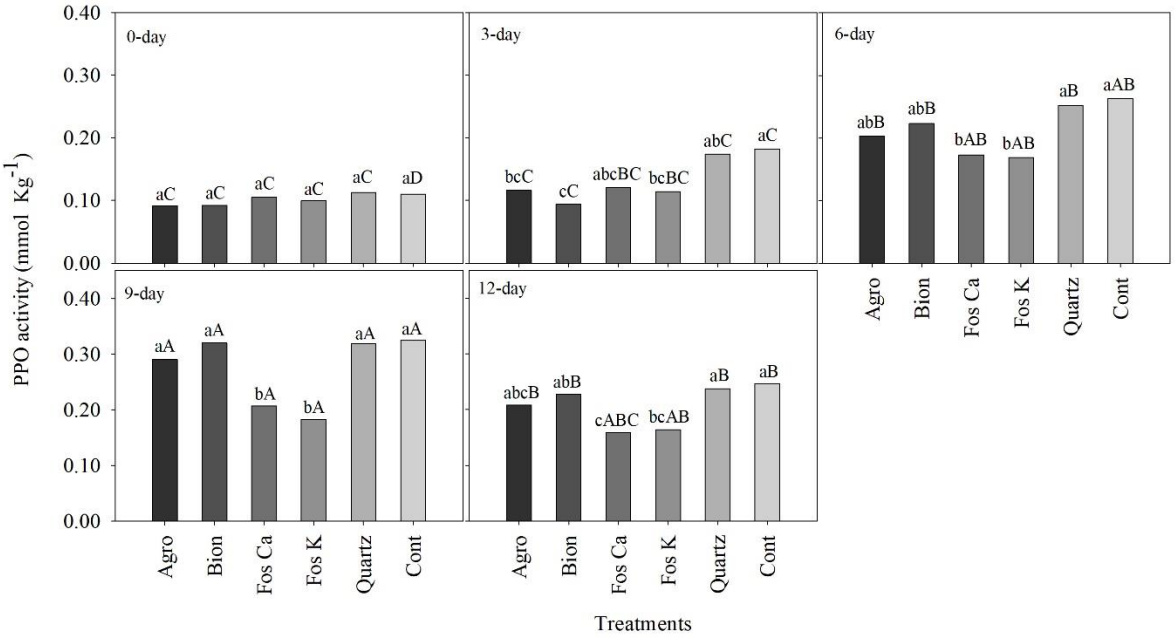

Figure 7. Polyphenoloxidase (PPO) activity in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.6. Superoxide dismutase (SOD, EC:1.15.1.1) activity

Statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. SOD activity in *H. psittacorum* flower stems tended to increase over the vase life, with occasional reductions on the third vase life, for the stems in the control, Agro-Mos® and Quartz® treatments and on the 12th day for the stems treated with Bion®. However, it was observed that, from the third day of evaluation, the stems treated with the Fosfitotal® and Bion® always presented higher SOD activity in relation to the control (Figure 8).

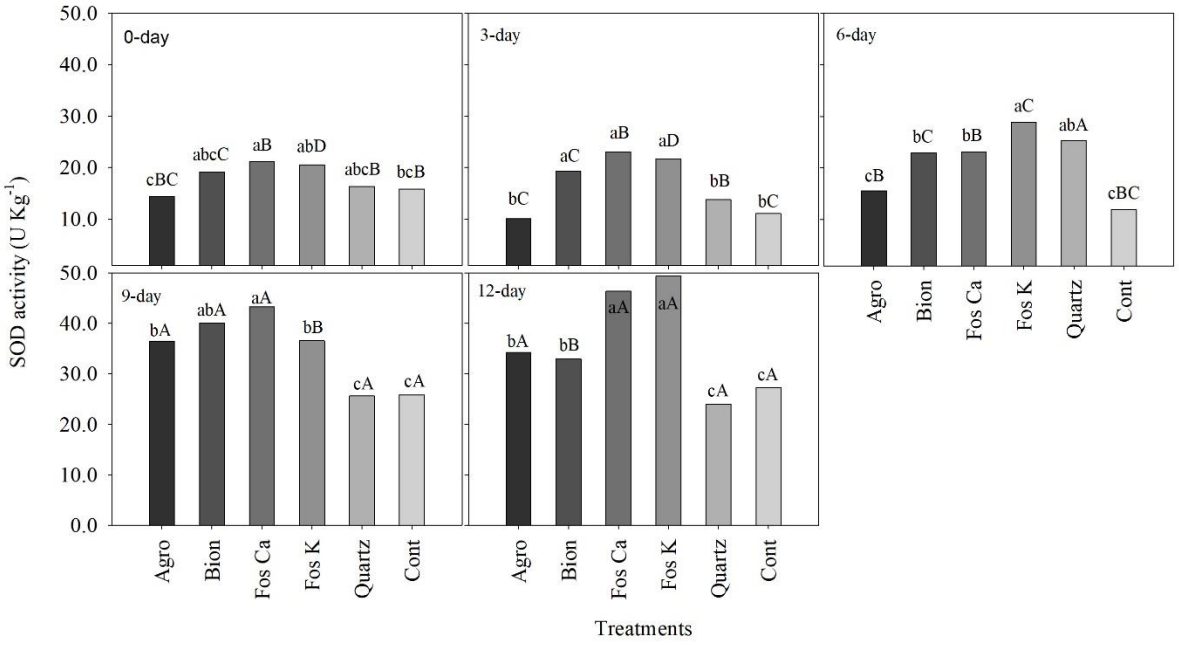

Figure 8. Superoxide dismutase (SOD) activity in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

3.7. Total soluble phenols

It was not possible to characterize peaks for total soluble phenolic contents in floral stems of *H. psittacorum*. The applied statistics showed that there was a significant interaction between treatments (plot) and time (subplot). However, the treatments influenced this parameter from the sixth day of vase life (Figure 9).

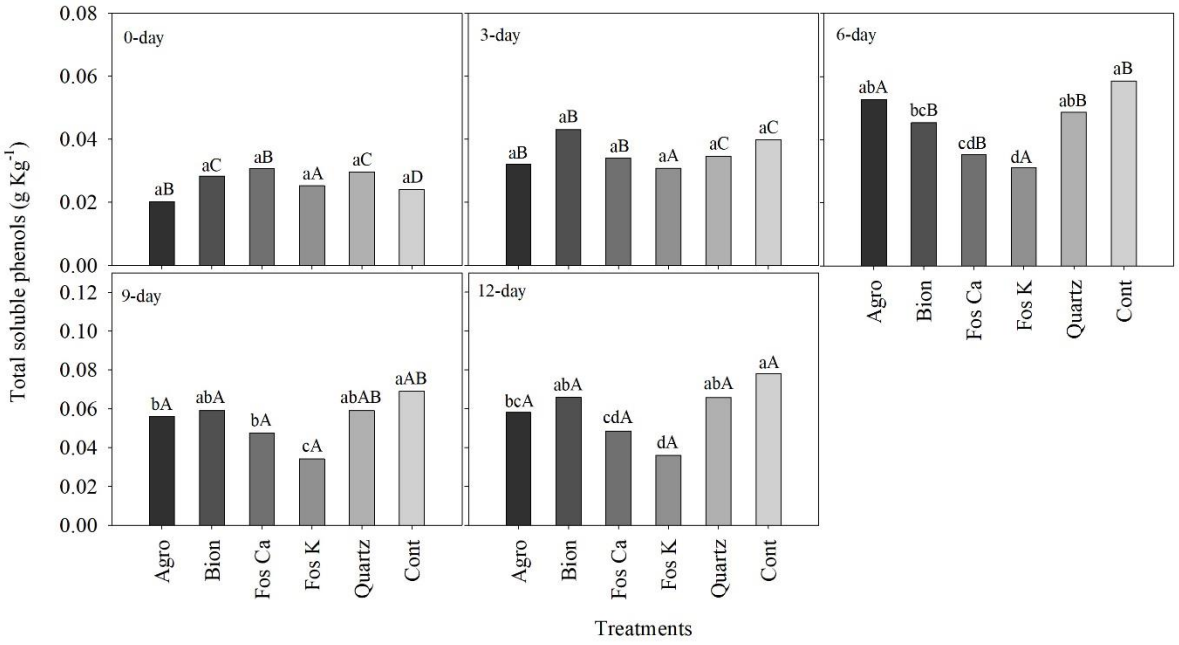

Figure 9. Total soluble phenols in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

Starting on the sixth vase life the stems treated with the potassium and calcium Fosfitotal® differed from the control, presenting lower phenol compound levels that did not alter until the end of the assessment period.

3.8.Lipid peroxidation – TBARS

The statistical analysis identified a significant interaction between treatments (plot) and time (subplot) for the parameter evaluated. At the beginning of the experiment lipid peroxidation increased but over time remained stable for all treatments. From the third day of vase life, a difference was observed between the treatments, which was shown in favor of the

Fosfitotal®, as compared to the control, from the sixth day in the vase (Figure 10). The results observed on the 9th and 12th days in the vase showed that the Fosfitotal® treatments had the lowest lipid peroxidation values, especially when compared to the control treatment.

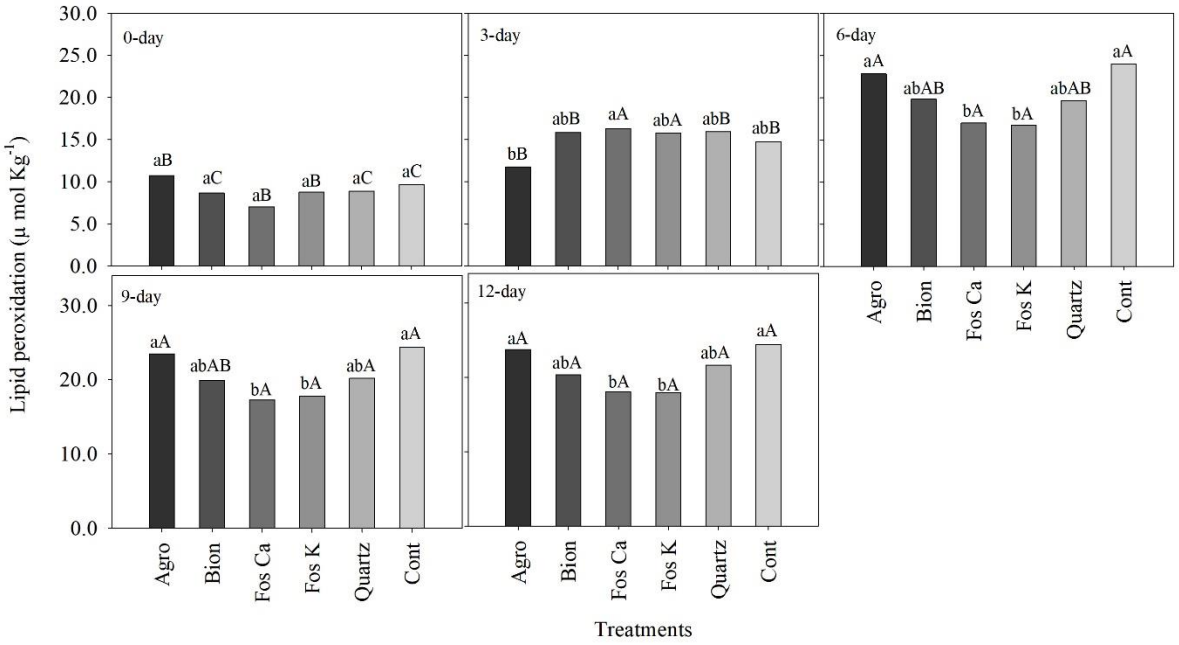

Figure 10 - Lipid peroxidation in *Heliconia psitacorum* stems, treated with Agro-Mos® (Agro), Bion®, Quartz®, K - Fosfitotal® (Fos K), Ca - Fosfitotal® (Fos Ca) and distilled water (Cont). The means of the treatments were compared by the Tukey test ($P < 0.05$). Lowercase letters compare the treatments on the day of the evaluation and uppercase letters compare the treatment over the vase life.

4. DISCUSSION

The visual scores attributed to the treatments demonstrated that the Fosfitotal®, especially K - Fosfitotal®, enabled a greater preservation of stem quality during vase life, possibly by acting on the plant metabolism. Maganve et al. (2013) reported that *H. psittacorum* plants not treated in post-harvesting had their vase life limited to 9 days and these results were

also observed in the present study but the stems treated with Fosfitotal® presented higher quality on the 12th day.

The reduction in visual scores during vase life is acceptable, because the flowers are in the process of senescence. Costa et al. (2011) stated that the visual quality of *H. bihai* flowers diminished during the useful vase life, due to damage or senescence. The results expressed by electrolyte extravasation corroborate with those observed in the visual analysis and indicate the better quality of Fosfitotal® - treated stems. The treatments with calcium and potassium Fosfitotal® may have increased tolerance to water loss and as a consequence prolonged the flower stem quality. According to Demidchik et al. (2014) ROS have been shown to activate gens GORK, SKOR, and annexins. ROS-activated K^+ efflux through GORK channels results in dramatic K^+ loss from plant cells, which stimulates proteases and endonucleases, and promotes programmed cell death. This mechanism is likely to trigger plant programmed cell death under severe stress. However, in moderate stress conditions, K^+ efflux could play an essential role as a metabolic switch in anabolic reactions, stimulating catabolic processes and saving 'metabolic' energy for adaptation and repair needs. From this assumption we can suggest that the Fosfitotal® supplying K^+ and Ca^+ cations interfere in these cellular mechanisms, since there is as yet no consensus that phosphite alone is metabolizable by plants (Gómez-Merino and Trejo-Téllez, 2015). Burra et al. (2014) reported that phosphite interfere in processes associated to the primary and secondary metabolisms, inducing an increase in gene expression related to abiotic stress, including the early response to dehydration.

Fresh matter maintenance is usually reflected in higher visual scores and stems with bigger fresh matter content are of better quality. However, in the present experiment, the treatments did not show reduction in fresh matter. According Mangave et al. (2013) fresh matter loss is a process expected during the vase life and can vary according to specific post-harvest treatments, such as spray application. In addition, fresh matter loss probably occurrs due to the

senescence process caused by physiological changes in the plant, such as water loss (Costa et al., 2011).

The soluble carbohydrate content was bigger in the stems treated with Fosfitotal® until the last storage day, when all the treatments were equal for soluble carbohydrates. These results suggest an association between carbohydrate content and bract quality because the Fosfitotal® - treated stems obtained better visual scores compared to the other treatments used. We can raise two hypotheses from these results, the first linked to the natural senescence process, that may have accelerated in the control, Agro-Mos®, Bion® and Quartz® treatments or been delayed in the treatments with Fosfitotal®. According to Woltering (2017), decreasing carbohydrate levels may trigger the senescence process. The second hypothesis is that increased lipid peroxidation leads to greater membrane damage. According to Bañuelos-Hernández et al. (2017), the bigger the damage, the bigger will be the release of non-selective ions and soluble sugars and amino acid loss. In the present case, it is possible to associate the bigger electrolyte extravasation with the bigger the soluble carbohydrate degradation and consequent reduction in the physiological functions necessary to maintain the flower stem quality.

The results expressed for POD when associated to the visual scores showed that plants with less POD activity obtained better scores and may represent an indication of less oxidative stress, for the stems treated with Fosfitotal® and Bion®. Rapid Bion® absorption by the plant stimulated resistance by interfering in physiological and biochemical processes (Debona et al., 2009). It is also frequently associated to the salicylic acid metabolic paths because it activates genes referent to resistance signaling (Thakur and Sohal, 2013). According to Bañuelos-Hernández et al. (2016), *H. psittacorum* stems with higher POD levels show greater damage and stress at cell level and this damage is represented by dark marks and visible desiccation on the bract, that was confirmed by the lowest scores attributed to the control and Agro-Mos® treatments.

The PPO activity in the treatments with the Fosfitotal® was lower compared to the control treatment and was probably related to the smaller membrane damage associated to the treatment, and there was also less tissue darkening and better visual scores at the end of vase life for the Fosfitotal® - treated flower stems. Tissue darkening may be a consequence of cell disorganization, caused by cell membrane rupture, because, according to Taranto et al. (2017) the PPO´s are oxidation reaction and cell disorganization catalyzers, caused by membrane rupture that places PPO´s in contact with phenol compounds, resulting in tissue darkening, a fact observed more often in the control treatment. The results found for PPO and POD activity contradict other studies that indicated an increase in the activity of these enzymes in plants under stress caused by *Pythium ultimum* in cucumber plants (Mofidnakhaei et al., 2016), but our results were obtained in the absence of biotic factors, that is, absence of phytopathogens. In this sense the phosphites may have acted differently in the metabolism of *H. psittacorum* in response to the pre-harvest treatment, including stimulating other antioxidant mechanisms.

Regarding the total soluble phenols, it can be inferred that low visual scores are associated to increase in total soluble phenol production and consequent raise in the PPO and POD enzyme activity. Based on these data is it can be suggested that the control treatment showed more damage, possibly caused by ROS, especially in the second half of the vase life when there were statistical differences between the treatments. Considerations by Araujo et al. (2015) support our views by stating that plants respond to physical lesions, infections by pathogens, abiotic and biotic elicitors or different types of stress by increasing the pre-existing total soluble phenol concentration or producing ones, using various metabolic paths. In this case, the heliconia stems with bigger associated damage increased production of these compounds to reduce damage caused by ROS, but without success.

The results expressed for SOD show that the Fosfitotal® - treated stems presented higher activity of this enzyme, compared to the control, throughout the vase life. These results,

associated to the visual scores attributed to the Fosfitotal® - treated flower stems, indicate that the high SOD activity may be associated to longer vase life of bract quality post-harvest. The SOD activity probably resulted in greater protection against reactive oxygen species and higher quality of Fosfitotal® - treated stems, because the SODs catalyze dismutation of two O_2^- radicals generating H_2O_2 and O_2 (Barbosa et al., 2014). These results suggest that the Fosfitotal® can induce antioxidant defense mechanisms because the stems were under similar stress conditions. However, further studies are needed on the effect of this product post-harvest, especially on tropical flowers. According to Gómez-Merino and Trejo-Téllez (2015) there are few studies on phosphites relating their effects to abiotic stress tolerance. In spite of the scarcity of these studies, Mofidnakhaei et al. (2016) reported increase in SOD activity in cucumber plants treated with potassium phosphite and considered that phosphite ions activated the defense system of the plant against biotic and abiotic stresses, that partly confirmed our (suspicions) hypotheses.

The results expressed by lipid peroxidation showed that the Fosfitotal® guarantee greater cell membrane integrity and consequently reduced electrolyte extravasation. It can also be stated, based on the SOD enzyme activity, that the treatments with phosphites maintained a better balance between ROS production and elimination, possibly reducing these deleterious effects when compared to the control treatment, that resulted in higher visual scores for the heliconia flower stems grown with this phytostimulant. Mangave et al. (2013) reported in a study on heliconia that increase in lipid peroxidation was accompanied by reduced membrane integrity and increase in electrolyte extravasation, as observed in the present experiment. These associated factors would confer higher quality to flowers treated with Fosfitotal®.

The products Agro-Mos®, Bion® and Quartz®, phytostimulants recognized as biochemical mechanism elicitors (Araujo et al., 2015; Gomes et al., 2016; Melo et al., 2016), in the present experiment did not stimulate sufficiently positive responses to the protective

enzyme activity in *H. psittacorum* treated flower stems, compared to the potassium and calcium Fosfitotal®.

5. CONCLUSION

Applications of phosphite during cultivation resulted in better conservation in post-harvest with quality gain to the *Heliconia psittacorum* stems and may have activated as yet unknown physiological and/or biochemical mechanisms. The results demonstrated that the plants treated with phosphites had reduced damage associated to oxidative stress, increased superoxide dismutase activity, a key enzyme in the protection process against reactive oxygen species and delayed lipid peroxidation.

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Considerações finais

Considerações Finais

As flores são artigos de contemplação e quando adquiridos por consumidores precisam ser vistosas, coloridas e perfeitas. Durante a produção, o ataque de fitopatógenos pode danificar a flor, reduzindo as qualidades que atraem o consumidor.

Os resultados da tese propiciaram ao produtor soluções viáveis para o controle de fitopatógenos durante a produção, além de manutenção da qualidade durante o período de armazenamento da flor.

Outro aspecto importante desta tese é a contribuição científica em uma área pouco explorada, sobretudo no Maranhão, e que muitos produtores demandam informações técnicas que subsidiem uma produção com qualidade.

Espera-se que os resultados aqui apresentados possam incentivar pesquisadores a ingressar nesta área de pesquisa e contribuir para o desenvolvimento da cadeia produtiva de flores tropicais.

ANEXOS

Anexo I

Normas de submissão para a Postharvest Biology and Technology (Capítulo III)

POSTHARVEST BIOLOGY AND TECHNOLOGY

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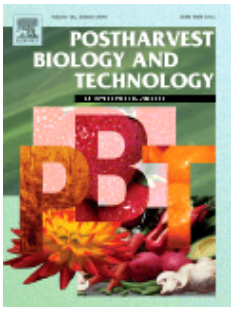

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The journal is devoted exclusively to the publication of original papers, review articles and frontiers articles on **biological** and **technological postharvest research**. This includes the areas of postharvest storage, treatments and underpinning mechanisms, quality evaluation, packaging, handling and distribution of fresh horticultural crops including fruit, vegetables, flowers and nuts, but excluding grains and forages.

Articles on their postharvest treatment as affecting the eventual quality of processed product may be included, but articles on processing of such crops beyond refrigeration, packaging and minimal processing will not be considered. Papers reporting novel insights from fundamental and interdisciplinary research will be particularly encouraged. These disciplines include systems biology, bioinformatics, entomology, plant physiology, plant pathology, (bio)chemistry, engineering, modelling, and technologies for nondestructive testing.

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1. Research Papers (regular papers)
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