UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

FCA
FACULDADE DE CIÊNCIAS AGRONÔMICAS
UNESP | BOTUCATU

VANESSA ALVES GOMES

TAIOBA *(Xanthosoma sagittifolium)* NO MANEJO DE NEMATOIDE-DAS-GALHAS

Botucatu
2021

VANESSA ALVES GOMES

TAIOBA *(Xanthosoma sagittifolium)* NO MANEJO DE NEMATOIDE-DAS-GALHAS

Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Doutora em Agronomia – Proteção de Plantas.

Orientadora: Silvia Renata Siciliano Wilcken

Botucatu

2021

G633t

Gomes, Vanessa Alves

Taioba (*Xanthosoma sagittifolium*) no manejo de nematoide-das-galhas) / Vanessa Alves Gomes. -- , 2021

64 p.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agronômias, Botucatu,

Orientadora: Silvia Renata Siciliano Wilcken

1. Nematoda. 2. Fitopatologia. 3. Patógenos. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Farmacêuticas, Araraquara. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Botucatu

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: TAIOBA (*Xanthosoma sagittifolium*) NO MANEJO DE NEMATOIDES-DAS-GALHAS"

AUTORA: VANESSA ALVES GOMES
ORIENTADORA: SILVIA RENATA SICILIANO WILCKEN

Aprovada como parte das exigências para obtenção do Título de Doutora em AGRONOMIA (PROTEÇÃO DE PLANTAS), pela Comissão Examinadora:

Prof.ª Dr.ª SILVIA RENATA SICILIANO WILCKEN (Participaçao Virtual)
Proteção Vegetal / Faculdade de Ciências Agronômicas de Botucatu - UNESP

Prof.ª Dr.ª REGIANE CRISTINA DE OLIVEIRA (Participaçao Virtual)
Proteção Vegetal / Faculdade de Ciências Agronômicas de Botucatu - UNESP p/

Prof. Dr. FERNANDO BROETTO (Participaçao Virtual)
Química e Bioquímica / Instituto de Biociências de Botucatu - UNESP p/

Prof. Dr. EVERALDO ANTÔNIO LOPES (Participaçao Virtual)
Produção Vegetal / Universidade Federal de Viçosa p/

Prof. Dr. JÚLIO CARLOS PEREIRA DA SILVA (Participaçao Virtual)
Defesa Fitossanitária / Universidade Federal de Santa Maria p/

Botucatu, 06 de agosto de 2021

Dedico esta tese aos meus amados pais,
Helenice e Belmiro, e a minha irmã, Carolina, meus
maiores incentivadores.

Meus exemplos de vida.

AGRADECIMENTOS

Agradeço a Deus e a Nossa Senhora por sempre guiarem meus estudos e minhas tomadas de decisão. Agradeço também ao Espírito Santo por me conceder o dom da sabedoria. Agradeço todas as bençãos divinas que me foram concedidas, dando-me força, paciência e resiliência, dia após dia.

Agradeço aos meus amados pais, Helenice e Belmiro, pelo apoio incondicional e todas as palavras de incentivo, mesmo nos momentos mais difíceis.

Agradeço a minha irmã, Carolina, por toda a paciência, pelos conselhos, pelo companheirismo e por estar sempre presente.

Agradeço a minha orientadora, professora Dr. Silvia Renata, por todos os ensinamentos compartilhados e por todas as oportunidades que me foram oferecidas durante o meu doutorado.

Agradeço ao professor colaborador, Dr. Fernando Broetto, por auxiliar na minha pesquisa e ceder o Laboratório de Química e Bioquímica – Unesp/Rubião para a confecção do extrato.

Agradeço ao professor colaborador, Dr. Márcio Pedroso, juntamente com a minha amiga, Fabíola, por auxiliar na minha pesquisa, realizando a análise de cromatografia gasosa na Central de Análise e Prospecção Química – UFLA.

Agradeço aos meus colegas do Laboratório de Nematologia Agrícola que me ajudaram no desenvolvimento dos meus experimentos.

Agradeço aos meus amigos de verdade, por estarem sempre presentes, independentemente da situação e/ou distância física.

Agradeço a toda a Unesp/FCA, todos os professores, coordenadores, biblioteca, secretaria, funcionários e prestadores de serviços. Todos os setores foram fundamentais para eu conquistar o tão sonhado título de Doutora em Agronomia - Proteção de Plantas.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

A todos vocês, os meus sinceros agradecimentos!

RESUMO

Meloidogyne spp. é um gênero relevante devido sua alta capacidade de causar danos às plantas e ser disseminado no mundo todo. Como o controle do nematoide-das-galhas é difícil, algumas medidas foram criadas para complementar o combate deste patógeno de forma eficiente. Dentre elas temos o controle alternativo simulando a técnica de biofumigação e por meio de extratos vegetais. Deste modo, objetivou-se nesta tese testar a resistência e a suscetibilidade da taioba (*Xanthosoma sagitifolium* (L.) Shott) aos nematoides *Meloidogyne incognita, M. javanica* e *M. enterolobii*, bem como o seu efeito no controle de *M. enterolobii* por meio da técnica de biofumigação e extrato aquoso. O teste de reação apresentou como tratamentos 0 (controle), 333, 999, 3000, 9000 e 27000 ovos e eventuais juvenis. Para validar o inóculo foram utilizadas plantas de tomateiros suscetíveis, cv. Santa Clara. *X. sagittifolium* se comportou como resistente a *Meloidogyne* spp., mesmo sob alta população do nematoide. Folhas de *X. sagittifolium* incorporadas nas concentrações de 0 (controle), 0,45; 0,9; 1,8 e 3,6 g foram utilizadas para simular a técnica de biofumigação. O número de galhas e ovos de *M. enterolobii* foi reduzido a partir da concentração de 1,8 g e, na concentração máxima, o número de galhas foi menor que 15 galhas/g de raiz e o número de ovos foi menor que 200 ovos/g de raiz. Os macerados liberaram compostos orgânicos voláteis capazes de reduzir a infectividade e reprodução dos nematoides. Na avaliação *in vitro* do extrato aquoso de *X. sagittifolium* em *M. enterolobii,* foram avaliadas as concentrações de 2,5; 5; 10; 15 e 25% (m:v), como controle negativo foi utilizado água e como controle positivo foi utilizado um produto a base de *Bacillus amyloliquefaciens.* Na concentração de 25% não houve eclosão de juvenis e apresentou mortalidade de 100% dos juvenis. Já na avaliação do extrato aquoso em casa de vegetação foram testadas as concentrações de 10; 15 e 25% do extrato, como controle negativo foi utilizado água e como controle positivo foi utilizado *B. amyloliquefaciens.* Na concentração de 25%, houve redução do número de galhas e ovos de 21 e 29%, respectivamente, sendo eficientes quando comparados com o *B. amyloliquefaciens*. *X. sagittifolium* não é hospedeira de *Meloidogyne* spp. e o controle alternativo fazendo uso de suas folhas é eficiente por meio da técnica de biofumigação e extrato aquoso.

Palavras-chave: Araceae; controle alternativo; *Meloidogyne* spp.; patógenos de solo, resistência.

ABSTRACT

Meloidogyne spp. are the most relevant plant-parasitic nematodes worldwide causing enormous losses in many crops. As their control is very challenging, some methods are increasingly gaining space to control these pathogens efficiently. The alternative control can be used simulating the technique of biofumigation and with plant extracts. That way, this work aimed to study the reaction of *Xanthosoma sagitifolium* (L.) Shott to *Meloidogyne incognita, M. javanica* and *M. enterolobii* and its effect on *M. enterolobii* control through soil biofumigation and aqueous extract. The reaction test presented as treatments 0 (control), 333, 999, 3000, 9000, 27000 eggs and eventual juveniles. To validate the inoculum, susceptible tomato plants cv. Santa Clara were used. *X. sagittifolium* did not host the *Meloidogyne* spp., even in a high population. *X. sagittifolium* leaves incorporated in soil at concentrations 0 (control), 0.45, 0.9, 1.8, 3.6 g were also used to simulate the technique of biofumigation. The number of galls and eggs of *M. enterolobii* has been reduced from the concentration of 1.8g, and at maximum concentration, the number of galls was less than 15 galls/g of root and the number of eggs was less than 200 eggs/g of root. The macerates emitted volatile organic compounds able to reduce the infectivity and reproduction of nematodes. In *in vitro* evaluation of aqueous extract of *X. sagittifolium* in *M. enterolobii* were evaluated the concentrations 2.5; 5; 10; 15% (m:v). Water was the negative control and *Bacillus amyloliquefaciens*-based product the positive control. In 25% concentration, there was no hatching of juveniles and there was no juvenile alive. In the greenhouse tests, *X. sagittifolium* leaf extract was evaluated at concentrations 10, 15 and 25% of extract. Water was the negative control and *B. amyloliquefaciens* the positive control. In 25% concentration, had a reduction in the number of galls and eggs of 21 and 29%, respectively, being efficient when compared to the *B. amyloliquefaciens*. *X. sagittifolium* is not a host plant of *Meloidogyne* spp., and the alternative control with theirs leaves is efficient through soil biofumigation and aqueous extract.

Keywords: Araceae; alternative control; *Meloidogyne* spp.; resistance; soil pathogens.

SUMÁRIO

INTRODUÇÃO GERAL..... 15

CAPÍTULO 1 - *Xanthosoma sagittifolium* **IS RESISTANT TO** *Meloidogyne* spp. **AND CONTROLS** *Meloidogyne enterolobii* **BY SOIL BIOFUMIGATION 19**

1.1 INTRODUCTION 19
1.2 MATERIALS AND METHODS 22
1.3 RESULTS AND DISCUSSION 26
ACKNOWLEDGMENTS 34
REFERENCES 35

CAPÍTULO 2 - *Xanthosoma sagittifolium* **EXTRACTS AGAINST** *Meloidogyne enterolobii* **..... 44**

2.1 INTRODUCTION 42
2.2 MATERIALS AND METHODS 44
2.3 RESULTS 47
2.4 DISCUSSION 50
ACKNOWLEDGMENTS 52
REFERENCES 52

CONSIDERAÇÕES FINAIS 60

REFERÊNCIAS 63

INTRODUÇÃO GERAL

Áreas infestadas com fitonematoides são capazes de gerar grandes prejuízos, principalmente em regiões tropicais e subtropicais. O gênero *Meloidogyne* é considerado o de maior importância econômica e mais de inúmeras espécies já foram relatadas parasitando diversos hospedeiros (NICOL et al., 2011; MACHADO, 2015). As espécies deste gênero, conhecidos como causadores de galhas em raízes, prejudicam a absorção de água e nutrientes, levando a um menor crescimento da parte aérea e redução na produtividade (ARAUJO et al., 2012). No campo, plantas infectadas demonstram sintomas visuais de amarelecimento e menor crescimento, principalmente em áreas localizadas, reboleiras (CHARCHAR, 1995).

Os danos causados pelos nematoides estão em função da interação entre o ambiente favorável, hospedeiro suscetível e o patógeno agressivo e virulento. Apenas em condições propícias o patógeno se instala, reproduz e causa dano (BERGAMIN FILHO, 1995). Além disso, a temperatura também exerce muita influência nos nematoides podendo favorecer ou não a eclosão dos juvenis. É possível observar também alterações no ciclo biológico afetando crescimento, desenvolvimento, distribuição, incubação, sobrevivência, reprodução, dentre outros fatores (KARSSEN & MOENS, 2006).

Um dos maiores problemas enfrentados em áreas com a presença destes patógenos é a sua dificuldade de manejo, gerando grandes perdas no campo. Regiões tropicais e subtropicais as perdas podem chegar a 80% da produção agrícola (SIKORA & FERNANDEZ, 2005; NICOL et al., 2011; JONES et al., 2013). As hortaliças estão entre as culturas mais atacadas por *Meloidogyne* spp. Um exemplo é a cultura da cenoura, que sofre deformações nas raízes e pode atingir 100% de perdas na presença de *M. incognita* raça 1, associado com *M. javanica* (CHARCHAR et al., 2000). Os danos variam a depender da cultura, condições ambientais, tipo de solo, densidade do plantio, cultivar utilizado, dentre outros. No caso do *M. enterolobii,* a sua importância cresce, cada vez mais, pela sua distribuição mundial em diversas culturas. É um nematoide que apresenta como hospedeiros a goiabeira, hortaliças, frutíferas, essências florestais, ornamentais e plantas daninhas (BITENCOURT & SILVA, 2010).

Uma medida de manejo bastante utilizada em fitonematoides é o uso de produtos químicos. No entanto, os nematicidas encontrados no mercado são escassos, além da possibilidade de causar danos ao meio ambiente e efeitos adversos em seres humanos e animais (SOUSA et al., 2015). Dessa forma, surge a necessidade de moléculas nematicidas menos tóxicas e mais sustentáveis para serem utilizadas associadas às demais medidas já existentes (SILVA et al., 2011). Com isso, surgiram vários estudos fazendo uso de plantas e seus metabólitos secundários. Sabe-se que algumas espécies vegetativas apresentam compostos com atividade antimicrobiana e inseticida, podendo ter ação nematicida (ISMAN, 1999; TOPALOVIĆ et al., 2020). As plantas apresentam em sua composição moléculas químicas, no entanto, estas moléculas se decompõem no meio ambiente rapidamente gerando compostos menos tóxicos, quando comparado com os produtos sintéticos (JARDIM et al., 2017).

Diversas plantas estão sendo estudas no manejo de patógenos. A família Araceae, por exemplo, pertence à ordem Alismatales que possui outras 14 famílias, todas elas ocupam uma posição chave na compreensão da evolução das monocotiledôneas (TOBE, 2010). Mesmo apresentando espécies diversas e distribuídas no mundo todo, a origem da família Araceae ainda é pouco conhecida e estudada. Plantas desta família apresentam uma certa toxicidade fazendo com que sejam estudadas no manejo de fitopatógenos. No entanto, os compostos químicos responsáveis pelos efeitos tóxicos ainda são pouco estudados.

Muitos autores atribuem sua toxicidade a presença de ráfides de oxalato de cálcio. Atualmente, sabe-se que este composto está diretamente relacionado com o mecanismo tóxico presente nas plantas. Plantas da família das aráceas são comestíveis e a toxicidade em seres humanos não acontece ao se alimentar destas espécies após a cocção. No entanto, a ingestão ou o contato com algumas espécies *in natura* pode causar alguns efeitos colaterais. Como exemplo, temos o contato com a mucosa bucal, causando sintomas de queimaduras e desconforto na cavidade oral, podendo desencadear edemas nos lábios e língua, além de uma salivação excessiva. Quando em contato com os olhos, os sintomas podem ser dor intensa, inflamação da pálpebra, contração do músculo ocular, lacrimejamento, bem como fotofobia. Em casos mais severos, é possível observar dificuldade de respiração e da fala, evoluindo para um maior edema na glote, provocando morte

por asfixia (ILARSLAN et al., 1997; LAINETTI et al., 1999; OLIVEIRA & PASIN, 2016).

A taioba, *Xanthosoma sagittifolium* (L.) Schott, pertence à família Araceae e tem origem na América tropical (SAVAGE et al., 2009). É uma espécie que apresenta boa adaptação em climas tropicais, no entanto, se desenvolve bem em locais com condições climáticas adversas, solos pobres, áreas sombreadas e solos acima da capacidade campo. É uma planta que se adapta facilmente ao ambiente e ainda apresenta resistência a pragas e doenças. Além disso, é uma cultura com baixo custo de produção e elevado rendimento área (PEREIRA et al., 2004). *X. sagittifolium* pode ser consumida pela população após cocção, apresentando em sua composição proteínas, carboidratos, vitaminas, tiamina, riboflavina, niacina, fibras, entre outros. No entanto, ela pode apresentar também fatores antinutricionais, substâncias como oxalatos, inibidores de proteinases, fitatos, taninos, alcaloides, esteroides e glicosídeos cianogênicos, causando em algumas pessoas dificuldade na digestão. Estes compostos são interessantes do ponto de vista agronômico por poderem se revelar uma boa estratégia no manejo de fitonematoides (PUIATTI, 2002; LEWU et al., 2010).

Produtos naturais, podem atuar no controle de fitonematoides a partir de extratos vegetais (raiz, folha, galho, fruto e semente), biofumigação, compostos orgânicos voláteis (COVs), óleos essenciais, macerados vegetais, tortas vegetais, farinhas, bem como resíduos oriundos do processamento de plantas pela indústria. É possível observar, nestas diferentes formas, plantas com propriedades nematicidas e/ou nematostáticas. No caso da *X. sagittifolium,* além das ráfides de oxalato de cálcio, esta espécie pode apresentar outros compostos tóxicos voláteis ou não voláteis capazes de atuar no manejo de fitonematoides. O teste dos componentes presentes nas plantas, de forma isolada, pode ser promissor na busca de novos nematicidas sustentáveis para o mercado.

Deste modo, o presente estudo avaliou a resistência e a suscetibilidade da *X. sagittifolium* aos nematoides *Meloidogyne incognita, M. javanica* e *M. enterolobii*, o efeito da biofumigação e do extrato foliar aquoso da planta no controle de *M. enterolobii*. Tendo como objetivos específicos, no primeiro capítulo, avaliou-se a hospedabilidade de *X. sagittifolium* aos nematoides *M. incognita, M. javanica* e *M. enterolobii;* além do efeito da parte vegetativa da *X. sagittifolium* no controle de *M. enterolobii* aplicando a técnica de biofumigação, com análise dos COVs emitidos

pela parte aérea da *X. sagittifolium* por cromatografia gasosa acoplada a espectrometria de massas (GC-MS). Já no segundo capítulo, avaliou-se o potencial nematicida do extrato de *X. sagittifolium* sobre *M. enterolobii* em laboratório e em casa de vegetação.

CAPÍTULO 1

Xanthosoma sagittifolium IS RESISTANT TO *Meloidogyne* spp. AND CONTROLS *Meloidogyne enterolobii* BY SOIL BIOFUMIGATION[1]

Abstract

Meloidogyne is a relevant plant-parasitic nematode that causes enormous damage. It is very challenging to control, and there are not many chemicals available on the market for that. As an alternative method of nematode control, biofumigation is increasingly gaining space. This research aimed to study the reaction of *Xanthosoma sagittifolium* to *Meloidogyne enterolobii, M. incognita* and *M. javanica* and soil biofumigation with *X. sagittifolium* leaves for *M. enterolobii* control. The reaction test was performed in the populations 0 (control), 333, 999, 3000, 9000, 27000 eggs and eventual juveniles. *X. sagittifolium* did not host the *Meloidogyne* species studied, even in a high population. *X. sagittifolium* leaves incorporated in soil at concentrations 0 (control), 0.45, 0.9, 1.8, 3.6 g were also studied to control *M. enterolobii*, and they were able to reduce galls and eggs. The number of galls and egg masses was reduced to a concentration of 1.8 g. In the maximum concentration, the number of galls was less than 15 galls, and the eggs were also reduced to less than 200 eggs. As these macerates emitted nematicidal volatile organic compounds (VOCs) against *M. enterolobii*, it reduced the infectivity and reproduction of nematodes.

Keywords: Alternative control. Araceae. Host-parasitic relationship. Resistance. Root-knot nematode. Volatiles.

1.1 INTRODUCTION

Plant-parasitic nematodes cause significant damage in tropical and subtropical agriculture. Among them, the *Meloidogyne* species, also known as root-knot nematode, affect agricultural production (Jones et al., 2011). The biggest problem in areas infested with these nematodes is the difficulty of managing and controlling them. Many nematicidal products have been withdrawn from the market due to their high toxicity to human beings and the environment (Sousa et al., 2015).

[1] Capítulo 1 - Publicado na revista Journal of Nematology, a formatação do texto segue as normas da revista.

Consequently, there is an increasing demand for lower-cost, high-efficiency, and sustainable new control methods (Subbotin & Chitambar, 2018).

Antagonistic plants have proved to be efficient in controlling plant-parasitic nematodes when used in crop rotation, extracts or seed cakes in direct application to the soil, or soil biofumigation (Caboni et al., 2013; Babaali et al., 2017; Xie et al., 2017; Chetia et al., 2019). Biofumigation is an effective method to control soil pathogens, such as nematodes. This technique consists of macerating the plant and putting this macerate in contact with the infested soil. When biofumigation is performed, it is possible to potentiate the effect of compounds in the plants. Macerate of broccoli, cauliflower, papaya, crambe, castor bean, neem, and mustard showed nematicidal potentials (Ploeg and Stapleton, 2001; Barros et al., 2014; Tavares-Silva et al., 2017; Pedroso et al., 2019a; Gomes et al., 2020).

Natural compounds in some plant species may control nematodes. Several plant compounds have antimicrobial, insecticidal, or nematicidal action (Isman, 1999). These compounds include volatile organic compounds (VOCs), which consist of molecules with up to 20 carbon atoms with a high vapour pressure that cross membranes freely and release the vapours into the atmosphere or soil without a diffusion barrier (Dudareva et al., 2006). Plants produce several compounds known as secondary metabolites (Pichersky and Gang, 2000), with 1% being VOCs such as terpenoids, phenylpropanoids, benzenoids, fatty acid derivatives, and amino acid derivatives (Dudareva & Pichersky, 2008). Brassicas produce isothiocyanates by glucosinolate degradation (Price et al., 2005); garlic has allicin emission (Slusarenko et al., 2008) and neem, peas, mustard, velvet bean, and jack bean emit toxic VOCs to *Meloidogyne* (Barros et al., 2014). One advantage of using plants in nematode control is that they have fewer toxic substances than chemical control. Using plants to control nematodes instead of synthetic chemicals can be an effective and sustainable option. Usually, plants have no harmful effects on non-target organisms, and they are not persistent in the environment. Therefore, they are indicated for phytopathogens control (Jardim et al., 2018; Pedroso et al., 2019a, Gomes et al., 2020; Silva et al., 2020a, 2020b).

The Araceae family has a wide variety of species, including the *Xanthosoma sagittifolium*. There is much confusion within this genus, with the same plant being

given more than one species common name depending on the location where the plant is. These plants are native to South America, which grow mainly in regions of tropical and subtropical climate. Their cultivation is widespread, and they are intensively grown and consumed in Central American, Africa, and Asia (Seganfredo et al., 2001). Many species in this family are highly toxic, such as *Xanthosoma sagittifolium, Epipremnum pinnatum, Colocasia esculenta, Monstera deliciosa Colocasia antiquorum*, and *Dieffenbachia picta,* due to the presence of calcium oxalate (Sena et al., 2016). This substance participates in the toxic mechanism, causing injury and exposing the organism of the individual to the toxic substance (Ilarslan et al., 1997; de Oliveira & Pasin, 2017). The *X. sagittifolium* is composed of proteins, carbohydrates, vitamins, thiamine, riboflavin, niacin, fibres, among others. However, it can also present antinutritional factors, substances such as oxalates, proteinase inhibitors, phytates, tannins, alkaloids, steroids, and cyanogenic glycosides (Puiatti, 2002; Lewu et al., 2010). A study with rhizome extract from *X. sagittifolium* was efficient in reducing hatching and causing mortality of *M. megadora* (Galhano et al., 1997).

M. enterolobii is a relevant nematode because it causes damage to several hosts, most of them resistant to other nematode species (Bitencourt and Silva, 2010). This species has higher virulence and reproductive potential than other *Meloidogyne* species (Brito et al., 2015). *M. enterolobii* has been reported in many countries such as Mexico, in watermelon plants (Ramírez-Suárez et al., 2014), in jackfruit, tomato and pepper in Florida, in the USA (Brito et al., 2015) and yam in Nigeria, with irreversible damage to these crops (Kolombia et al., 2016). In Brazil, this nematode has been found in several states, parasitizing guava (Silva et al., 2008), and in vegetables, mainly tomatoes and peppers (Carneiro et al., 2006). The nematode can break the resistance conferred by Mi-1 gene to *M. arenaria, M. incognita* and *M. javanica* in tomato (Fargette et al., 1994), besides soybean 'Forrest' and sweet potato 'CDH' (Carneiro et al., 2001; Cantu et al., 2009; Bitencourt and Silva, 2010; Melo et al., 2011) and, 'Prata' bell pepper (Carneiro et al., 2006).

Considering the great potential of crop rotation and soil biofumigation for plant-parasitic nematodes control, this study aimed to verify the reaction of *X. sagittifolium* to root-knot nematode species, *Meloidogyne incognita*, *Meloidogyne javanica* and *M. enterolobii*; and the effectiveness of soil biofumigation with *X. sagittifolium* in

controlling *M. enterolobii*. Furthermore, a gas chromatography analysis coupled to mass spectrometry was performed to identify the possible VOCs emitted by *X. sagittifolium* leaves, which can be involved in nematode control.

1.2 MATERIALS AND METHODS

The experiments were carried out at the Laboratories of Nematology and greenhouses of São Paulo State University - UNESP/FCA – Botucatu, SP, Brazil. The nematode populations used as inoculums were obtained from pure populations of *Meloidogyne enterolobii, M. incognita* and *M. javanica* maintained in tomato plants cv Santa Clara in greenhouse. To extract the eggs, infected roots were washed in tap water, chopped into 1 to 2 cm pieces, and grounded in a blender with 0.5% NaClO solution for approximately 20 seconds. Finally, the material was placed into a 60-mesh screen over a 500-mesh screen, where the eggs were collected in water (Hussey and Barker, 1973 modified by Boneti and Ferraz, 1981).

X. sagittifolium rhizomes, obtained from Jardim de Minas Company, were partly used in experiments of *X. sagittifolium* suitability to *M. enterolobii, M. incognita* or *M. javanica,* and the remaining was cultivated in a greenhouse to obtain leaves used in biofumigation experiments with *M. enterolobii* to represent this genus. Hence, the leaves were taken from the plants, sterilized using water solution with 2.0 % bleach for 1 minute, and double washed in distilled sterile water. After removing the excess water with a paper towel, they were macerated in a blender to be used immediately in the experiments.

X. sagittifolium reaction to Meloidogyne spp.:

The experiment was carried out in spring-summer in a greenhouse under controlled temperature conditions so as not to exceed 30º C, and in this period, there was no occurrence of low temperatures. The plants were subjected to daily irrigation and fertilization, according to the crop needs. Regarding diseases and pests, no problems were observed during the experiments, as the greenhouse was disinfected, and offered suitable conditions for the crop. The rhizomes were soaked in water for 12 h and then transplanted to pots containing 2 L of autoclaved substrate composed of a mixture of soil, sand and organic matter (1: 2: 1). *Meloidogyne* spp. eggs

suspension was prepared as described above. After one month, when the thaw had already developed, the researchers drilled holes in the soil with a glass stick around the plants' roots, and inoculated the eggs' suspension with a pipette for each treatment. The treatments were: 0 (control), 333, 999, 3,000, 9,000, 27,000 eggs and eventual juveniles and five replications, each plot consisting of one plant. 'Rutgers' tomato plants were used to attest the viability of the *M. enterolobii, M. incognita* or *M. javanica* inoculum (1,000 eggs and eventual juveniles). The experiments consisted of a completely randomized experimental design with six different treatments and five repetitions. The experiments were evaluated 60 days after nematode inoculation. The galls and egg masses were evaluated according to the grade scale proposed by Taylor and Sasser (1978), where grade 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = more than 100 galls or egg masses. The root systems were washed and processed separately, where the roots were chopped, and the eggs were extracted by Hussey and Barker's (1973) technique, modified by Boneti and Ferraz (1981). The collected eggs were counted under an inverted light microscope (Leica, model DME). Finally, the reproduction factors of the nematodes were calculated (RF = final population / initial population). Plants with RF <1 are considered resistant, and RF ≥ 1 are considered susceptible (Oostenbrink, 1966). The experiments were conducted separately and followed the same methodology described above for each nematode species, *M. enterolobii*, *M. incognita* and *M. javanica*. The biofumigation test was performed only with the species *M. enterolobii* to represent the root-knot nematodes.

Biofumigation with macerated X. sagittifolium leaves:

M. enterolobii was used to represent root-knot nematodes species at biofumigation tests. The experiment was carried out in spring-summer in a greenhouse under controlled temperature conditions so as not to exceed 30º C, and in this period, there was no occurrence of low temperatures. The plants were subjected to daily irrigation and fertilization according to the crop needs. Regarding diseases and pests, no problems were observed during the experiments, as the greenhouse was disinfected and offered suitable conditions for the crop. The experiment was conducted with five treatments and five repetitions. Macerated *X. sagittifolium* leaves, obtained as described above, were incorporated into the commercial substrate (Carolina Soil – Pardinho, SP, Brazil) in amounts of about 0 (control), 0.45, 0.9, 1.8, 3.6 g corresponding the percentage of 0, 1, 2, 4, 8% and put

at plastic cups (45 g). 1,000 eggs of *M. enterolobii* in 2.0 ml of water was placed in the plastic cups together with the substrate and the macerated *X. sagittifolium* leaves and shaken with a glass stick until homogenization. The moisture of mixture in the plastic cups was adjusted to 60% of the field capacity (20 ml). The surface of the mixture was completely covered with plastic caps, thereby forming a gas chamber between the plastic caps and the substrate by emissions of VOCs (Volatile Organic Compounds) from *X. sagittifolium* leaves macerates mixed to the substrate. The eggs were left exposed to the VOCs released from the mixture for three days. After that, the plastic caps were removed, and this mixture was placed in a styrofoam tray (76 cells, 120 cm^3 each cell) with a 20-day-old tomato seedling cv. Santa Clara. Each cell in the styrofoam tray represents an experimental unity, with five treatments and five repetitions too. After 60 days, each plant was removed. The substrate was carefully removed from the roots by water, and the root system was placed on a paper towel to remove excess water, weighed, and the number of galls per gram of root was counted. Then, the roots were chopped, and the eggs were extracted by Hussey and Barker's technique (1973), modified by Boneti and Ferraz (1981). The collected eggs were counted in Peters slide under an inverted light microscope. Finally, the reproduction factor of the nematodes was calculated (RF = final population/ initial population).

Gas chromatography analysis of VOCs emitted by Xanthosoma sagittifolium leaves:

Volatile identification was conducted at the Center of Analysis and Chemical Prospecting (CAPQ – Department of Chemistry/UFLA). Gas chromatography (GC-MS) was carried out, first, with the macerate of leaves of *X. sagittifolium*, and second, with the water exposed to the volatiles emitted by the macerate of the leaves of *X. sagittifolium* to verify whether the volatile organic compounds (VOCs) can be retained in water. Six replicates of 1.5 g of macerated *X. sagittifolium* leaves and six replicates of water in contact with *X. sagittifolium* leaves were prepared for analysis of VOCs. Samples were placed in 20 ml SPME Supelco vial, closed and kept in an incubation chamber at 28° C for three days. Three vials containing only sterile sand and three empty tubes were also analyzed as controls. Three days later, the volatiles from macerated *X. sagittifolium* leaves were extracted by SPME technique in headspace mode, using DVB/CAR/PDMS fiber (Divinylbenzene, Carboxen, Polydimethylsiloxane). The temperature and extraction time were 55° C to 250 rpm

for 35 minutes, respectively. The GC-MS QP 2010 Ultra mass spectrometer (Shimadzu, Japan), equipped with AOC-5000 automatic liquid and gas injector (Shimadzu, Japan) and HP-5 column, was used for the separation and identification of VOCs (5% phenyl-95% dimethylsiloxane) of dimensions 30 m × 0,25 mm × 0,25 µm. The temperature of the injector was 250° C, the interface was 240° C, and the detector ion source was 200° C. The injector was operated in splitless mode or split 1: 2 mode, according to the peak intensity in the samples. As carrier gas He 5.0 was used as 1,0 ml.min^{-1}. The temperature setting of the GC oven was 40° C to 130° C at 3° C min^{-1} and then up to 240° C at 10° C min^{-1}. To identify the VOCs in the samples, the mass spectrum of each chromatogram peak was extracted through the Automated Mass Spectral Deconvolution and Identification System (AMDIS) v. 2.63. The VOC identification was done by comparing the mass spectra of the sample peaks and the NIST library spectra by the Mass Spectral Search Program v. (NIST, Washington, DC, USA), and by comparing the experimentally obtained retention indices (RI Exp.) and the retention indices of the literature (RI Lit.) (Adams, 2007; Nist, 2013). The experimental retention indices were obtained by injecting a homologous series of alkanes. The comparison among the mass spectra was made only for peaks in which the similarity was greater than 80%.

Statistical analysis:

All experiments were repeated twice. The trials were arranged in a completely randomized design with five replicates per treatment. The data were previously submitted to normality tests (Shapiro-Wilk test) and homogeneity of variance of the errors (Bartlett's test). Once the assumptions were met, the F test was applied through analysis of variance (ANOVA). The experiments were analyzed separately. When the significance level ($P < 0.05$) occurred in biofumigation experiments, regression analysis was performed with the aid of SigmaPlot software (SigmaPlot 12.0, Systat Software Inc.) where the graphs were generated too. In *X. sagittifolium* reaction to *M. enterolobii, M. incognita* e *M. javanica*, the variables were transformed by $\sqrt{x+1}$ and when the significance level ($P < 0.05$) occurred, made Scott-Knott Test was performed with the aid of Sisvar software (Ferreira, 2014).

1.3 RESULTS AND DISCUSSION

We studied whether *X. sagittifolium* can be host the root-knot nematode. Due to its resistance characteristics we also studied if *X. sagittifolium* can control *M. enterolobii*. Biofumigation with leaves of *X. sagittifolium* effectively suppressed *M. enterolobii* in tomato roots. Many species in Araceae family have highly toxic in their composition, and there are few studies about these plants. Based on the toxic composition of *X. sagittifolium* in these experiments and the study by Galhano (1997), the authors believe in the potential of using this practice in the field, but further studies are needed to verify whether these plants could be used in agriculture to crop rotation and soil biofumigation.

In reaction tests, the researchers observed that there was no formation of galls and external egg masses of the nematodes. *X. sagittifolium* was unfavorable for *M. enterolobii, M. incognita* and *M. javanica* multiplication, with reproductive factors less than one, or even equal to zero, being considered resistant to these species of root-knot nematodes (Tables 1-3). The plant continued to show resistance patterns even with the inoculation of highest population. Plants with a high number of eggs had few root systems in the presence of the nematode and these changes may occur as a plant survival strategy (Tables 1-3) (Chen et al., 2004). The viability of the inoculum is proven in the tomato treatment, where the reproduction factor was 13.9, 12.35 and 10.6 for *M. enterolobii, M. incognita* and *M. javanica,* respectively, being the only treatment that behaved as susceptible (Tables 1-3).

Table 1. Reaction test on *Xanthosoma sagittifolium* with *Meloidogyne enterolobii* under the six treatments and the tomato (indicator plant) showing susceptibility or resistance in the treatments based on the reproduction factor (RF).

Plant	Inoculated eggs[1]	*M. enterolobii*				Classification
		Galls	Egg mass	Eggs	RF[2]	
X. sagittifolium	0	0 b	0 b	0 b	0 b	Resistant
	333	0 b	0 b	0 b	0 b	
	999	0 b	0 b	0 b	0 b	
	3,000	0 b	0 b	0 b	0 b	
	9,000	0 b	0 b	316 b	0.03 b	
	27,000	0 b	0 b	1,076 b	0.04 b	
Tomato	1,000	4.80 a	4.80 a	13,876 a	13.9 a	Susceptible
CV (%)	-	3.00	3.00	42.78	10.41	-

[1] Number of inoculated eggs.

[2] RF = final population/ initial population. Plants with RF <1 are considered resistant and RF ≥ 1 susceptible (Oostenbrink, 1966).

Table 2. Reaction test on *Xanthosoma sagittifolium* with *Meloidogyne incognita* under the six treatments and the tomato (indicator plant) showing susceptibility or resistance in the treatments based on the reproduction factor (RF).

Plant	Inoculated eggs[1]	*M. incognita*				Classification
		Galls	Egg mass	Eggs	RF[2]	
X. sagittifolium	0	0 b	0 b	0 b	0 b	Resistant
	333	0 b	0 b	0 b	0 b	
	999	0 b	0 b	0 b	0 b	
	3,000	0 b	0 b	0 b	0 b	
	9,000	0 b	0 b	0 b	0 b	
	27,000	0 b	0 b	0 b	0 b	
Tomato	1,000	4.80 a	4.80 a	12,350 a	12.35 a	Susceptible
CV (%)	-	3.00	3.00	40.86	14.86	-

[1] Number of inoculated eggs.

[2] RF = final population/ initial population. Plants with RF <1 are considered resistant and RF ≥ 1 susceptible (Oostenbrink, 1966).

Table 3. Reaction test on *Xanthosoma sagittifolium* with *Meloidogyne javanica* under the six treatments and the tomato (indicator plant) showing susceptibility or resistance in the treatments based on the reproduction factor (RF).

Plant	Inoculated eggs[1]	*M. javanica*				Classification
		Galls	Egg mass	Eggs	RF[2]	
X. sagittifolium	0	0 b	0 b	0 b	0 b	Resistant
	333	0 b	0 b	0 b	0 b	
	999	0 b	0 b	240 b	0.24 b	
	3,000	0 b	0 b	326 b	0.11 b	
	9,000	0 b	0 b	360 b	0.04 b	
	27,000	0 b	0 b	582 b	0.02 b	
Tomato	1,000	4.80 a	4.80 a	10,592 a	10.6 a	Susceptible
CV (%)	-	3.00	3.00	56.59	14.19	-

[1] Number of inoculated eggs.
[2] RF = final population/ initial population. Plants with RF <1 are considered resistant and RF ≥ 1 susceptible (Oostenbrink, 1966).

The biofumigation with *X. sagittifolium* had a significant effect on the number of *M. enterolobii* galls and eggs. So, *X. sagittifolium* has the potential to control *M. enterolobii*. By increasing the amount of the macerated *X. sagittifolium* leaves, the number of galls and eggs per gram of root reduced significantly ($p < 0.05$) (Fig. 1A,B). The reduction in the number of galls was more evident at the 1.8 g concentration, about 25 galls/g of roots. The reduction in the galls continued by increasing the *X. sagittifolium* macerate amount, reaching about 15 galls/g of roots with 3.6 g of macerate (Fig. 1A). Also, the reduction in the number of eggs became more expressive at the concentration of 0.9 g, about 600 eggs / g root. The reduction in the eggs number was observed at 0.45 g of macerate, reaching significant reductions, less than 200 eggs/g of roots in 3.6 g of macerate (Fig. 1B). Both results were compared to the values obtained in the control (0 g) (Fig. 1A,B).

When the reproduction factor was evaluated in biofumigation experiments, the value started to reduce in 0.45 g, reaching a reproduction factor lower or equal to one in the highest concentration (3.6 g). Significant reduction when compared to the

control (0 g) with reproduction factor around eight (Fig. 1C). Regarding root weight, the analysis revealed that with increasing concentrations, there was an increase in root weight, when compared with de control (0 g) (Fig. 1D). Some studies have verified the efficiency of biofumigation in the control of nematodes with vegetable residues and even papaya seeds (Ploeg & Stapleton 2001; Wang et al. 2009; Ntalli et al., 2011; Gomes et al., 2020). The reduction in gall and egg formation by applying 1.8 g.45 mL^{-1} of macerate represents about 60-ton ha^{-1} (1 ha×15 cm depth=1500 m^3). Generally, to fumigate the soil with plants, the incorporation of 50-ton ha^{-1} is recommended (Ploeg and Stapleton 2001). Although the *X. sagittifolium* application was higher than the general recommendation, these plants produce a sizeable aerial part that is still a good option for biofumigation.

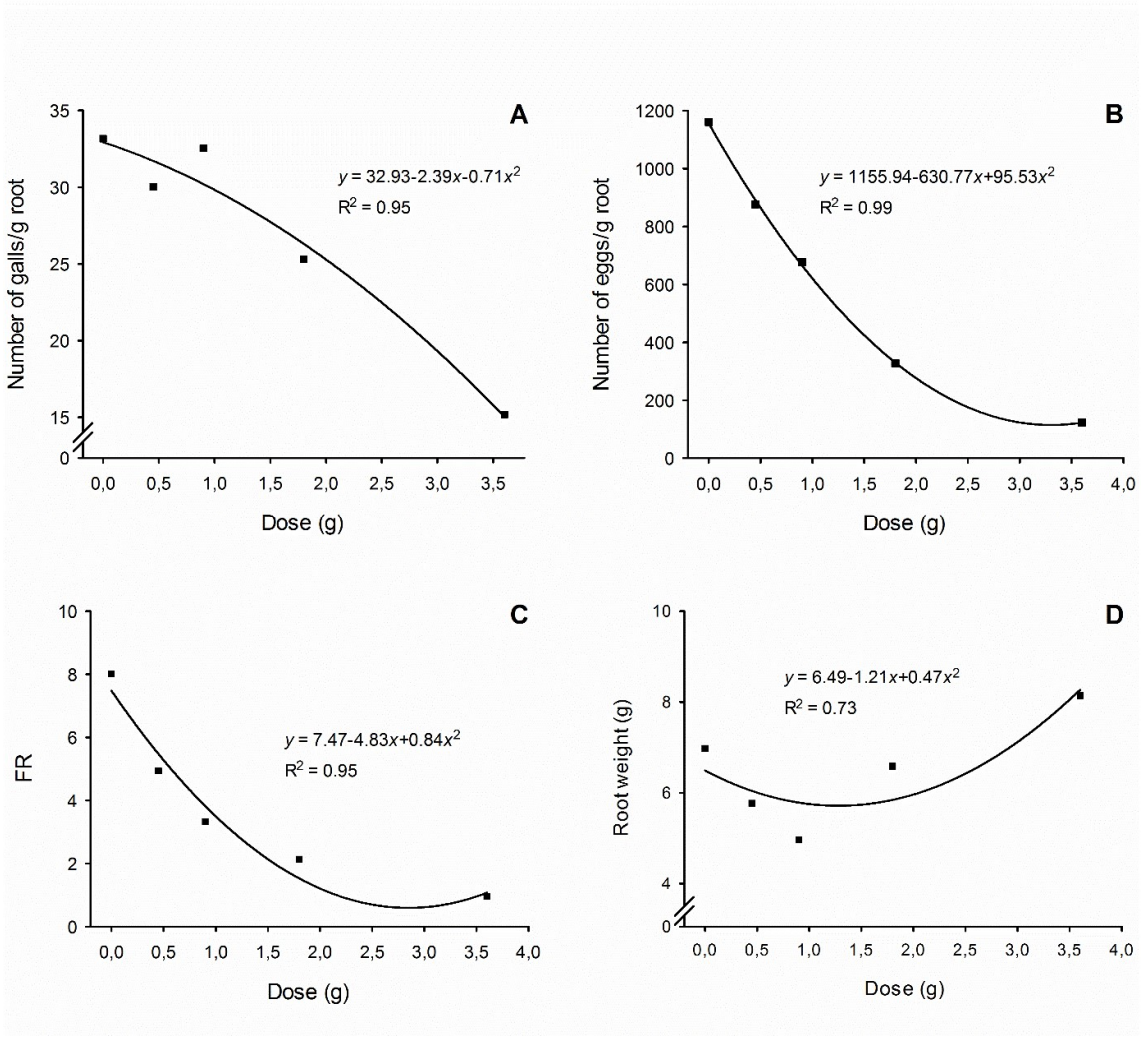

Fig. 1. Biofumigation with *Xanthosoma sagittifolium* leaves macerates against *Meloidogyne enterolobii*. These experiments were done by incorporating *X. sagittifolium* leaves at different concentration (g/45g) along with *M. enterolobii* eggs and planting tomato after three days of biofumigation. A: Number of galls. B: Number of eggs. C: Reproduction factor. D: Root weight. The experiments were done twice. Quadratic polynomial regression models, $p<0.01$.

As concentrations of *X. sagittifolium* leaves macerate increased, there was an increase in root weight. This happens because, with the reduction of the number of galls, the roots develop more and increase their root weight. Some plant VOCs have the potential to interfere with the infectivity and reproduction of root-knot nematode, reducing theirs galls and eggs (Jardim et al., 2018; Kihika et al., 2017; Gomes et al., 2020; Silva et al., 2020a, 2020b) and in this work, the same effect is observed. Brassicas can be used in biofumigation as a feasible practice to reduce plant-

parasite nematodes in crop fields (Ploeg, 2008; Lord et al., 2011; Chetia et al., 2019). However, further studies are needed to verify the best methodology in the field and the way that brassica works in this cultural control practice. *X. sagittifolium* leaves studied in this work showed toxic activity against *M. enterolobii* that was comparable to the activity shown from broccoli. Thus, additional trials should be conducted using the *X. sagittifolium* to confirm whether their use as biofumigants is feasible, because it is not a host to *M. enterolobii* (RF <1), as we can see in the study of the host suitability of *X. sagittifolium* (Tables 1-3).

The GC-MS of the VOCs emitted by macerated *X. sagittifolium* leaves revealed 19 compounds in total. The compounds identified were categorized as low ("+"), and medium to high ("++"), by the intensity of the peaks detected in the chromatograms. The compounds identified with medium to high intensities were ethanol, acetone, 3-methyl-butanol, and 3-hexen-1-ol. The compounds propanol, ethyl acetate, 2-methyl-propanol, 3-penten-1-ol, 3-pentanone, acetoin, 2-methyl-butanol, 2,3-butanediol, ethyl 2-methyl-butanoate, hexanol, 3-methyl-butyl acetate, 3-octanone, 2-ethyl-hexanol, and dodecane occurred in small intensities in macerates of *X. sagittifolium* leaves, as well as a not identified compound (Table 4).

Table 4. Volatilome of *Xanthosoma sagittifolium* leaves by gas chromatography.

Compounds	RI exp[a]	RI Lit[b]	Intensity[c] Macerated	Intensity[c] Water
Ethanol	-	-	XX	XX
Acetone	-	-	XX	XX
Propanol	-	-	X	X
Ethyl acetate	609	628	X	X
2-methyl-propanol	615	622	-	X
3-penten-1-ol	687	686	X	X
3-pentanone	700	696	X	X
Acetoin	720	718	X	-
3-methyl-butanol	737	734	XX	XX
2-methyl-butanol	740	738	X	X
2,3-butanediol	811	811	X	-
Ethyl 2-methyl-butanoate	849	846	X	-
3-hexen-1-ol	856	857	XX	XX
Hexanol	871	867	X	X
3-methyl-butyl acetate	875	880	X	-
3-octanone	985	984	X	-
2-ethyl-hexanol	1033	1029	X	X
Not identified	1189	-	X	-
Dodecane	1199	1200	X	-

[a] Calculated retention indices by injecting a homologous series of alkanes.
[b] Theoretical retention index according to the literature (ADAMS, 2007).
[c] X: Low intensity; XX: Medium to high intensity.

The chromatographic analysis revealed several compounds of the alcohol group: ethanol, propanol, 3-penten-1-ol, 3-methyl-butanol, 2-methyl-butanol, 2,3-butanediol, 3-hexene-1-ol, hexanol and 2-ethyl-hexanol. It is known that ethanol could result from the fermentation of plants (Olsson and Hahn-Hägerdal, 1996). However, the ethanol (EtOH), when tested by applying the isolate compound, is known to have a nematicidal potential (Silva et al., 2017; Pedroso et al., 2019b). Thus, regardless of the origin of the compound, it behaves as toxic to nematodes. Concentrations of EtOH below 5% by volume had a toxic effect on the *Caenorhabditis elegans,* and this nematode is the model nematode for biology studies and development of the nematode (Patananan et al., 2015). Other studies have shown that aqueous EtOH solutions (70% volume) and their vapours reduced hatching of J2 of *M. incognita*. There was also large reduction of galls and eggs in the root system when 40 ml of EtOH (40 and 70%) were applied in the soil with *M. incognita*. Water exposed to EtOH vapours for 1 h became toxic and caused 100% J2 mortality in 12 hours (Silva et al., 2017). Another study reveals that ethanol was responsible for the control of *Heterodera glycines*. Soil infested by *H. glycines* with ethanol reduced infectivity by almost 100% and number of eggs by about 67% at ethanol concentrations of 48% and 72%, respectively. Ethanol concentration at 48% can reduce the lipid content of J2. So, the ethanol is toxic to *H. glycines* in low concentrations, and affects their pathogenic behavior, instead of merely reducing lipids (Pedroso et al., 2019b). Thus, these compounds may present toxicity to nematodes and act to control nematodes, not only of the genus *Meloidogyne*.

Acetone, 3-pentanone and octanone are from the ketone class. Studies show that aliphatic ketones from *Ruta chalepensis* (Rutaceae) induce paralysis on root-knot nematodes (Ntalli et al., 2011). This suggests that this group has some phytonematoid toxicity. We also found dodecane and esters, such as ethyl acetate, ethyl 2-methyl-butanoate, 3-methyl-butyl acetate in the *X. sagittifolium* leaves. Little is known about the toxicities of these molecules and whether their contact with phytonematoids may cause any control. Further studies on these secondary compounds found in GC-MS are needed to determine and prove which indeed have *M. enterolobii* nematode toxicity and can be used to control this plant-parasite nematode.

In conclusion, this research demonstrated, for the first time, the performance of *X. sagittifolium* in the control of *M. enterolobii*. The life cycle of *M. enterolobii* was interrupted when had *X. sagittifolium* plants. *X. sagittifolium* is toxic to this nematode by reducing eggs and galls and this can be due the secondary compounds present in its composition, but tests with the secondary compounds are necessary to prove. Further studies of this interaction of the compounds with the soil microbiota are necessary to verify whether they are altered by it before acting on the nematode control. Even though *X. sagittifolium* is little known globally, this is an ordinary plant that adapts to many environments and proliferates rapidly. Therefore, the fact of *X. sagittifolium* leaves are toxic to *M. enterolobii* in low amounts could allow crop rotation with this plant. Then, after the crop rotation, it is possible to incorporated this plant into infested soils with *Meloidogyne* spp. Furthermore, the exploitation of VOCs from this plant could reveal novel toxic compounds able to be applied as nematicidal products. To prove it, these compounds should be tested separately in subsequent studies.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support and fellowships from: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

The authors would like to thank the Central of Analysis and Chemical Prospecting of the Federal University of Lavras – UFLA – Lavras, MG, Brazil, for supplying the equipment and technical support for chromatographic analysis.

REFERENCES

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream: Allured Publishing Corporation.

Babaali, D., Roeb, J., Hammache, M., and Hallmann, J. 2017. Nematicidal potential of aqueous and ethanol extracts gained from *Datura stramonium, D. innoxia* and *D. tatula* on *Meloidogyne incognita*. Journal of Plant Diseases and Protection 124(4):339-348.

Barros, A. F., Campos, V. P., Silva, J. C. P., Pedroso, M. P., Medeiros, F. H. V., Pozza, E. A., and Reale, A. L. 2014. Nematicide activity of volatile organic compounds emitted by *Brassica juncea, Azadirachta indica, Canavalia ensiformis, Mucuna pruriens* and *Cajanus cajan* against *Meloidogyne incognita*. Applied Soil Ecology 80:34-43.

Bitencourt, N. V., and Silva, G. S. 2010. Reprodução de *Meloidogyne enterolobii* em Olerícolas. Nematologia Brasileira 34(3):181-183.

Boneti, J. I. S., and Ferraz, S. 1981. Modificação do método de Hussey and Barker para extração de ovos de *Meloidogyne exigua* de cafeeiro. Fitopatologia Brasileira 6(3):553.

Brito, A., Smith, T., and Dickson, D. W. 2015. First report of *Meloidogyne enterolobii* infecting *Artocarpus heterophyllus* Worldwide. Plant Disease 99(9):1284.

Caboni, P., Saba, M., Tocco, G., Casu, L., Murgia, A., Maxia, A., Menkissogolu-Spiroudi, U., and Ntalli, N. 2013. Nematicidal activity of mint aqueous extracts against the root-knot nematode *Meloidogyne incognita*. Journal of Agricultural and Food Chemistry 61:9784–9788.

Carneiro, R. M. D. G., Almeida, M. R. A., Braga R. S., Almeida, C. A., and Gloria, R. 2006. Primeiro registro de *Meloidogyne mayaguensis* parasitando plantas de tomate e pimentão resistentes à meloidoginose no estado de São Paulo. Nematologia Brasileira 30(1):81-86.

Carneiro, R. M. D. G., Moreira, W. A., Almeida, M. R. A., and Gomes, A. C. M. M. 2001. Primeiro registro de *Meloidogyne mayaguensis* em goiabeira no Brasil. Nematologia Brasileira 25(2):223-232.

Cantu, R. R., Wilcken, S. R. S., Rosa, J. M. O., and Goto, R. 2009. Reação de porta-enxertos de tomateiros a *Meloidogyne mayaguensis.* Suma Phytopathologica 35(3):216-218.

Chetia, A., Gogoi, P., Roy, P., and Goswani, M. 2019. Antagonistic plants as a tool of nematode management. Journal of Medicinal Plants Studies 7(6):113-116.

Dudavera, N., and Pichersky, E. 2008. Metabolic engineering of plant volatiles. Current Opinion in Biotechnology 19(2):181-189.

Dudavera, N., Negre, F., Nagegowda, D. A., and Orlova, I. 2006. Plant volatiles: recent advances and future perspectives. Critical Reviews in Plant Science 25(5):417-440.

Fargette, M., Davies, K. G., Robinson, M. P., and Trudgill, D. L. 1994. Characterisation of resistance breaking *Meloidogyne incognita*-like populations using lectins, monoclonal antibodies and spores of *Pasteuria penetrans*. Fundamental and Applied Nematology 17(6):537-542.

Ferreira, D. F. 2014. Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia 35:1039-1042.

Galhano, C. I. C., Ryan, M. F., Santos, M. S. N., and Starisky, G. 1997. Interactions between Tannia (*Xanthosoma sagittifolium*) and the root-knot nematodes, *Meloidogyne megadora* and *M. javanica.* Nematropica 27(1):7-17.

Gomes, V. A., Campos, V. P., Silva, J. C. P., Silva, F. J., Silva, M. F., and Pedroso, M. F. 2020. Activity of papaya seeds (*Carica papaya*) against *Meloidogyne incognita* as a soil biofumigant. Journal of Pest Science 93(2):783-792.

Hussey, R. S., and Barker, K. R. A. 1973. Comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57(12):1025-1028.

Ilarslan, H., Palmer, R. G., Imsande, J., and Horner, H. T. 1997. Quantitative determination of calcium oxalate and oxalate in developing seeds of soybean (Leguminosae). American Journal of Botany 84(8):1042-1046.

Isman, M. 1999. Pesticides based on plant essential oils. Pesticide Outlook 10:68-72.

Jardim, I. N., Oliveria, D. F., Silva, G. H., Campos, V. P., and Souza, P. E. 2018. (E)-cinnamaldehyde from the essential oil of *Cinnamomum cassia* controls *Meloidogyne incognita* in soybean plants. Journal of Pest Science 91(1):479-487.

Jones, J., Gheysen, G., and Fenoll, C. 2011. Genomics and molecular genetics of plant-nematode interactions. Pp. 632-636 i*n* J. M. Nicol, ed. Current nematode threats to world agriculture. Dordrecht: Springer Science.

Kihika, R., Murungi, L. K., Coyne, D., Hassanali, A., Teal, P. E., and Torto, B. 2017. Parasitic nematode *Meloidogyne incognita* interactions with different *Capsicum annum* cultivars reveal the chemical constituents modulating root herbivory. Scientific Reports 7:2903.

Kolombia, Y. A., Kumar, P. L., Claudius-Cole, A. O., Karssen, G., Viane,N., Coyne, D., and Bert, W. 2016. First report of *Meloidogyne enterolobii* causing tuber galling damage on white yam (*Dioscorea rotundata*) in Nigeria. Plant Disease 100(10):2173.

Lewu, M. N., Yakubu, M. T., Adebola, P. O., and Afolayan, A. J. 2010. Effect of accessions of *Colocasia esculenta*-Based diets on the hepatic and renal functional indices of weanling wistar rats. Journal of Medicinal Food 13(5):1210-1215.

Lord, J. S., Lazzeri, L., Atkinson, H. J., and Urwin, P. E. 2011. Biofumigation for control of pale potato cyst nematodes: activity of brassica leaf

extracts and green manures on *Globodera pallida in vitro* and in soil. Journal of Agricultural and Food Chemistry 59(14):7882-7890.

Melo, O. D., Maluf, W. R., Gonçalves, R. J. S., Gonçalves Neto, A. C., Gomes, L. A. A., and Carvalho, R. C. 2011. Triagem de genótipos de hortaliças para resistência a *Meloidogyne enterolobii*. Pesquisa Agropecuária Brasileira 46(8):829-835.

Nist. 2013. Wnt signaling. (September 20, 2019). Chemistry Webook-National Institute of Standards and Technology, ed Wormbook.doi.org/10.18434/T4D303, http://webbook.nist.gov/chemistry/.

Ntalli, N. G., Manconi, F., Leonti, M., Maxia, A., and Caboni, P. 2011. Aliphatic ketones from *Ruta chalepensis* (Rutaceae) induce paralysis on root-knot nematodes. Journal of Agricultural and Food Chemistry 59(13):7098-7103.

de Oliveira, R. R., and Pasin, L. A. A. P. 2017. Ocorrência de oxalato de cálcio em plantas não relatadas como tóxicas. Revista Científica da FEPI - Revista Científica Universitas 6(3):1-4.

Olsson, L., and Hahn-Hägerdal, B. 1996. Fermentation of lignocellulosic hydrolysates for ethanol production. Enzyme and Microbial technology 18(5): 312-331.

Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Mendelingen Landbouwhogeschool Wageningen 66:1-46.

Patananan, A. N., Budenholzer, L. M., Eskin, A., Torres, E. R., and Clarke, S. G. 2015. Ethanol-induced differential gene expression and acetyl-CoA metabolism in a longevity model of the nematode *Caenorhabditis elegans*. Experimental Gerontology 61:20-30.

Pedroso, L. A., Campos, V. P., Barros, A. F., Justino, J. C., and Paula, L. L. 2019a. Activity against *Meloidogyne incognita* of volatile compounds produced during amendment of soil with castor bean cake. Journal of Nematology 1:1-10.

Pedroso, L. A., Campos, V. P., Barros, A. F., Silva, J. C. P., ASSIS, G. M., and Ribeiro, C. R. 2019b. Nematicidal activity of ethanol solutions on soybean cyst nematode *Heterodera glycines*. Journal of Nematology 1:1-11.

Pichersky, E., and Gang, D. R. 2000. Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends in Plant Science 5(10):439-445.

Ploeg, A. T., and Stapleton, J. J. 2001. Glasshouse studies on the effects of time, temperature and amendment of soil with broccoli plant residues on the infestation of melon plants by *Meloidogyne incognita* and *M. javanica*. Journal of Nematology 3(8):855-861.

Ploeg, A. 2008. Biofumigation to manage plant-parasitic nematodes. Pp. 239-248 *in* A. Ciancio, ed. Integrated Management and Biocontrol of Plant Pests and Diseases, vol. 2. Dordrecht: Springer Science.

Price, A. J., Charron, C. S., Saxton, A. M., and Sams, C. E. 2005. Allyl isothiocyanate and carbon dioxide produced during degradation of *Brassica juncea* tissue in different soil conditions. HortScience 40(6):1734-1739.

Puiatti, M. 2002. Manejo da cultura do taro. Pp. 203-252 *in* C. A. S. Carmo, ed. Inhame e taro: sistema de produção familiar. Vitória: Incaper.

Ramírez-Suárez, A., Rosas-Hernández, L., Alcasio-Rangel, S., and Powers, T. 2014. First report of the root-knot nematode *Meloidogyne enterolobii* parasitizing watermelon from Veracruz, Mexico. Plant Disease 98(3):428.

Seganfredo, R., Finger, F. L., Barros, R. S., and Mosquim, P. R. 2001. Influência do momento de colheita sobre a deterioração pós-colheita em folhas de taioba. Horticultura Brasileira 19:184-187.

Sena, S. B., Rocha, C. L. D., Santana, D. A. O., Aguiar, L. R., and Souza, A. C. R. 2017. Plantas tóxicas: análise *in loco* da existência no bairro areal em Porto Velho-Ro. Saber Científico V:1-13.

Silva, J. C. T., Oliveira, R. D. L., Jham, G. N., and Aguiar, N. D. C. 2008. Effect of neem seed extracts on the development of the soybean cysts nematode. Tropical Plant Pathology 33(3):171-179.

Silva, J. C. P., Campos, V. P., Freire, E. S., Terra, W. C., and Lopez, L. E. 2017. Toxicity of ethanol solutions and vapours against *Meloidogyne incognita*. Journal of Nematology 19:271-280.

Silva, M. F., Campos, V. P., Barros, A. F., Silva, J. C. P., Pedroso, M. P., Silva, F. J., Gomes, V. A., and Justino, J. C. 2020a. Medicinal plant volatiles applied against the root-knot nematode *Meloidogyne incognita*. Crop Protection 130:105057.

Silva, M. F., Campos, V. P., Barros, A. F., Terra, W. C., Pedroso, M. P., Gomes, V. A. Ribeiro, C. R., and Silva, F. J. 2020b. Volatile emissions of watercress (*Nasturtium officinale*) leaves and passion fruit (*Passiflora edulis*) seeds against *Meloidogyne incognita*. Pest Management Science 76(4):1413-1421.

Slusarenko, A. J., Pastel, A., and Portz, D. 2008. Control of plant disease by natural products: allicin from garlic as a case study. European Journal of Plant Pathology 121:313-322.

Sousa, R. M. O. F., Rosa, J. S., Silva, C. A., Almeida, M. T. M., Novo, M. T., Cunha, A. C., and Fernandes-Fereira, M. 2015. Larvicidal, molluscicidal and nematicidal activities of essential oils and compounds from *Foeniculum vulgare*. Journal of Pest Science 88(2):413-426.

Subbotin, A. S., and Chitambar, J. J. 2018. Plant parasitic nematodes in sustainable agriculture of North America. Northeastern: Springer.

Taylor, A. L., and Sasser, J. N. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Raleigh: North Carolina State University.

Tavares-Silva, C. A., Dias-Arieira, C. R., Puerari, H. H., Silva, E. J. D., and Izidoro Jr., A. 2017. Crambe–soybean succession on the management of

Pratylenchus *brachyurus* and *Meloidogyne javanica*. Summa Phytopathologica 43(4):316-320.

Wang, D., Rosen, C., Kinkel, L., Cao, A., Tharayil, N., and Gerik, J. 2009. Production of methyl sulfde and dimethyl disulfde from soil-incorporated plant materials and implications for controlling soilborne pathogens. Plant Soil 324:185-197.

Xie, G., Cui, H., Dong, Y., Wang, X., Li, X., Deng, R., Wang, Y., and Xie, Y. 2017. Crop rotation and intercropping with marigold are effective for root-knot nematode (*Meloidogyne* sp.) control in angelica (*Angelica sinensis*) cultivation. Canadian Journal of Plant Science 97:26-31.

CAPÍTULO 2

Xanthosoma sagittifolium EXTRACTS AGAINST *Meloidogyne enterolobii*[2]

Abstract

The application of *X. sagittifolium* extract directly on the field could be an interesting alternative control of root-knot nematodes. In the present study, we evaluated first the *X. sagittifolium* extract ability to inhibit hatching and kill *M. enterolobii* juveniles *in vitro*. Subsequently, we studied the nematicidal activity of this extract reducing the infectivity and reproduction in tomato plants. *X. sagittifolium* extract was prepared at a concentration of 25% (m:v) and diluted to 2.5; 5; 10; 15% (m:v). Water was the negative control and product based on *Bacillus amyloliquefaciens* the positive control. In *M. enterolobii* hatching tests, at extract in 25% concentration, there was no hatching of juveniles. The mortality of J_2 was 100% in the maximum concentration of the extract, there was no juveniles alive. In the greenhouse tests, *X. sagittifolium* leaf extract had a significant effect on the number of galls and eggs of *M. enterolobii.* There was a reduction of 17%, 24% and 21% in the number of galls per gram of root in the concentrations of 10, 15 and 25% of the extract, respectively. There was a 14% and 29% reduction in the number of eggs per gram of root in the concentrations of 15 and 25% of the extract, respectively. Treatment with product based on *B. amyloliquefaciens*, reduced the number of galls and eggs by 54% and 53%, respectively. Leaf extract of *X. sagittifolium* controlled *M. enterolobii*, as well as, *B. amyloliquefaciens*. Thus, extract represents a good control management that can be used.

Keywords: Alternative control. Araceae. Root-knot nematodes. Soil pathogens.

2.1 INTRODUCTION

Root-knot nematodes (RKN) (*Meloidogyne* spp.) are one of the most destructive and widespread plant-parasitic nematodes. They infest several plant species worldwide in tropical and subtropical fields (Jones *et al.* 2011). RKN can interfere with crop development and bring significant losses (Yao *et al.* 2010). Among the RKN the *Meloidogyne enterolobii* (Yang; Eisenback, 1983) is becoming an important species due to its high virulence and reproductive potential in important crops. Also, it can overcome the genetic resistance usually applied against RKN in many crops, like tomatoes and sweet-pepper (Bitencourt and Silva 2010; Brito *et al.* 2015).

[2] Capítulo 2 - Submetido na revista Journal of Plant Pathology, a formatação do texto segue as normas da revista.

The development of alternative and sustainable methods to control RNK constantly a challenge. Chemicals are usually used to control nematodes, whereas, alternative practices like biological control or amendments incorporation are less harmful to the environment and equally efficient (Dong 2012; Silva *et al.* 2018). The alternative control can be accomplished with seed cakes, oils essentials, soil biofumigation, crop rotation, volatile compounds, plant extracts, for example (Ntalli *et al.* 2013; Babaali *et al.* 2017; Xie *et al.* 2017; Chetia *et al.* 2019; Pedroso *et al.* 2019; Gomes *et al.* 2020a 2020b). The nematicidal activities of many plant compounds have been largely studied (Isman 1999; Topalović *et al.* 2020). Plants produce many secondary metabolic and volatile organic compounds (VOCs), such as terpenoids, phenylpropanoids, benzenoids, alcohols, fatty acid and amino acid derivatives (Pichersky and Gang 2000; Dudareva *et al.* 2006; Dudareva and Pichersky 2008). The alternative control using plant incorporation has the benefits of less interference in the soil microbiota and any negative effects are not persistent in the environment. Then, plant residues or extracts are a good option for control plant pathogens, like RKN (Jardim *et al.* 2018; Pedroso *et al.* 2019; Gomes *et al.* 2020a 2020b; Silva *et al.* 2020a 2020b).

Xanthosoma sagittifolium (L) Schott belongs to Araceae family and it is native from South America. This plant is known for different common names according to the region. *X. sagittifolium* grows without many climatic and nutritional requirements. Many people consume this plant as an eatable vegetable in Central American, Africa, and Asia due to its nutritional characteristics like proteins, carbohydrates, vitamins, thiamine, riboflavin, niacin, fibres, among others (Seganfredo *et al.* 2001). However, some species in Araceae family are highly toxic for some pathogens due to the calcium oxalate rapids, such as *Xanthosoma sagittifolium, Epipremnum pinnatum, Colocasia esculenta, Monstera deliciosa Colocasia antiquorum*, and *Dieffenbachia picta* (Sena *et al.* 2017). This compound is not toxic for humans, but it can be toxic for several organisms and act in their control (Ilarslan *et al*. 1997; de Oliveira and Pasin 2017). *X. sagittifolium* presents in its composition antinutritional factors, such as oxalates, proteinase inhibitors, phytates, tannins, alkaloids, steroids and cyanogenic glycosides (Puiatti 2002; Lewu *et al.* 2010). Several secondary compounds are detected in *X. sagittifolium* leaves by gas chromatography/mass spectrometry (GC-MS) due to their volatil. The main volatile organic compounds

(VOCs) from *X. sagittifolium* are alcohols, and ketones (Gomes *et al.* 2020b). Furthermore, *X. sagittifolium* is not a host plant for *Meloidogyne incognita, M. javanica* and *M. enterolobii*, which supports the idea of using these plants in infested areas for soil biofumigation (Gomes *et al.* 2020b).

There are few studies with *X. sagittifolium*. Galhano *et al.* (1997) reported that rhizome extract of this plant was efficient in reducing hatching and causing mortality of *M. megadora* (Whitehead, 1968). Therefore, considering the great potential of crop rotation and soil biofumigation for RKN control due *X. sagittifolium* low host suiability and high toxicity against *M. enterolobii* (Gomes *et al.* 2020b), this work aimed to verify if leaves aqueous extracts of *X. sagittifolium* also control *M. enterolobii*. The application of *X. sagittifolium* extracts directly on the field could be an interesting alternative control of plant-parasitic nematode. To verify these effects, we first, evaluated *X. sagittifolium* extract's ability to inhibit hatching and kill J_2 of *M. enterolobii* in laboratory. Subsequently, we studied the nematicidal activity of these extracts reducing the infectivity and reproduction in *Solanum lycopersicum* (L.) under greenhouse conditions.

2.2 MATERIALS AND METHODS

The experiments were carried out at the Laboratories of Nematology and greenhouses and Laboratory of Chemistry and Biochemistry of São Paulo State University - UNESP/FCA – Botucatu, SP, Brazil.

Nematode inoculum

The nematodes used in this work were obtained from pure populations of *M. enterolobii* maintained in tomato plants cv Santa Clara in a greenhouse. To extract the eggs, we used Hussey and Barker's technique (1973), modified by Boneti and Ferraz (1981). Iinfected roots were washed in tap water, chopped into 1 to 2 cm pieces, and grounded in a blender with 0.5% NaClO water solution for approximately 20 seconds. Finally, the material was placed into a 60-mesh screen over a 500-mesh screen, where the eggs were collected in water. Hatching chambers were prepared to obtain second-stage juveniles (J_2) (Southey 1970). The number of eggs and juveniles was adjusted using Peters chamber under an inverted microscope.

Xanthosoma sagittifolium extract preparation

Xanthosoma sagittifolium rhizomes, obtained from Jardim de Minas Company, were cultivated in a greenhouse to obtain leaves used in aqueous extract preparation at different concentrations. The extract was used against *M. enterolobii* in laboratory and greenhouse. Rhizomes were cultivated in spring-summer in a greenhouse under controlled temperature conditions so as not to exceed 30º C, and in this period, there was no occurrence of low temperatures (less than 20º C). The plants were subjected to daily irrigation and fertilization, according to their needs. No diseases and pests were observed during the experiments. The rhizomes were kept under water for 12 h and then transplanted to pots containing 2 L of an autoclaved substrate composed of a mixture of soil, sand and organic matter (1: 2: 1). Sixty days later, the leaves and stems of *X. sagittifolium* were collected, sterilized with 2.0 % NaClO water solution for 1 minute followed by a double wash in distilled sterile water. The excessive water was removed by a dried paper towel. To obtain the aqueous extract 200g of leaves of *X. sagittifolium* were crushed in an industrial blender with 600 mL of distilled sterile water, until all the material was completely ground, providing a final concentration of 25% (m:v). Then, 300 µg of Polyvinylpolypyrrolidone (PVPP) was added to this mixture to prevent oxidation of the plant material. The whole mixture was filtered through a voile cloth. The filtrates were kept in capped tubes and submerged in ice during all the process. Right after, the filtrate was centrifuged for 15 minutes at 6500 rpm. The supernatant was collected and represented the aqueous extract at 25% (m:v). The other concentrations were done by dilutions of 25% with distilled sterile water giving rise to a concentration of 2.5; 5; 10; and 15 (v:v). In the centrifugation process, the PVPP used is deposited on the bottom of the tube without interfering with the extract components. The extract was stored in a freezer at -70º C until use.

Effect of aqueous extract of *Xanthosoma sagittifolium* against *Meloidogyne enterolobii in vitro*

The hatching and mortality effects of *X. sagittifolium* aqueous extract against *M. enterolobii* was carried out by using the concentrations of 0 (control); 2,5; 5; 10; 15 and 25% of extract of *X. sagittifolium*. The experiments consisted of six treatments and five repetitions. To investigate the anti-hatching activity and mortality effect of different concentrations of extracts, 100 µL of nematode suspension, containing 30

eggs or J_2, were placed in a well of a polypropylene plate (ELISA). Each cell on the ELISA plate corresponds to an experimental unit. The cells were then filled with 900 µL of aqueous extract. In the control, the extract was replaced by 900 µL of distilled water. The number of eggs and juveniles per cell was evaluated soon after setting the experiment, in a light microscope with inverted objectives. The plates were then sealed with film paper and kept in an incubator at 27º C (±2 °C). In the plates with J_2 to evaluate the mortality numbers of mobile and immobile J_2 were evaluated shortly after 48 hours. NaOH (1 M) solution was used to prove the mortality of these nematodes (Chen and Dickson 2000). In the plates containing eggs to evaluate the hatching, after 15 days, the number of hatched J_2 was evaluated. Finally, the lethal concentrations (LC50 and LC95) were found.

Nematicidal effect of aqueous extract of *Xanthosoma sagittifolium* against *Meloidogyne enterolobii*

The nematicidal effect of aqueous extract of *X. sagittifolium* against *M. enterolobii* was carried out. The experiment consisted of five treatments and five repetitions. The treatments were 0 (negative control); 10; 15 and 25% of extract of *X. sagittifolium* and the biological nematicidal based on *Bacillus amyloliquefaciens* (isolated BV03, 42 g/L) (positive control). All the concentrations of leaf extract of *X. sagittifolium* were diluted from the extract of 25%. To investigate the nematicidal effect of the extract in greenhouse experiments, was used styrofoam trays with 76 holes of 120 cm^3 each cell containing commercial substrate (Carolina Soil – Pardinho, SP, Brazil) and a tomato seedling. To simulate an application via drench in the field, a dose of 3L / ha and a spray volume of 400L / ha was used. The tomato seedlings after completing 20 days received the inoculum and the treatments. 1 mL of 1000 eggs and eventual juveniles of *M. enterolobii* were inoculated by drilling holes in the soil with a glass stick around the roots of the tomato seedlings and injecting with a pipette the suspension of eggs and eventual juveniles. Then, were used 0.2 mL of treatments (extracts 10; 15; 25% or distilled water or *Bacillus amyloliquefaciens*). Each cell on the styrofoam tray represented an experimental unity, with five treatments and five repetitions too. The styrofoam trays with the plants were kept in a greenhouse for 60 days. The experiment was carried out in spring-summer in a greenhouse under controlled temperature conditions so as not to exceed 30º C, and in this period, there was no occurrence of low temperatures (less

than 20º C). The plants were subjected to daily irrigation and fertilization according to the crop needs. Regarding diseases and pests, no problems were observed during the experiments, as the greenhouse was disinfected and offered suitable conditions for the crop. 60 days after, the aerial part and the root system of tomatoes were evaluated. The aerial parts of the tomatoes were cut and weighed, corresponding to the fresh mass of the aerial part (FMAP). Right after, the aerial parts of tomatoes were dried in a forced air oven for 48 h at 65 °C and weighed, corresponding to the dry mass of the aerial part (DMAP). Then, the roots were removed, carefully washed in a beaker with water, dried on paper towels and weighed. The number of galls was estimated and the roots were chopped, and the eggs were extracted by Hussey and Barker's technique (1973), modified by Boneti and Ferraz (1981). The collected eggs were counted in Peters slide under an inverted light microscope (Leica, model DME). Finally, the reproduction factor of the nematodes was calculated (RF = final population/ initial population).

Statistical analysis

All experiments were carried out twice. The trials were arranged in a completely randomized design with five replicates per treatment. The data were previously submitted to normality tests (Shapiro-Wilk test) and homogeneity of variance of the errors (Bartlett's test). Once the assumptions were met, the F test was applied through analysis of variance (ANOVA). The experiments were analyzed separately. When the significance level ($P < 0.05$) occurred in greenhouse experiments, the means of each treatment were grouped and differentiated by the Tukey test, performed in Sisvar software (Ferreira 2014). When the significance level ($P < 0.05$) occurred in experiments *in vitro*, regression analysis was performed and the graphs were generated. For the assay of lethal concentrations (LC50 and LC95), in mortality experiments, Probit analysis was applied using Finney's method in Excel software by a Probit spreadsheet (Srinivasan 2004).

2.3 RESULTS

In the *in vitro* experiment, it was possible to observe a significant effect on the use of *X. sagittifolium* leaf extract in the hatching and mortality tests of *M. enterolobii*

J_2. As the concentration of the extract increased, the hatching was lower and the J_2 mortality rate was higher. In *M. enterolobii* hatching tests, all concentrations of the extract significantly reduced hatching compared to concentration 0 (negative control) ($P < 0.05$). At the concentration of 10% of the extract, only 10% of hatched J_2. Hatching was reduced by 95% at a concentration of 15% and at maximum concentration, 25% of the extract, there was no J_2 hatching. The hatch reduction was significant when compared to the control values, water treatment, with hatch around 80% (Figure 1-A).

In mortality tests, from the concentration of 5% of the extract, all concentrations caused mortality of juveniles. At a concentration of 5%, there was mortality of more than 80% of the J_2. In the concentration of 10 and 15% the mortality values were around 90% and in the maximum concentration of the extract, 25%, there were no J_2 alive. The increase in mortality was significant ($P < 0.05$) when compared to the control values, treatment with water, with 0% mortality of J_2 (Figure 1-B). Through Probit analysis, the mean LC_{50} and CL_{95} (average of experiments 1 and 2) of *X. sagittifolium* leaf extracts were 4.2 and 9.1%, respectively (Table 1).

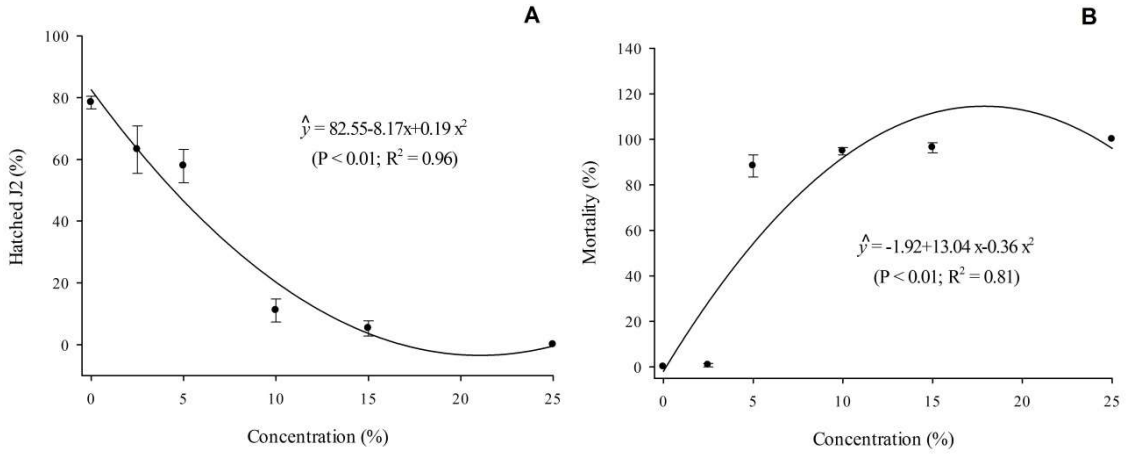

Figure 1. Effect of concentrations of *Xanthosoma sagitiifolium* leaf extract on the hatching (A) and mortality (B) of J_2 of *Meloidogyne enterolobii* under *in vitro* conditions.

Table 1. Lethal concentration (CL_{50} and CL_{95} - %) of *Xanthosoma sagittifolium* leaf extract against *Meloidogyne enterolobii*.

Experiments	LC^a_{50} (95% FL[c])	LC^b_{95} (95% FL[c])	Slop±SEM[d]	Degree of free-dom	Chi square
Experiment 1	4.2 (3.8-4.6)	9.3 (8.2-10.5)	5.3±0.7	4	59.0
Experiment 2	4.2 (3.9-4.7)	8.9 (8.0-10.0)	5.5±0.7	4	43.8
Average	4.2 (3.8-4.6)	9.1 (8.1-10.3)	5.4±0.7	4	51.4

[a] Concentration necessary to kill 50% of *M. enterolobii* J_2
[b] Concentration necessary to kill 95% of *M. enterolobii* J_2
[c] 95% lower and upper fiducial limits are shown in parenthesis
[d] SEM represents the standard error of the mean

In the greenhouse tests, *X. sagittifolium* leaf extract had a significant effect on the formation of galls and eggs of *M. enterolobii* ($P < 0.05$). By increasing the concentration of the extract, the reduction of gall and reduction also increased (Table 2). All concentrations of the extract reduced the number of galls. Comparing to water control, the number of galls per gram of root was reduced by 17%, 24% and 21% in the concentrations of 10, 15 and 25% of the extract, respectively. The positive control with *B. amyloliquefaciens* proved to be efficient, reducing galls by 54% (Table 2). Regarding the number of eggs, there was a 14% and 29% reduction in the concentrations of 15 and 25% of the extract, respectively. Treatment with *B. amyloliquefaciens* (positive control) proved to be efficient, reducing the number of eggs by 53% (Table 2).

The RF for the extract at a concentration of 25% and *B. amyloliquefaciens* (positive control) was very similar. The 25% extract obtained an RF = 14.5 and *B. amyloliquefaciens* (positive control) obtained an RF = 13. Both were significantly lower when compared to water (negative control) of RF = 20.6 (Table 2). Regarding the weight of the roots, *B. amyloliquefaciens* improved the root system. In this treatment, root production was 35% higher when compared to the other treatments (Table 2). The concentrations of 15 and 25% of the extract increased the aerial part dry mass (APDM) of the plant infested by the nematode by 30 and 28%, respectively. In the treatment with *B. amyloliquefaciens* (positive control), there was an increase in the APDM by 1% (Table 2).

Table 2. Different concentrations of *Xanthosoma sagittifolium* leaf extract controlling *Meloidogyne enterolobii* in a greenhouse experiment.

Treatments	Root (g)	Galls	Galls/g	Eggs	Eggs/g	RF	APFM (g)	APDM (g)
Water	4.44 a	224.40 c	50.80 c	20,634 b	4,662 c	20.63 b	11.96 a	6.56 a
Extract 10%	4.74 a	199.40 b	42.21 b	20,358 b	4,308 c	20.35 b	15.63 ab	7.09 a
Extract 15%	4.73 a	181.60 b	38.65 b	18,920 b	4,015 bc	18.92 b	16.60 b	8.42 b
Extract 25%	4.42 a	177.60 b	40.19 b	14,476 a	3,298 b	14.47 a	14.83 ab	8.60 b
B. amyloliquefaciens	5.97 b	137.40 a	23.44 a	13,032 a	2,224 a	13.03 a	27.76 c	9.93 c
CV (%)	11.01	7.00	10.68	7.19	10.78	7.19	12.55	6.49

2.4 DISCUSSION

Leaf extract of *X. sagittifolium* controlled *M. enterolobii*, as well as, *Bacillus amyloliquefaciens* (positive control). Thus, the use of extracts represents a good control measure that can be used, associated with other control measures, in areas with nematodes. The search for alternative methods for the control of nematodes has usually been done by certain specific groups of plants. However, several plant species, from different families, are known to present nematicidal properties (Sukul 1992; Ferris and Zheng 1999). The main compounds found in plants with nematicidal activity are polyethylene, acetylenes, alkaloids, fatty acids, and derivatives phenolic, like terpenoids, glucosinolates, isothiocyanates, sesquiterpenes and thienyls (Gommers and Bakker 1988; Chitwood 1992; Ferraz and deFreitas 2004). In this study, the tests were performed with a plant of non-conventional studied family (Araceae) against RKN.

Aqueous extract of *X. sagittifolium,* at different concentrations, was tested by *in vitro* and *in vivo* experiments against *M. enterolobii*. Despite the biofumigation in our previous works controlling RKN (Gomes *et al.* 2020b) there are no reports of extracts of *X. sagittifolium* controlling *M. enterolobii*. In addition, the previous study has shown that this plant species does not host *Meloidogyne* spp., even submitted to high RKN (Gomes *et al.* 2020b). The choice *X. sagittifolium* leaves for the extract was since they already have an effect in biofumigation tests, preparing aqueous extract

may be also applied as a sustainable alternative to control RLN. It is also believed that the secondary metabolites present in the leaves have a good potential for nematode control. Other studies have found that leaf extracts are often more efficient when compared to extracts from other organs (Moqrich *et al.* 2005; Saxena and Sharma 2005; Babaali *et al.* 2017).

In the present study, only the aqueous extract was used and it is possible to observe its efficiency in controlling *M. enterolobii* in tomatoes. The choice of making the aqueous extract came from using only water as a solvent and presenting a simple and easy to reproduce methodology. In addition, it is a methodology that poses no risk to the operator because it does not use a toxic solvent. There are very dangerous solvents, such as methanol, a substance that has high toxicity by inhalation or dermal absorption (Beattie *et al.* 2019). From this, we can conclude that the components present in *X. sagittifolium*, which act in the control of nematodes, can be extracted easily, using only water as a solvent. Another benefit found is that the aqueous extract is more suitable for applications in the field. The fact that it does not contain toxic organic solvents in its composition, it offers greater protection to crops (Caboni *et al*. 2013).

Plants of the Araceae family naturally present large amounts of calcium oxalate in their composition. The presence of calcium oxalate raffids makes these plants highly toxic (Ilarslan *et al.* 1997; de Oliveira and Pasin 2017). Vegetative species from other families may present this toxic compound in smaller quantities, such as *Medicago littoralis* (Fabaceae). When this species has calcium oxalate in its composition, it is less attractive to insects, thus reducing their diet. In this way, it was possible to verify that the chewing insects prefer to feed on the mutations of plants that do not have calcium oxalate. Thus, in plants without this compound, the damage is much greater (Oldach *et al.* 2014).

In addition to f calcium oxalate raffids in *X. sagittifolium* (Ilarslan *et al.* 1997; de Oliveira and Pasin 2017), 19 secondary metabolites were found in its volatilome by GC-MS analyzes (Gomes *et al.* 2020b). It is believed, then, that the extracts of *X. sagittifolium* were efficient in controlling 80% of hatching, as well as about 100% of the mortality of J_2 from *M. enterolobii* due to all these diversities of compounds found in this species. Then, it was possible to observe that the extracts of leaves of *X.*

sagittifolium also showed activity in tomato plants infected with *M. enterolobii*. Therefore, we can say that the methodology used for the extracts was able to preserve the major toxic components of the plant, enabling the control of these nematodes.

Besides RKN toxic effects, the tomato plants submitted to the extracts showed better development. With the increase in the concentration of extract, there was a greater growth and development of tomato plants infested with *M. enterolobii*. Extracts of other plant species had the same behaviour controlling RKN and estimulating plant growth by increasing the concentrations (Rafiee *et al*. 2019).

Therefore, the use of aqueous extract of *X. sagittifolium* is an efficient the management of control *M. enterolobii*. It is an alternative capable of reducing the pathogen populations in the area in a sustainable way. It is also a cost-effective technique that can be applied in the field in the presence of RKN.

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support and fellowships from: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

REFERENCES

Abdel-Salam MS, Ameen H, Soliman G, Elkelany U, Asar A (2018) Improving the nematicidal potential of *Bacillus amyloliquefaciens* and *Lysinibacillus sphaericus* against the root-knot nematode *Meloidogyne incognita* using protoplast fusion technique. Egyptian Journal of Biological Pest Control 28 (1): 31. DOI: https://doi.org/10.1186/s41938-018-0034-3

Andrade Filho A, Campolina D, Dias MD (2001) Toxicologia na prática clínica. Belo Horizonte: Folium.

Babaali D, Roeb J, Hammache M, Hallmann J (2017) Nematicidal potential of aqueous and ethanol extracts gained from *Datura stramonium, D. innoxia* and *D. tatula* on *Meloidogyne incognita*. Journal of Plant Diseases and Protection 124 (4): 339-348. DOI: https://doi.org/10.1007/s41348-017-0079-7

Beattie M, Beattie C, Morrison ED, Bayasi G (2019) Methanol Toxicity From Occupational Exposure: A Case Report. Annals of internal medicine 171 (2): 143-144. DOI: https://doi.org/10.7326/L18-0584

Becker JO, Zavaleta-Mejia E, Colbert SF, Schroth MN, Weinhold AR, Hancock JG, Van Gundy SD (1988) The effect of rhizobacteria on root-knot nematode and gall formation. Phytopathology 78: 1466–1469. DOI: https://doi.org/10.1094/Phyto-78-1466

Bitencourt NV, Silva GS (2010) Reprodução de *Meloidogyne enterolobii* em Olerícolas. Nematologia Brasileira 34 (3): 181-183.

Boneti JIS, Ferraz S (1981) Modificação do método de Hussey and Barker para extração de ovos de *Meloidogyne exigua* de cafeeiro. Fitopatologia Brasileira 6 (3): 553.

Brito JA, Smith T, Dickson DW (2015) First report of *Meloidogyne enterolobii* infecting *Artocarpus heterophyllus* Worldwide. Plant Disease 99 (9): 1284. DOI: https://doi.org/10.1094/PDIS-12-14-1292-PDN

Caboni P, Saba M, Tocco G, Casu L, Murgia A, Maxia A, Menkissoglu-Spiroud U, Ntalli N (2013) Nematicidal activity of mint aqueous extracts against the root-knot nematode *Meloidogyne incognita*. Journal of Agricultural and Food Chemistry 61: 9784–9788. DOI: https://doi.org/10.3389/fpls

Chen SY, Dickson DW (2000). A technique for determining live second-stage juveniles of *Heterodera glycines*. Journal of Nematology 32: 117-121.

Chetia A, Gogoi P, Roy P, Goswami M (2019). Antagonistic plants as a tool of nematode management. Journal of Medicinal Plants Studies 7 (6): 113-116.

Chitwood DJ (1992) Nematicidal compounds from plants. In Phytochemical resources for medicine and agriculture, eds. H. N. Nigg and D. Seigler, pp. 185-204. Plenum Press, New York.

de Oliveira RR, Pasin LAAP (2017) Ocorrência de oxalato de cálcio em plantas não relatadas como tóxicas. Revista Científica da FEPI - Revista Científica Universitas 6 (3): 1-4.

Dong S (2012) A review on root-knot nematode control with compost. Biol. Disa. Sci. 3: 245–248.

Dudavera N, Pichersky E (2008) Metabolic engineering of plant volatiles. Current Opinion in Biotechnology 19 (2): 181-189. DOI: https://doi.org/10.1016/j.copbio.2008.02.011

Dudavera N, Negre F, Nagegowda DA, Orlova I (2006) Plant volatiles: recent advances and future perspectives. Critical Reviews in Plant Science 25 (5): 417-440. DOI: https://doi.org/10.1080/07352680600899973

Ferraz S, de Freitas LG (2004) Use of antagonistic plants and natural products. In Nematology-Advances and Perspectives, Vol. II: Nematode Management and Utilization, eds. Z. X. Chen, S. Y. Chen and D. W. Dickson, pp. 931-977. CABI Publishing, Wallingford, UK.

Ferreira DF (2014) Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia 35: 1039–42.

Ferris H, Zheng L (1999) Plant sources of Chinese herbal remedies: effects on *Pratylenchus vulnus* and *Meloidogyne javanica*. Journal of Nematology 31: 241-263.

Galhano CIC, Ryan MF, Santos MSNA, Staritsky G (1997) Interactions between Tannia (*Xanthosoma sagittifolium*) and the root-knot nematodes, *Meloidogyne megadora* and *M. javanica*. Nematropica 27: 7–17.

Gomes VA, Campos VP, Silva JCP, Silva FJ, Silva MF, Pedroso MP (2020a) Activity of papaya seeds (*Carica papaya*) against *Meloidogyne incognita* as a soil biofumigant. Journal of Pest Science 93 (2): 783-792. DOI: https ://doi.org/10.1007/s1034 0-020-01192-z

Gomes VA, Silva FJ, Baquião EM, Faria VF, Ferreira JCA, Pedroso MP, Broetto F, Wilcken, SRS (2020b) *Xanthosoma sagittifolium* is resistant to *Meloidogyne* spp. and controls *Meloidogyne enterolobii* by soil biofumigation. Journal of Nematology 52: 1-12. DOI: https://doi.org/10.21307/jofnem-2020-107

Gommers FJ, Bakker J (1988) Physiological diseases induced by plant responses or products. In Diseases of Nematodes, Vol. I. eds. F. J. Gommers and J. Bakker, pp. 3- 22. CRS Press, Boca Raton, FL, USA.

Holdaway-Clarke TL, Weddle NM, Kim S, Robi A, Parris C, Kunkel JG, Hapler PK (2003) Effect of extracellular calcium, pH and borate on growth oscillations in *Lilium formosanum* pollen tubes. Journal of Experimental Botany 54: 65-72. DOI: https://doi.org/10.1093/jxb/erg004.

Hussey RS, Barker KRA (1973) Comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* 57 (12): 1025-1028.

Ilarslan H, Palmer RG, Imsande J, Horner HT (1997) Quantitative determination of calcium oxalate and oxalate in developing seeds of soybean (Leguminosae). American Journal of Botany 84(8):1042-1046. DOI: https://doi.org/10.2307/2446147.

Isman M (1999) Pesticides based on plant essential oils. Pesticide Outlook 10: 68–72.

Jardim IN, Oliveira DF, Silva GH, Campos VP, Souza PE (2018) (E)-cinnamaldehyde from the essential oil of Cinnamomum cassia controls *Meloidogyne incognita* in soybean plants. Journal of Pest Science 91:479–87. DOI: https://doi.org/10.1007/s10340-017-0850-3

Jones J, Gheysen G, Fenoll C (2011) “Genomics and molecular genetics of plant-nematode interactions”, In Nicol, J. M. (Ed.), Current Nematode Threats to World Agriculture. Dordrecht: Springer Science, pp. 632–6.

Lewu MN, Yakubu MT, Adebola PO, Afolayan AJ (2010) Effect of Accessions of *Colocasia esculenta*-Based Diets on the Hepatic and Renal Functional Indices of

Weanling Wistar Rats. Journal of Medicinal Food 13 (5): 1210-1215. DOI: https://doi.org/10.1089/jmf.2009.0211

Mercer CF, Greenwood DR, Grant JL (1992) Effect of plant and microbial chitinases on the eggs and juveniles of *Meloidogyne hapla* Chitwood. Nematologica 38: 227–236. DOI: https://doi.org/10.1163/187529292X00199

Messerli MA, Créton R, Jaffe LF, Robinson KR (2000) Periodic Increases in elongation rate precede increase in cytosolic Ca2+ during pollen tube growth. Developmental Biology 222: 84-98. DOI: https://doi.org/10.1006/dbio.2000.9709

Moqrich A, Hwang SW, Earley TJ, Petrus MJ, Murray AN, Spencer KSR, Andahazy M, Story GM, Patapoutian A (2005) Impaired Thermosensation in Mice Lacking TRPV3, a Heat and Camphor Sensor in the Skin. Science 307: 1468-1472. DOI: https://doi.org/10.1126/science.1108609

Navarro LA (2007) Caracterização dos cristais de oxalato de cálcio nas partes reprodutivas e vegetativas aéreas de *Ilex paraguariensis* A. St. Hil. (Aquifoliaceae). Revista Brasileira de Biociências 5 (S1): 762-764.

Ntalli NG, Nasiou E, Spiroudi U (2013) Evaluation of essential oils from rosemary, orange, lavandula and false yellowhead on hatching and motility of root-knot nematode. Journal of Agricultural Science and Technology 3: 603–616.

Oldach KH, Peck DM, Nair R, Sokolova M, Harris J, Bogacki P, Ballard R (2014) Genetic analysis of tolerance to the root lesion nematode *Pratylenchus neglectus* in the legume Medicago littoralis. BMC plant biology 14 (1): 100. DOI: https://doi.org/10.1186/1471-2229-14-100

Pedroso LA, Campos VP, Barros AF, Justino JC, de Paula LL (2019) Activity against *Meloidogyne incognita* of volatile compounds produced during amendment of soil with castor bean cake. Journal of Nematology 1: 1-10. DOI: https://doi.org/10.1163/15685411-00003319

Pichersky E, Gang DR (2000) Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends in Plant Science 5 (10): 439-445. DOI: https://doi.org/10.1016/s1360-1385(00)01741-6

Puiatti M (2002) Manejo da cultura do taro. Pp. 203-252 *in* C. A. S. Carmo, ed. Inhame e taro: sistema de produção familiar. Vitória: Incaper.

Rafiee F, Charehgani H, Abdollahi M (2019) Evaluation of burdock and Mountain almond leaf extracts against Meloidogyne javanica on tomato. Archives of Phytopathology and Plant Protection 52 (11-12): 1035-1047. DOI: https://doi.org/10.1080/03235408.2019.1689736

Raven JA, Smith FA (1976) Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytology 76: 415-431. DOI: https://doi.org/10.1111/j.1469-8137.1976.tb01477.x

Saharan BS, Nehra V (2011) Plant growth promoting Rhizobacteria: a critical review. Life Sciences and Medicine Research 1–30 LSMR-21.

Saxena R, Sharma R (2005) Efficacy of aqueous extracts of wild medicinal plants on J2 mortality of root knot nematode, *Meloidogyne incognita*. Environment and Ecology 23: 164–167.

Seganfredo R, Finger FL, Barros RS, Mosquim PR (2001) Influência do momento de colheita sobre a deterioração pós-colheita em folhas de taioba. Horticultura Brasileira 19: 184–7.

Sena SB (2017) Plantas tóxicas: análise in loco da existência no bairro areal em Porto Velho-ro.

Silva MF, Campo VP, Barros AF, Silva JCP, Pedroso MP, Silva FJ, Gomes VA Justino JC (2020a). Medicinal plant volatiles applied against the root-knot nematode *Meloidogyne incognita*. Crop Protection 130: 105057. DOI: https://doi.org/10.1016/j.cropro.2019.105057

Silva MF, Campo VP, Barros AF, Terra WC, Pedroso MP, Gomes VA, Ribeiro CR, Silva FJ (2020b) Volatile emissions of watercress (*Nasturtium officinale*) leaves and passion fruit (*Passiflora edulis*) seeds against *Meloidogyne incognita.* Pest Management Science 76:1413–21. DOI: https://doi.org/10.1002/ps.5654

Silva JCPS, Medeiros FHV, Campos VP (2018) Building soil suppressiveness against plant-parasitic nematodes. Biocontrol Science and Technology 28(5): 423-445. DOI: 10.1080/09583157.2018.1460316

Southey JF Laboratory methods for work with plant and soil nematodes. 5a ed. London: Ministery of Agriculture, Fischeries and Food, 1970. ISBN 0112427545.

Srinivasan MR (2004) Probit analysis. In: Palaniswamy S, Kuttalam S, Chandrasekaran S, Kennedy JS, Srinivasan MR (eds) Electronic manual on pesticides and environment department of agricultural entomology. TNAU, Coimbatore.

Sukul NC (1992) Plant antagonistic to plant-parasitic nematodes. Indian Revist Life Science 12: 23-52.

Topalović O, Bredenbruch S, Schleker ASS and Heuer H (2020) Microbes attaching to endoparasitic phytonematodes in soil trigger plant defense upon root penetration by the nematode. Frontiers Plant Science 11: 138. DOI: 10.3389/fpls.2020.00138

Xie G, Cui H, Dong Y, Wang X, Li X, Deng R, Wang Y, Xie Y (2017) Crop rotation and intercropping with marigold are effective for root-knot nematode (*Meloidogyne* sp.) control in angelica (*Angelica sinensis*) cultivation. Canadian Journal of Plant Science 97: 26-31. DOI: https://doi.org/10.1139/cjps-2016-0071

Whitehead AG (1968) Taxonomy of Meloidogyne (Nematoda: Heteroderidae) with descriptions of four new species. Transactions of the Zoological Society of London 31: 263-401.

Woo-Jin J, Soon-Ju J, Kyu-Nam A, Yu-Lan J, Ro-Dong P, Kil-Yong K, Bo-Kyoon S, Hwan-Tae K (2002) Effect of chitinase-producing *Baenibacillus illinoisensis* KJA 424 on egg hatching of root knot nematode (*Meloidogyne incognita*). Journal of Microbiology Biotechnology 12 (6): 865–871.

Yang B, Eisenback JD (1983) *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara earpode tree in China. Journal of Nematology 15 (1): 381-391.

Yang J, Liang L, Li J, Zhang K (2013) Nematicidal enzymes from microorganisms and their applications. Applied Microbiology and Biotechnology 97: 7081–7095. DOI: https://doi.org/10.1007/s00253-013-5045-0

Yao RY, Chen Z, Li Z, Yu S, Yong X, Fan L, Yang W (2010) Identification of the root-knot nematode disease on *Angelica sinensis* (Oliv.) *Diels*. Journal of Yunnan Agricultural Unversity 25: 45–48.

CONSIDERAÇÕES FINAIS

Os resultados apresentados nesta tese contribuem para o aprofundamento dos estudos de um manejo alternativo ao químico à nematologia agrícola. Crescem, cada vez mais, os problemas encontrados na agricultura causados por nematoides fitoparasitas. Diante da dificuldade de controle destes patógenos e a falta de produtos comerciais registrados no mercado, surge a necessidade de buscar alternativas para conter este problema no campo. Deste modo, com a cobrança da sociedade por medidas mais sustentáveis, métodos alternativos têm ganhado mais força nos estudos. Como exemplo, temos a utilização de plantas e seus metabólitos secundários, que podem ser utilizados associados a outras medidas de controle, sendo capazes de trazer inúmeras contribuições para o manejo de nematoides.

Plantas como *Xanthosoma sagittifolium* são pouco estudas e escassos são os trabalhos científicos realizados com elas. Neste trabalho foi possível observar a *X. sagittifolium* se comportando como resistente a *Meloidogyne incognita, M. javanica* e *M. enterolobii*. Por serem plantas resistentes, rústicas, de fácil propagação e cultivo, os trabalhos continuaram em relação a sua composição. *X. sagittifolium* apresentou metabólitos secundários majoritários pertencentes dos grupos dos álcoois e cetonas. Estas substâncias se mostraram eficientes no controle de *M. enterolobii*, quando testadas incorporadas no solo, na técnica de biofumigação, bem como quando testadas em extrato aquoso.

Pelo fato de serem resistentes e atuarem no controle de nematoides de grande importância econômica no Brasil, é possível utilizar esta planta no manejo de nematoides. A biofumigação, no geral, consiste em incorporar na área valores em torno de 50 t/ha de material vegetal na profundidade de 15 cm. No caso da *X. sagittifolium,* após os estudos realizados em casa de vegetação aplicando a técnica, infere-se que ao incorporar 60 t/ha é possível observar reduções significativas do fator de reprodução de *M. enterolobii*.

O custo de produção da cultura da *X. sagittifolium* varia a depender da região e disponibilidade do material vegetativo no mercado. No entanto, após implantada a cultura as exigências nutricionais da planta não encarecem o cultivo. A planta se desenvolve em diversos tipos de solos, apresenta boa adaptabilidade a diferentes condições climáticas e ambientais. Assim, a medida se torna eficiente em pequenas/médias áreas, o qual o produtor consegue realizar a compra do material

propagativo da *X. sagittifolium* para realizar a rotação de culturas em um talhão infestado por nematoides-das-galhas. Outra opção é o produtor mesmo multiplicar os rizomas na sua propriedade para uso futuro em áreas infestadas pelo patógeno.

A *X. sagittifolium* é uma planta de clima tropical, no entanto, se desenvolve bem em todas as estações do ano. A sua exigência está mais relacionada ao solo úmido, principalmente no período de implantação da cultura. O ciclo da *X. sagittifolium* dura cerca de 8 meses. Durante o período que a cultura se encontra na área, o produtor consegue comercializar as folhas em comércio local a partir do segundo mês, pois fazem parte da alimentação humana, gerando uma fonte de renda extra. Cabe ao produtor determinar quanto tempo ele pretende ficar com a cultura no talhão com fins de reduções populacionais dos nematoides fitoparasitas. É necessário levar em consideração a quantidade de massa fresca presente na área, sendo sugerido um período médio de 4 meses.

Após este período em que a planta está sendo cultivada em áreas infestadas, estima-se uma redução populacional pelo fato da espécie utilizada não ser hospedeira de nematoides-das-galhas. Além disso, ao realizar a incorporação do material vegetal no solo, possibilita a liberação dos compostos voláteis e não voláteis presentes nas plantas. Os compostos liberados atuam no controle destes patógenos.

Já em relação aos resultados encontrados nos testes fazendo uso de extratos de *X. sagittifolium* no controle de nematoides, verificamos outra possibilidade de controle. Os produtores que desejarem podem cultivar esta espécie vegetal e confeccionar seus próprios extratos para aplicar nas áreas infestadas. No entanto, outra opção viável são as indústrias desenvolverem novos produtos a base destes compostos presentes nestas plantas. As indústrias podem partir destes estudos preliminares e dar continuidade neste processo desenvolvendo novos produtos visto que produtos oriundos de plantas são menos persistentes no ambiente, atuando no controle do patógeno de forma mais sustentável.

Os testes realizados com os extratos *in vitro* mostraram resultados mais eficazes no controle de *M. enterolobii*. Isto provavelmente acontece, pois após a aplicação dos extratos no solo há volatilização dos compostos voláteis. Deste modo, temos atuando no controle apenas a presença dos compostos não voláteis. Um fator que pode auxiliar neste processo é a reaplicação dos extratos nas áreas infestadas. Assim, é possível introduzir constantemente o princípio ativo que atua no controle

dos nematoides e otimizar o controle. Outra medida que pode contribuir para reduzir a volatilização é a utilização de veículos para estes extratos. Ao encapsular ou utilizar um biopolímero, você possibilita a preservação dos compostos voláteis presentes nos extratos e os disponibiliza no solo, de forma lenta e gradual, fazendo com que o composto atinja o alvo e impeça a infectividade e/ou reprodução dos nematoides.

Quando comparados os custos das técnicas sugeridas nesse trabalho, com as medidas de manejo existentes, é possível verificar a sua aplicabilidade no campo. Atualmente, há uma necessidade de utilizar medidas associadas no manejo de nematoides para otimizar as reduções populacionais na área. Como existem poucos produtos químicos disponíveis no mercado, medidas como controle biológico, controle cultural, por meio de rotação de culturas, adubação verde e plantas não hospedeiras, são fundamentais para otimizar este processo.

O uso de plantas no controle de nematoides possibilita melhorias nas reduções populacionais do patógeno, bem como atua indiretamente melhorando a química, física e biologia do solo. Além disso, os extratos podem trazer melhorias às plantas, sendo considerados bons promotores de crescimento e desenvolvimento do sistema radicular e parte aérea. De modo geral, os resultados obtidos nesse estudo foram bastante promissores e relevantes contribuindo para a nematologia agrícola, área de grande importância na agricultura do Brasil e no mundo.

REFERÊNCIAS

ARAUJO, F.F.; BRAGANTE, R.J.; BRAGANTE, C.E. Controle genético, químico e biológico de meloidoginose na cultura da soja. **Pesquisa Agropecuária Tropical**, v. 42, n.2, p. 220-224, 2012.

BERGAMIN FILHO, A. Epidemiologia: conceitos e benefícios. In: BERGAMIN FILHO, A.; KIMATI, H.; AMORIM, L. (Ed.). **Manual de Fitopatologia: Princípios e Conceitos,** v. 1, p. 540-553, 1995.

BITENCOURT, N.V. & SILVA, G.S. Reprodução de *Meloidogyne enterolobii* em Olerícolas. **Nematologia Brasileira**, Piracicaba, v. 34, n. 3, p. 181-183, 2010.

CHARCHAR, J.M. Nematoides em Hortaliças. Concórdia: **Embrapa Hortaliças**, p. 12, 1995. (Embrapa Hortaliças. Circular Técnica, 18).

CHARCHAR, J.M.; VIEIRA, J.V.; FACION C.E. Controle de nematoides das galhas em cenoura através de rotação. **Fitopatologia Brasileira.** 25 (suplemento): 335 (Resumo). 2000.

ILARSLAN, H.; PALMER, R.G.; IMSANDE, J.; HORNER, H.T. Quantitative determination of calcium oxalate and oxalate in developing seeds of soybean (Leguminosae). **American Journal of Botany**, v. 84: p. 1042-1046, 1997.

ISMAN, M. Pesticides based on plant essential oils. **Pesticide Outlook**, v.10, p. 68-72, 1999.

JARDIM, I.N.; OLIVEIRA, D.F.; SILVA, G.H.; et al. (E)-cinnamaldehyde from the essential oil of *Cinnamomum cassia* controls *Meloidogyne incognita* in soybean plants. **Journal of Pest Science**, v. 91, p. 479-487, 2017.

JONES, J.T.; HAEGEMAN, A.; DANCHIN, E.G.; GAUR, H.S.; HELDER, J.; JONES, M.G.; KIKUCHI, T.; MANZANILLA-LÓPEZ, R.; PALOMARES-RIUS, J.E.; WESEMAEL, W.M.; PERRY, R.N. Top 10 plant-parasitic nematodes in molecular plant pathology. **Molecular Plant Pathology**, v.57, p. 946–961, 2013.

KARSSEN, G. & MOENS, M. Root-knot nematodes. In: PERRY, R. N.; MOENS, M. (Eds.). **Plant nematology**. Wallingford: CAB International, p. 59-90, 2006.

LAINETTI, R.; VIEIRA, A. C. M.; PEREIRA, N. A. **Revista Brasileira de Farmácia**, v. 80, p. 64, 1999.

LEWU, M.N.; YAKUBU, M.T.; ADEBOLA, P.O.; AFOLAYAN, A.J. Effect of Accessions of *Colocasia esculenta*-Based Diets on the Hepatic and Renal Functional Indices of Weanling Wistar Rats. **Journal of Medicinal Food**, v. 13, n. 5, p. 1210-1215, 2010.

MACHADO, A.C.Z. Nematoides devastam lavouras de soja. **Revista Campos e Negócios,** 2015. http://www.revistacampoenegocios.com.br/nematoides-devastam-lavouras-de-soja/. Accessed 10 April 2021.

NICOL, J.M.; TURNER, S.J.; COYNE, D.L.; et al. Current nematode threats to world agriculture. **Genomics and molecular genetics of plant – nematodes interactions**, Cham (ZG), Switzerland: Springer Science, cap. 2, 2011.

OLIVEIRA, R. R. & PASIN, L.A.A.P. Ocorrência de oxalato de cálcio em plantas não relatadas como tóxicas. **VII Congresso de Iniciação Científica da FEPI**, p. 1-4, 2016.

PEREIRA, F.H.F.; PUIATTI, M.; MIRANDA, G.V.; SILVA, D.J.H.; FINGER, F.L. Divergência genética entre acessos de taro. **Horticultura Brasileira**, Brasília, v.22, n.1, p. 55-60, 2004.

PUIATTI, M. Manejo da cultura do taro. In: CARMO, C.A.S., (Ed.) Inhame e taro: sistema de produção familiar. Vitória, ES: Incaper, p. 203-252, 2002.

SAVAGE, G.P.; MARTENSSON, L.; SEDCOLE, J.R. Composition of oxalates in baked taro (*Colocasia esculenta* var. Schott) leaves cooked alone or with additions of cows milk or coconut milk. **Journal of Food Composition and Analysis**, v. 22, p. 83-86, 2009.

SIKORA, R.A. & FERNANDEZ, E. Nematode parasites of vegetables. In: LUC, M., SIKORA, R. A., BRIDGE, J. (Ed.). **Plant parasitic nematodes in subtropical and tropical agriculture.** 2nd edition, CABI publishing, p. 319-392, 2005.

SILVA, G.A. **Métodos alternativos de controle de fitonematoides**. RAPP, v. 19, 2011.

SOUSA, R.M.O.F.; ROSA, J.S.; SILVA, C.A.; et al. Larvicidal, molluscicidal and nematicidal activities of essential oils and compounds from *Foeniculum vulgare*. **Journal of Pest Science**, v. 88, p. 413–426, 2015.

TOBE, H. & KADOKAWA, T. Endosperm development in the Araceae (Alismatales) and evolution of developmental modes in monocots. **Journal of Plant Research**, v. 123, p. 731, 2010.

TOPALOVIĆ, O.; BREDENBRUCH, S.; SCHLEKER, A.S.S.; HEUER, H. Microbes attaching to endoparasitic phytonematodes in soil trigger plant defense upon root penetration by the nematode**. Frontiers Plant Science**, v. 11, p. 138 2020.