



**UNIVERSIDADE FEDERAL DO CEARÁ
CENTRO DE CIÊNCIAS AGRÁRIAS
DEPARTAMENTO DE FITOTECNIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA - FITOTECNIA**

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**LETALIDADE E SUB-LETALIDADE DE ACARICIDAS UTILIZADOS NA
CULTURA DO COQUEIRO SOBRE *Amblyseius largoensis* (MUMA) (ACARI:
PHYTOSEIIDAE)**

FORTALEZA

2021

MARIA EDVÂNIA NEVES BARROS

LETALIDADE E SUB-LETALIDADE DE ACARICIDAS UTILIZADOS NA CULTURA
DO COQUEIRO SOBRE *Amblyseius largoensis* (MUMA) (ACARI: PHYTOSEIIDAE)

Tese apresentada ao Programa de Pós-Graduação em Agronomia/Fitotecnia da Universidade Federal do Ceará, como requisito parcial para obtenção do título de Doutora em Agronomia/Fitotecnia. Área de concentração: Entomologia/Acarologia.

Orientador: Prof. Dr. José Wagner da Silva Melo.

FORTALEZA

2021

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Sistema de Bibliotecas

Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

B2791 Barros, Maria Edvânia Neves.
Letalidade e sub-letalidade de acaricidas utilizados na cultura do coqueiro sobre Amblyseius largoensis
(MUMA) (Acari: Phytoseiidae) / Maria Edvânia Neves Barros. – 2021.
66 f. : il. color.

Tese (doutorado) – Universidade Federal do Ceará, Centro de Ciências Agrárias, Programa de Pós-Graduação em Agronomia (Fitotecnia), Fortaleza, 2021.
Orientação: Prof. Dr. José Wagner da Silva Melo.

1. Seletividade. 2. Controle químico. 3. Controle biológico. 4. Raoiella indica. 5. Efeito subletal. I. Título.
CDD 630

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Aprovada em: 21 / 12 / 2021.

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À Deus pela vida e coragem de seguir em frente, por me proporcionar saúde e sabedoria para chegar até aqui. À Nossa Senhora de Fátima por interceder e cuidar de mim.

Aos meus pais Aureny Barros da Silva e Francisco Neves Florindo por acreditarem em mim e investirem nos meus estudos. Aos meus irmãos: Evaneide, Edvalda, Edcleide, Edcláudia, Edson e Evanio por todo apoio, incentivo e ensinamentos.

Ao meu esposo Alfredo Mendonça de Sousa, por todo amor, paciência e compreensão.

AGRADECIMENTOS

Primeiramente agradeço a Deus, que mesmo em um momento pandêmico, ele me guardou e me livrou de todo mal. Por ter me dado forças, esperança e perseverança para chegar até aqui.

Aos meus pais Aureny e Francisco, minha fonte de luz, sabedoria e fé, por serem minha base com a qual posso contar, pelo apoio, incentivo e afeto. Em especial minha mãe, por seu trabalho como costureira e lavadeira, para investir suas economias nos meus estudos.

Às minhas irmãs Edclaudia, Edvalda, Edcleide e Evaneide, e aos meus irmãos Edson e Evânio, por me incentivarem nos estudos, por acreditarem e fazerem muito por mim. Também agradeço aos meus demais parentes: avós, sobrinhos, sobrinhas, tios, tias, primos e primas, os quais me ajudam com boas energias me incentivando sempre a buscar minhas conquistas.

Aos meus filhos peludos Bela e Belo, pelo amor que transmitem, pela calmaria e grandes lambeijos.

Agradeço a Universidade Federal do Ceará (UFC), por ter me proporcionado tantos momentos bons, realização pessoal e profissional. Agradeço também ao Programa de Pós-Graduação em Agronomia/ Fitotecnia da UFC pela acolhida e ensinamentos, juntamente com o Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela concessão da bolsa de estudo.

Agradeço aos amigos, que me acompanham desde a graduação como a Janequelle, Ricardo Pereira, Alfredo, Luana Soares, Rosana, Valsérgio, Israel e Berg, pelas risadas e tristezas que passamos juntos.

Agradeço ao meu amado, amigo e esposo Alfredo Mendonça, pelo apoio, conselhos, orientações e calmaria, quando minha pouca paciência acaba.

Agradeço aos companheiros (as) do Laboratório de Manejo de Ácaros e Insetos (LAMAI): Wesller, Manoel, Luana, Eduardo, Rosenya, Adson, Bruna, Felipe e Mateus pelos ensinamentos e troca de saberes. Aos amigos que já passaram pelo LAMAI: Josi, Neurilan, Neville, Jairo, Vivi, Jamison, Jackson e Júnior, pelos momentos de conversas e trabalho.

Obrigada em especial ao amigo Wesller por me ajudar nos experimentos e acolher minhas frustações, independente do dia e da hora.

Obrigada ao casal Wagner e Débora que tanto admiro, pelo respeito e apoio. Ao Prof.^o Dr. José Wagner S. Melo, por seu conhecimento genuíno e sua humildade, em transmitir aquilo que sabe. Por ser amigo, conselheiro e ter o dom de orientar. À doutora

Débora Barbosa a qual admiro e agradeço por suas conversas e ensinamentos.

Aos funcionários da Universidade Federal do Ceará pela competência e eficiência na prestação de serviços e a todos que, de alguma forma, contribuíram no desenvolvimento deste trabalho.

“Eu me identifico com os que erram, com os que se perdem, com os que duvidam, com os que não foram convidados. Durante boa parte de minha vida, eu pertenci ao grupo dos não desejados. Se hoje, por circunstâncias que nem julgo ser merecedor, desfruto de reconhecimento e de carinho, faço questão de acolher em meu coração os que estão nos mesmos lugares onde, um dia, por Deus eu fui encontrado” (Pe. Fábio de Melo)

“Seja forte e corajoso! Não se apavore nem desanime, pois o Senhor, o seu Deus, estará com você por onde você andar” (Josué 1:9)

RESUMO

O ácaro *Amblyseius largoensis* Muma tem sido citado como um importante agente de controle biológico do ácaro-praga *Raoiella indica* Hirst. Uma vez que *A. largoensis* não se encontra disponível comercialmente, produtores de coco têm utilizado produtos registrados para outro ácaro-praga na cultura do coqueiro (*Aceria guerreronis* Keifer) visando minimizar as perdas ocasionadas por *R. indica*. No entanto, não se conhece o efeito de tais produtos sobre as populações de *A. largoensis*, as quais ocorrem naturalmente em folíolos de coqueiro infestados com *R. indica*. A contribuição natural (predação) deste predador pode ser explorada através de estratégias de controle que possibilitem sua conservação, destacando-se, nesse sentido, a necessidade de utilização de produtos seletivos. O presente estudo teve como objetivo avaliar o efeito de acaricidas registrados para a cultura do coqueiro (azadiractina, abamectina, clorfenapir e fenpiroximato) sobre a sobrevivência, consumo, oviposição, eficiência de conversão de alimento em ovos e sobre as respostas, funcional e numérica do ácaro predador *A. largoensis*. Os acaricidas abamectina, fenpiroximato e clorfenapir reduziram a sobrevivência de fêmeas de *A. largoensis*. Abamectina foi o único acaricida a afetar a predação, oviposição e eficiência de conversão de alimento de *A. largoensis*. *Amblyseius largoensis* exibiu resposta funcional tipo II quando alimentado com ovos de *R. indica*, não sendo o tipo de resposta funcional alterado pela exposição aos acaricidas. Apesar de não alterar o tipo de resposta funcional, a exposição à abamectina resultou em um decréscimo no número médio de presas consumidas pelo predador. A exposição aos acaricidas elevou o tempo de manipulação de presas em 67%, 25%, 38% e 35% para abamectina, azadiractina, fenpiroximato e clorfenapir, respectivamente. A exposição à abamectina reduziu a taxa de ataque em 52%. A resposta numérica foi afetada apenas pela exposição à abamectina, onde apenas 60% das fêmeas realizaram oviposição e independentemente da densidade de presas ofertadas o número médio de ovos/fêmea/dia foi sempre inferior a 0,36. A eficiência de conversão do alimento em biomassa de ovos reduziu com o aumento das densidades de presas, e essa tendência não foi alterada pela exposição aos acaricidas. Apesar de não alterar a tendência de eficiência de conversão do alimento em biomassa de ovos, a exposição à abamectina comprometeu drasticamente a oviposição de *A. largoensis* não sendo observado um acréscimo na produção de ovos com o aumento da densidade de presas.

Palavras-chave: Seletividade; controle químico; controle biológico; *Raoiella indica*; efeito subletal.

ABSTRACT

The mite *Amblyseius largoensis* Muma has been cited as an important biological control agent for the pest mite *Raoiella indica* Hirst. Since *A. largoensis* is not commercially available, coconut producers have used products registered for another pest mite in coconut (*Aceria guerreronis* Keifer) in order to minimize the losses caused by *R. indica*. However, the effect of such products on populations of *A. largoensis*, which naturally occur in coconut leaflets infested with *R. indica*, is not known. The natural contribution (predation) of this predator can be explored through control strategies that allow its conservation, highlighting, in this sense, the need to use selective products. The present study aimed to evaluate the effect of registered acaricides for coconut (azadirachtin, abamectin, chlorfenapyr and fenpyroximate) on survival, consumption, oviposition, efficiency of food conversion into eggs and on the functional and numerical responses of the predatory mite *A. largoensis*. The acaricides abamectin, fenpyroximate and chlorfenapyr reduced the survival of females of *A. largoensis*. Abamectin was the only acaricide to affect predation, oviposition and food conversion efficiency of *A. largoensis*. *Amblyseius largoensis* exhibited a type II functional response when fed with *R. indica* eggs, and the type of functional response was not altered by exposure to acaricides. Despite not altering the type of functional response, exposure to abamectin resulted in a decrease in the average number of prey consumed by the predator. Exposure to acaricides increased prey handling time by 67%, 25%, 38% and 35% for abamectin, azadirachtin, fenpyroximate and chlorfenapyr, respectively. Exposure to abamectin reduced the attack rate by 52%. The numerical response was affected only by exposure to abamectin, where only 60% of the females performed oviposition and, regardless of the density of prey offered, the average number of eggs/female/day was always lower than 0.36. The efficiency of food conversion into egg biomass decreased with increasing prey densities, and this trend was not altered by exposure to acaricides. Despite not altering the trend of efficiency of conversion of food into egg biomass, exposure to abamectin drastically compromised the oviposition of *A. largoensis*, not being observed an increase in egg production with increasing prey density.

Keywords: Selectivity; chemical control; biological control; *Raoiella indica*; sublethal effects.

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1 CAPÍTULO 1 – INTRODUÇÃO GERAL

O principal grupo de inimigos naturais de ácaros fitófagos é formado por ácaros predadores da família Phytoseiidae (MORAES, 2002). Essa família contém mais de 2.700 espécies (DEMITE *et al.*, 2021), as quais são comumente encontradas sobre plantas em associação com diferentes grupos de artrópodes como ácaros fitófagos, tripes e moscas-brancas (HELLE; SABELIS, 1985; GERSON *et al.*, 2003; ZHANG, 2003; MCMURTRY *et al.*, 2013). Pouco mais de 200 espécies de fitoseídeos já foram relatadas no Brasil (DEMITE *et al.*, 2021). Algumas espécies de fitoseídeos são produzidas em laboratório e liberados em casa de vegetação e/ou campo para controle de ácaros fitófagos (MCMURTRY *et al.*, 1978; PICKETT; GILSTRAP, 1986; RASMY; ELLAITHY, 1988; PEÑA; OSBORNE, 1996). Dentre as principais características relacionadas para que estes organismos sejam amplamente utilizados em programas de controle biológico pode-se citar o seu baixo requerimento alimentar, rápido desenvolvimento, alta persistência em plantas com baixa infestação de presas e capacidade de sobrevivência em substratos alternativos (MORAES; FLECHTMANN, 2008).

Dentre os fitoseídeos destaca-se o ácaro *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) por sua ocorrência em regiões tropicais e subtropicais do mundo, frequentemente associados a plantas perenes (DEMITE *et al.*, 2021), como o coqueiro (*Cocos nucifera L.*) (LAWSON-BALAGBO *et al.*, 2008; NEGLOH *et al.*, 2011; MELO *et al.*, 2015). Quanto a amplitude da dieta, *A. largoensis* é classificado como ácaro predador generalista do tipo III, podendo se alimentar de ácaros de várias famílias, incluindo eriofiídeos, tarsonemídeos, tetraniquídeos e tídeídeos, bem como de várias espécies de insetos, como tripes e moscas brancas (GERSON; WEINTRAUB, 2012; MCMURTRY *et al.*, 2013). Indivíduos de *A. largoensis* podem também utilizar exsudados vegetais (JAMES, 1989; KREITER *et al.*, 2002; NOMIKOU *et al.*, 2003; GNANVOSSOU *et al.*, 2005), néctar (VAN RIJN; TANIGOSHI, 1999), e pólen (BROUFAS; KOVEOS, 2000; ABDALLAH *et al.*, 2001; NOMIKOU *et al.*, 2001; VANTORNHOUT *et al.*, 2005) como fonte de alimento. No coqueiro, essa espécie é encontrada principalmente em folhas ou superfície de frutos e raramente sob as brácteas dos frutos de coco (LAWSON-BALAGBO *et al.*, 2008; REI *et al.*, 2008; MELO *et al.*, 2015).

Amblyseius largoensis é considerado um importante inimigo natural de duas pragas do coqueiro: o ácaro-da-necrose-do-coqueiro, *Aceria guerreronis* Keifer (CARRILLO *et al.*, 2012; MELO *et al.*, 2015) e o ácaro-vermelho-das-palmeiras, *Raoiella indica* Hirst

(Acari: Tenuipalpidae) (CARRILLO *et al.*, 2014). *Aceria guerreronis* reside sob o perianto dos frutos, onde se alimenta do tecido meristemático que é protegido por brácteas florais (HOWARD; ABREU RODRIGUEZ, 1991; SABELIS; BRUIN, 1996; AMBILY; MATHEW, 2003; FERNANDO *et al.*, 2003; SIRIWARDENA *et al.*, 2005; ARATCHIGE *et al.*, 2007; LAWSON-BALAGBO *et al.*, 2007; SILVA *et al.*, 2017). As brácteas do fruto atuam como uma barreira física, dificultando o acesso ao microhabitat ocupado pelo ácaro (*A. guerreronis*) (ARATCHIGE *et al.*, 2007; LAWSON-BALAGBO *et al.*, 2007; MELO *et al.*, 2014; MELO *et al.*, 2015). Uma vez que *A. largoensis* não pode acessar a área sob as brácteas, provavelmente se alimente de *A. guerreronis* quando estes saem da região meristemática para se dispersarem (MELO *et al.*, 2015). Colônias de *R. indica* se desenvolvem na parte abaxial das folhas alimentando-se das células do mesófilo foliar e causando um amarelecimento gradual, seguido de bronzeamento e necrose dos folíolos (CARRILLO *et al.*, 2012; OCHOA *et al.*, 2011). Dentre os artrópodes associados a *R. indica*, *A. largoensis* tem sido o inimigo natural mais frequentemente e abundante (GALLEGO *et al.*, 2003; ETIENNE; FLECHTMANN, 2006; HOY *et al.*, 2006; PEÑA *et al.*, 2009; ZANNOU *et al.*, 2010; TAYLOR *et al.*, 2011; GONDIM *et al.*, 2012; MORAES *et al.*, 2012). Estudos demonstraram que: (i) *A. largoensis* é capaz de se alimentar, desenvolver e reproduzir em uma dieta constituída unicamente pelo *R. indica* (CARRILLO *et al.*, 2010; CARRILLO; PEÑA, 2011); (ii) a dinâmica populacional de *R. indica* e *A. largoensis* estão relacionadas (GONDIM JR *et al.*, 2012; BARROS *et al.*, 2020), sugerindo interação presa-predador; e (iii) há incremento populacional de *A. largoensis* após a colonização das plantas de coqueiro por *R. indica* (PEÑA *et al.*, 2009).

Apesar da importância dos ácaros predadores para a cultura do coqueiro, o principal método empregado para controle dos ácaros-pragas nesta cultura é o químico. No Brasil há atualmente 9 acaricidas registrados para a cultura do coqueiro (MAPA, 2021), sendo abamectina, azadiractina, fenpiroximato e clorfenapir os princípios ativos mais utilizados (LIMA *et al.*, 2013a; LIMA *et al.*, 2013b; LIMA *et al.*, 2015a; LIMA *et al.*, 2015b; LIMA *et al.*, 2016). Estes normalmente têm sido utilizados para controle de *A. guerreronis* com pulverizações direcionadas para os cachos em desenvolvimento (MELO *et al.*, 2012), pulverizações direcionadas para *R. indica* não tem sido amplamente praticada talvez devido a elevada área da copa da planta (entre 30 e 45 folhas). Ainda assim, as folhas devem receber cargas e derivas das pulverizações direcionadas aos cachos devido a própria arquitetura da planta, com cachos inseridos nas bases das folhas e emitidos a aproximadamente 120° um do outro (SOBRAL, 1994).

Independentemente da eficácia do uso dos acaricidas, em plantios onde os acaricidas são utilizados, organismos não alvos como ácaros predadores podem ser expostos aos acaricidas tanto através do contato direto durante o processo de pulverização quanto por contato com superfícies contaminadas durante o forrageamento ou ainda através da alimentação com presas contaminadas (JEPSON, 1989; AHMAD *et al.*, 2003; HUA *et al.*, 2004; TORRES; RUBERSON, 2004). Após esta exposição podem ser observados efeitos letais ou subletais sobre os organismos (DESNEUX *et al.*, 2007; GUEDES *et al.*, 2016; GUEDES *et al.*, 2017). Tais efeitos têm sido usados na avaliação da segurança de acaricidas para ácaros predadores (BOLLER *et al.*, 2005), e incluem desde a mortalidade até impactos na longevidade, fecundidade, taxa de desenvolvimento, razão sexual e comportamento (POLETTI *et al.*, 2007; NADIMI *et al.*, 2009; TEODORO *et al.*, 2009; HAMEDI *et al.*, 2011; LIMA *et al.*, 2013; LIMA *et al.*, 2015; LIMA *et al.*, 2016). No entanto, ainda não existe uma compreensão completa do impacto dos acaricidas sobre o potencial dos ácaros predadores em suprimir as populações de pragas.

A supressão da população de uma praga por um predador é dependente de dois componentes da interação presa-predador: a resposta funcional e a resposta numérica do predador (HOLLING, 1959; HOLLING, 1965). A resposta funcional é definida como a relação entre o número de presas atacadas por um único predador durante um determinado intervalo de tempo e densidade da presa (SOLOMON, 1949). Holling (1959), ao estudar a resposta funcional de predadores invertebrados em detalhes, propôs tipos distintos de respostas funcionais: sendo a resposta funcional do tipo I caracterizada pela morte de presas independente da densidade; resposta funcional do tipo II caracterizada pelo decréscimo percentual de presas mortas com o aumento da densidade de presas; e resposta funcional do tipo III, onde, ao longo de uma certa faixa de densidades crescentes de presas, uma porcentagem crescente de presas é morta que então se estabiliza sob a influência do tempo de manipulação ou saciedade do predador. A resposta numérica representa a mudança na densidade do predador em função da mudança na densidade de presas (SOLOMON, 1949; HASSEL, 1978). Segundo Hassell (1966), a resposta numérica de um predador é dividida em dois componentes, a resposta reprodutiva e a resposta agregativa. A resposta reprodutiva de um predador está relacionada a alteração de sua densidade devido a oviposição e sobrevivência de seus descendentes em diferentes densidades de presas, enquanto que a resposta agregativa representa a alteração na distribuição do predador mediadas pelas alterações nas densidades de presas (HASSEL, 1966).

Dentre os diversos fatores que podem afetar a resposta funcional e numérica de

ácaros predadores, tem-se a exposição a acaricidas (POLLETI *et al.*, 2007; LIMA *et al.*, 2015; TEODORO *et al.*, 2020; SOUZA NETO *et al.*, 2020). Tal exposição tem comumente alterado parâmetros importantes associados a resposta funcional tais como a taxa de ataque (determina a capacidade de captura de um predador dentro de uma determinada área) e o tempo de manipulação (tempo necessário para um predador identificar, atacar, subjugar e consumir uma presa específica) (POLLETI *et al.*, 2007; LIMA *et al.*, 2015; SOUZA NETO *et al.*, 2020). Entretanto, alteração no tipo de resposta funcional de ácaros predadores mediante a exposição a acaricidas tem sido recentemente demonstrada (TEODORO *et al.*, 2020). Apesar dos diversos estudos, os efeitos dos acaricidas sobre a resposta funcional de muitos ácaros predadores importantes ainda não foram investigados. A exposição a acaricidas pode afetar também a resposta numérica em ácaros estimulando (hormese) (CORDEIRO *et al.*, 2013) ou limitando a produção de ovos (HAMEDI *et al.*, 2011; PARK *et al.*, 2011; LIMA *et al.*, 2016; TEODORO *et al.*, 2020; SOUZA NETO *et al.*, 2020). A eficiência na conversão de biomassa de presas em progênies do predador por meio do aumento da oviposição em altas densidades de presas pode determinar o sucesso de um ácaro predador em um programa de controle biológico (FATHIPOUR *et al.*, 2020).

O presente estudo teve como objetivo avaliar o efeito de acaricidas registrados para a cultura do coqueiro (azadiractina, abamectina, clorfenapir e fenpiroximato) sobre a sobrevivência, consumo, oviposição, eficiência de conversão de alimento em ovos e sobre as respostas, funcional e numérica do ácaro predador *A largoensis*.

NOTA¹.

¹Esta tese segue normas da Revista: Systematic and Applied Acarology, com adaptações para as normas do guia de normalização de trabalhos acadêmicos da Universidade Federal do Ceará (UFC).

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2 CAPÍTULO 2 – BIOPESTICIDE AND ACARICIDES IMPAIR SURVIVAL, PREDATION, OVIPOSITION, AND CONVERSION OF FOOD INTO EGGS OF A PHYTOSEID MITE, *Amblyseius largoensis* (ACARI: PHYTOSEIIDAE)

ABSTRACT

Amblyseius largoensis (Muma) is a predatory mite that occurs naturally in plants infested with the pest mite, *Raoiella indica* Hirst, and has been reported as a potential predator for the control of *R. indica*. Since *A. largoensis* is not available commercially, coconut producers have used products registered for another coconut pest mite (*Aceria guerreronis* Keifer) against *R. indica* with the aim of minimizing economic losses caused by this pest. However, it is possible to explore the natural contribution of this predator using control strategies that allow its conservation. Thus, in the present study, the effects of pesticides registered for the coconut crop (azadirachtin, abamectin, chlorfenapyr, and fenpyroximate) on the survival, consumption, oviposition, and feed conversion efficiency of *A. largoensis* were evaluated to determine the compatibility between acaricides and the predatory mite. The results showed that the acaricides abamectin, fenpyroximate, and chlorfenapyr reduced the survival of *A. largoensis* females. Abamectin was the only acaricide that reduced predation, oviposition, and the feed conversion efficiency of *A. largoensis*. Azadirachtin did not affect any of the assessed parameters. These results suggest a potential compatibility *A. largoensis* with the pesticides azadirachtin, fenpyroximate, and chlorfenapyr.

Keywords: Sublethal effects; chemical control; biological control; selectivity; conservation biological control.

2.1 Introduction

The predatory mite, *Amblyseius largoensis* Muma (Acari: Phytoseiidae), is found in different parts of the world and is considered a pantropical species (DEMITE *et al.*, 2021; GÓMEZ-MOYA *et al.*, 2018). This predator has been reported in 59 countries and associated with over 250 plant species (GÓMEZ-MOYA *et al.*, 2018). Many such plant species are represented by perennial plants, such as the coconut plant (*Cocos nucifera* L.) (LAWSON-BALAGBO *et al.*, 2008; NEGLOH *et al.*, 2011; MELO *et al.*, 2015; GÓMEZ-MOYA *et al.*, 2018). In the coconut plant, *A. largoensis* is found mainly on the leaflets and on the surface of

fruits (LAWSON-BALAGBO *et al.*, 2008; REIS *et al.*, 2008; LIMA *et al.*, 2012), but it is also seen (less frequently) on the meristematic region of the fruit (the region covered by flower bracts) (MELO *et al.*, 2015).

Amblyseius largoensis is an important predator of two pest mites that occur in the coconut crop: *Aceria guerreronis* Keifer (Acari: Eriophyidae) (GALVÃO *et al.*, 2007; GALVÃO *et al.*, 2008; MELO *et al.*, 2015; GÓMEZ-MOYA *et al.*, 2018) and *Raoiella indica* Hirst (Acari: Tenuipalpidae) (CARRILLO *et al.*, 2012a; DOMINGOS *et al.*, 2013; NAVIA *et al.*, 2014; MENDES *et al.*, 2018; LIMA *et al.*, 2018). The association of *A. largoensis* with *R. indica* has been reported in several countries, including Brazil (CARRILLO *et al.*, 2012b; VASQUÉS e DE MORAES, 2012; GONDIM JR. *et al.*, 2012; DOMINGOS *et al.*, 2013). The potential of this predator to control *R. indica* has been demonstrated in its ability to feed, develop, and reproduce on a diet composed solely of *R. indica* (GALLEGO *et al.*, 2003; RAMOS *et al.*, 2010; BOWMAN e HOY, 2012; TAYLOR *et al.*, 2011; DOMINGOS *et al.*, 2013). Releases of *A. largoensis* have also resulted in a marked reduction in the population of *R. indica* (CARRILLO *et al.*, 2014). Moreover, changes in functional response parameters have been shown in this predator, but not in the type of response when it had previous experience of feeding on *R. indica*. *Amblyseius largoensis*, therefore, can potentially be used as a biological control agent of *R. indica* in recently colonized areas, especially at low pest densities (MENDES *et al.*, 2018).

The natural occurrence of *A. largoensis* in plants infested with *R. indica* makes Biological control a promising strategy for the management of this pest. Because *A. largoensis* is not available commercially, strategies involving releases of this predator are still not viable. However, it is possible to explore the natural contribution of this predator using control strategies that allow its conservation (survival and persistence) in areas infested with *R. indica*. Although there are no products registered against *R. indica* in Brazil, coconut producers have used products registered for *A. guerreronis* to control *R. indica*, with the aim of minimizing economic losses caused by this pest. Assis *et al.* (2013) demonstrated that some products registered for use against *A. guerreronis* may also be effective against *R. indica*, albeit at different doses. Based on a comparison of the lethal concentrations estimated for *R. indica* and *A. largoensis*, they concluded that some product registered for *A. guerreronis* are selective for *A. largoensis*. Despite the importance of the results obtained by Assis *et al.* (2013), they are not sufficient to guarantee the selectivity of the tested products. Lethality is only one of the effects observed after an organism has been exposed to a pesticide; sublethal effects also occur and are probably more frequent in non-target organisms

(e.g. natural enemies). The potential sublethal effects on predatory mites include: changes in walking activity (LIMA *et al.*, 2013b; VÉLEZ *et al.*, 2019) or dispersal (MONTEIRO *et al.*, 2018); compromised foraging and predation (LIMA *et al.*, 2015a; SOUSA NETO *et al.*, 2020); impaired mating (LIMA *et al.*, 2015b); reduction in instantaneous growth rate (LIMA *et al.*, 2013a); delayed oviposition (LIMA *et al.*, 2015b); and/or compromised longevity (HAMEDI *et al.*, 2010).

The identification of pesticides that cause minimal effects on natural enemies is necessary for the integration of biological control with chemical control (HASSAN *et al.*, 1991). The present study assessed the effects of acaricides, registered for the coconut crop against *A. guerreronis*, on the survival, consumption, oviposition, and feed conversion efficiency (FCE) of *A. largoensis*, an important natural enemy of *R. indica*.

2.2 Material and Methods

Collection and rearing of mite species

Coconut leaflets infested with *R. indica* were collected at the campus of the Federal University of Ceará, Fortaleza, Ceará, Brazil. The leaflets were inspected under a stereoscopic microscope and *A. largoensis* individuals (~200 females) were collected for the establishment of rearing units. These units consisted of 10 x 10 cm flexible polyvinyl chloride (PVC) discs that were placed on a polyethylene foam disc (15 cm diameter and 1 cm thickness) inside plastic trays (16 cm diameter and 2.5 cm depth). The edges of the PVC discs were covered with absorbent cotton wool and moistened with distilled water to prevent mites from escaping. The predators were fed a diet of castor bean (*Ricinus communis* L.) pollen and *R. indica* eggs. The eggs of *R. indica* were offered on fragments of coconut leaflets infested with approximately 100 eggs per leaflet. Food was provided daily. The rearing units were kept at $27.5 \pm 1.0^{\circ}\text{C}$, $70 \pm 10\%$ RH, and a photoperiod of 12:12 LD.

Tested pesticides

In Brazil, no pesticide has been registered for the control of *R. indica* (AGROFIT, 2020). Therefore, products commonly used for the coconut crop and registered for the control of a different species of pest mite, *A. guerreronis* (AGROFIT, 2020; LIMA *et al.*, 2013a), were used in the present study. The following acaricides were used: abamectin (Abamex, 18 g

a.i. L⁻¹, emulsifiable concentrate, Nufarm, Maracanaú, Ceará, Brazil); azadirachtin (AzaMax, 12 g a.i. L⁻¹, emulsifiable concentrate, UPL do Brasil Indústria e Comércio de Insumos Agropecuários S.A., Ituverava, São Paulo, Brazil); fenpyroximate (Ortus 50 SC, 50 g a.i. L⁻¹, suspension concentrate, Nichino do Brasil Agroquímicos Ltda., Barueri, São Paulo, Brazil), and chlorfenapyr (Pirate, 240 g a.i. L⁻¹, suspension concentrate, Basf S.A., São Paulo, Brazil). The acaricides were used at concentrations recommended for the coconut crop: 13.5 mg a.i. L⁻¹ for abamectin; 30 mg a.i. L⁻¹ for azadirachtin; 100 mg a.i. L⁻¹ for fenpyroximate; and 120 mg a.i. L⁻¹ for chlorfenapyr.

Survival of Amblyseius largoensis

One hundred newly laid eggs of *A. largoensis* were collected from the rearing units and transferred to a new rearing unit. The units were checked daily to monitor the development of the immature forms to the adult stage of the predator. The immature individuals were fed castor oil pollen and eggs of *R. indica*. Seven days later, corresponding to the start of the reproductive period (DOMINGOS *et al.*, 2013), gravid females (7 days) were selected to conduct the experiment.

PVC plates (3 x 3 cm) were immersed for 5 s in distilled water (as control) or in the acaricide solutions. After immersion, the plates were left to dry for 30 min (27.5 ± 1 °C, 70 ± 10% RH, and a photoperiod of 12:12 LD). These plates were then used to establish the experimental units, similar to those used for the rearing, but differ only in size and the presence of acaricide residues on their surface. The gravid females of *A. largoensis* (7 days) were individually isolated in the experimental units; 20 females were used in each treatment, with each female corresponding to one repetition. Daily, one hundred eggs of *R. indica* were transferred to each experimental unit and served as food for the females. The amount of food and the developmental stage of the prey offered to *A. largoensis* females were based on studies by Mendes *et al.* (2018) and Carrillo & Penã (2012), who demonstrated that *A. largoensis* females preferred eggs to other stages of *R. indica* and that each female consumed, on average, 46 eggs/day. Food was provided daily. The experimental units were kept under the same environmental conditions as those used for rearing.

The evaluations started 24 h after isolation of the *A. largoensis* females in the experimental units, with or without acaricide residues, and continued at 12-hour intervals until the predator females died. The total numbers of live and dead predators were recorded during

the evaluations. The predators were considered dead when they did not walk at least the length of their body, after being touched with a soft-bristled brush.

Predation, oviposition, and feed conversion efficiency of Amblyseius largoensis

PVC plates (10 x 10 cm) were immersed for 5 s either in distilled water (as control) or in the acaricide solutions. After immersion, the plates were left to dry for 30 min (27.5 ± 1 °C, 70 ± 10% RH, and a photoperiod of 12:12 LD). The plates then formed the arenas where the gravid female predators were isolated, at ~seven days (the start of their reproductive period). The females were exposed to acaricide residues or distilled water (as control), without available food for 4 h. A 4-h exposure period was chosen to minimize a potentially lethal effect and to focus the assessment on the acute effect on survival, resulting from exposure to the pesticide (LIMA *et al.*, 2017). After the confinement period, the females were transferred and isolated in new arenas, similar to those used before, but of a different size (3 x 3 cm), and without any residues of acaricides. Twenty females were used per treatment (acaricide residues or distilled water), with each female corresponding to one repetition. One hundred eggs of *R. indica* were offered as food to each female at daily intervals. The number of prey eggs consumed and number of eggs deposited by the females were assessed every 24 hours for 4 days. In addition, the oviposition data obtained in each repetition of each treatment were used to calculate conversion efficiency of feed into eggs (FCE), using the formula:

$$\frac{\text{Number of eggs deposited}}{\text{Number of prey consumed}} \times 100. \quad \text{Eq. 1}$$

Statistical analysis

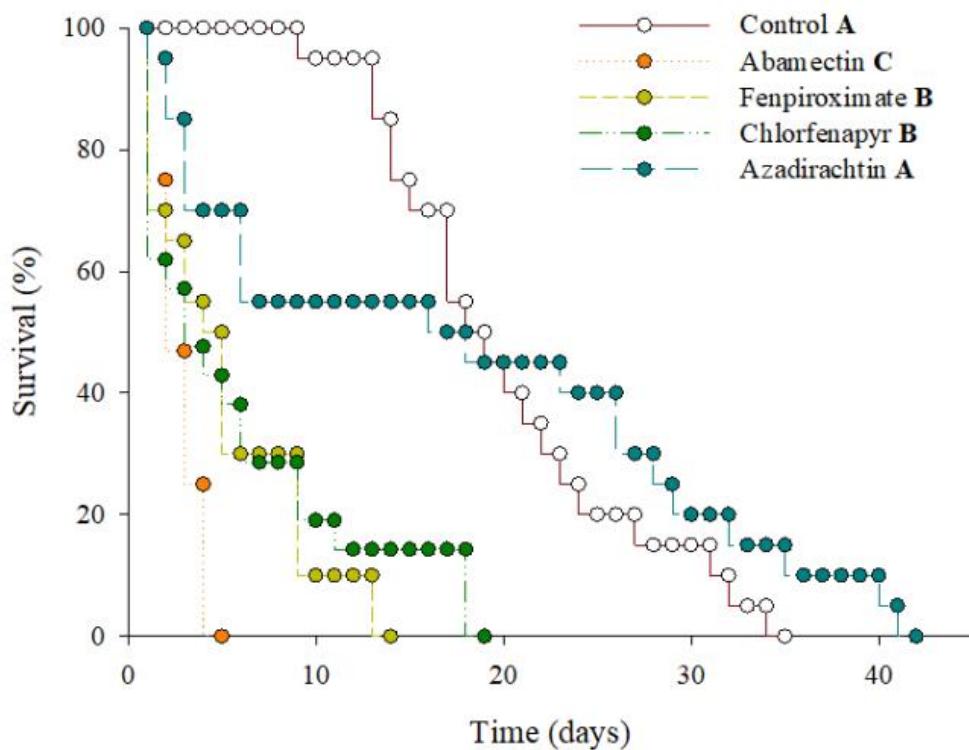
Survival data were used to build survival curves, using Kaplan-Meier estimators, and were compared using the log rank test. The mean survival times of mites were subjected to normality and homogeneity tests and then compared using the Tukey HSD test. The data on predation, oviposition, and FCE were subjected to normality and homogeneity tests and then analyzed using generalized linear models. Because time did not have a significant effect on predation, oviposition, and FCE, the data were combined, using the mean of the four days for each repetition, and compared using the Tukey HSD test ($P=0.05$). The software SAS (SAS Institute, Cary, NC, USA) was used in all analyses.

2.3 Results

Survival of Amblyseius largoensis

The survival of the predator was affected by the presence of acaricide residues ($\chi^2 = 124.19$; $df = 4$; $P < 0.0001$). Females that were not exposed to acaricide residues (control) survived for a maximum of 35 days. A similar result was obtained for the predators exposed to residues of azadirachtin. Abamectin was the most effective acaricide in reducing the survival of the predators, which survived for a maximum of 5 days. Intermediate survival times were observed for the females exposed to residues of fenpyroximate and chlorfenapyr (survival for 14 and 19 days, respectively), with no difference between the two (Figure 1).

Figure 1 - Survival curve of *Amblyseius largoensis* when exposed to residues of the acaricides abamectin, azadirachtin, chlorfenapyr, and fenpyroximate, or distilled water (as control). Curves with the same letter are not significantly different, as per the log rank test ($\chi^2 = 124.19$; $df = 4$; $P < 0.0001$).

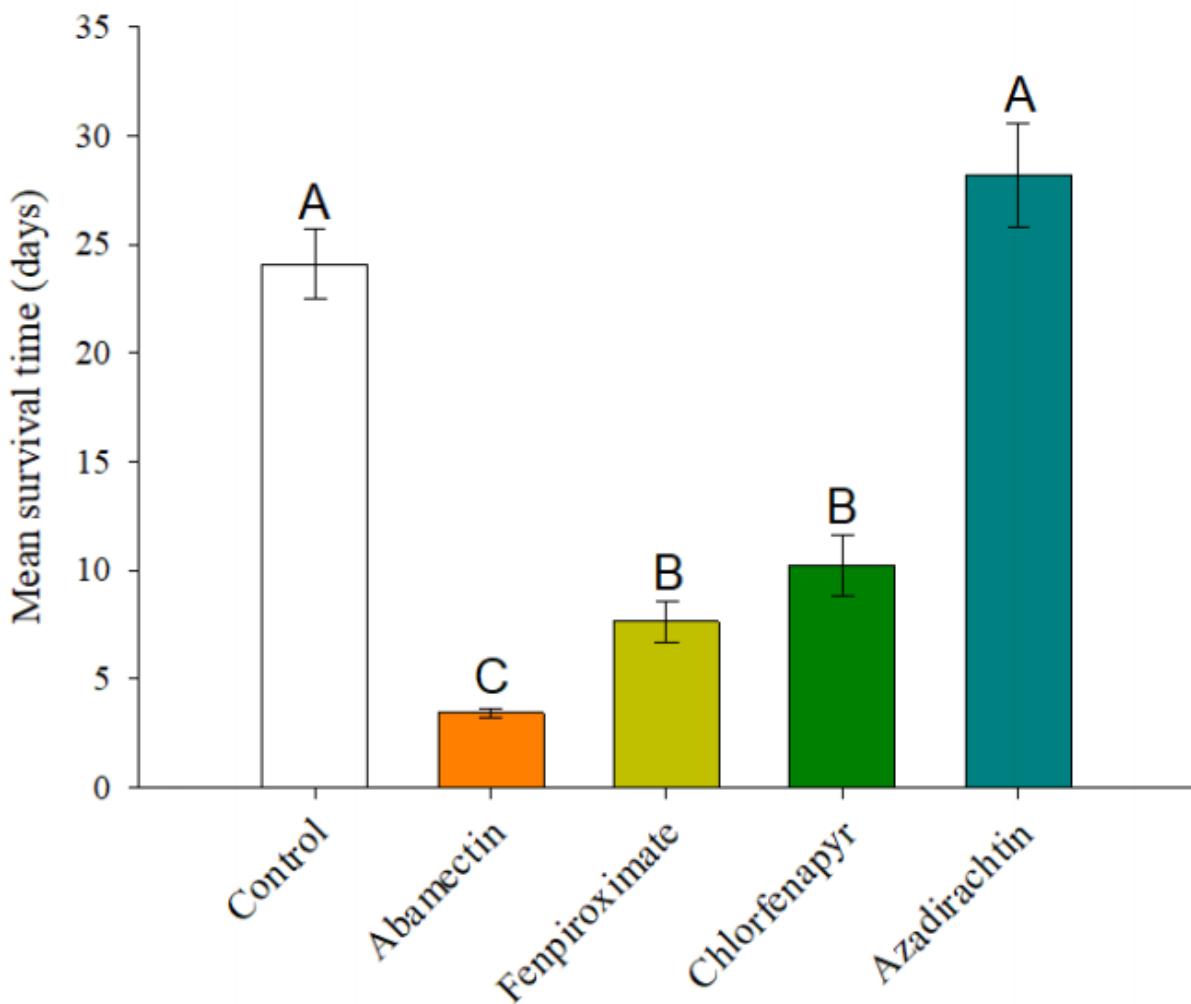


Source: Elaborated by the Author.

The mean survival time of the predators was also affected by the acaricide residues ($F_{4,108} = 26.91$; $P < 0.0001$), showing a similar pattern to that of the survival curves. Azadirachtin residues did not affect the mean survival time (28.01 ± 2.37 days) of the females compared to the control (24.10 ± 1.60 days). Abamectin was the most effective acaricide in

reducing the mean survival time of females (3.47 ± 0.20 days). Intermediate mean survival times were obtained for females exposed to chlorfenapyr (10.24 ± 1.40 days) and fenpyroximate (7.64 ± 0.94 days), with no difference between the two (Figure 2).

Figure 2 - Mean survival time (\pm SE) of *Amblyseius largoensis* when exposed to residues of the acaricides abamectin, azadirachtin, chlorfenapyr, and fenpyroximate, or distilled water (as control). Bars with the same letter are not significantly different, as per the Tukey HSD test ($F_{4,108} = 26.91$; $P < 0.0001$).



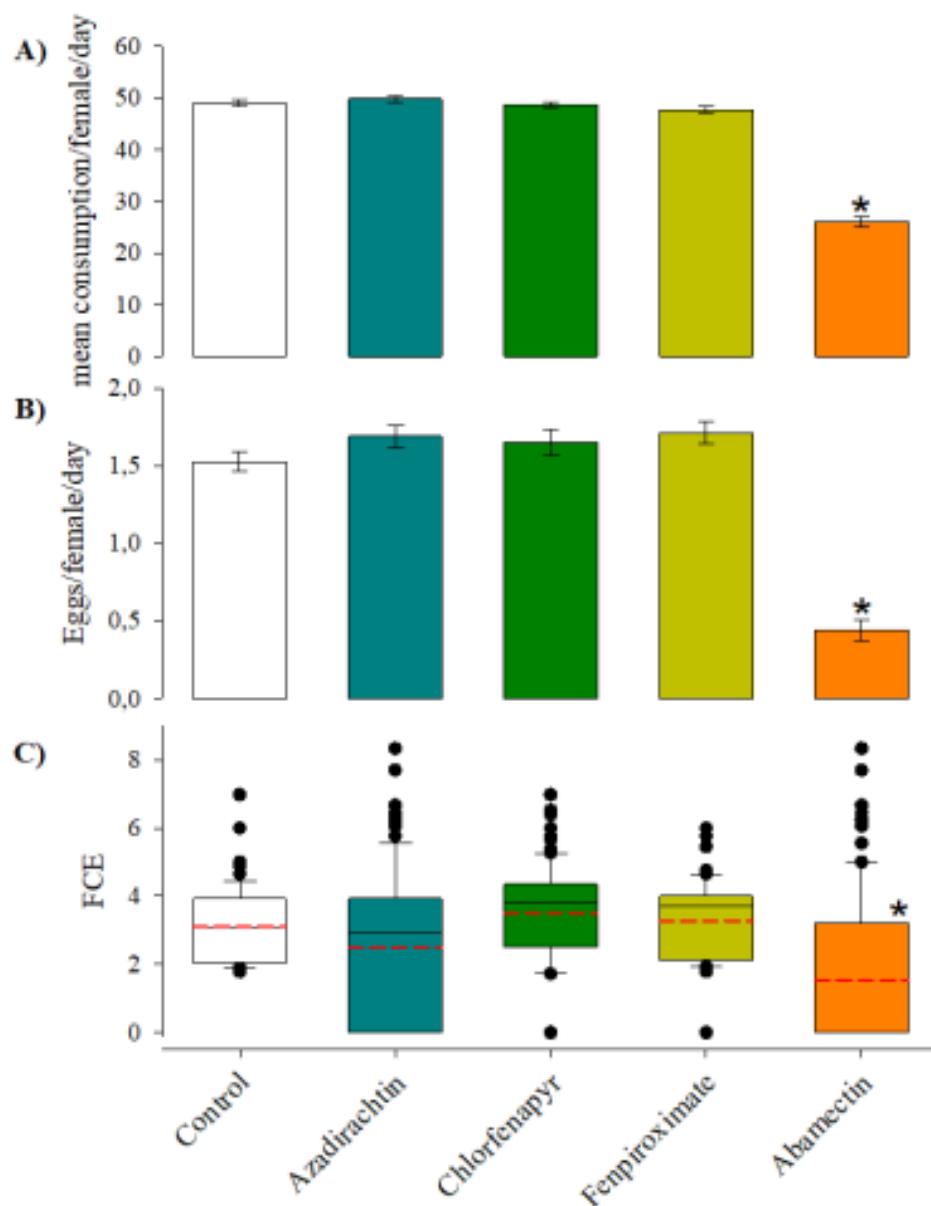
Source: Elaborated by the Author.

Predation, oviposition, and feed conversion efficiency of Amblyseius largoensis

The predators were successfully feed and reproduce at all treatments (Figure 3A and B). Abamectin was the only pesticide that reduced both the predation rate (46.71% reduction, compared to the control) ($F_{4,95} = 166.50$; $P < 0.0001$; Figure 3A) and the oviposition rate (71.48% reduction compared to the control) ($F_{4,95} = 40.08$; $P < 0.0001$; Figure 3B). Females of *A. largoensis*, not exposed to acaricide residues (control) and exposed

to residues of fenpyroximate, chlorfenapyr, and azadirachtin exhibited a mean egg consumption of 48.8 eggs (ranging from 26.14 to 49.81) per female and deposited, on average, 1.6 eggs per day (ranging from 0.44 to 1.69). Abamectin was also the only pesticide that altered the FCE of *A. largoensis* females (52% reduction, compared to the control).

Figure 3 - Mean consumption of *R. indica* eggs (\pm SE) (A), mean number of eggs (\pm SE) (B), and (C) feed conversion efficiency (FCE) to eggs of *A. largoensis* females when exposed to residues of the acaricides abamectin, azadirachtin, chlorfenapyr, and fenpyroximate, or distilled water (as control). The asterisk indicates a significant reduction relative to the other treatments (Tukey HSD test, $P < 0.05$).



Source: Elaborated by the Author.

2.4 Discussion

Abamectin is one of the most widely used acaricides worldwide (RIGA *et al.*, 2014). The present study showed that this pesticide drastically reduced the longevity of *A. largoensis* females (85% reduction). Other authors have also demonstrated a reduction in the survival of predatory mites of the Phytoseiidae family after exposure to abamectin (LIMA *et al.*, 2013a; FERNÁNDEZ *et al.*, 2017; DOKER e KAZAK, 2020), including the species in the genus *Amblyseius* (DOKER; KAZAK, 2019). Assis *et al.* (2013) considered abamectin non-selective for *A. largoensis* after comparing the toxicity curves of this pesticide to the predator and its prey, *R. indica*. These authors showed that the LC50 for *A. largoensis* was 0.092 mL/L, which is well below the concentration recommended for *A. guerreronis* and that used in the present study (0.75 mL/L). This fact explains the significant reduction in the survival of the predator.

Exposure to residues of abamectin also affected the rates of predation, oviposition, and, consequently, the FCE of *A. largoensis*, with this acaricide being the only one that affected these parameters. The predatory behavior of arthropods involves multiple processes: prey identification, foraging, capture/subjugation, acceptance, and consumption (HOLLING, 1959; HOLLING, 1961; HAYNES, 1988). Therefore, some pesticide capable of altering one of these processes can interfere with predation. A change in the predatory behavior of *A. largoensis* after exposure to abamectin was expected because this pesticide exerts a neurotoxic effect through its interaction with GABA receptors at inhibitory synapses (YU, 2008). The avermectins (which include abamectin) block chloride channels, which remain open, causing an ion imbalance that results in the paralysis of na organism (JANSSON; DYBES, 1998). Results, similar to those obtained in the present study, have been observed for other Phytoseiidae, such as *Neoseiulus baraki* (Athias-Henriot) (LIMA *et al.*, 2013b; LIMA *et al.*, 2015) and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) (MONJARÁS-BARRERA *et al.*, 2019), exposure to abamectin affected the attack rate and resulted in reduced prey capture. Such a change in predatory behavior may explain the reduction in oviposition and, consequently, in the FCE of *A. largoensis* females after exposure to abamectin. According to Sabelis (1985a, b), oviposition in phytoseiid mites is nutritionally demanding, not only due to the high number of eggs produced, but also the amount of resources invested per egg. Reduced oviposition after exposure to abamectin has also been observed in the predatory mites, *N. idaeus* Denmark & Mumma (Acari: Phytoseiidae)

(SOUSA NETO *et al.*, 2020) and *N. longispinosus* (Evans) (Acari: Phytoseiidae) (IBRAHIM; YEE, 2000).

Fenpyroximate and chlorfenapyr resulted in intermediate effects on survival and did not alter the predation, oviposition, or FCE of *A. largoensis* females. The pesticide fenpyroximate has been shown to be selective for *A. largoensis* (ASSIS *et al.*, 2013). However, Assis *et al.* (2013) conducted a study in which only the lethal effects were investigated. The low impact of fenpyroximate and chlorfenapyr on the evaluated parameters may be associated with several factors, including high activity of detoxification enzymes (KIM *et al.*, 2004; Lima *et al.* 2013), differences in the size of the pest or the predator or cuticle thickness and composition (HORNSBY *et al.*, 1995), susceptibility to the target site, or even a combination of all these factors. Moreover, the evaluation of pesticide effects based solely on treated individuals would have incomplete end points. Hamedi *et al.* (2010) demonstrated that fenpyroximate-treated females of the predatory mite species *Phytoseius plumifer* (Canestrini & Fanzago) (Acari: Phytoseiidae) had a decreased longevity period, compromising its fertility and that of the subsequent generation. Lima *et al.* (2016) also reported that this product interfered with the offspring of the treated females of the *N. baraki*, resulting in reductions in net reproductive rate (R_0) and intrinsic rate of population growth (rm) as well as an increase in doubling time (DT). Such information is not available for chlorfenapyr.

Unlike the other acaricides, azadirachtin did not affect any of the assessed parameters (survival, predation, oviposition, and FCE of *A. largoensis*). Compatibility between this pesticide and some Phytoseiidae species, such as *N. californicus* (McGregor) (Acari: Phytoseiidae) (Castagnoli *et al.* 2005) and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) (COTE *et al.*, 2002), have been reported. The factors that explain the minor effect of fenpyroximate and chlorfenapyr may also explain the absence of sublethal effects of azadirachtin. It is necessary, however, to investigate Other behavioral and physiological parameters, even in subsequent generations, before it can be concluded that fenpyroximate, chlorfenapyr, and azadirachtin have low or no impact on *A. largoensis*. Moreover, there are no studies on azadirachtin toxicity to *R. indica*, based on our best knowledge.

The present study suggests a potential compatibility between the acaricides azadirachtin, fenpyroximate, and chlorfenapyr and the predatory mite, *A. largoensis*. However, sublethal effects are not always perceptible in individuals exposed to acaricides; such effects often become evident in subsequent generations (HAMEDI *et al.*, 2010; LIMA *et*

al., 2016). Further studies, therefore, are necessary before a conclusion may be drawn regarding the compatibility between the evaluated products and the predatory mite, *A. largoensis*.

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3 CAPÍTULO 3 – ACARICIDE-IMPAIRED FUNCTIONAL AND NUMERICAL RESPONSES OF THE PREDATORY MITE, *AMBLYSEIUS LARGOENSIS* (ACARI: PHYTOSEIIDAE) TO THE PEST MITE *RAOIELLA INDICA* (ACARI: TENUIPALPIDAE)

ABSTRACT

The suppression of pest populations by a predator depends on two basic components of the predator-prey interaction: the functional and the numerical responses of the predator. Such responses can be affected by exposure to acaricides. In the present study, the effects of acaricides (abamectin, azadirachtin, fenpyroximate, and chlорfenапyr) on the functional and numerical responses of the predatory mite, *Amblyseius largoensis* (Acari: Phytoseiidae) an important natural enemy of the pest mite, *Raoiella indica* (Acari: Tenuipalpidae), were investigated. The exposure of *A. largoensis* to acaricides occurred through contact with a surface contaminated with dried acaricide residue. Subsequently, *A. largoensis* exhibited a type II functional response, which was not altered by exposure of any acaricides. However, exposure to abamectin resulted in a decrease in the average mean numbers of prey consumed by a predator. Exposure to acaricides increased prey handling time by 67%, 25%, 38%, and 35% for abamectin, azadirachtin, fenpyroximate, and chlорfenапyr, respectively. Exposure to abamectin reduced the attack rate of *A. largoensis* by 52%. The numerical response of *A. largoensis* was only affected by exposure to abamectin, where just 60% of the females oviposited, and regardless of the prey density, the average mean numbers of eggs/female/day was always less than 0.4. The food conversion efficiency into biomass of *A. largoensis* eggs decreased with increasing prey density, and this trend was not altered by exposure to any acaricides. However, exposure to abamectin drastically compromised the oviposition of *A. largoensis*, showing no increase in egg production with increasing prey density.

Keywords: Phytoseiidae. Tenuipalpidae. Pesticide. Sublethal effect. Integrated pest management.

3.1 Introduction

Acaricides are widely used for the control of pest mites and play an important role in implementation of integrated pest management strategies for many crops (CROFT, 1990). In crops where acaricides are used, predators can be exposed to acaricides through direct

contact during the spraying process, contact with contaminated surfaces during foraging or through feeding on contaminated prey (JEPSON, 1989; AHMAD *et al.*, 2003; HUA *et al.*, 2004; TORRES e RUBERSON, 2004). After exposure, lethal or sublethal effects on organisms can be observed (DESNEUX *et al.*, 2007; GUEDES *et al.*, 2016; GUEDES *et al.*, 2017). Such effects have been used in the assessment of the safety of acaricides against predatory mites (BOLLER *et al.*, 2005), including mortality, longevity, fecundity, development rate, sex ratio, and behavior (POLETTI *et al.*, 2007; NADIMI *et al.*, 2009; TEODORO *et al.*, 2009; HAMEDI *et al.*, 2011; LIMA *et al.*, 2013, 2015a, 2015b, 2016). However, the impact of acaricides on the ability of predatory mites to suppress pest populations is still unclear.

The suppression of the population of any pest by a predator depends on two basic components of the predator-prey interaction, namely the functional and numerical response of the predator (HOLLING, 1959, 1965).

The functional response is defined as the relationship between the number of prey attacked by a single predator in response to prey density during a given time interval (SOLOMON, 1949). The numerical response, however, represents the change in predator density as a function of the change in prey density. However, with an increase in prey density, predators have more food and offspring and therefore also increase in number (SOLOMON, 1949; HASSEL, 1978). HOLLING (1959) proposed three types of functional responses: type I, resulting in the death of prey regardless of density; type II, where with increasing prey density, a decreasing percentage of prey is consumed (negative density-dependent response); type III, where, along a certain range of increasing prey densities, an increasing percentage of prey is killed that then stabilizes under the influence of handling time or satiety (positive density-dependent response). Among the types of functional responses, type II functional responses are most frequently reported in predators (HOLLING, 1959; MURDOCH, 1973; SABELIS, 1985a).

Exposure to acaricides is one of the various factors that can affect the functional response of predatory mites (POLETTI *et al.*, 2007; LIMA *et al.*, 2015; TEODORO *et al.*, 2020; SOUZA NETO *et al.*, 2020). This exposure has commonly altered important parameters associated with functional responses, such as the attack rate (defined as the capture capacity of a predator within a given area) and the handling time (time required for a predator to identify, attack, subdue, and consume a specific prey) (POLETTI *et al.*, 2007; LIMA *et al.*, 2015; SOUZA NETO *et al.*, 2020). However, changes in the type of functional response of predatory mites after exposure to acaricides have recently been demonstrated (TEODORO *et*

al., 2020). Despite several studies, the effects of acaricides on the functional response of many important predator mites have not yet been investigated.

Studies on the numerical response of the predator are as important as functional response studies (HOLLING, 1959); however, numerical response studies are less common. According to Hassell (1966), the numerical response of a predator is composed of two components: the reproductive response and aggregative response. The reproductive response of a predator is related to changes in its density due to the oviposition and survival of its offspring at different prey densities. The aggregative response represents the change in the distribution of the predator mediated by changes in prey densities (HASSEL, 1966). Exposure to acaricides may also affect the numerical response to mite stimulation (hormesis) (CORDEIRO *et al.*, 2013) or limit egg production (HAMEDI *et al.*, 2011; PARK *et al.*, 2011; LIMA *et al.*, 2016; TEODORO *et al.*, 2020; SOUZA NETO *et al.*, 2020). The efficiency of the conversion of prey biomass into predator progeny by increasing oviposition at high prey densities can determine the success of a predator mite in a biological control program (FATHIPOUR *et al.*, 2020).

The predatory mite, *Amblyseius largoensis* Muma (Acari: Phytoseiidae) has been reported as a promising species to be used in biological control programs against the mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae) (CARRILLO e PENA, 2012; CARRILLO *et al.*, 2012a, 2012b, 2014; DOMINGOS *et al.*, 2013; NAVIA *et al.*, 2014; MENDES *et al.*, 2018; LIMA *et al.*, 2018), a pest that arrived in the Americas in 2006 (ETIENE e FLETCHMANN, 2006). *A. largoensis* also stands out for several reasons, such as its ability to feed, develop, and reproduce feeding exclusively on *R. indica* (GALLEGOS *et al.*, 2003; RAMOS *et al.*, 2010; BOWMAN, 2010; TAYLOR *et al.*, 2011; DOMINGOS *et al.*, 2013), presenting a type II functional response (CARRILLO e PENA, 2012; MENDES *et al.*, 2018), and an increase in reproductive response resulting from an increase in the density of *R. indica* (CARRILLO e PENA, 2012; CARRILLO *et al.*, 2012a). In addition, releases of *A. largoensis* resulted in significant reductions (ranging from 43% to 92% at predator:prey ratios of 1:30 and 1:10, respectively) of the population of *R. indica* in coconut plants (Carrillo *et al.* 2014). *R. indica* has dispersed rapidly in the Americas, reaching high population numbers and expanding the number of host species, including species of economic importance, such as coconut palm (*Cocos nucifera* L.) (COCCO e HOY, 2009; LIMA *et al.*, 2011; CARRILLO *et al.*, 2012b; GONDIM *et al.*, 2012; MELO *et al.*, 2018). To date, there is no information on the possible impacts of acaricides on the functional and numerical responses (basic components for suppressing the population of a pest) of *A. largoensis*.

Thus, in the present study, the effects of abamectin, azadirachtin, fenpyroximate, and chlorfenapyr, acaricides with different modes of action commonly used in coconut tree crops, on the functional and numerical responses of *A. largoensis* fed on *R. indica* were investigated.

3.2 Material and Methods

Collection and rearing predatory mites

Coconut leaflets infested with *R. indica* were collected from the campus of the Federal University of Ceará (UFC) in Fortaleza, Ceará, Brazil. Each leaflet was inspected under a stereo microscope, collecting adult predatory mites of the species *A. largoensis* (~200 specimens) for the establishment of rearing units. The rearing units were constructed from flexible PVC boards (10 × 10 cm), which were placed on a polyethylene foam disc (15 cm in diameter and 1 cm thick) inside plastic trays containing water (16 cm in diameter and 2.5 cm in depth). The edge of the polyethylene disc was surrounded with cotton wool moistened with distilled water to prevent the mites from escaping. Predators were fed using castor bean pollen, *Ricinus communis* L. and *R. indica* eggs. The eggs of *R. indica* were collected from coconut leaflets originated from the field by using a soft bristle brush. Food was replenished daily. Rearing units were maintained at 27.5 ± 1.0 °C, 70 ± 10% relative humidity (RH), and 12:12 light: day (L:D) photoperiod.

Acaricides tested

In Brazil, there is no acaricide registered for the control of *R. indica* (Ministério da Agricultura, Pecuária e Abastecimento 2020). Thus, in the present study, products commonly used in coconut tree crops registered for another species of pest mite, *Aceria guerreronis* Keifer (Acari: Eriophyoidea) were selected (LIMA *et al.*, 2013a; LIMA *et al.*, 2013b; LIMA *et al.*, 2015a; LIMA *et al.*, 2015b; LIMA *et al.*, 2016).

Consequently, we tested abamectin (Abamex, 18 g a.i. 1-1, emulsifiable concentrate, Nufarm, Maracanaú, Ceará, Brazil), azadirachtin (AzaMax, 12 g a.i. 1-1, emulsifiable concentrate, UPL do Brasil Indústria e Comércio de Insumos Agropecuários S.A., Ituverava, São Paulo, Brazil), fenpyroximate (Ortus 50 SC, 50 g a.i. 1-1, suspension concentrate, Nichino do Brasil Agroquímicos Ltda., Barueri, São Paulo, Brazil), and chlorfenapyr (Pirate, 240 g a.i. 1-1, suspension concentrate, Basf S.A., Av. das Nações Unidas,

São Paulo, Brazil).

*Testing for the effect of acaricides on functional and numerical response of *A. largoensis**

The experiments were conducted under the same environmental conditions used to rear the mites. The acaricides were diluted in 1 L of distilled water at the following concentrations recommended by the manufacturer: 0.75 mL/1 L water (abamectin); 2.5 mL/1 L water (azadirachtin); 2.0 mL/1 L water (fenpyroximate); 0.5 mL/1 L water (chlorfenapyr). PVC plates (3×3 cm) were immersed for 5 s in distilled water (control) or acaricide solutions. After immersion, the plates were set to dry for 30 min (27.5 ± 0.5 °C, $70 \pm 10\%$ RH, and 12:12 LD photoperiod). Then arenas of Petri dishes (1.5 cm high, Ø 9 cm) containing polyethylene foam (1 cm thick and Ø 9 cm), filter paper (Ø 9 cm) and the PVC plate formerly immersed in water or acaricide solution. In each experimental unit, the edge of the PVC plate was covered with hydrophilic cotton moistened with distilled water to prevent mites from escaping.

Later, gravid *A. largoensis* females (~ 7 days old) were removed from the rearing unit and kept under food deprivation for 4 h in a rearing unit equal to the model used in the rearing of mites. After the period of food deprivation, the females were transferred to the experimental units, one female per experimental unit, which were then subjected to treatments with densities of 30, 60, 90, 120, and 150 eggs of *R. indica*. Twenty replicates were used for each treatment, except for the last two densities, with 10 replicates each.

The evaluations were carried out every 24 h for 4 days, with egg replacement at the end of the day, and computing the consumption and oviposition to verify the functional and numerical responses. Only females alive at the end of the experimental time were considered to analysis.

Statistical analysis

The functional responses were analyzed at two stages of data analyze. In the first stage, the type of functional response was determined by regression analysis of the proportion of prey consumed in relation to the initial density (HOLLING, 1959). The significance of the linear parameter of the equation were used to distinguish between the types of functional response: if the linear coefficient is not significant, it indicates a type I functional response; if negative and significant it indicates a type II response, and if positive and significant it indicates a type III response (JULIANO, 1993).

In the second stage, prey handling time (Th) and the attack rate (a') parameters were determined. Once the experiments were conducted with prey replacement, Holling's disc equation ($N_e = \frac{aNt}{1+aNT_h}$) was used to estimate Th and a' . In the equation, N indicates the initial number of prey, and a , Th , and T represent attack rate, prey handling time, and total evaluation time, respectively.

Differences in these parameters between treatments were verified using a 95% confidence interval (95% CI). The numerical response (variation in the oviposition rate of predators as a function of prey density) was estimated using polynomial regression. The conversion efficiency of ingested food (ECI) into egg biomass, or growth efficiency, was calculated using the equation: $ECI = (No \times 100) / Nc$, where No is the average number of eggs per female in each density, and Nc is the average number of prey consumed by each female in each density (OMKAR e PERVEZ, 2004). The data were then subjected to polynomial regression analysis. All analyses were performed using the SAS software (SAS INSTITUTE, CARY, NC, USA).

3.3 Results

Exposure to acaricides did not alter the type of functional response displayed by *A. largoensis* fed with *R. indica* eggs. A type II functional response (significantly negative linear coefficient in Holling's disc equation, Table 1) were observed regardless of acaricide applications.

Table 1 - Coefficients estimated by a logistic regression of proportion of prey consumption of adult female, *Amblyseius largoensis* fed with *Raoiella indica* eggs when exposed to acaricides.

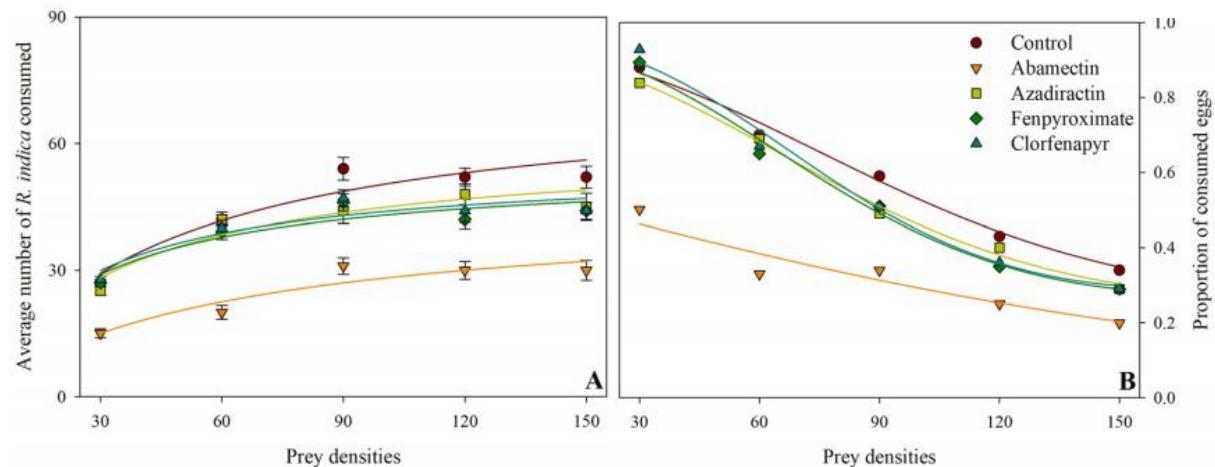
Treatments	Holling's Disc Equation	χ^2	DF	P	Logistic regression coefficient			
					$I^1(P)$	$L^2(P)$	$Q^3(P)$	Type ⁴
Control	$y = \frac{\exp [(0.00008x^2) - (0.036x) + 2.86]}{1 + \exp [(0.00008x^2) - (0.036x) + 2.86]}$	161.46	87	<.001	2.86 (<.001)	-0.036 (<.001)	0.00008 (<.001)	II
Abamectin	$y = \frac{\exp [(0.000006x^2) - (0.01x) + 0.18]}{1 + \exp [(0.000006x^2) - (0.019x) + 0.18]}$	114.67	87	0.02	0.18 (0.22)	-0.01(0.001)	0.000006 (0.07)	II
Azadirachtin	$y = \frac{\exp [(0.0001x^2) - (0.039x) + 2.76]}{1 + \exp [(0.0001x^2) - (0.039x) + 2.76]}$	231.83	87	<.001	2.76 (<.001)	-0.039 (<.001)	0.00010 (<.001)	II
Fenpyroximate	$y = \frac{\exp [(0.00014x^2) - (0.049x) + 3.22]}{1 + \exp [(0.00014x^2) - (0.049x) + 3.22]}$	176.64	87	<.001	3.22 (<.001)	-0.049 (<.001)	0.00014 (<.001)	II
Clorfenapyr	$y = \frac{\exp [(0.00017x^2) - (0.056x) + 3.64]}{1 + \exp [(0.00017x^2) - (0.056x) + 3.64]}$	211.03	87	<.001	3.64 (<.001)	-0.056 (<.001)	0.00017 (<.001)	II

¹ Intercept; ²Linear; ³Quadratic; ⁴Type of functional response.

Source: Elaborated by the Author.

Similar trends were also obtained for both the number and the proportion of prey consumed (Figures 4A and 4B). The number of prey consumed increased with increasing prey density, stabilizing at the highest density (90 to 150 prey), while the proportion of prey consumed decreased with increasing prey density. However, despite similar trends, the consumption of prey by the predator at the highest densities (where the highest consumption was observed) when exposed to abamectin was lower than that observed in the lowest densities (where the lowest consumption was observed) for other treatments (Figure 4A). When exposed to abamectin, the proportion of prey consumed by the predator was always less than 0.5% (Figure 4B).

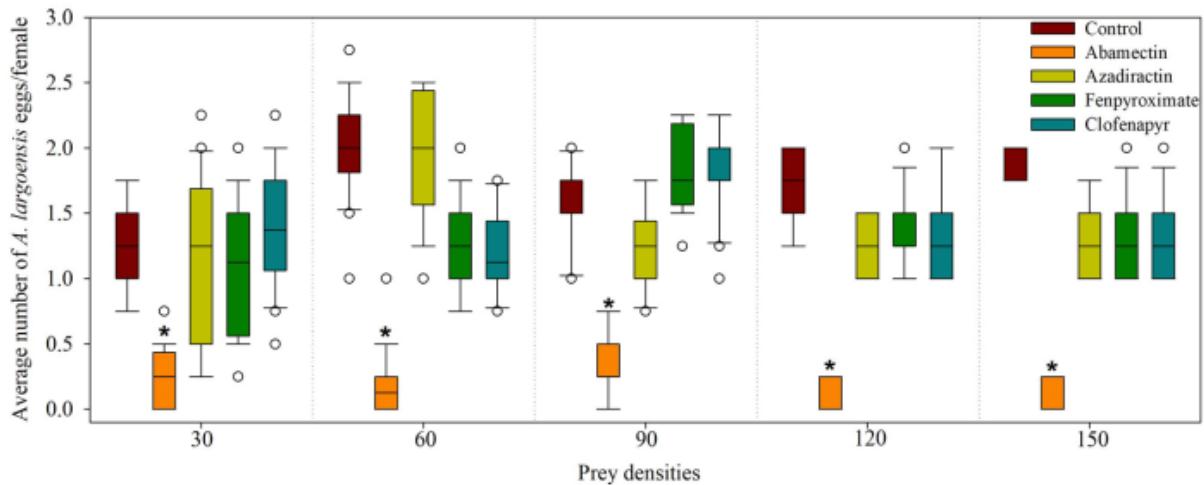
Figure 4 - Functional response curves of *Amblyseius largoensis* preying on increasing densities of *Raoiella indica* eggs after exposure to acaricides (A) and proportions of egg consumption by the predator (B).



Source: Elaborated by the Author.

Abamectin was the only acaricide able to change the attack rate of *A. largoensis*, reducing it by approximately 52% in comparison to the control (Figure 5A). However, exposure to acaricide impacted the prey handling time (*Th*) of *A. largoensis*, increasing it by approximately 67%, 25%, 38%, and 35% for abamectin, azadirachtin, fenpyroximate, and chlorfenapyr, respectively (Figure 5B).

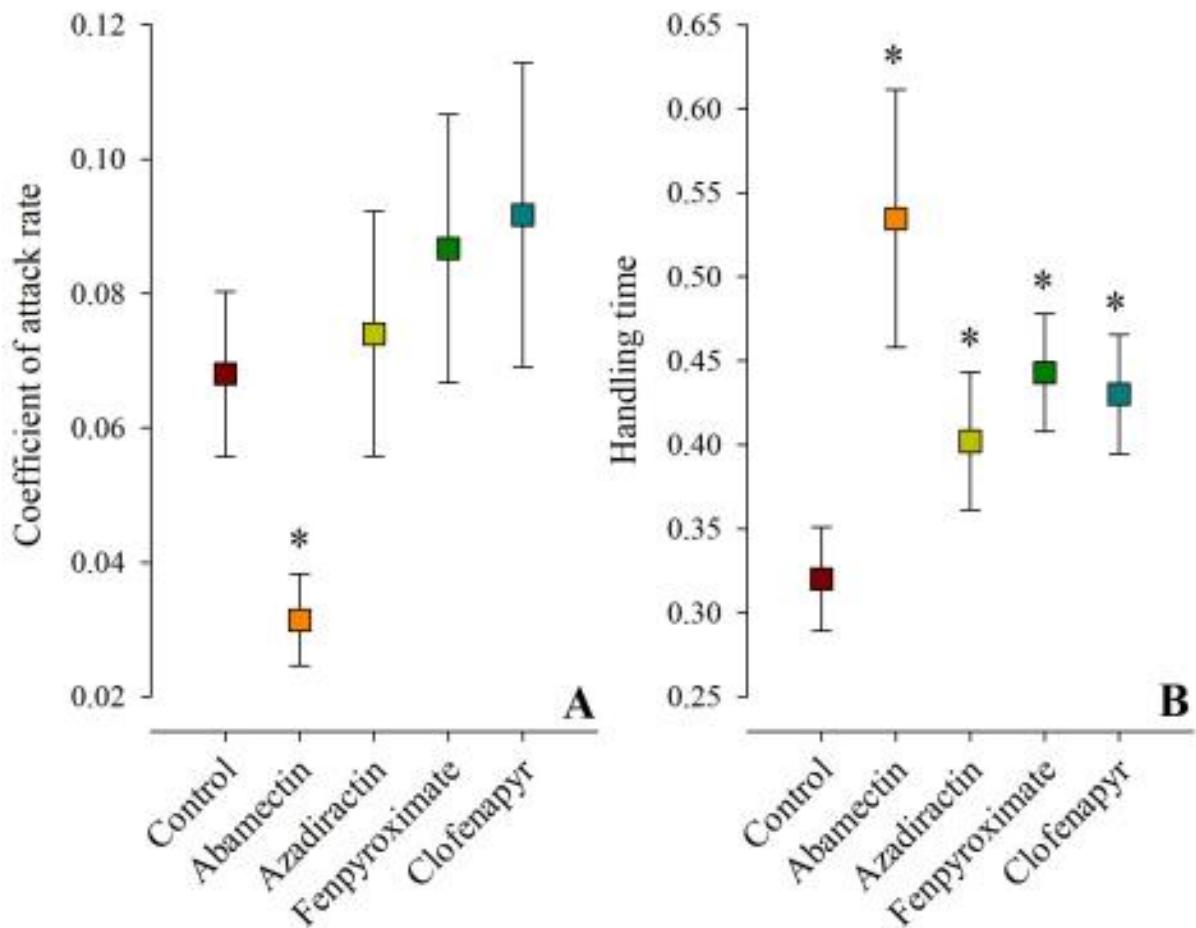
Figure 5 - (A) Attack rate coefficient a' (proportion of prey captured by the predator per unit of search time) and (B) handling time (proportion for the exposure period of 24 h). Values followed by asterisks (*) are significantly different from the control based on the 95% confidence intervals.



Source: Elaborated by the Author.

Even the lowest densities of prey were sufficient to stimulate the oviposition of *A. largoensis* (Figure 6). However, the average mean numbers of eggs/female/day was affected by exposure to acaricides ($\chi^2 > 40.2$; $df=4$; $P < 0.0001$). All *A. largoensis* females exposed to azadirachtin, fenpyroximate, and chlорfenапyr as well as the control performed oviposition and showed similar trends. For these treatments, an increasing trend was observed, followed by stabilization in the average number of eggs/female/day with increasing prey density. The average number of eggs/ female/day ranged from 1.1 to 2.0. Only 60% of *A. largoensis* females exposed to abamectin oviposited, where regardless of the density of prey, the average number of eggs/female/day was always less than 0.4.

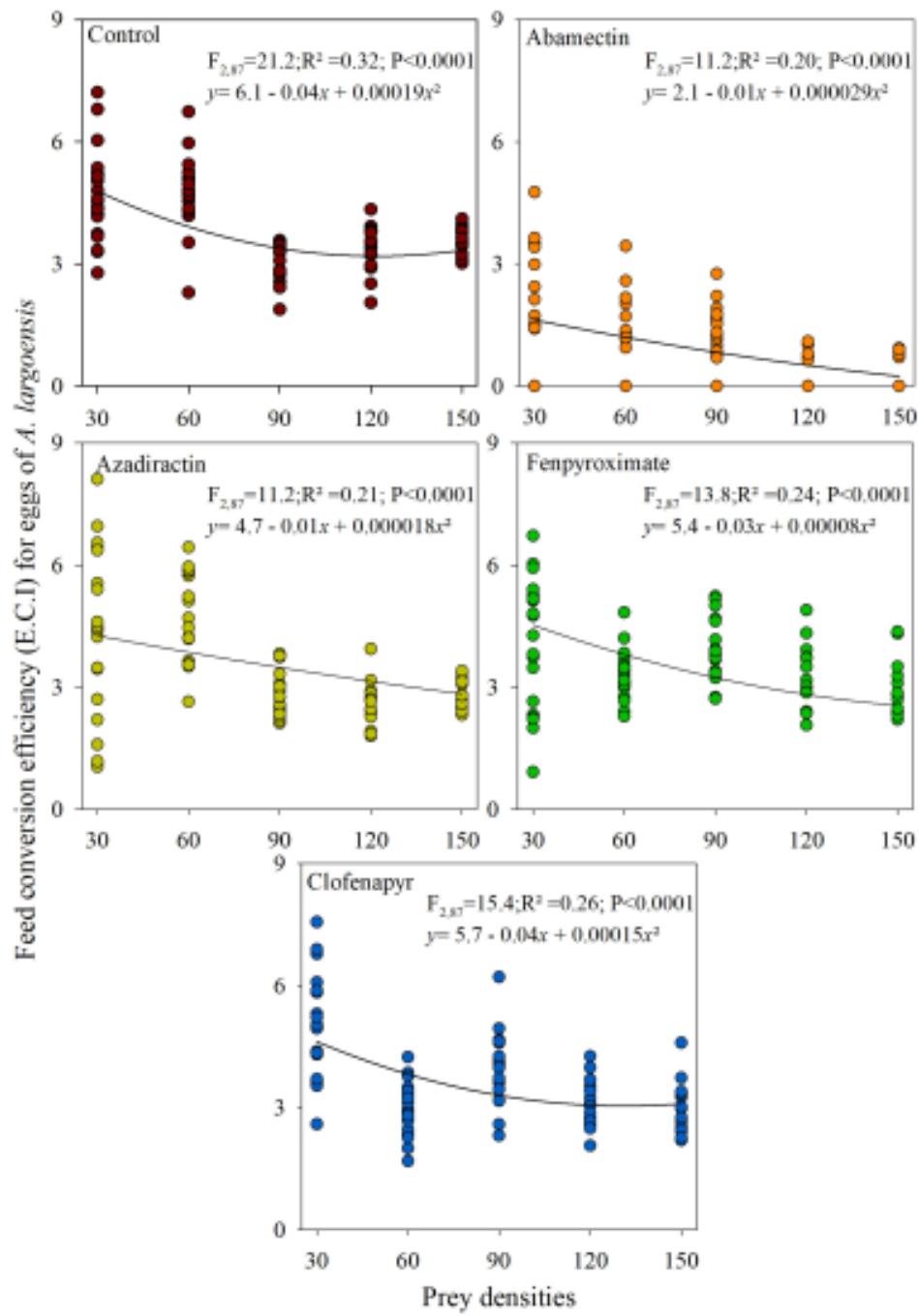
Figure 6 - Average mean numbers of eggs laid per *Amblyseius largoensis* female exposed to acaricides and provided with different densities of *Raoiella indica* eggs when exposed to acaricides. Asterisks indicate significant differences.



Source: Elaborated by the Author.

Similar trends in the conversion efficiency of ingested food into egg biomass (ECI) were observed in all treatments. The data fit the same regression model: (Figure 7). The ECI decreased as prey density increased. Despite similar trends, the maximum ECI of *A. largoensis* when exposed to abamectin (1.7% observed in the lowest prey density) was lower than the minimum ECI of the other treatments (approx. 2.9 in the highest prey densities) (Figure 7).

Figure 7 - Food conversion efficiency (E.C.I) for eggs of *Amblyseius largoensis* females when exposed to abamectin, azadirachtin, chlufenapyr and fenpyroximate, or distilled water (control).



Source: Elaborated by the Author.

3.4 Discussion

Exposure to acaricides (abamectin, fenpyroximate, azadirachtin, and chlorfenapyr) did not change the type of functional response of *A. largoensis*. Similar results were obtained for other species of Phytoseiidae mites, such as *Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks) (POLETTI *et al.*, 2007), *Neoseiulus baraki* (Athias-Henriot) (LIMA *et al.*, 2015), *Phytoseiulus persimilis* (Athias-Henriot) (MONJARÁS-BARRERA *et al.*, 2019) and *Neoseiulus idaeus* (Denmark & Muma) (SOUSA NETO *et al.*, 2020). Although it did not change the type of functional response, exposure to abamectin resulted in a decrease in the average number of prey consumed per predator. This reduction can be explained by a change in both the attack rate of the predator (reduction of approximately 52%) and the time of prey handling (increase of approximately 67%). These changes suggest both a reduction in prey capture potential by the predator, as well as more time spent manipulating prey, slowing the start of the search for new prey. Changes in the rate of attack and time of prey handling have been observed in mites after exposure to neurotoxic products, such as abamectin (LIMA *et al.*, 2015; SOUSA NETO *et al.*, 2020). Abamectin binds to the chloride channels in nerve synapses and may negatively affect locomotion, feeding, and orientation by sensory stimuli (WOLSTENHOLME, 2012).

Abamectin also affected the numerical response of *A. largoensis*, where only 60% of the females exposed to this acaricide were able to oviposit, and the average number of eggs per female was less than 0.4, regardless of the number of prey available. Egg production by predatory mites requires a lot of feeding, not only for the number of eggs produced, but also for the amount of energy invested per egg (SABELIS, 1985b). Thus, the low consumption of prey by *A. largoensis* females exposed to abamectin explains the impairment of oviposition. Impaired fecundity of females after exposure to abamectin has been observed in other phytoseiid species, such as *Phytoseius plumifer* (Canestrini & Fanzago) (HAMEDI *et al.*, 2011) and *N. idaeus* (SOUSA NETO *et al.*, 2020). Females of *A. largoensis* exposed to abamectin exhibited an egg-like structure in the distal part of its opisthosoma, which was also observed in *N. idaeus* (SOUSA NETO *et al.*, 2020). Sousa Neto *et al.* (2020), proposed two hypotheses to explain this observation: (i) females exposed to abamectin retain their eggs avoiding oviposition, and (ii) or they have compromised mobility, preventing them from expelling all eggs produced. This situation should be studied in detail in further studies.

Only part of the energy derived from the prey biomass is converted into eggs, while the rest is used to maintain the vital activities of the organism such as metabolic costs

and respiration (BAUMGARTNER *et al.*, 1987). In the present study, we demonstrated that the efficiency of the conversion of food into egg biomass (ECI) of *A. largoensis* decreases with increasing prey density. These results suggest that at low prey densities, *A. largoensis* females allocate most of the energy obtained via feed to egg production, while at high densities of prey, despite the greater number of eggs, the energy investment towards eggs is lower. Exposure of *A. largoensis* females to azadirachtin, fenpyroximate, and chlорfenапyr acaricides did not affect the efficiency of food conversion into egg biomass. This suggests that the energetic expenditure toward detoxification is very low or non-existent. The exposure of *A. largoensis* females to abamectin did not change the efficiency of conversion of food into egg biomass; however, it drastically compromised the oviposition of females and there was no increase in egg production with increasing prey density. The impairment of oviposition in *A. largoensis* females can be explained by the hypotheses raised by Sousa Neto *et al.* (2020), and mentioned above but may also reflect a physiological trade-off between fecundity and longevity (GUEDES *et al.*, 2016, 2017). Mites can use resources intended for oviposition to increase their longevity (GOTOH *et al.*, 2006). In addition, several studies have shown that the fecundity of some phytoseiid species decreases after exposure to different acaricides, including abamectin (HAMEDI *et al.*, 2011; SOUSA NETO *et al.*, 2020).

Functional and numerical response studies help to understand the ability of a predator to reduce pest density. Since functional responses (response type and average prey intake) and Numerical responses (average number of eggs per female) of *A. largoensis* were not affected by the acaricides azadirachtin, fenpyroximate, and chlорfenапyr, it is possible to hypothesize that when used together (predators and acaricides), they will be able to reduce the pest population considerably, and perhaps sufficiently. Abamectin considerably reduced both predation and oviposition of *A. largoensis*, suggesting negative impacts on the predator mites resulting from exposure to this acaricide. Although the results of the present study identify acaricides that can be prioritized or avoided for the conservation of the predator mite, *A. largoensis* and its consequent action as a biological control agent, additional studies are necessary for a better understanding of the possible impacts of such products on the activity of the predator. Some acaricides may cause irritation or may repel predators (LIMA *et al.*, 2013) or may have transgenerational effects (HAMEDI *et al.*, 2010; LIMA *et al.*, 2016) or may induce hormoligosis effects (CORDEIRO *et al.*, 2013), which have potential effects not known to the predator and acaricides tested.

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