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DEPARTAMENTO DE CIÊNCIAS AGRÁRIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA DE ALIMENTOS

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DETECÇÃO DE FEROMÔNIO DE *Euschistus heros* (F.) UTILIZANDO NANOSSENSORES DE CANTILEVER

ERECHIM - RS
2021

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DETECÇÃO DE FEROMÔNIO DE *Euschistus heros* (F.) UTILIZANDO NANOSSENSORES DE CANTILEVER

Tese de Doutorado apresentada como requisito parcial à obtenção de grau de Doutora, pelo Curso de Pós-Graduação em Engenharia de Alimentos, Departamento de Ciências Agrárias da Universidade Regional Integrada do Alto Uruguai e das Missões – Campus de Erechim.

Orientadoras: Drª. Clarice Steffens
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Erechim, 16 de março de 2021.

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“Há uma força motriz mais poderosa que o vapor, a eletricidade e a energia atômica: a vontade.”

(Albert Einstein)

Resumo da Tese apresentada ao Programa de Pós-Graduação em Engenharia de Alimentos como parte dos requisitos necessários para a obtenção do Grau de Doutora em Engenharia de Alimentos.

DETECÇÃO DE FEROMÔNIO DE *Euschistus heros* (F.) UTILIZANDO NANOSSENSORES DE CANTILEVER

Janine Martinazzo
Março/2021

Orientadores: Prof. Dr^{a}. Clarice Steffens
Prof. Dr^{a}. Juliana Steffens

O uso de diferentes técnicas para o controle de pragas na agricultura busca uma maior segurança e preservação ambiental. O percevejo marrom *Euschistus heros* é uma destas pragas que acomete várias culturas agrícolas. O inseto libera feromônio em baixas concentrações para acasalamento. Uma alternativa promissora e eficiente para a detecção deste composto volátil é o uso de sensores. O objetivo deste estudo foi desenvolver nanossensores de cantilever com diferentes camadas sensoras utilizando polianilina (PANI), poliestireno sulfonato de sódio (PSS) e nanohíbrido de polianilina e prata (PANI.Ag) e aplicá-los na detecção do feromônio sexual (2,6,10-trimetiltridecanoato de metila) de *E. heros* na forma sintética, *in vivo* e em septo de borracha. Os resultados mostram que as camadas sensoras apresentaram espessura na escala nanometrica de 98,36 nm para PANI/PSS e de 20,03 nm para PANI.Ag. Ambos os nanossensores apresentaram mudança de frequência de ressonância quando expostos ao feromônio de *E. heros* com tempo de resposta, limite de detecção e sensitividade, respectivamente de 32,94 s, 0,01454 µg/mL e 164,97 Hz.mL/µg para o nanossensor e PANI/PSS e 29,81 s, 0,01291 µg/mL e 185,84 Hz.mL/µg para o de PANI.Ag. Os nanossensores foram considerados estáveis durante o armazenamento (360 dias) e também detectar o composto 2,6,10-trimetiltridecanoato de metila em septos de borracha. Os compostos interferentes tridecano e (Z)-3-acetato de hexenila não demonstraram interações significativas com as camadas sensoras, diferente do que acontece com o composto feromonal. Os experimentos *in vivo* confirmaram a interação da camada sensora de PANI.Ag com os voláteis liberados pelos percevejos além de não mostrar interação com outra espécie de percevejo, *Diceraeus furcatus* (F.). Conclui-se que os nanossensores de cantilever desenvolvidos podem detectar o feromônio sexual de *E. heros*, apresentando potencial de serem utilizados na detecção dos percevejos, visando o seu manejo com a utilização da nanotecnologia.

Palavras-chave: Polianilina. Nanotecnologia. Percevejo. Frequência de ressonância. Compostos interferentes.

Abstract of Thesis presented to Food Engineering as a partial fulfillment of the requirements for the Degree of Doctor in Food Engineering.

DETECTION OF PHEROMONE *Euschistus heros* (F.) USING CANTILEVER NANOSENSORS

Janine Martinazzo
March/2021

Advisors: Prof. Dr^{a}. Clarice Steffens
Prof. Dr^{a}. Juliana Steffens

The use of different techniques for pest control in agriculture seeks for safety and environmental preservation. The stinkbug *Euschistus heros* is one of these pests that affects various agricultural crops. This insect releases pheromone in order to mate in low concentrations. A promising and efficient alternative for the detection of this volatile compound is the use of sensors. The aim of this study was to develop cantilever nanosensors with different sensitive layers using polyaniline (PANI), polystyrene sodium sulfonate (PSS) and polyaniline and silver nanohybrid (PANI.Ag) and apply to detect the sexual pheromone (2,6,10 -methyl trimethyltridecanoate) of *E. heros* in synthetic form, *in vivo* and in rubber septum. The results show that the sensitive layers had a nanoscale thickness of 98.36 nm for the PANI/PSS and 20.03 nm PANI.Ag. Both nanosensors showed a change in resonance frequency when exposed to *E. heros* pheromone with response time, detection limit and sensitivity, respectively, of 32.94 s, 0.01454 μg/mL and 164.97 Hz.mL/μg for the nanosensor and PANI/PSS and 29.81 s, 0.01291 μg/mL and 185.84 Hz.mL/μg for the PANI.Ag. The nanosensors were stable during storage (360 days) and also detect the compound 2,6,10-methyl trimethyltridecanoate in rubber septa. The interferents compounds tridecane and hexenyl (Z) -3-acetate did not demonstrate significant interactions with the sensing layers, differently from what happens with the sexual pheromone compound. The *in vivo* experiments confirmed the interaction of the PANI.Ag sensor layer with the volatiles released by the stinkbugs, not showing interaction with the species *Diceraeus furcatus* (F.). It is concluded that the developed cantilever nanosensors can detect the sexual pheromone of *E. heros*, demonstrating its potential to monitor populations in order to manage them, use of nanotechnology.

Keywords: Polyaniline. Nanotechnology. Stinkbug. Resonance frequency. Interferents compounds.

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LISTA DE ABREVIATURAS E SIGLAS

AFM - Microscopia de Força Atômica (do inglês, *Atomic Force Microscope*)
Ag - Prata
$AgNO_3$ - Nitrato de prata
AgNPs - Nanopartículas de prata
ATR-FTIR - Espectroscopia no Infravermelho por Transformada de Fourier com medida de Refletância Total Atenuada (do inglês, *attenuated total reflectance Fourier transform infrared spectroscopy)*
C_4H_9NO - n, n-dimetilacetamida
f_{res} - Frequência de ressonância
HCl - Ácido clorídrico
H_2SO_4 - Ácido sulfúrico
IUPAC - International Union of Pure and Applied Chemistry
k - Constante de mola
$KMnO_4$ - Permanganato de potássio
LbL - *Layer by Layer*
LOD - Limite de detecção
LOQ - Limite de quantificação
MIP - Manejo Integrada de Pragas
$NaNO_3$ - Nitrato de sódio
nm - Nanômetro
N_2 - Gás nitrogênio
PANI - Polianilina
PCA - Análise dos Componentes Principais (do inglês, *Principal Component Analysis*)
PPB - Partes por bilhão
PPM - Partes por milhão
PPT - Partes por trilhão
PPQ - Partes por quatrilhão
PSS - Poli (estireno sulfonato de sódio)
RMS - Raiz quadrada da média da rugosidade (do inglês, *root mean square*)
UR - Umidade Relativa
UV-Vis - Espectroscopia no Ultravioleta - Visível (do inglês, *ultraviolet–visible spectroscopy*)
XRD - Difratometria de Raios X (do inglês, *X-ray Diffraction*)
λ - Comprimento de onda

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CAPÍTULO I

PERCEVEJO MARROM *Euschistus heros* (F.): IMPORTÂNCIA ECONÔMICA, FEROMÔNIOS E OBJETIVOS DA TESE

1.1 Introdução

O uso de diferentes técnicas que sejam eficientes para o controle de pragas na agricultura vem tomando o espaço do uso descontrolado de agroquímicos, buscando uma maior segurança e preservação ambiental. Isso se deve a presença de diversas pragas nas lavouras que trazem prejuízos e o elevado uso de agroquímicos faz com que a busca pelo compromisso com a saúde e bem-estar e a conservação do ambiente evidenciem alternativas para seu controle.

O crescimento populacional e a preocupação com a elevada demanda de alimentos no passar dos anos faz com que aumente o interesse em manter os grãos e as sementes colhidos nas lavouras com o mínimo de perdas até o consumo final, além de que seja garantida a preservação de alimentos de culturas agrícolas para seu consumo direto ou seu consumo após a transformação industrial, garantindo a segurança alimentar da humanidade (LORINI et al., 2015; HODAIFA; NIETO; SEYEDSALEHI, 2018).

Um inseto só pode ser considerado praga quando atinge um determinado índice de dano econômico para a cultura plantada. Dependendo da espécie, do tamanho populacional da praga, da fase de desenvolvimento, estrutura vegetal atacada e da duração do ataque, pode haver maior ou menor prejuízo, em quantidade e em qualidade (AGEITEC, 2019).

Dentre essas pragas, o *Euschistus heros* (F.), também conhecido por percevejo marrom, é comumente encontrado em plantações de soja, feijão, ervilha, alfafa e algodão (SORIA; DEGRANDE; PANIZZI, 2010; PANIZZI; BUENO; SILVA, 2012). Por sugarem o grão para se alimentarem, provocam redução na produção e na qualidade das sementes (BLASSIOLI-MORAES et al., 2012; SILVA et al., 2014).

O levantamento populacional de pragas como o *E. heros* é conduzido por meio do uso do pano-de-batida, que consiste em bater as plantas sobre um pano com o objetivo de deslocar os insetos para fazer a contagem. Na sequência, as plantas voltam à posição original, e os insetos sobre o pano são contados e registrados (CORRÊA-FERREIRA, 2012). A utilização de agroquímicos para o controle das pragas, estes causam muitos problemas para a saúde da população devido a sua permanência no ambiente, podendo levar a contaminação das águas, além dos solos e ar (SEKHON, 2014). Neste sentido, algumas armadilhas estão sendo estudadas para a identificação das pragas, onde o próprio feromônio sexual do inseto é utilizado para atração dos mesmos com eficiência (BORGES et al. 2011).

O desenvolvimento e a integração de estratégias com métodos alternativos no manejo dessas pragas é de extrema importância, a fim de minimizar os danos causados na agricultura além de ter em vista a eficácia no controle. Conforme Borges et al. (2007), o uso de métodos semioquímicos, especialmente agregação e feromônios sexuais, é uma abordagem de manejo ambiental favorável. A nanotecnologia na agricultura pode gerar redução de danos ambientais beneficiando a agricultura de precisão, além de aprimorar a intervenção humana por meio de dispositivos sensores, facilitando a produtividade. Deste modo, o uso do feromônio do próprio inseto na construção de nanossensores é uma tecnologia que se destaca como uma alternativa promissora para uma agricultura sustentável, direcionando para uma conceituação e validação de novas ferramentas e abordagens.

O feromônio sexual do inseto é um tipo de substância volátil secretada por um indivíduo e recebida por outro da mesma espécie ou não, a fim de fazer algum tipo de comunicação entre eles (ZARBIN; RODRIGUES; LIMA, 2009; BREZOLIN et al., 2018). Em especial, este tipo de feromônio produzido por um sexo do inseto para atração do sexo oposto tem o objetivo de reprodução, consideradas substâncias capazes de causar respostas em concentrações muito baixas (MORAES et al., 2008a; WEBER et al., 2017).

Nanossensores de cantilever podem ser utilizados para detecção de moléculas voláteis ou adsorção de moléculas com extrema sensitividade, uma vez que apresentam a capacidade de mudar a frequência vibracional ou sofrer deflexão devido a adsorção de moléculas sobre a superfície, tornando-os excelentes transdutores e aptos para serem utilizados como sensores químicos, físicos ou biológicos (STEFFENS et al., 2014, BREZOLIN et al., 2019; BREZOLIN et al., 2020a; BREZOLIN et al., 2020b). Trazem como vantagem a miniaturização, rápido tempo de resposta, são específicos e detectam analitos com elevada sensitividade.

Assim, o desenvolvimento de nanossensores para detecção de feromônios visa uma alternativa promissora e eficiente, com detecção em tempo real de traços do composto volátil, com alta sensitividade, versatilidade e menor agressão ao meio ambiente.

1.2 Objetivos

1.2.1 Objetivo Geral

Desenvolver nanossensores de cantilever com diferentes camadas sensoras para detecção do feromônio sexual de *E. heros* sintético, *in vivo* e em septo de borracha.

1.2.2 Objetivos Específicos

- Desenvolver a funcionalização do cantilever com diferentes camadas sensoras, utilizando polianilina (PANI), poliestireno sulfonato de sódio (PSS) e nanohíbrido de polianilina e prata (PANI.Ag).
- Avaliar a eficiência da funcionalização por meio das alterações na rugosidade, espessura, morfologia, topografia, deposição de massa e investigar a composição da amostra.
- Avaliar a resposta de frequência de ressonância (f_{res}) dos nanossensores de cantilever em contato com diferentes concentrações de feromônio do *E. heros*, o composto 2,6,10-trimetiltridecanoato de metila e a sua detecção em septo de borracha.
- Avaliar a resposta dos nanossensores de cantilever em contato com compostos interferentes e outra espécie de percevejo o *Diceraeus furcatus* (F.).
- Avaliar a resposta dos nanossensores de cantilever com percevejos *E. heros* (teste *in vivo*) em ambiente contendo separadamente machos, fêmeas ou ambos.
- Caracterizar as respostas dos nanossensores de cantilever quanto ao tempo de resposta, limite de detecção, limite de quantificação, sensitividade, repetibilidade e estabilidade.

1.3 Referências

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CAPÍTULO II

REFERENCIAL TEÓRICO

2.1 Feromônios em percevejo marrom *Euschsitus heros* (F.)

Pentatominae é a principal subfamília de percevejos com aproximadamente 2800 espécies exclusivamente fitófagos, usando mais de 10 famílias de plantas como hospedeiros (PANIZZI e LUCINI, 2017; ESQUIVEL et al., 2018). Dentre as espécies, o percevejo marrom *Euschistus heros* (F.) é nativo da América tropical e está bem adaptado aos climas mais quentes, sendo mais abundante no centro-oeste do Brasil (GODOY et al., 2010; DEGRANDE e VIVAN, 2012). *Euschistus heros* (Figura 2.1) é predominante em lavouras de soja, mas também podem se alimentar de diversas plantas como o feijão, ervilha, alfafa (PANIZZI e SLANSKY JUNIOR, 1985; PANIZZI, 1997) e em plantações de algodão (PANIZZI; BUENO; SILVA, 2012, WEBER et al., 2018).

Figura 2.1: Percevejo marrom *E. heros* adulto (Adaptado de PANIZZI; BUENO; SILVA, 2012).

A soja tem grande importância no cenário mundial, tanto no âmbito alimentar como econômico, uma vez que seus grãos atendem o mercado de produção de farelo, farinhas, óleo, bebidas à base de soja entre outros (OLIVEIRA et al., 2015). O percevejo marrom *E. heros* pode ocasionar muitos danos à cultura, pois suga diretamente os grãos de soja para se alimentar, ocasionando redução na produção e na qualidade das sementes (DEPIERI e PANIZZI, 2011; BLASSIOLI-MORAES et al., 2012; SILVA et al., 2012; SILVA et al., 2014). Em plantações de algodão, o *E. heros* se alimenta das cápsulas de maturação, provocando perdas na qualidade dos fios e na produtividade (GREENE et al., 2001; WILLRICH et al., 2004).

O Manejo Integrado de Pragas (MIP), é caracterizado pelo uso de diversas técnicas que são empregadas harmonicamente visando solucionar um problema específico. O objetivo primário do MIP é reduzir as perdas causadas por pragas de modo efetivo, economicamente viável e ecologicamente compatível com o meio ambiente, chegar a soluções mais duradouras, ao invés de saídas a curto prazo (DEGRANDE e VIVAN, 2012). O pano de batida é o método mais utilizado para monitoramento do *E. heros*. Esse método consiste em um pano ou plástico branco de 1 metro de comprimento por 1 metro de largura, tendo nas bordas uma bainha onde são inseridos dois cabos de madeira. O pano, devidamente enrolado e sem perturbar as plantas, é introduzido entre duas fileiras adjacentes de soja e estendido sobre o solo. Rapidamente, as plantas das duas fileiras de soja são inclinadas sobre o pano e batidas vigorosamente, com o objetivo de deslocar os insetos das plantas para o pano. Na sequência, as plantas voltam à posição original, e os insetos sobre os panos são contados e registrados em fichas de monitoramento (CORRÊA-FERREIRA, 2012). Segundo Panizzi et al. (1977), se considera como nível de controle a presença de dois percevejos adultos por metro linear, para a produção de grãos, e de um percevejo, para a produção de sementes. O uso de pano de batida requer mão de obra qualificada, além da exigência de tempo devido as extensas áreas agrícolas, uma vez que é indicado realizar dez panos de batida a cada 100 ha (CORRÊA-FERREIRA et al., 2009). Com isso, nem sempre esta técnica é realizada ou então, realizada de forma correta (PIRES et al., 2006), levando, muitas vezes, a aplicações ineficientes de inseticidas. Uma alternativa para este método a fim de monitorar ou então detectar a presença de *E. heros* seria a utilização de armadilhas ou dispositivos com seu feromônio sexual.

Diversos estudos comprovando a eficiência do uso do feromônio sexual do *E. heros* em armadilhas foram publicados. Dentre eles, no trabalho de Borges et al. (1998b), a mistura racêmica sintética de 2,6,10-trimetiltridecanoato de metila, (componente do feromônio masculino de *E. heros*) foi atraente para outras espécies de pentatomídeos usando armadilha caseira. No estudo de Borges et al. (2011), os resultados mostraram que armadilhas com 1 mg de feromônio sexual (2,6,10-trimetiltridecanoato de metila) capturavam insetos com eficiência. Silva et al. (2014), avaliaram a eficiência das formulações em septo de borracha e lure do feromônio sexual do percevejo *E. heros* e compararam ao método do pano de batida. Durante o período crítico do ataque dos percevejos à soja, as armadilhas com feromônio foram mais eficientes para captura e monitoramento desses insetos no campo do que a técnica do pano de batida.

O termo feromônio foi proposto por Karlson e Lüscher em 1959, para definir substâncias secretadas por um indivíduo e recebidas por um segundo indivíduo da mesma espécie (BREZOLIN et al., 2018). São moléculas orgânicas voláteis de baixo peso molecular e que pode ser usado para comunicação entre membros do mesmo sexo ou do sexo oposto. (PHILLIPS, 1997). A produção e liberação de feromônios de insetos é governada por uma variedade de fatores ambientais e mecanismos fisiológicos. Além disso, a quantidade de feromônios que os insetos liberam é extremamente baixa e varia de alguns nanogramas a microgramas por unidade de tempo, dependendo da espécie (PIÑERO e RUIZ-MONTIEL, 2012). Fatores que podem ter impacto no lançamento de feromônios incluem ritmo circadiano, temperatura, presença de fontes alimentares e idade dos insetos (TEWARI et al., 2014). Os feromônios estão incluídos na comunicação intraespecífica, onde tanto o emissor quanto o receptor pertencem à mesma espécie, diferente dos aleloquímicos, que desempenham papéis interespecíficos nos quais o emissor e o receptor são de diferentes espécies (JURENKA, 2004; ZARBIN; RODRIGUES; LIMA, 2009).

Existem diferentes tipos de feromônios e, dependendo da função biológica, apresentam propriedades químicas diferentes (LAW e REGNIER, 1971). Os feromônios de agregação são compostos emitidos para atrair tanto machos quanto fêmeas (BAKKE; FROYEN; SKATTABOL, 1977; KRAWIELITZKI et al. 1977), já os feromônios repelentes são compostos que atuam como sinais de alerta para evitar fontes de colonização ou alimentação (ROBINSON et al. 2005; ROBINSON et al. 2008). Feromônios de alarme são compostos que são iniciados por um comportamento agressivo ou podem causar dispersão como uma resposta aos predadores e são usados principalmente por insetos (WILSON, 1965). Os feromônios de trilha são compostos usados por muitos insetos para marcar o caminho para um ninho ou fontes de alimento (TAI; MATSUMURA; COPPEL, 1969). Além destes, destacam-se também os feromônios sexuais que são sinais químicos produzidos por um inseto para atração do inseto do sexo oposto, com o objetivo de reprodução, e são consideradas substâncias muito ativas, capazes de causar respostas em concentrações muito baixas (MORAES et al., 2008a; WEBER et al., 2017). Formando parte de um sistema de MIP, o uso de feromônios e outros semioquímicos estão sendo usados para monitorar o controle de pragas em milhões de hectares de terra (WITZGALL; KIRSCH; CORK, 2010) podendo ser uma ferramenta alternativa útil quando se trata de estimar os níveis de população de insetos por meio de amostragem de campo (WONG et al., 2012; HARRIS et al., 2015). O uso de feromônios tem-se desenvolvido seguindo três caminhos principais, o monitoramento de populações de insetos utilizando armadilhas com feromônios, captura massal usando um grande número de armadilhas, e confusão sexual que interfere na comunicação e impede o acasalamento dos insetos (ZARBIN; RODRIGUES; LIMA, 2009).

Na espécie *E. hero*s foram identificados três acetatos específicos do macho, o 2,6,10-trimetiltridecanoato de metila, (*E*, *Z*) -2,4-decadienoato de metila e 2,6,10-trimetildodecanoato de metila. Bioensaios em laboratório e testes de campos mostraram que fêmeas de *E. heros* são atraídas principalmente pelo componente 2,6,10-trimetiltridecanoato de metila sendo a presença dos outros dois componentes desnecessária para atração (ALDRICH et al., 1994; BORGES et al., 1998a, 1998b, 1999). Diversos estudos foram realizados monitorando o feromônio sexual de *E. heros*, como no trabalho de Silva et al. (2014), que avaliaram a eficiência das formulações do feromônio sexual do *E. heros* em septo de borracha e lure, comparando ao método do pano de batida para o monitoramento em lavouras de soja. As amostragens foram realizadas semanalmente, durante a fase reprodutiva da soja. As formulações do feromônio foram eficientes na captura de *E. heros*, e a formulação em lure capturou mais percevejos. O controle precoce da população com feromônio resultou em sementes com maior qualidade do que as provenientes das parcelas monitoradas pelo pano de batida. Borges et al. (2011) avaliaram armadilhas desenvolvidas com feromônio de *E. heros* em campo e observaram que estas atraíram continuamente insetos fêmeas por mais de 30 dias. As armadilhas foram eficazes quando comparadas ao método do pano de batida, facilitando o monitoramento do inseto.

De acordo com Brezolin et al. (2018), a detecção de voláteis como os semioquímicos por meio do uso de sensores gera interesse em diversas áreas. Geralmente a identificação destes compostos pode ser avaliada em detalhes com a cromatografia gasosa ou com o equipamento de espectrometria de massa. Porém, o elevado tempo de análise e a exigência de pessoal capacitado para esta operação estão entre algumas desvantagens, fazendo com que outros métodos ganhem destaque, como o uso de sensores químicos específicos, capaz de reconhecer odores simples ou complexos (YEW e CHUNG, 2015). Já a utilização de sensores tem como vantagem a detecção em tempo real, sensitividade, versatilidade e menor agressão ao meio ambiente, reconhecendo analitos específicos (JOHNSON e MUTHARASAN, 2012).

A importância do desenvolvimento de novos dispositivos para detecção de feromônios traz como uma alternativa promissora os nanossensores de cantilever com a microscopia de força atômica (AFM), pois estes, apresentam-se como dispositivos miniaturizados e extremamente sensitivos (na ordem de partes por bilhão (ppb) e, até mesmo, em partes por trilhão (ppt) a diversos compostos) (STEFFENS et al., 2014a).

2.2 Sensores para detecção de feromônios

O sensor é definido como um dispositivo que identifica e transforma uma informação em sinal mensurável a ser usado (HULANICKI; GLAB; INGMAN, 1991). A geração do sinal ocorre quando as moléculas do analito interagem com a camada sensitiva por meio de processos físicos e químicos promovendo mudanças, geralmente nas propriedades elétricas ou ópticas, que são detectadas por um transdutor apropriado e convertidas em um sinal elétrico de saída (TEWARI et al., 2014). Os sensores para detecção de feromônios tem mostrado grande interesse, pois é uma técnica promissora no manejo de insetos-praga além da pequena quantidade liberada pelos insetos e da dificuldade de comprovar a atividade biológica através de bioensaios (STEFFENS et al., 2014b). Os sensores de voláteis funcionam como transdutores, convertendo em sinais, as respostas da interação de um material ativo com moléculas odoríferas. Os principais sensores utilizados para a detecção dos compostos odoríferos são eletroquímicos, principalmente condutométricos e os piezoelétricos (JANATA e JOSOWICZ, 2003; BREZOLIN et al., 2018). Nos sensores condutométricos, os eletrodos convertem em um sinal elétrico a resposta da interação do composto volátil com o material ativo e um aquecedor é necessário para aumentar a temperatura de detecção. Os sensores piezoelétricos se baseiam na medição de mudanças na frequência de ressonância devido a alterações de massa e/ou microviscosidade (THÉVENOT et al., 2001). De acordo com a camada sensora, podem ser classificados como sensores semicondutores baseados em óxidos metálicos e sensores semicondutores com uma camada de polímeros condutores orgânicos (JANATA e JOSOWICZ, 2003; BARSAN; KOZIEJ; WEIMAR, 2007). Os sensores semicondutores são simples, robustos e apresentam excelente resposta, reagindo a pequenas concentrações do analito. Normalmente, os dispositivos são fabricados a partir de óxido de zinco, estanho, titânio, tungstênio e irídio, e dopados com uma camada metálica ou filmes finos de ligas de paládio ou platina. São sensores de grande sensitividade (5 a 500 ppm) e rápido tempo de resposta. Como inconveniente estão sujeitos à saturação mesmo em baixas concentrações de voláteis (MEDEIROS et al., 2012).

Com o desenvolvimento de tecnologias de sistemas microeletromecânicos (DEAN e LUQUE, 2009; LIAO e TSAI, 2009), cantileveres ressonantes foram fabricados para detecção de moléculas químicas (LAVRIK; SEPANIAK; DATSKOS, 2004). Além da sensitividade e seletividade, são vantajosos em tamanho de dispositivo miniaturizado (YU et al., 2009). Assim, sensores de cantilever são promissores para aplicações industriais de detecção e monitoramento de gases. Na agricultura, a nanotecnologia agrega valor à agricultura de precisão, aprimorando a intervenção humana com o uso de sensores no monitoramento e redução de danos ambientais, identificando compostos, a fim de melhorar a eficiência da produção (EMBRAPA, 2019).

2.2.1 Sensores de cantilever

Avanços da nanotecnologia permitiram o desenvolvimento de dispositivos miniaturizados que podem atuar como sensores de alta sensitividade, com resposta em tempo real e reconhecimento específico (CARRASCOSA et al., 2006; JOHNSON e MUTHARASAN, 2012). A invenção, em 1986, do equipamento de microscopia de força atômica - AFM (do inglês *Atomic Force Microscope*), introduziu a possibilidade da criação de uma nova ferramenta de detecção para vários analitos por meio de funcionalização de cantilever, que então operam como sensores (SANG; ZHANG; ZHAO, 2013). Os sensores desenvolvidos em nanoescala apresentam aumento da área de superfície específica (MALIK et al., 2013) além de outras propriedades interessantes, como as propriedades físicas, químicas, mecânicas diferenciadas dos mesmos materiais em grande escala, como por exemplo, um aumento significativo da sensitividade e do limite de detecção, possibilitando, assim, que sejam necessárias poucas moléculas de analito ou espécie alvo para garantir a sua detecção (ZHAO e JIANG, 2010; DURÁN e MARCATO, 2013; SANTOS et al., 2014). Os sensores nanomecânicos baseados em cantileveres, podem ser aplicados para detectar muitas substâncias específicas, como pesticidas (MARTINAZZO et al., 2018; MUENCHEN et al., 2018), metais pesados (CHEN et al., 2012; RIGO et al., 2019), hormônios (DE CEZARO et al., 2019), feromônios de insetos (BREZOLIN et al., 2019; BREZOLIN et al., 2020a; BREZOLIN et al., 2020b), entre outras. Os cantileveres são os dispositivos microeletromecânicos (MEMs) mais simples que podem ser produzidos em massa. Oferecem uma resposta dinâmica aprimorada, tamanho bastante reduzido, alta precisão e confiabilidade (PINNADUWAGE et al., 2004). Os cantileveres consistem em um dispositivo que pode atuar como um sensor físico, químico ou biológico, detectando alterações na deflexão ou na frequência vibracional. Eles são compostos de hastes finas e flexíveis. Um ou mais bastões de silício ou bastões de nitreto de silício são usados para construção, material de baixo custo, e geralmente têm comprimento e espessura da ordem de micrômetros, podendo sofrer deformações quando uma massa específica de analito é adsorvida em sua superfície (VASHIST 2007; RAITERI et al., 2001; LANG et al., 2016). A detecção de substâncias por meio de cantileveres permite obter resoluções em nanograma (10^{-9}), picograma (10^{-12}), femtograma (10^{-15}), attograma (10^{-18}) e até zeptograma (10^{-21}) (SWIERCZEWSKA et al., 2012; UZUNLAR et al., 2013; TOUHAMI, 2014; OKAN e DUMAN, 2018). O sensor de cantilever pode ser operado em gases ou em meio líquido, podendo analisar amostras diretamente no seu ambiente natural e em diferentes temperaturas (GADEGAARD, 2006; TAMAYO et al., 2013; VAHABI; SALMAN; JAVANMARD, 2013). Pode ser utilizado para medir forças entre o cantilever (constante de mola) e a amostra a ser analisada, para isso, a sensitividade dos nanossensores irá depender da interação da camada ativa com o elemento a ser analisado (STEFFENS et al., 2014b).

Dentre as várias formas de detecção, para utilização como sensor, o método óptico é o mais utilizado, pois a luz visível que parte de um diodo de laser de baixa potência, é focada na extremidade do cantilever que atua como um espelho. O feixe refletido atinge uma posição no fotodetector, onde a distância percorrida do feixe sobre o detector é proporcional à deflexão da haste ou a variação da frequência de ressonância, de acordo com as moléculas adsorvidas no cantilever, que depende da natureza do ligante químico (BERGER et al., 1997) (Figura 2.2).

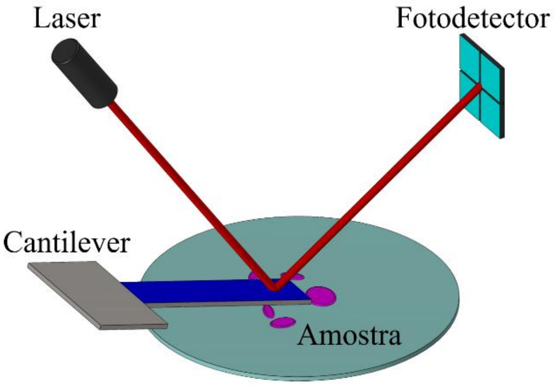

Figura 2.2: Diagrama do funcionamento de nanossensor de cantilever, com a incidência de feixe de laser em sua extremidade e sua reflexão para o fotodetector de quatro quadrantes.

Dentre os diferentes modos de operação, o modo estático e o modo dinâmico são os mais utilizados (HEGNER e ARNTZ, 2004). No modo estático, apenas um lado da superfície do cantilever é funcionalizado, ocorrendo uma interação molecular (reconhecimento do analito) causando uma alteração na tensão, ocorrendo a deflexão (mudança de posição na direção z). O modo dinâmico depende de alterações de frequência de ressonância causadas por adsorção de massa ou rigidez do cantilever (constante de mola). A frequência de ressonância diminui conforme aumenta a massa no cantilever devido a interações existentes com a amostra (WANG et al., 2007; JOHNSON e MUTHARASAN, 2012; TAMAYO et al., 2013; SANG et al., 2014).

Para que sejam alcançadas grandes deflexões no modo estático, o cantilever deve ter uma haste longa e uma constante de mola baixa. Para que elevadas frequências de ressonância sejam alcançadas no modo dinâmico, utiliza-se um cantilever com haste curta e constante de mola grande, na faixa de 1 a 40 N/m (WANG et al., 2007; MANZOLI et al., 2010).

Os parâmetros mecânicos básicos de um cantilever são a constante de mola e a frequência de ressonância. A constante de mola (k) (N/m) é um fator de proporcionalidade entre a força (F) (em Newton) aplicada e a deflexão vertical resultante do cantilever (nm) (ΔS) ao sofrer uma força, onde está relação é chamada de Lei de Hooke, de acordo com a Equação 1 (YEW; CODY; KRAVITZ, 2008):

$$F = k.\Delta S \qquad \text{Equação 1}$$

A constante de mola (k) produz a rigidez de um cantilever. Sua expressão é dada em função das dimensões geométricas e parâmetros do material (Equação 2) (WANG; ZHANG; HE, 2007).

$$k = \frac{E.w.t^3}{4.l^3} \qquad \text{Equação 2}$$

onde: E é o módulo de *Young* (E = 1,3 x 10^{11} N/m^2 para silício no plano <100> de sua estrutura cristalina) e, *t*, *w* e *l* são, respectivamente, a largura, espessura e comprimento do cantilever (LANG; HEGNER; GERBER, 2010).

A constante de mola de um cantilever é relacionada ao módulo de *Young*, o qual depende das propriedades do material, definindo assim a sensitividade do cantilever (NADDEO et al., 2012).

A frequência de ressonância (f_{res}) para um cantilever retangular simples pode ser expressa pela Equação 3 (CHEN et al., 1995):

$$f_{res} = \frac{1}{2\pi}\sqrt{\frac{k}{m}} \qquad \text{Equação 3}$$

onde: m é a massa de um cantilever (kg), a qual pode ser expressa como (Equação 4):

$$m = \rho.t.l.w \qquad \text{Equação 4}$$

onde: ρ é a densidade do material (kg/m^3), t, w e l denotam a espessura, largura e o comprimento do cantilever, respectivamente.

Uma vez que as moléculas do analito alvo são especificamente capturadas pela camada sensora, a massa do cantilever aumentará, provocando uma diminuição na frequência de ressonância do cantilever (YU et al., 2012). Segundo Mirza et al. (2014), foi observado alteração no comportamento do cantilever, onde a mudança na frequência de ressonância está diretamente relacionada com a massa adicionada. Ainda, a frequência de ressonância de um cantilever pode ser alterada devido a uma mudança na constante de mola ocasionada pelo estresse induzido por flexão (CHERIAN e THUNDAT, 2002).

A sensitividade durante a medição, não depende somente das propriedades do cantilever. Os ruídos e a resolução são fortemente influenciados pelo desempenho do sistema de instrumentação e das condições do ambiente externo, e por serem frágeis, devem ser operados cuidadosamente (TAMAYO et al., 2013; BAUSSELS, 2015). Filenko (2008), afirma que uma melhoria na sensitividade (cerca de 25 vezes) pode ser alcançada com a ausência de ruído durante a operação de cantileveres.

2.2.2 Funcionalização de sensores de cantilever

Todas as substâncias sólidas (adsorventes) são capazes de atrair moléculas de gases (adsorvatos) para suas superfícies. A superfície sólida de um transdutor de cantilever é tipicamente modificada, ou seja, funcionalizada, para apresentar afinidade com o analito de interesse, o que vai depender da particularidade do material sólido e do volátil (FILENKO, 2008).

Para servir como sensores, os cantileveres devem ser revestidos com uma camada sensora que seja altamente específica e capaz de reconhecer moléculas alvo. Um método de funcionalização para alcançar a uniformidade dos compostos utilizados em nanoescala, preservando suas características únicas, é a montagem camada por camada, do inglês *Layer-by-Layer* (LbL), que permite a montagem de materiais na escala nanométrica, com simplicidade e qualidade (LEE et al., 2015; MICHEL et al., 2012). Além disso, resulta em estruturas organizadas, com maior probabilidade de ser alcançada na direção perpendicular ao substrato, devido à estrutura em camadas. A técnica LbL é muito interessante para a produção de estruturas supramoleculares devido à sua capacidade de combinar diferentes materiais de forma sinérgica (DECHER, 1997; ARIGA et al., 2015) além de ser possível diversas aplicações, especialmente para sensoriamento (OLIVEIRA et al., 2011; XI et al., 2012; BARROS et al., 2017).

Com base em um simples passo de imersão, a técnica LbL permite a fabricação de filmes finos de sensores e fornece excelente controle sobre as propriedades da superfície (DETSRI e POPANYASAK, 2015). Técnica bastante barata, uma vez que não requer salas limpas ou artigos de vidro sofisticados, além de ser conduzida à temperatura ambiente. Apesar de sua simplicidade, a LbL fornece um controle da espessura do filme e estrutura interna (SOLER, 2018). Esta técnica é obtida através da adsorção sequencial de componentes de carga oposta por forças atrativas tais como interações eletrostáticas, ligações de hidrogênio, entre outras (LEE et al., 2015). O substrato a ser modificado é imerso numa solução de um polieletrólito de carga oposta (por exemplo, um substrato carregado negativamente é imerso em solução de um policátion). O polieletrólito adsorve na superfície do substrato, revertendo sua carga original. O substrato é enxaguado e imerso numa solução de um polieletrólito de carga oposta (solução de um poliânion), que também adsorve e inverte a carga superficial (DECHER, 1997; TORASSO et al.; 2017). Assim, as arquiteturas altamente ordenadas e de múltiplas camadas da LbL podem ser fabricadas de forma reprodutível, permitindo controle em nanoescala da espessura e composição de materiais compósitos híbridos (LEE et al., 2015). O método de deposição LbL não só pode ser aplicado a polímeros, mas também em combinações de polímeros e partículas ou com nanopartículas interagindo mutuamente (LEE; RUBNER; COHEN, 2006). A enorme versatilidade dos métodos de deposição LbL aparece ainda mais quando se consideram as diferentes possibilidades para depositar esses filmes em interfaces de líquido sólido. Dois passos de adsorção devem ser separados por enxágue com o solvente a fim de não apenas remover polieletrólitos fracamente adsorvidos, mas também para evitar contaminação cruzada das soluções contendo as moléculas para ser depositado (BUNGENBERG de JONG, 1952; MICHEL et al., 2012).

Outros métodos para revestir cantileveres também são empregados, como colocação manual (BERGER et al., 1996; BERGER et al., 1997), por soluções de pipetagem direta das moléculas da sonda nos cantileveres (LANG et al., 1998) ou empregando spray de pulverização (BALLER et al., 2000; LANG; HEGNER; GERBER, 2010). O método *dip coating* consiste em imergir um substrato em uma solução de polimerização química, onde parte do polímero será depositado em sua superfície (CHO et al., 2005). Este processo ocorre em diferentes substratos e a espessura do filme é geralmente controlada pelo tempo de imersão. Outro processo similar envolve, alternativamente, a imersão de um substrato nas soluções de monômero e oxidante. O monômero adsorvido será polimerizado na superfície do substrato (SILVERSTEIN et al., 2005; BAI e SHI, 2007). A vantagem da deposição por *dip coating* é a deposição de solução de baixo custo usada principalmente para revestimento uniforme de grandes áreas (MITZI, 2009) e para sintetizar filmes finos de alta qualidade (CHAKI; CHAUDARY; DESHPANDE, 2016). Os filmes de *dip coating* são obtidos devido ao crescimento de camada por camada durante cada imersão do substrato na solução aquosa. Em cada imersão do substrato, a camada individual é formada por adsorção iônica. Assim, a formação de película fina de revestimento por imersão é por adsorção íon-por-íon levando a deposição de camada por camada em cada mergulho, consequentemente à possibilidade de minimização ou até mesmo eliminação de defeitos e imperfeições nos filmes finos sintetizados. Outras vantagens são o controle sobre a taxa de deposição e a espessura do filme por meio de controle no tempo de imersão durante cada mergulho e regulando o número de afundamentos, respectivamente. Outros parâmetros como velocidade de imersão, velocidade de retirada, duração de secagem, tempo que o substrato fica sem solução entre mergulhos controlam a deposição do filme (CHAKI et al., 2017).

2.3 Polímeros condutores

Sensores baseados em polímeros condutores (polipirrol, polianilina e politiofeno) apresentam algumas vantagens como a facilidade de sintetizar, baixo custo, versatilidade, apresentam variedade de métodos de fabricação (dependendo do tipo de dopante utilizado e do grau de dopagem) e alta sensitividade a compostos voláteis (10 a 100 ppm) (BAI e SHI, 2007; OSTWAL; SAHIMI; TSOTSIS, 2009; DHIBAR et al., 2013; HUO; ZHANG; ZHANG, 2014). Polímeros condutores destacam-se por apresentarem propriedades elétricas, ópticas e magnéticas de materiais condutores e/ou semicondutores mantendo as mesmas propriedades mecânicas de polímeros convencionais (ACEVEDO et al., 2005; BHADRA et al., 2009). São formados por uma cadeia principal contendo ligações simples e duplas alternadas, ou conjugação. A utilização de polímeros condutores no desenvolvimento de sensores traz vantagens como a fácil sintetização além da grande sensitividade a compostos voláteis (BAI e SHI, 2007; KAPLAN, 2012).

O polímero condutor pode responder a uma ampla gama de gases e vapores, dependendo de vários mecanismos de interação. Algumas interações que ocorrem entre as moléculas de gás e o polímero condutor são descritas a seguir (BĂNICĂ, 2012):

- A molécula de analito afeta a transferência de carga entre o polímero e o contato do eletrodo;

- O analito provoca oxidação ou redução da cadeia polimérica, alterando assim a densidade dos veículos de carga;

- O analito pode interagir com os veículos de carga móvel na estrutura do polímero, alterando assim a mobilidade do transportador ao longo da cadeia;

- O analito altera a probabilidade de transporte e movimento entre cadeias, afetando assim a resistividade do filme.

2.3.1 Polianilina (PANI)

A PANI é um polímero condutor intrínseco, que pode ser dopado por protonação, ou seja, a adição de uma espécie fornecedora de H+, pode conferir um aumento da condutividade deste material. Este polímero também apresenta diferentes estados de oxidação, na qual a forma esmeraldina, que é 50% oxidada, é a mais estável, porém é isolante, no entanto se a mesma reagir com um ácido como ácido clorídrico (HCl) ocorre a formação de um sal de esmeraldina que é condutor (KANG; NEOH; TAN, 1998; VARGAS et al., 2018). A síntese química convencional possui a grande vantagem de produzir um polímero de alta massa molecular e de elevada pureza, que pode ser obtido diretamente no estado dopado, em grandes quantidades, na forma de um pó verde (estado de oxidação sal esmeraldina) (MEDEIROS et al., 2012). Conforme Fratoddi et al. (2015), a PANI apresenta-se como um material heterogêneo, possuindo regiões altamente cristalinas condutoras e regiões amorfas isolantes. A dopagem com ácido melhora a organização e consequentemente, sua condutividade. Nanoestruturas de polímeros condutores (nanobastões, fios, fibras e tubos) vêm sendo estudadas, baseadas na expectativa de que a combinação de polímeros orgânicos condutores e nanoestruturas permitiriam obter novas propriedades ou superiores (EFTEKHARI; LI; YANG, 2017).

A interação das moléculas com a camada sensora depositada na superfície do sensor pode ocorrer de duas formas: por adsorção química, onde as moléculas e átomos voláteis interagem com a superfície por ligações químicas, ou por adsorção física, relacionada com as forças de Wan der Waals entre átomos e voláteis de superfície, a energia associada a essas interações é muito baixa. Nessa situação específica, as moléculas podem ser adsorvidas quando atingem a superfície do substrato, mas não sofrem reações químicas e mantêm suas características (GHEORGHE et al., 2017). De acordo com Rozemarie et al. (2017), a PANI isolada ou associada a diferentes polímeros ou substâncias minerais, tem muitas aplicações como sensor de gás, devido à sua capacidade de converter uma interação química (uma variação na sua protonação ou estado de oxidação) em um sinal elétrico. Os sensores baseados em PANI são eficazes à temperatura ambiente em detectar muitas substâncias perigosas minerais ou orgânicas, mostrando boa seletividade e estabilidade e resposta rápida e tempo de recuperação.

Um sensor cantilever nanoestruturado com o nanocompósito de PANI e nanotubos de carbono foi desenvolvido e aplicado na detecção de 2,6,10-trimetiltridecanoato de metila, principal composto do feromônio sexual de *E. heros*. O sensor apresentou uma ligação específica ao composto e o limite de detecção foi de 2,18 fg/mL (BREZOLIN et al., 2020a).

2.3.2 Poliestireno sulfonato de sódio (PSS)

O poliestireno sulfonato de sódio (PSS) é um polímero sintético, que pode ser obtido por meio da sulfonação do poliestireno ou por meio da polimerização a partir do seu monômero sulfonato de estireno (WITT, 2012). O PSS possui como grupos laterais anéis benzênicos contendo grupos sulfônicos dissociados, o que proporciona carga negativa quando dissolvido em água (LIMA; PEREIRA; FONSECA 2017).

Como relatado por Decher (1997), a metodologia de LbL é baseada na atração eletrostática onde a deposição de poliânions entrelaçados e policátions sobre uma superfície carregada fornece um filme de multicamadas. Nesta técnica, o PSS é utilizado como solução aniônica. Mudanças estruturais na camada são introduzidas por deposição de polieletrólito. As regiões de cargas positivas e negativas são capazes de formar potenciais locais de ligação para sondas carregadas multivalentes e determinadas pela carga da camada de polieletrólito mais externo (SCHLENOFF et al., 1998).

Graboski et al. (2019) desenvolveram sensores de gás utilizando eletrodos interdigitados de grafite, os quais foram impressos em substrato de papel vegetal e eletrodos litografados de ouro confeccionados sobre substrato de vidro, utilizando PANI e PSS pelo método LbL, para detecção de diferentes aromas. Os resultados mostraram que os sensores foram eficientes na discriminação de aromas, demonstrando excelente potencial para análise de alimentos em aplicações industriais.

Brezolin et al. (2019) desenvolveram um sensor nanoestruturado de cantilever para detecção do composto 2,6,10-trimetiltridecanoato de metila, principal componente do feromônio sexual de *E. heros* utilizando PANI e PSS pelo método LbL. O sensor demonstrou bom desempenho e alto limite de detecção (1,44 fg/mL).

2.4 Nanopartículas de prata (AgNPs)

Nanopartículas metálicas são aquelas produzidas a partir de um átomo metálico desde que atendem as definições de tamanho preconizado pela nanotecnologia (BLACKMAN, 2009). Dentre os inúmeros tipos de metais utilizados na preparação de nanopartículas, é de grande interesse a prata, especialmente por suas propriedades catalíticas. Esse metal apresenta propriedades atribuídas aos metais nobres, como por exemplo, ótima condutividade e estabilidade química. Na forma de nanopartículas, no entanto, a prata apresenta uma superfície de contato muito maior e consequentemente suas propriedades são aumentadas (FRATTINI et al., 2005). Estudos mostram que as nanopartículas demonstraram o grande potencial como camada sensora devido ao seu tamanho extremamente pequeno, área específica alta e outras propriedades inerentes, como alta condutividade elétrica e propriedades ópticas únicas. AgNPs já foram estudadas em sistemas de nanossensores para detecção de gases (YANG; LI; YAN, 2010; GUO e WANG, 2011; CHEN; MORRIS; WHITMORE, 2013). Segundo Castillo-Castro et al. (2007), as nanopartículas poderiam atuar como junções condutoras entre as cadeias de PANI, resultando em um aumento da condutividade elétrica dos compósitos. Muitas pesquisas sobre compósitos de nanopartículas de metal e polianilina mostram capacidades de sensoriamento, elétricas e catalíticas aprimoradas, quando comparadas com PANI somente (NASERI; FOTOUHI; EHSANI, 2018). A existência de vários grupos funcionais de oxigênio em OG permite que AgNPs interajam com as folhas de OG através de ligações eletrostáticas ou de transferência de carga (MUSZYNSKI; SEGER; KAMAT, 2008), melhorando o desempenho de sensores (KAVINKUMAR; SASTIKUMAR; MANIVANNAN, 2015; KAVINKUMAR e MANIVANNAN, 2016). Já em matrizes de polímeros condutores a incorporação de AgNPs melhoram o funcionamento óptico, mecânico, térmico, de condução além das propriedades eletroquímicas (DHIBAR e DAS, 2015).

2.5 Nanohíbridos

À junção de dois ou mais materiais com propriedades diferentes e complementares para a construção de um novo material dá-se o nome de compósito, com o objetivo de se obter um novo material com propriedades únicas, impossíveis de serem encontradas em seus componentes individuais. Quando pelo menos uma das fases do material resultante se encontra em escala nanométrica, este se denomina nanocompósito. Estes podem ser formados pela combinação de materiais de diferentes tipos, como orgânico-orgânico, inorgânico-inorgânico e orgânico-inorgânico, que são conhecidos como materiais híbridos (MATHEWS e RAWLINGS, 1999; JOSÉ e PRADO, 2005). Contudo, as propriedades finais de um material híbrido são determinadas predominantemente em função da natureza da interface interna entre as fases orgânico-inorgânica (JOSÉ e PRADO, 2005).

Nanocompósitos de PANI e metais são considerados materiais atrativos, apresentando maiores propriedades ópticas, condutoras, químicas e mecânicas, podendo serem utilizados como sensores (GARAI; CHATTERJEE; NANDI, 2010; JIA et al., 2012). Estudos de nanocompósitos baseados em polímeros condutores geraram interesses de pesquisa enormes devido às suas propriedades excitantes. Destes metais, a prata (Ag) é um metal importante para a produção de nanopartículas devido à sua alta condutividade e também alta estabilidade térmica. A introdução de Ag em várias matrizes poliméricas melhora as propriedades térmicas, ópticas, mecânicas, e conduzindo propriedades resultando em uma nova classe de importantes materiais adequados para suas aplicações em dispositivos de memória e sensores (KUILA; GARAI; NANDI, 2007; DHIBAR e DAS, 2014). Os compósitos contendo nanopartículas metálicas e polímeros condutores são materiais muito interessantes devido à sua alta condutividade e boa estabilidade ambiental, bem como suas propriedades catalíticas (HNIDA; SOCHA; SULKA, 2013; SAID et al., 2015). A combinação de polímeros condutores com partículas metálicas oferece uma maneira promissora com a possibilidade de introduzir propriedades elétricas baseadas na sinergia entre os dois componentes e, ao reduzir o tamanho dos materiais para a escala nanométrica, as propriedades aumentam ainda mais (MA et al., 2015; SOPHIA e MURALIDHARAN, 2015).

Em nanohíbridos, as atividades eletrocatalíticas aumentaram com a redução dos tamanhos de material (HNIDA; SOCHA; SULKA, 2013; LI et al., 2015). Os polímeros condutores podem impedir a agregação de nanopartículas metálicas, aliviando a transferência de elétrons, o que aumenta a sensitividade e a seletividade (BIAN et al., 2010).

Quando a oxidação de anilina e a redução do íon metálico ocorreram juntos durante a reação, a PANI e o metal elementar são obtidos simultaneamente. As principais vantagens desta técnica são a ausência de iniciadores químicos externos e a possibilidade de polimerização em massa. Estes híbridos representam uma nova classe de materiais que podem combinar propriedades físicas desejáveis, características de seus componentes orgânicos e metálicos dentro de um único composto (LEYVA et al., 2011). Os híbridos de PANI e Ag podem ser obtidos de diferentes formas: pela síntese da PANI na presença de AgNPs, utilizando a PANI na forma esmeraldina como agente redutor de sais de prata ou pela oxidação da anilina na presença de sais de prata. A obtenção de híbridos de PANI e Ag se dá, de forma geral, pela redução de sais de prata na presença da PANI previamente sintetizada (BLINOVA et al., 2009; FUJII et al., 2010).

Recentemente, os sensores de gás baseados em nanocompósitos de PANI combinados com diferentes catalisadores de metais nobres têm sido objetos de considerável interesse. A razão para incorporar partículas metálicas na matriz polimérica condutora é aumentar a área específica destes materiais e assim melhorar a eficiência catalítica (CHOUDHURY, 2009). Jing et al. (2007) sintetizaram nanopartículas de prata com PANI. O nanocompósito Ag/PANI foi sintetizado com sucesso via polimerização *in situ* por meio da oxidação da anilina com base em colóide de AgNPs retidas com ácido mercaptocarboxílico. Stejskal et al. (2009) utilizaram o nitrato de prata, sal precursor das AgNPs também como agente oxidante para a polimerização do monômero anilina à PANI. O estudo da influência do ácido utilizado como dopante nas características do compósito formado mostrou um tamanho médio obtido para as AgNPs de 40 a 80 nm. Bober et al. (2010) sintetizaram um compósito Ag/PANI utilizando como dopante da PANI o ácido fórmico, oxidando a anilina com o nitrato de prata. Foram obtidos materiais constituídos por nanofios de prata revestidos com PANI com o uso de baixas concentrações de ácido fórmico e partículas esféricas com altas concentrações de ácido fórmico.

Nanocompósitos em LbL podem ser projetados na superfície de qualquer tipo de suporte sólido, variando de suave a áspero, em qualquer formato, como planos ou hastes e em qualquer tamanho de suporte sólido, de macro a nanoescala (PATERNO e SOLER, 2013; SEO; LEE; PARK, 2016). As nanoestruturas montadas não apenas combinam funcionalidades atraentes de cada componente, mas também mostram características sinérgicas, permitindo aplicações múltiplas, como revestimentos, eletrodos de capacitores e sensores químicos (ALCANTARA et al., 2013; SOLER, 2018).

Brezolin et al. (2020b) desenvolveram um sensor microeletromecânico com o nanocompósito de PANI/OG para detecção de feromônio sintético de *E. heros*, obtendo uma resposta linear, com sensitividade e limite de detecção de 6,24 Hz/fg e 0,91 fg/mL, respectivamente, demonstrando resultados promissores na detecção dessa substância na agricultura.

2.6 Considerações finais

O uso de feromônios de insetos para monitoramento e controle de pragas agregando a utilização da nanotecnologia é uma alternativa valiosa, trazendo avanços tecnológicos. A exagerada utilização de agroquímicos para combater as pragas podem trazer sérios problemas ao meio ambiente e também a saúde da população. Com isso iniciativas promissoras que identifiquem e controlam as pragas nas lavouras podem diminuir o uso dessas substâncias químicas. Em vista disto, o desenvolvimento de nanossensores de cantilever utilizando o feromônio sexual de *E. heros* pode ser aplicado na detecção desta praga nas lavouras, uma vez que os nanossensores são específicos, com alta sensitividade, podendo ser aplicados em tempo real. Isso deverá possibilitar estratégias mais efetivas de controle e monitoramento, refletindo na saúde dos seres vivos e também do meio ambiente sem a elevada utilização de agroquímicos.

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CAPÍTULO III

DEVELOPMENT OF NANOSENSORS WITH PANI.Ag NANOHYBRID AND PANI/PSS NANOCOMPOSITE AND APPLIED DETECTION OF SEXUAL PHEROMONE FROM *Euschistus heros*

Abstract:

In this study, polyaniline/poly (styrene sulfonate) (PANI/PSS) nanocomposite and polyaniline.silver (PANI.Ag) nanohybrid thin films were obtained in cantilever nanosensors surface. Their use as sensing element for pheromone detection were investigated. The developed films were characterized in relation to topography, roughness, thickness, height, and structural properties. The topography study revealed that both films have a globular morphology with thickness and height in nanoscale. The gas sensing performance was investigated for sexual pheromone from the neotropical brown stink bug, *Euschistus heros* (F.). The sensitivities of nanosensors based on PANI/PSS nanocomposite and PANI.Ag nanohybrid films were similar. A high limit of detection (LOD) of 3.16 ppq, and the limit of quantification (LOQ) of 10.05 ppq were obtained for PANI.Ag nanohybrid nanosensor. The nanosensor layer were supported by UV-Vis, FTIR analyses showing the incorporation of Ag nanoparticles in the nanohybrid. The developed cantilever nanosensors showed a reduction in the resonance frequency when exposed to the *E. heros* pheromone, showing high results of LOD, LOQ, sensitivity and repeatability, presenting great potential for detection of the *E. heros* sexual pheromone.

Keywords: Polyaniline; Sensor; Thin films; Thickness; Height.

3.1. Introduction

Sex pheromones are volatile organic molecules of low molecular weight, used for communication between members of the same sex or the opposite sex. In particular, the insect pheromone produced by one sex to attract the opposite sex has the objective of reproduction, considered substance capable of causing responses in very low concentrations [1]. Male of *E. heros* insects liberate as volatile compounds three specific acetates: methyl 2,6,10-trimethyltridecanoate, methyl (E, Z) -2,4-methyl decadienoate, and methyl 2,6,10-trimethyldodecanoate. Laboratory bioassays and field tests showed that *E. heros* females are mainly attracted by the methyl 2,6,10-trimethyltridecanoate component, the presence of the other two components being unnecessary for attraction [2,3].

Pheromones are used to monitor pest control, as part of an Integrated Pest Management (IPM) system [4]. Monitor and identify *E. heros* population accurately is very important to control and made proper decisions in conjunction with action thresholds, especially when pesticides are used in your control. Monitoring eliminates the use of pesticides when they are not really needed. Thus, indicate the right time of applications, reducing the production cost in soybean, bean, pea, cotton, and alfalfa crops, where *E. heros* can be a prevalent pest. This insect can cause a lot of damage to the culture, because it sucks directly the grains to feed, reducing the production and quality of the seeds. In this way, new tools for insect monitoring and detection are desired for future applications in agricultural being a useful alternative to estimating insect population levels [5].

The development of new devices for pheromones detection brings as a promising alternative the cantilever gas nanosensors, since they are extremely sensitive devices (in the order of parts per billion (ppb) until parts-per-quadrillion (ppq)) [6].

Gas sensors coated with polymeric nanocomposites and nanoparticles have received considerable attentions due to many outstanding properties (mechanical and physical) caused by the large surface area to volume ratio, and great interfacial reactivity of the nanoparticles. Nanomaterials including silver nanoparticles (Ag NP) are chemically stable and present high surface to volume ratio of metal. These materials have been used in sensors showing ultra sensitivity, fast and easy operation. Conducting polymer matrices incorporated with Ag nanoparticles can improve some properties as optical, mechanical, and thermal [7,8]. Between the conducting polymers, the polyaniline (PANI) has the ability to detect volatile molecules, because of its excellent chemical, ease polymerization, low monomer cost, thermal and environmental stability [9-11]. In previous work cantilever sensors coated with PANI/PSS [12], PANI/graphene oxide nanocomposite [13], and PANI/multi-wall carbon [14] were used for the detection of methyl 2,6,10-trimethyltridecanoate, the main component of *E. heros* sexual pheromone.

This study aims to develop a simple and efficient cantilever nanosensor to detect *E. heros* pheromone using PANI.Ag nanohybrid and PANI/PSS nanocomposite film. PANI.Ag was synthesized through a chemical oxidation method, which has relatively low cost and high stability. The performances of cantilever gas nanosensors such as sensitivity, limit of detection (LOD), limit of quantitation (LOQ), coefficient of determination (R^2), response time, repeatability, and accuracy were investigated in details.

3.2 Materials and Methods

3.2.1 Chemical synthesis

PANI was prepared by conventional chemical synthesis of interfacial polymerization as described in previous work [12]. The oxidant (ammonium persulfate, 98%, Sigma-Aldrich, USA) was dissolved in 1 mol/L of hydrochloric acid (HCl, 99%, Merck, Kenilworth, NJ). The aniline (99.5%, Sigma Aldrich, USA) was dissolved in dichloromethane (99.5%, Synth, Diadema, Brazil) in a ratio 1:2 (v/v). Next, this solution was placed slowly into a round bottom flask (protected from light) containing the aqueous solution. The reaction was maintained at room temperature (25°C) for 2 h. After, the reaction product was filtered, washed three times with methanol and distilled water. The filtrate was dried in a desiccator under vacuum for 20 h, obtaining the PANI powder (emeraldine salt). The powder (10 mg) was diluted in N,N dimethylacetamide (99.5%, Sigma Aldrich, USA), and maintained in ultrasound bath equipment (Unique model USC-1800, Brazil) for 1 h. Next, HCl solution was added in the dilution until it reached pH 3.0.

The PANI.Ag synthesis was obtained by the reduction of silver nitrate in the PANI powder (emeraldine base). Initially, 0.25 g of aniline (monomer) was dispersed in 15 mL N,N dimethylacetamide and disposed in ultrasound for 15 min. Next, was added 10 mL of HCl solution (1 mol/L) and 25 mL of silver nitrate aqueous solution (0.08 g/mL, Synth, Brazil). The reaction occurred under agitation (Fisaton, 752^a, Brazil) for 24 h at room temperature (25°C) [15]. Then, the precipitates were filtered, washed with distilled water, and dried under a dynamic vacuum for 48 h. The powder was diluted in N,N dimethylacetamide and doped with HCl (1 mol/L), and maintained under sonication followed by vigorous. The solution was used to obtain PANI.Ag film.

3.2.2 Films deposition

Two gas nanosensors were obtained using PANI/PSS nanocomposite and PANI.Ag nanohybrid films. PANI obtained by convectional chemical synthesis was deposited on the sensor surface by layer by layer (LbL) technique, and PANI.Ag by dip coating technique.

PANI/PSS nanocomposite: The PANI/PSS nanocomposite film was deposited alternate the polycation (PANI) and polyanion (polystyrene sulfonate solution - PSS) layer at the cantilever surface [12]. The cantilever was immersed in the polycation solution for 3 min. Then, washed with HCl (1 mol/L) and dried for 15 min at room temperature (25 °C). After drying, was immersed in the polyanion solution for 3 min, then washed with HCl, and dried for 15 min. This process was repeated until obtaining seven bilayers. All details regarding the film deposition can be found in our recent publication [12].

PANI.Ag nanohybrid: The PANI.Ag nanohybrid film was achieved by immersion of the cantilever in PANI.Ag solution, remaining in contact for 3 min. Then, it was washed with HCl (1 mol/L), and dried in a desiccator at room temperature (25 °C).

3.2.3 Characterization methods

Surface topography, roughness, thickness, and height of PANI/PSS nanocomposite and PANI.Ag nanohybrid films were investigated using atomic force microscopy (AFM, Nanosurf, C3000 Controller, Switzerland). The root-mean-square average (RMS) was calculated in different locals of the image. The thickness of the sensor layer was obtained in area of 50 μm^2. The images, roughness, thickness, and height were processed using the Gwyddion software. Measurements were done in three different regions.

The structural analyses of the composites were performed by UV-Vis spectrophotometer (Agilent, Model 8453E, USA), using a quartz cuvette. The attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) were obtained in a FT-IR spectrophotometer (Perkin-Elmer Spectrum 100S, USA). The spectra was recorded from 4000 to 800 cm^{-1} at the transmittance mode. X-ray diffraction (Mini flex II, Rigaku, Tokyo, Japan) patterns were obtained using powder diffraction techniques on a diffractometer with CuK(alpha) radiation, to observe the composition, structure and physical properties of the PANI and PANI.Ag. The samples were scanned from 1.35 to 70.

3.2.4 Gas-Sensing Measurements

The gas nanosensors’ responses of pheromone were monitored through resonance frequency measurement. The experimental apparatus includes a polypropylene chamber (6.3 cm^3), with two side holes (Figure 3.1a), for entry and exit the gases, docked over the AFM scanner, and sealed with two rubber rings. The chamber had a 25 μm filter paper inside (Millipore, Brazil) for better dispersion of the volatile. The cantilever was placed in the support and inserted in the scanner of AFM equipment (Nanosurf, C3000 Controller, Switzerland) (Figure 3.1b). The laser was adjusted, positioned at the end and central position center of the cantilever, followed by the adjusting of the signal strength in the photodetector in AFM equipment [14].

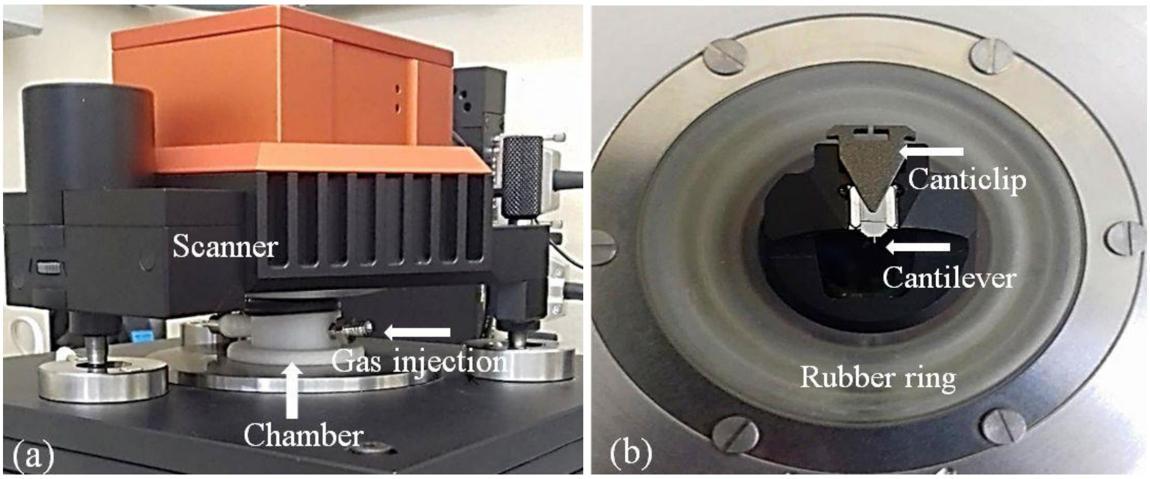


Figure 3.1: Illustration of gas chamber connected to the AFM scanner to assess the resonance frequency (a) and cantilever inner of the gas chamber inserted in the canticlip (b).

The resonance frequency was evaluated for all developed cantilever nanosensors and for non-functionalized cantilevers (control). Initially, the nanosensor was kept in contact with synthetic air (White Martins, Brazil, 99%) for 2 min, to ensure stable data collection of the resonance frequency signal. Once, the nanosensors were stabilized, a baseline was initially obtained for two minutes. Next, the pheromone was inserted into the chamber using a micro syringe (Hamilton, USA) and evaluated the resonance frequency every 20 s for 5 min. In this way, resonance frequency values were measured alternately tree times in synthetic air and pheromone volatile compounds. After the measurements, in each concentration, the gas chamber was cleaned with dry nitrogen gas.

The 2,6,10-methyl trimethyltridecanoate volatile compound, sex pheromone of *E. heros*, has 0.9 ± 0.1 g/cm^3 density and 270 g/mol molecular mass. Different concentrations (0.005, 0.01, 0.05, 0.1, 0.2, and 0.3 µg/mL) were studied based on the volume of the chamber, density and molecular mass of the pheromone. The volume of the pheromone compound injected into the gas chamber to achieve a certain concentration was calculated according to Steffens et al. [16]. As the injected gas was initially in the liquid form, it was dropped on filter paper and evaporated to convert it to a gas state. After each concentration evaluated, the filter paper was removed, and synthetic air was added again inside the chamber. In all experiments, temperature and relative humidity (RH) inside the chamber were monitored with a commercial sensor (Sensirium, Kit EK-H5, resolution SHT2x, Brazil). The experiments were performed at in acclimatized room at 25 ± 2^{o}C.

3.2.5 Gas-Sensing Measurements

The performances of both gas nanosensors were evaluated in terms of sensitivity, linear correlation (R^2), LOD, LOQ, response time, repeatability, and accuracy. The response time of the nanosensors was defined as 90% time taken by the device to change resonance frequency and obtain the equilibrium [17] when exposed to the pheromone and then back to the original signal upon exposure to synthetic air. The detection limit (LOD) was calculated from the analytical curve considering the mean blank value plus three times the standard deviation. In this way, shows the smallest pheromone concentration able to distinguish by the developed nanosensors. The quantification limit (LOQ) was obtained by the mean blank value plus ten times the standard deviation. Sensitivity was obtained by the slope of the analytical curve. The repeatability of the nanosensors was evaluated by the capability of repeating similar characteristic behaviors. Therefore, the resonance frequency was evaluated in a concentration of 0.3 µg/mL of pheromone. A relative standard deviation lower than 1.9% indicates that the measurements are repeatable [18].

3.3 Results and Discussion

3.3.1 Characterization of thin films

The ultra-violet-visible spectra (UV-Vis) of PANI synthesis of aqueous solution (Figure 3.2a) present characteristic bands at about 240 and 300 nm. These peaks are attributed to the electron transition from the ligand orbital (π) to the anti-ligand orbital (π *), and π-π * transition in the benzoid rings, respectively, related to the benzoid and quinoid rings of the PANI chain [19]. The bands observed at 450 and 470 nm are responsible for the transition of the antibonding electron (π *) of the polaronic band [20]. Absorption at ~ 450 nm in PANI.Ag spectra (Figure 3.2b) may be characteristic of the AgNPs band. This strong absorption peak between 400 and 450 nm is due to the electron surface plasmon resonance in the conduction bands [21]. The wide shoulder at 800 nm is attributed to the π-polaron transition formed in the main PANI chain, so both synthesis (convectional synthesis and PANI.Ag) are in the conducting form (emeraldine salt) [22,23].

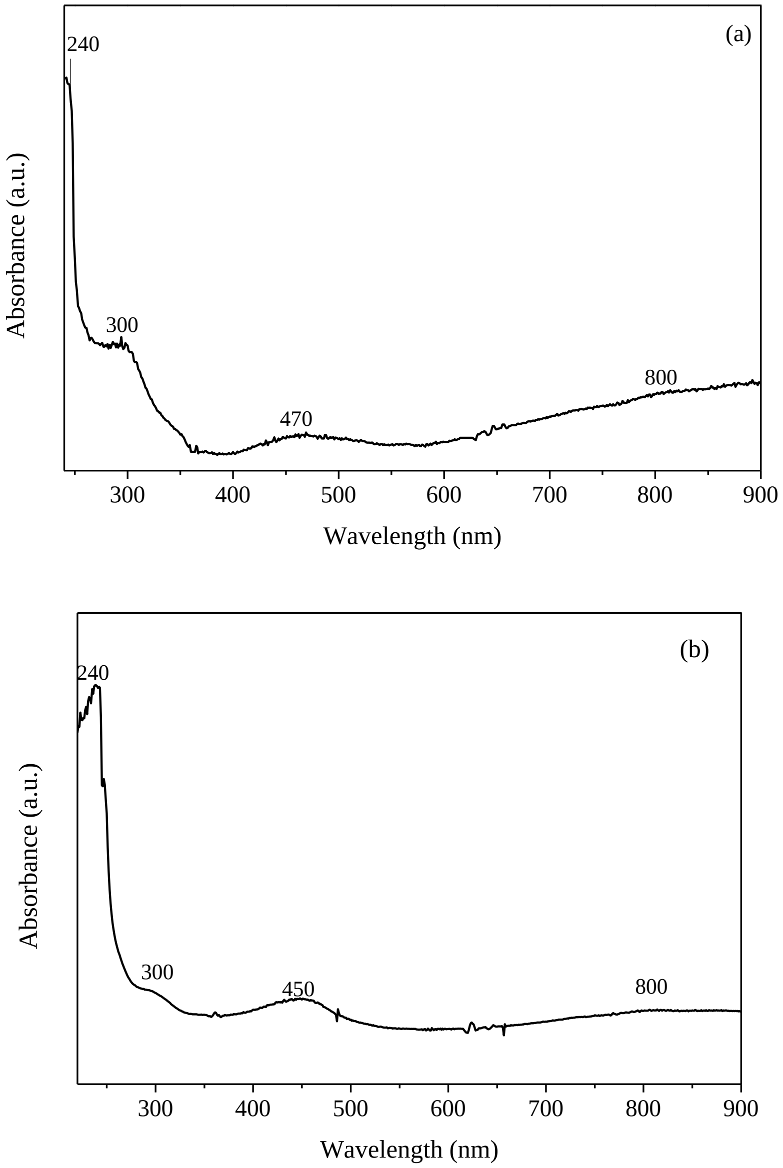


Figure 3.2: Ultra-violet-visible spectra (UV-Vis) of PANI (a) and PANI.Ag (b) powders.

The XRD pattern of PANI illustrated in Figure 3.3a shows the peak at 2θ = 15º, 20º, and 25º characteristic of the polymer in the form of emeraldine salt. The peaks at 15º and 20º are attributed to parallel and perpendicular repeat units of the PANI chains. The peak at 25º is attributed to periodicity between PANI chain. The PANI pattern is generally broader and less intense, which corresponds to the amorphous nature [24]. According to Mooss and Athawale [25] the peaks at 2θ, 19.96º (100) and 25.26º (110), with respective plans corresponding to the quinoid and benzoid units of PANI.

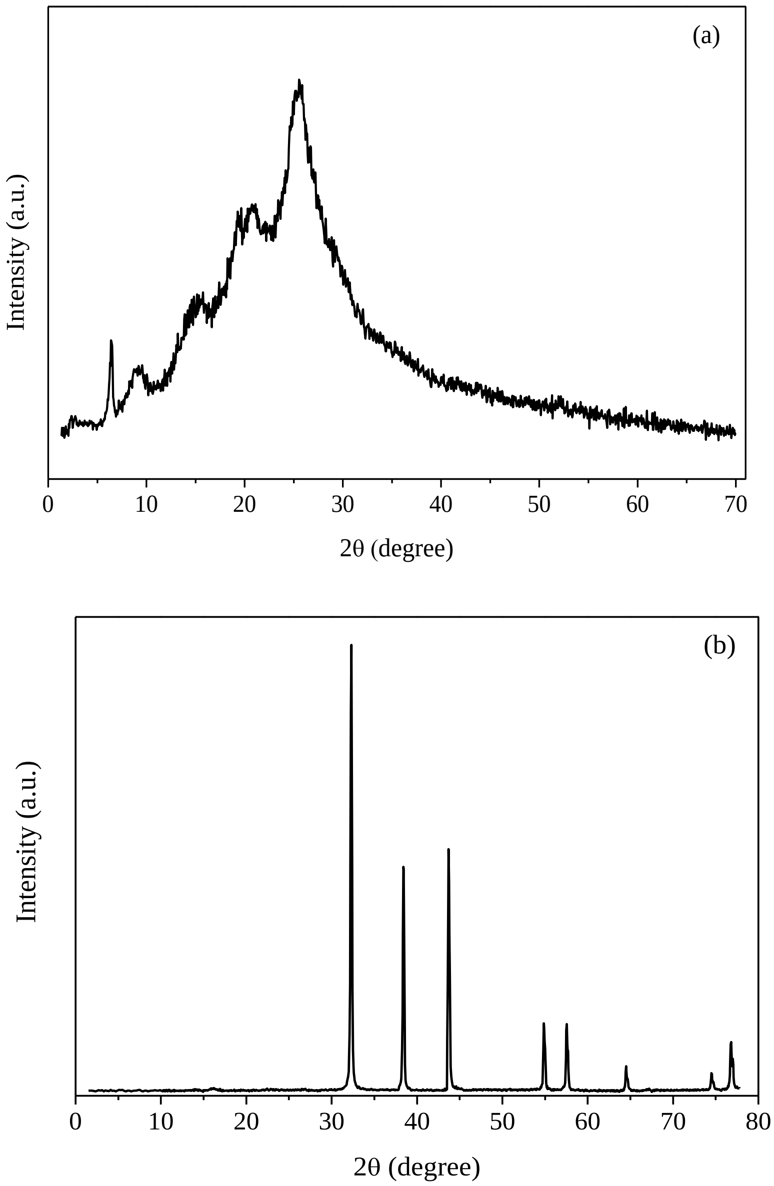
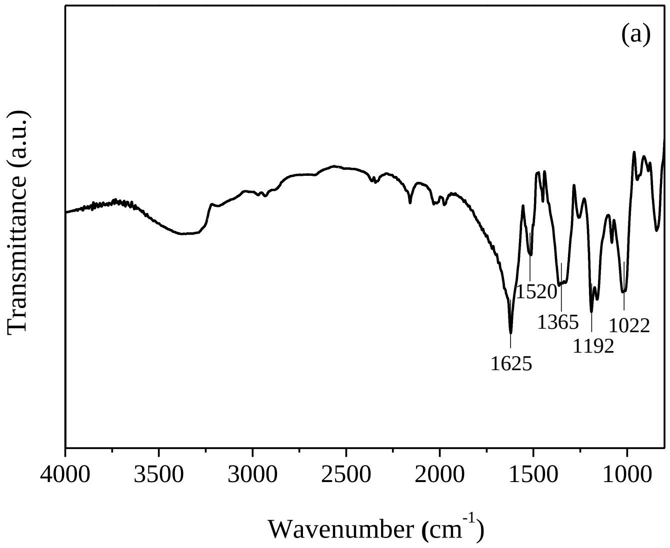


Figure 3.3: X-ray diffraction (XRD) patterns of PANI (a) and PANI.Ag (b) powders.

For the XRD pattern of PANI.Ag showed diffraction peaks around 2θ: 32º, 38º, 44º, 55º, 57º, 64º, 74º, and 77° (Figure 3.3b). The sharp and intense peaks in the PANI.Ag sample indicates the presence of AgNPs with a crystalline nature in the polymer chain. These results corroborate with the literature, where AgNPs present sharp peaks in values of 2θ around 38°, 44°, 64°, and 77°, corresponding to the cubic phase of centered silver face, and the Bragg reflection peaks are indexed to (111), (200), (220), and (311), respectively, crystalline plans of Ag [26- 28]. In addition to pure Ag, the AgCl phase is also present in the sample, at 2θ = 32º, which is attributed to the plan (200) according to Zhao et al. [29]. This peak can be formed in PANI.Ag films due to doping with HCl [30].

The ATR-FTIR spectrum of PANI/PSS (Figure 3.4a) shows characteristic peaks at about 1022, 1192, 1365, 1520, and 1625 cm^{-1}. In PANI.Ag (Figure 3.4b) were identified bands at 1120, 1290, 1394, 1477, and 1560 cm^{-1}. The bands in the region of 1000-1170 cm^{-1} are characteristic of aromatic C – H modes, and the vibration resulted in the HCl dopant of PANI [31]. The bands 1365-1394 cm^{-1} are attributed to the C – N stretch in the vicinity of the quinoid and benzenoid ring, 1520 cm^{-1} to elongation of quinoid rings, and 1625 cm^{-1} to the benzoid and quinodic units typical of doped PANI [32,33].

Is possible to observe that the intensities of the bands were reduced in PANI.Ag in relation to the PANI/PSS due to the presence of AgNPs in the polymeric matrix. Some peaks of composed polymer with particles are shifted to lower wavenumbers in relation to PANI, confirming the interaction between the nanoparticles and the polymer [34]. According to Leyva et al. [35], there is a change in the peaks associated with C = C and C = N of the quinoid ring, suggesting that AgNPs stay closer to the imine nitrogen in the PANI chain. Both syntheses (PANI, and PANI.Ag) showed broadband around 3300 cm^{-1} represents the N – H stretching mode [36].



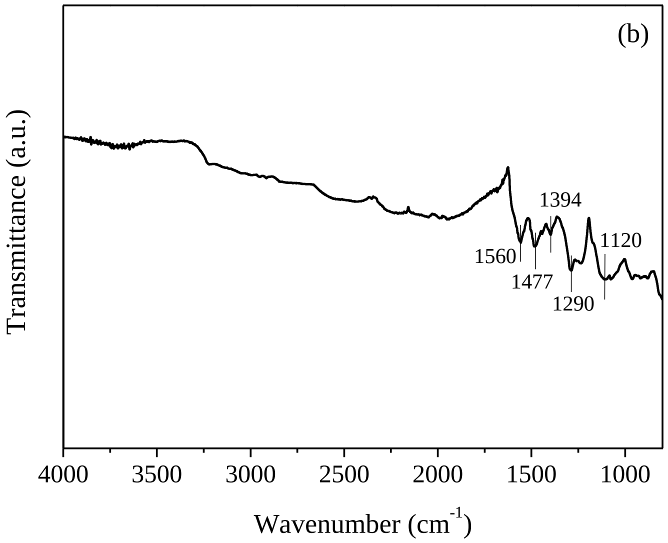


Figure 3.4: Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) of PANI (a) and PANI.Ag (b) powders.

AFM image in Figure 3.5a-b showed the topography and interface between silicon and the PANI/PSS and PANI.Ag films, and Figure 3.5c-d further illustrates the height profile. Table 1 demonstrate the surface roughness of the films. The film coated with PANI.Ag has a smooth film surface and thick between 5 to 10 nm. The PANI/PSS layer has a morphology with larger contours compared to the PANI.Ag. All images showed the typical PANI globular morphology. However, an irregularity, with an increase in the distance between the polymeric aggregates is observed in PANI.Ag film.

RMS value (4.65 nm) close to those found in this work for the PANI.Ag nanocomposite film (4.37 nm) was found by Biswas et al. [37], which relates this low value to the silver nanoparticles (AgNPs) being incorporated into the polymeric matrix. High RMS values obtained for the PANI/PSS film is due to the LbL deposition process, also observed by Graboski et al. [17]. Roughness of 79 nm was found in PANI film deposited on silicon substrate by the spin coating technique [36], and 87 nm for PANI and PSS film deposited by LbL method [12]. These results corroborate with the RMS value for PANI/PSS film found in the present work. The PANI/PSS nanocomposite film is 4.9 times thicker than PANI.Ag film (Table 1). However, both films are on nanoscale, favoring the development of sensing devices by the large surface area, which increases sensitivity to the analyte.

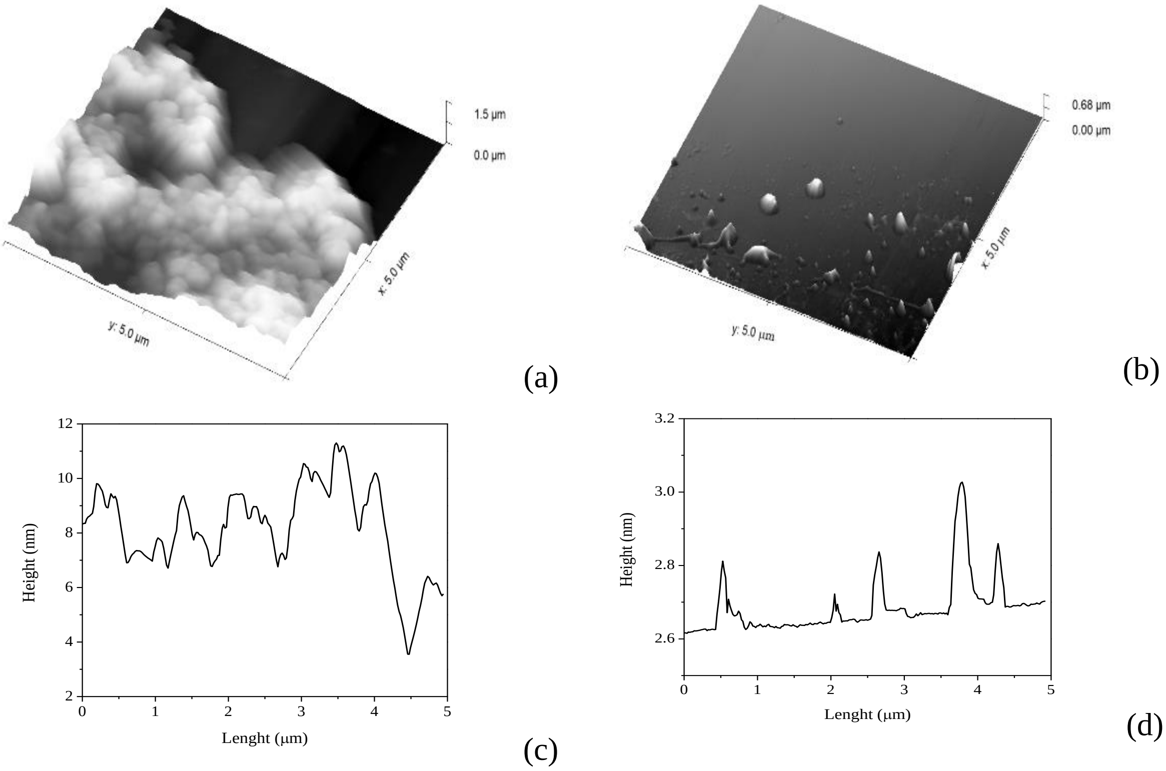


Figure 3.5: 3D Atomic Force Microscopy (AFM) images the interface between silicon and PANI/PSS (a) and PANI.Ag (b) films, and height profile of the film coating along the line of PANI/PSS (c) and PANI.Ag (d).

Table 1. Values of roughness (RMS), thickness, and height of PANI/PSS and PANI.Ag films on silicon surface.

Films	RMS (nm)	Thickness (nm)	Height (nm)
PANI/PSS	70.25±4.03	98.36±0.40	9.23±1.24
PANI.Ag	4.37±0.31	10.03±0.90	2.71±0.10

3.3.2 Application of cantilever nanosensors for *E. heros* pheromone detection

The resonance frequency response of the cantilever nanosensors was evaluated using different concentrations (0.005; 0.01; 0.05; 0.1; 0.2, and 0.3 µg/mL) of pheromone (Figure 3.6). The control sensor is the non-functionalized cantilever. The temperature inside the chamber remained at 25.7 °C (± 0.98) and the RH at 48.5% (± 5.56).

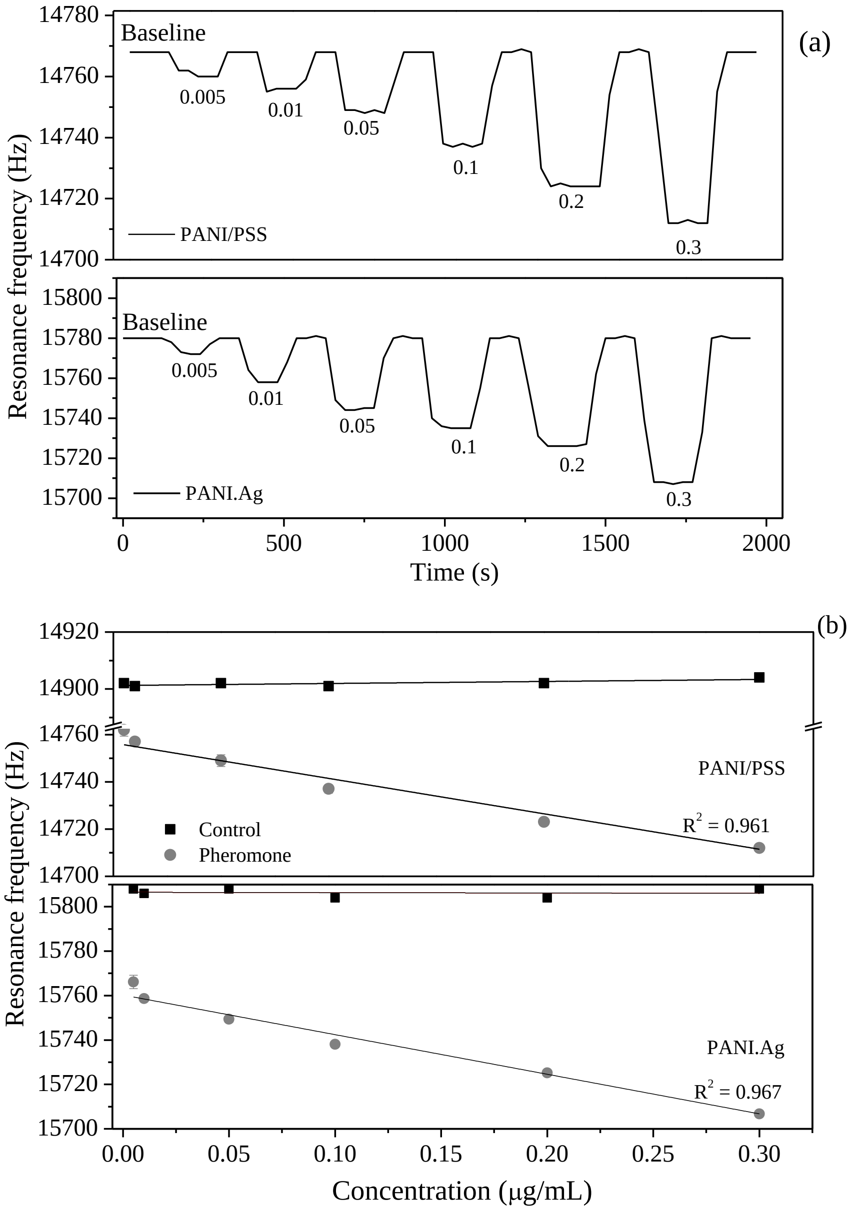


Figure 3.6: Response of the cantilever nanosensors coated with PANI/PSS nanocomposite and PANI.Ag nanohybrid films (a), and calibration curve (b) to *E. heros* pheromone.

According to the Figure 3.6, it is possible to observe the reduction of the resonance frequency response of both nanosensors with the increase of pheromone concentration. When the gas nanosensors are exposed to different concentrations of pheromone volatile molecules, the resonance frequency are rapidly decreasing and will be stable. When the gas nanosensors are exposed to synthetic air, the recovery response is also fast and gradually approaching the baseline. It was also found that the response was linear in the studied range, obtaining a correlation coefficient (R^2) above of 0.961 (Figure 3.6b). However, the non-functionalized cantilever (control) not showed variation in resonance frequency when exposed to the same concentrations of pheromone. These results suggest that the nanosensors can detect the pheromone quantitatively.

3.3.3 Sensing mechanism

The change in the resonance frequency response of the PANI/PSS and PANI.Ag cantilever nanosensors are due to the interaction of the sensor layer with the methyl 2,6,10-trimethyltridecanoate volatile compound of the pheromone. This compound has three chiral centers, leading to eight possible stereoisomers [38]. In contact with the PANI film the adsorption of the compound occurs in the sensor layer of the nanosensor, increasing the mass. Once, the resonant frequency of cantilever nanosensor is closely related to its effective mass, the increase of the mass resulted in a decrease of the resonance frequency with the increased concentration of the pheromone. Thus, the change of the resonance frequency can result from the mass change due to the physical adsorption of the volatile molecules. The detection mechanism of this adsorbed volatile may be related to the structural expansion of PANI, changing the mass of the cantilever nanosensor.

The interaction of *E. heros* pheromone can be explained by the diffusion of the methyl 2,6,10-trimethyltridecanoate ester at the interface of the nanocomposite or nanohybrid, with an increase in the mass, adsorption energy, and charge transfer, inducing a change in the resonance frequency with different concentrations of pheromone.

3.3.4 Characteristics of nanosensors

Response time, LOD, LOQ, sensitivity, and repeatability were evaluated for both cantilevers nanosensors (PANI/PSS, and PANI.Ag). The response times obtained were 32.94 s (±0.61) and 29.81 s (±0.45) for nanosensors of PANI/PSS nanocomposite and PANI.Ag nanohybrid film, respectively. The difference between the response times may have occurred due to the difference in adsorption in the sensor layers. PANI.Ag film showed less roughness and height, which may have facilitated the interaction with methyl 2,6,10-trimethyltridecanoate. However, both sensors presented a fast response due to nanostructured thin films. According to Suhail; Abdulahh and Kadhim [39], the response time depends on the rate of the diffusion and adsorption of gas molecules with the sensor layer.

The LOD of PANI/PSS nanosensor exposed to liquid pheromone was 3.78 fg/mL (ppq), with a sensitivity of 0.64 Hz.mL/fg. However, the nanohybrid nanosensor functionalized with PANI.Ag showed LOD of 3.16 fg/mL and sensitivity of 0.76 Hz.mL/fg. Cantilever nanosensors are miniaturized and extremely sensitive devices, being able to detect in the order of parts per billion (ppb) and even parts per trillion (ppt) [40]. The incorporation of Ag in three-dimensional porous structure of PANI allows for nanohybrid high LOD value. These values were similar than those obtained by nanosensor of PANI/multi-wall carbon for methyl 2,6,10-trimethyltridecanoate (2.18 fg/mL) [14] and nanosensor of PANI/PSS (1.44 fg/mL) [12].

High sensitivity values can be related to a larger surface area [41], of the sensing films observed by the globular morphology typical of PANI thin film (Figure 3.5) providing more active sites for the adsorption of gas molecules. In addition, the nanometer scale of roughness and thickness (Table 1) contributed for the high sensitivity. PANI matrix was important both for the nanocomposite and for the nanohybrid. Comparatively the nanocomposite provide high rough, and thickness in relation to nanohybrid. Thus, both characteristics provide a good adsorption, rapid diffusion, and interaction sites for pheromone volatile compound sensing. In this way, the nanosensors being able to detect in the order of ppq the *E. heros* pheromone. Comparing the sensing layers with the antennae of insect, it can be said that nanosensors are capable of mimicking the behavior, once the volatile pheromone was adsorbed in the antennae surface of the insect similarly of the sensing layer of nanosensors. The pheromone volatile molecules diffuse into insect antennae sensilla by microscopic pores and then in the chemosensory membranes interacts with receptor protein [42].

Microcantilever sensor coated with PANI in the emeraldine state by spin-coating method was applied in the detection of pheromone 2-heptanone specifically for honeybees. The device showed a LOD of 31 ppm and a sensitivity of 0.7 nm/ppm$_v$ [36]. Brezolin et al. [13] report that the increase in pheromone concentration there was an improvement in the interaction between the analyte and sensor, resulting in low values of resonance frequency, where an elastic tension is generated on the cantilever surface.

The LOQ value is the lowest concentration of the analyte that can be determined with an acceptable level of precision and accuracy [43]. PANI/PSS and PANI.Ag cantilever nanosensors showed LOQ of 12.59 and 10.05 fg/mL (ppq), respectively. Zhang et al. [44] report that *E. heros* adult male release around 2.5 μg/day of methyl 2,6,10-trimethyltridecanoate (pheromone). The LOQ values found for both nanosensors indicate that they are able of detecting this amount, making them a promising alternative for detecting insect pheromone in the field. By detecting the amount of pheromone, can be performed an estimation of *E. heros* population or infestation in the field. Thus, the nanosensors can be a powerful tool to reduce the use of agrochemicals and applied them if necessary in the right moment.

The repeatability results evaluated in a concentration of 0.3 µg/mL showed resonance frequency results of 14904.6 Hz (±0.02%) and 15705.4 Hz (±0.015%) for the nanosensors of PANI/PSS and PANI.Ag, respectively (Figure 3.7). All results obtained were lower than the maximum tolerable of 1.9% (reference value of AOAC [18]). Both cantilever nanosensors recurrent similar characteristic behaviors when the same environment is repeated several times, demonstrating that they are accurate when being used.

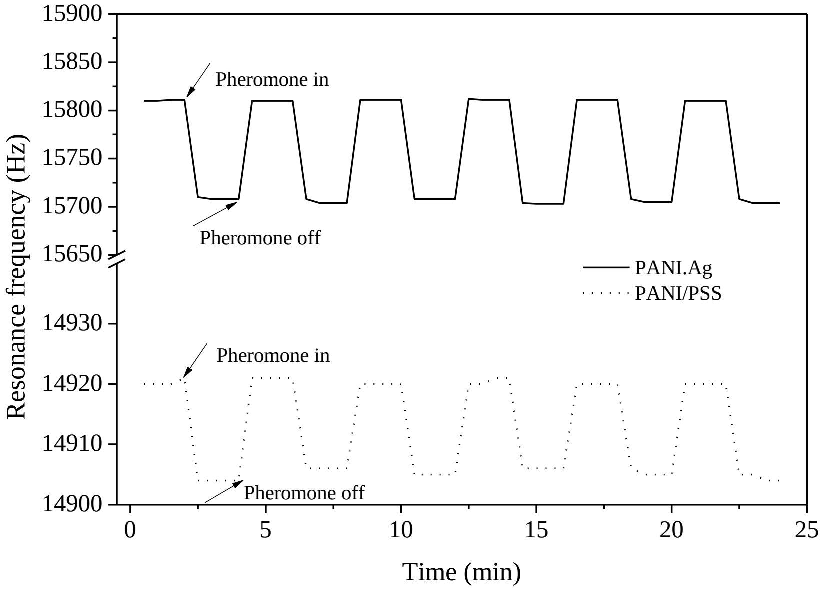


Figure 3.7: Repeatability results using both nanosensors of PANI/PSS and PANI.Ag.

3.4 Conclusion

The characterization of the PANI/PSS and PANI.Ag films by UV-Vis, XRD, and ATR-FTIR techniques showed bands and peaks characteristic of the polymer and Ag. The AFM images presented a globular morphology typical of PANI, with height and thickness in the nanometric scale. The PANI/PSS and PANI.Ag cantilever nanosensors presented a reduction in resonance frequency with the increased concentration of pheromone. High LOD, LOQ, and sensitivity were observed for both nanosensors. The response time of the nanosensors was 32.94 s (±0.61) for nanosensors of PANI/PSS nanocomposite and 29.81 s (±0.45) for PANI.Ag nanohybrid, with good repeatability. Thus, the nanocomposite PANI/PSS and PANI.Ag nanohybrid films demonstrate great potential for the detection of the *E. heros* sexual pheromone.

Acknowledgments

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Conflict of interest

The authors declare that have no competing interests.

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CAPÍTULO IV

LONG TERM STABILITY OF CANTILEVER GAS NANOSENSORS TO DETECT *Euschistus heros* (F.) PHEROMONE RELEASE BY RUBBER SEPTA

Abstract:

The application of cantilever gas nanosensors coated with PANI/PSS and PANI.Ag to monitor the release of the neotropical brown stink bug, *Euschistus heros* (F.) pheromone in rubber septa (insect pheromone dispenser). The rubber septa was impregnated with the 2,6,10-methyl trimethyltridecanoate compound, the main component of the sexual pheromone of *E. heros* stink bug. Studies were conducted to detect and determine the pheromone release ratio under laboratory conditions during two months. Both developed cantilever nanosensors showed a daily reduction in resonance frequency when exposed to the rubber septum containing the pheromone, which was not observed in the control cantilever. The relative humidity did not influence the resonance frequency of the nanosensors. A slight reduction of 1.4% in the resonance frequency for the nanosensor of PANI/PSS after 300 days of storage, and of 0.32% for the nanosensor PANI.Ag after 360 days, was observed.

Keywords: Rubber septa; Pheromone; Gas nanosensor; Stability.

4.1 Introduction

The growing population increase the demand for food production, however is estimated a lost to pests between 20 to 40% of global crop production annually [1]. The use of insecticides is a major challenge to achieve sustainable agriculture [2], because its overuse has become a global health concern due to the chemical compounds remaining in the environment, contaminating the soil, water and air [3].

Euschistus heros, known as brown stink bug, is one of the pests that suck the grain inserting their stylets to suck up nutrients causing a reduction in food production and quality [4]. It is extremely important to develop and integrate strategies with alternative methods in the management of these pests, in order to minimize the damage caused in agriculture, in addition to having effectiveness in control. The use of semiochemical methods, especially aggregation and sexual pheromones, is a favorable environmental management approach [5,6].

Control of insect populations is often accomplished through preventive applications of synthetic insecticides [7]. Pesticides benefit crops; however, they also impose a serious negative impact on the environment. Its excessive use can lead to the destruction of biodiversity, promote the selection of resistant bug populations, environmental contamination and risks to human health [8]. Different efficient techniques that do not use agrochemicals can be used in pest control. The pheromone of *E. heros*, being a natural, non-toxic and specific compound, can be used for the detection the insect itself in the field [6, 9].

Pheromone-containing traps have been widely used by many agricultural crops around the world to capture and monitor pest insects in the field [10, 11]. In view of the increase in agricultural grain production, biological control is more frequent and the diversification of environments as well as the use of semiochemical baits to attract pests in crops is an alternative to increase the effectiveness of control [12]. Rubber septa containing sexual pheromone are an alternative to monitor the presence of insects in crops, since the compound is natural, specific and has low environmental aggressiveness [6,11].

Traps of rubber septa impregnated with pheromone are effective for monitoring bug populations in soybean crops [9], where septa with 1 mg of methyl 2,6,10-trimethyltridecanoate attracted female *E. heros* insects for more than 30 days in field [6].

Nanotechnology in agriculture can reduce environmental damage, improving the precision agriculture, in addition enlightening human intervention through sensor devices, facilitating crop management. Thus, the detection of insect's pheromone by nanosensors technology stands out as a promising alternative for sustainable agriculture, leading to a conceptualization and validation of new tools and approaches [13].

Cantilever nanosensors can be used to detect volatile molecules with extreme limit of detection and sensitivity, since they have the ability to change the vibrational frequency or suffer deflection due to the adsorption of molecules on the surface, making them excellent transducers and suitable to be used as chemical, physical or biological sensors [14-16]. Cantilever nanosensor nanostructured with polyaniline (PANI) and sodium polystyrene sulfonate (PSS) was applied for the detection of methyl 2,6,10-trimethyltridecanoate, the main component of the sexual pheromone of *E. heros* demonstrated good performance and the ability to detect the semiochemical presenting high limits of detection (1.44 fg/mL) and sensitivity (5.28 Hz/fg) [17]. Microelectromechanical sensor with PANI and graphene oxide had a limit of detection of 0.91 fg/mL, with a sensitivity of 6.24 Hz/fg to *E. heros* pheromone [18]. In addition, nanostructured nanocomposite PANI/carbon multi-wall deposited in cantilever sensor showed 2.18 fg/mL limit of detection and 2.62 Hz/fg sensitivity in the detection of methyl 2,6,10-trimethyltridecanoate [19].

The cantilever nanosensors have the advantage of miniaturization, fast response time, to detect analytes with high sensitivity. In this sense, the objective of this work is to applied cantilever nanosensors to detect the *E. heros* sexual pheromone impregnated in rubber septum and evaluate its release over time. In addition, study the influence of relative humidity on the response of the developed nanosensors and stability during storage.

4.2 Material and Methods

4.2.1 Functionalization of cantilever nanosensors

Silicon (Si) cantileveres (Budget Sensors®, Bulgaria) with 13 ± 4 kHz resonance frequency and 0.2 N/m spring constant (k) were used in this work. The cantilevers were cleaned by immersion in piranha solution (3:1, H_2SO_4: H_2O_2, v/v) for 5 min, then rinsed several times with deionized water and dried. The cantilevers were coated by *layer by layer* technique using PANI/PSS as described in a previous work [17] and by *dip coating* technique using PANI.Ag nanohybrid. The PANI.Ag was obtained by *in situ* synthesis, next solubilized in dimethylacetamide and doped with HCl (1 M). The thin film of nanohybrid was obtained by immersion of cantilever surface on 100 µL PANI.Ag solution, remaining in contact for 3 min. Then, washed with 100 µL of HCl (1 mol/L) pH 3, and dried for 15 min at room temperature (25 °C). A control cantilever without functionalization also were used in the experiments.

4.2.2 Response of cantilever nanosensors

The sexual pheromone of *E. heros* was evaluated in commercial rubber septum (precision seal natural rubber) with 20 mm long and 9 mm diameter (9 mm OD, back rubber, Sigma Aldrich, USA). The septum was impregnated with 2 mg of 2,6,10-methyl trimethyltridecanoate the main compound of the *E. heros* pheromone [9]. The septum's with pheromone were supplied by Embrapa's Genetic and Biotechnology Laboratory of Semio-chemicals, Brasília/Brazil.

To analyze the release of volatiles, the rubber septum was stored in amber glass, with a screw cap containing a rubber seal for 60 days, at room temperature (25 °C). During storage, the bottle remained open in order to mimic the released pheromone in the field. To assess the resonance frequency of the nanosensors 1 h before the flask was closed for the concentration of the volatile. With a microsyringe (Hamilton, USA), 20 µL of the volatile was collected from inside the flask and placed in the gas chamber. For this purpose, a special gas chamber of polypropylene (6.38 cm^3) was designed, which consists of an inlet hole for insert the pheromone into the chamber, and an outlet hole for the exit the gas molecules. The chamber has an upper central hole 25 mm in diameter and 13 mm high to fit the AFM scanner and two side holes for the entry and exit of volatile compounds. Two rubber sealing rings were used at the top in order to seal (Figure 4.1). The experimental details of the gas-sensing setup are stated in previous work [18].

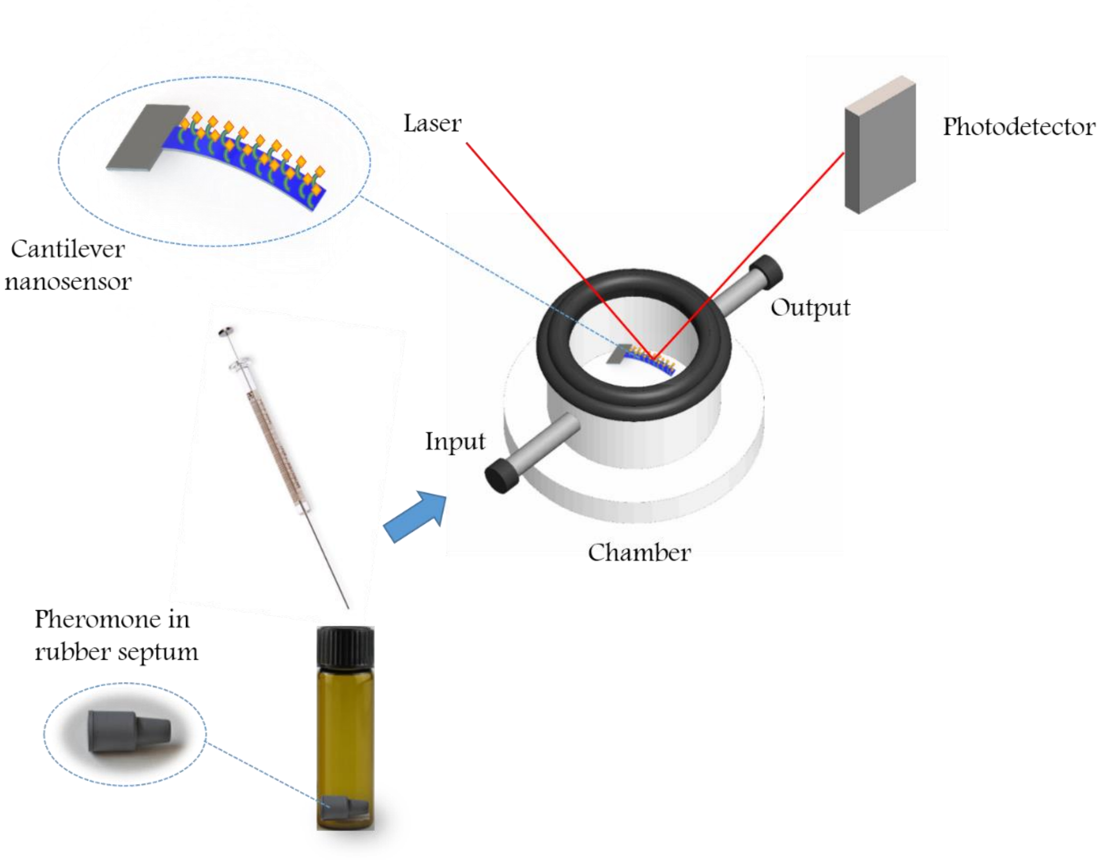


Figure 4.1: Schematic diagram of the cantilever nanosensor with laser beam and measurement of resonance frequency to the volatiles from rubber septum.

The resonance frequency was measured in AFM equipment (Nanosurf, C3000 Controller, Switzerland). The cantilever was placed in the holder (canticlip) and inserted into the AFM scanner. The laser was adjusted, that is, positioned at the end of the cantilever and in the central position, followed by the verification of the signal strength (65-68%) in the photodetector. The resonance frequency measurement was configured in dynamic mode. The chamber was placed over the cantilever and sealed. In the AFM the vibration frequency was recorded and saved the values.

The response of each nanosensor was evaluated for 60 days, and the measurements were taken every 24 h. This time was choose based on release rate of septum containing the *E. heros* pheromone with low variability over 63 days [6]. During this period, the same cantilever nanosensor was always used, and it was stored in a desiccator at room temperature (25ºC).

4.2.3 Stability of cantilever nanosensors

The stability of the cantilever nanosensors was evaluated for 365 days (twelve months), measuring the resonance frequency every 60 days, in contact with synthetic air. The cantilever nanosensors remained stored in a desiccator and at room temperature (25 °C). The experimental condition assures no humidity and temperature variation between the measurements. The analyses were performed in a chamber without odorless using reference air (dry synthetic air). The measures were performed using two identical nanosensors.

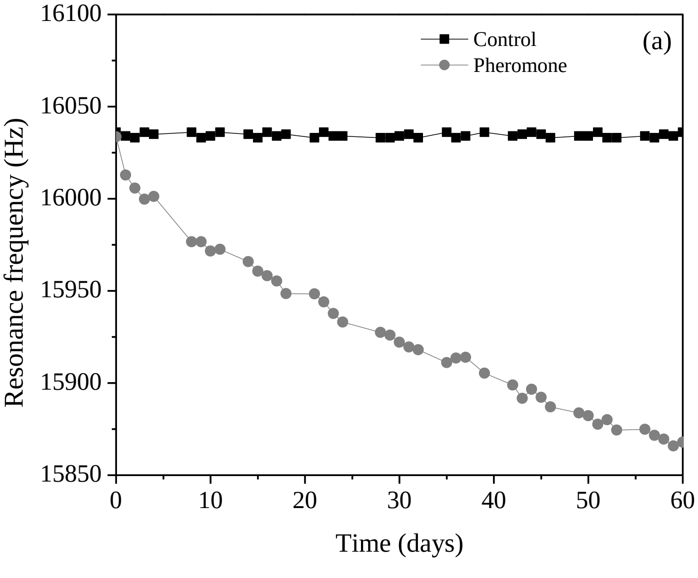
4.2.4 Response to relative humidity (%)

Nanosensors experiments are carried out in different relative humidities (30-70% RH). The tests were conducted in a gas chamber coupled over the AFM scanner. The relative humidity was controlled by dry nitrogen (99.998% purity, White Martins, Brasil) at flow rate of 0.1 L/min. The wet ambient inside the chamber was obtained by a gas bubbler tube containing water. Manual valves were used to control N_2 inlet and outlet. So, was possible obtain inside the chamber a relative humidity in the range of 30% to 70%, which are monitored by a commercial humidity sensor (Sensirium, Kit EK-H5, Brazil). The temperature of the gas chamber was maintained at 25.00 ± 0.02ºC, and also the ambient temperature in the room. After relative humidity stabilization, the pheromone was injected into the gas chamber with a micro syringe. The generated resonance frequency of sensing devices were collected at same way as describe for the gas sensing experiments.

4.3 Results and Discussion

4.3.1 Rubber septum response

The responses for each cantilever nanosensor (PANI/PSS and PANI.Ag) were analyzed for 60 days, once a day exposed to the volatiles from rubber septum containing the *E. heros* pheromone. The temperature remained at 23.28°C (± 0.73) and the RH at 50.10% (± 2.42) during the experiments. Figure 4.2 shows the resonance frequency responses for the PANI/PSS and PANI.Ag cantilever nanosensors. It was observed during 60 days of monitoring a daily reduction in the resonance frequency of the cantilever nanosensors exposed to the rubber septum volatiles from pheromone. On the other hand, there was no change in the resonance frequency of the nanosensor when exposed to synthetic air (white) during the experiment, which remained around 16034 Hz. After 60 days of evaluation, a difference of approximately 166 Hz and 323 Hz for PANI/PSS and PANI.Ag cantilever nanosensors, respectively, were observed in relation the to begin of the measurements.



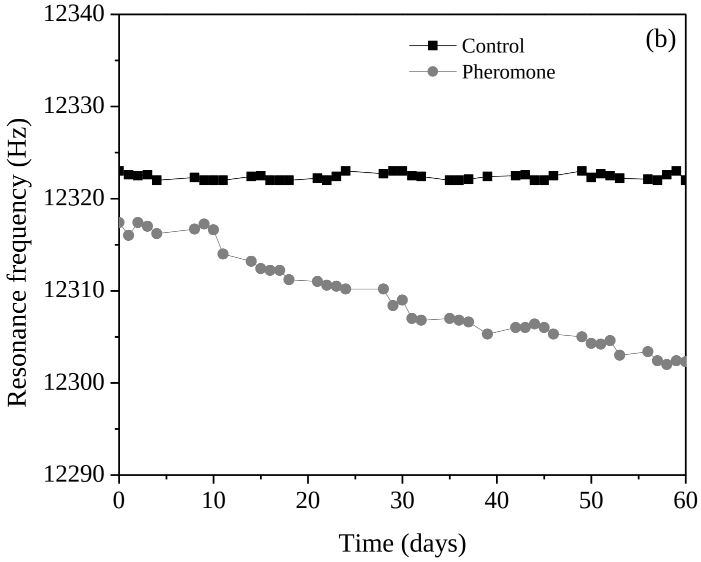


Figure 4.2: Resonance frequency response of the PANI/PSS (a) and the PANI.Ag (b) cantilever nanosensors exposed to the *E. heros* pheromone in a rubber septum.

To assess the amount of pheromone released by the rubber septum during the 60 days analyzed, the resonance frequency results obtained with the cantilever nanosensors were converted using the line equation of each nanossensor.

PANI/PSS $y = -2.4241x + 16002$ $R^2 = 0.9664$

PANI.Ag $y = -0.2732x + 12317$ $R^2 = 0.9593$

Where y represents the resonance frequency and x the concentration of pheromone.

Figure 4.3 shows the results for the PANI/PSS and PANI.Ag cantilever nanosensors of the amount of pheromone liberate by the rubber septum during 60 days. The cantilever nanosensors developed in this work showed similar detection in relation to the release of pheromone in the rubber septum monitored during 60 days. At the end of the analyzed period, the release detected was around 0.1 µg. In this way, the developed nanosensors are a promising tool for detection the sexual pheromone of *E. heros*, and can be an alternative to monitor the presence of the insect in crops.

These results are in agreement with those found by Borges et al. [6], where traps containing rubber septum impregnated with 1 mg of the sexual pheromone of *E. heros* attracted females for more than 30 days in the field, releasing approximately 0.05 µg. In the present study, as the rubber septum contained 2 mg of the compound, and the release found was about 0.1 µg after 60 days for both cantilever nanosensors.

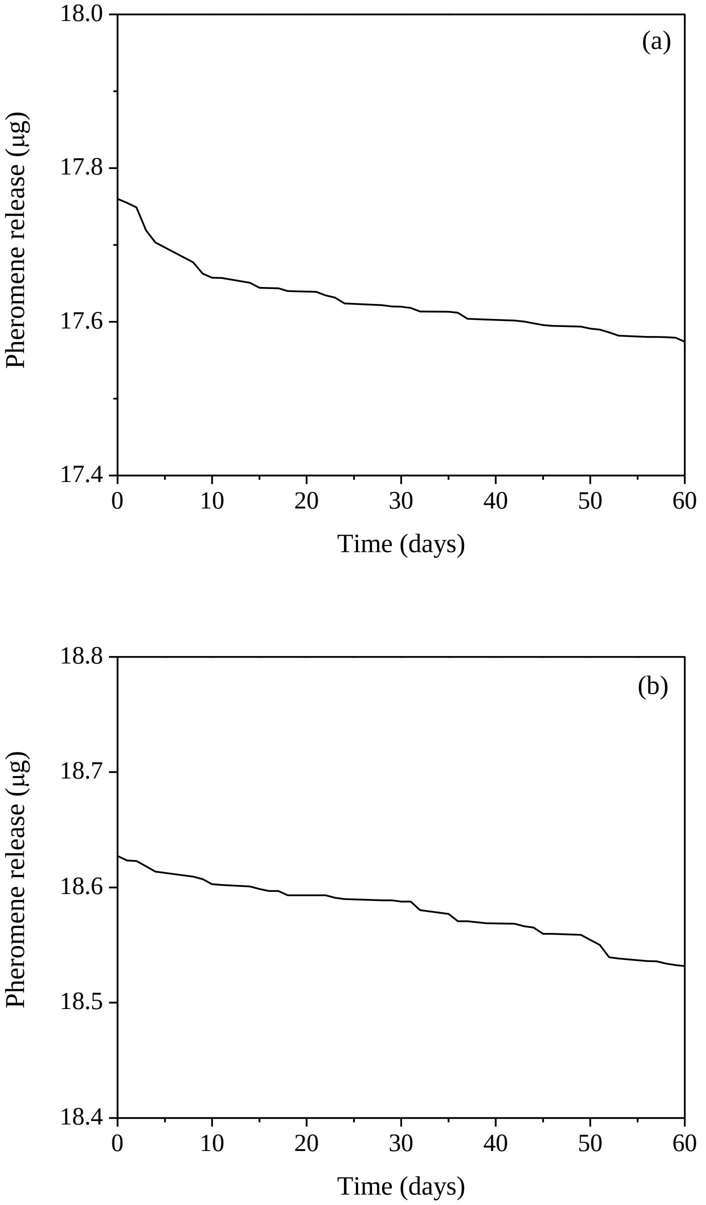


Figure 4.3: Amount (µg) of *E. heros* pheromone released from the rubber septum detected by the PANI/PSS (a) and PANI.Ag (b) cantilever nanosensor.

4.3.2 Effect of relative humidity

The nanosensors response in real-time monitoring the pheromone in environment is very important to evaluate the influencing factors as relative humidity. A variation of RH from 30 to 70% RH was studied in a concentration of 0.3 µg/mL pheromone. Figure 4.4 shows the resonance frequency response to RH during pheromone detection. The response of nanosensors coated PANI/PSS nanocomposite (Figure 4.4a) showed a similar behavior with the relative humidity from 30 to 70% RH. On the other hand, the nanosensor with PANI.Ag nanohybrid film (Figure 4.4b) showed a difference of 4 Hz in the response.

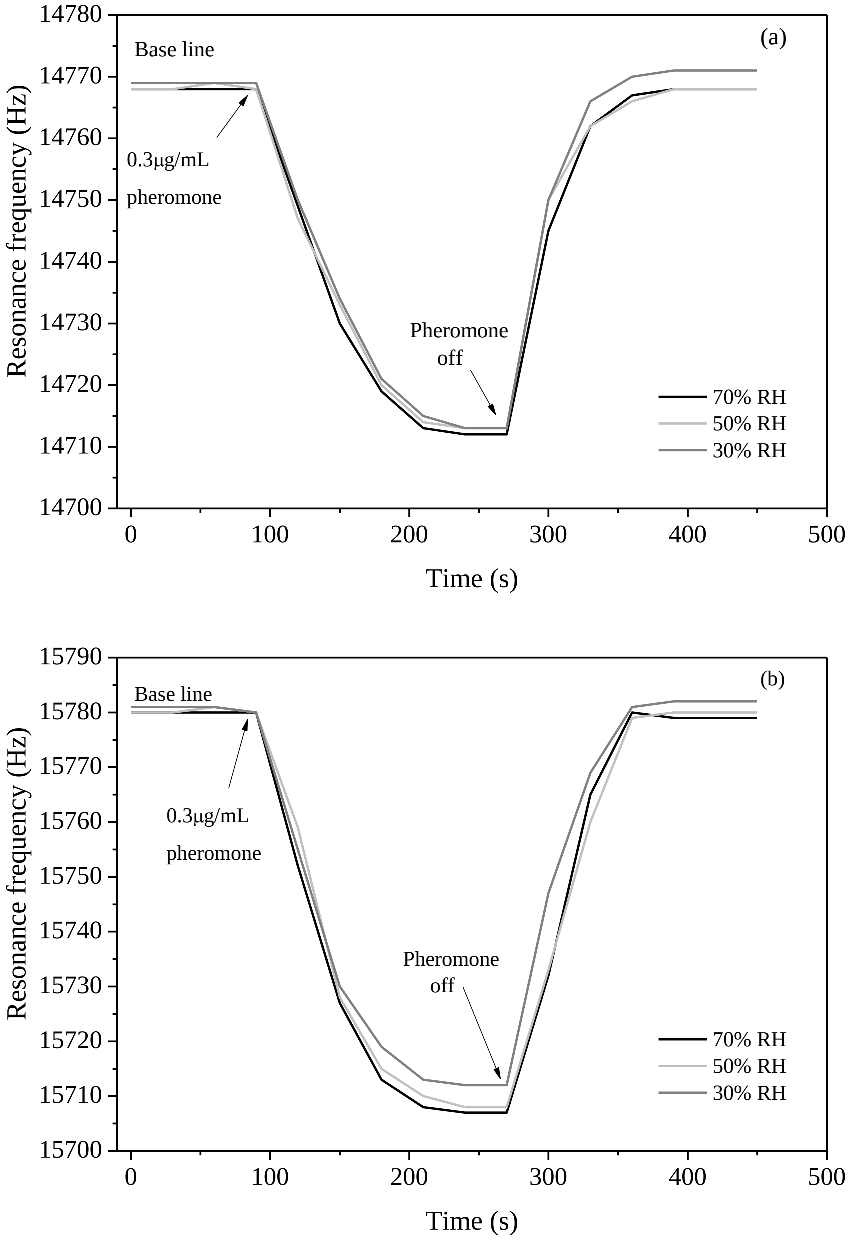


Figure 4.4: Response of cantilever nanosensors coated with PANI/PSS nanocomposite (a) and PANI.Ag nanohybrid films (b) to 0.3 μg/mL at different RH.

PANI in doped state has a hydrophilic characteristic [14] however, depending on the functionalization used; it can interfere in hydrophobicity of the sensitive layer, which is the case of the LbL technique. Brezolin et al. [17] using the LbL technique to obtain a nanostructured

PANI/PSS layer, it observed a hydrophobic characteristic in the film, where in the functionalization occur a reorganization of the chains in the layer. This result can be correlated those found in this work, where the possible hydrophobic characteristic of the film may have contributed to the absence of interaction with the different RHs, showing stable signal of resonance frequency (Figure 4.4a). As for the PANI.Ag nanosensor, where the interaction of the two components that form the nanohybrid, the adsorption of water molecules (in high relative humidity - 70%) on the surface increase the mass, in this way, reduce the resonance frequency response (Figure 4.4b). As the nanohybrid film is hydrophilic, it absorbs water molecules and expand the interparticular distances between the conducting fillers of the nanohybrid layer.

4.3.3 Stability response

The stability response for the developed cantilever nanosensors was evaluated for 360 days, where the results are shown in Figure 4.5. During approximately 180 days of storage, the PANI/PSS cantilever nanosensor remained stable, showing a reduction of 0.30% and in relation to the first day of analysis (Figure 4.5a). Already the PANI.Ag cantilever nanosensor showed a reduction in resonance frequency at 60 days (Figure 4.5b).

In 240 days of storage the PANI/PSS cantilever nanosensor showed a reduction of 0.77%, and with 300 days a reduction 1.40% in the resonance frequency (Figure 4.5a), and for PANI.Ag in 300 days presented a reduction of 0.23% (Figure 4.5b). In one year of storage, the PANI/PSS and PANI.Ag cantilever nanosensors showed a reduction of 5.22% and 0.32%, respectively, in relation to the initial value. These results demonstrate a stable nanosensor, so it can be used for a long period. In addition, it useful life is extremely important, ensuring its characteristics for detection the volatile from pheromone for long time. Commercial gas sensors, oxygen for example, show long term stability ones around 2% per year. So, the PANI.Ag cantilever nanosensors is very stable for one year (365 days), and the PANI/PSS cantilever nanosensor for 300 days under normal conditions, can expect the device’s readings to change by less than 2%.

Conducting polymers suffer instability during the storage, so the long long-term was achieved using hybrid composites as sensing elements for cantilever gas sensors [20]. Both hybrid composites (PANI/PSS and PANI.Ag) have functionalities and properties in the integrated structure with dimensions in the order of nanometers that showed high surface-to-volume ratio that provide good adsorption of pheromone volatile molecule.

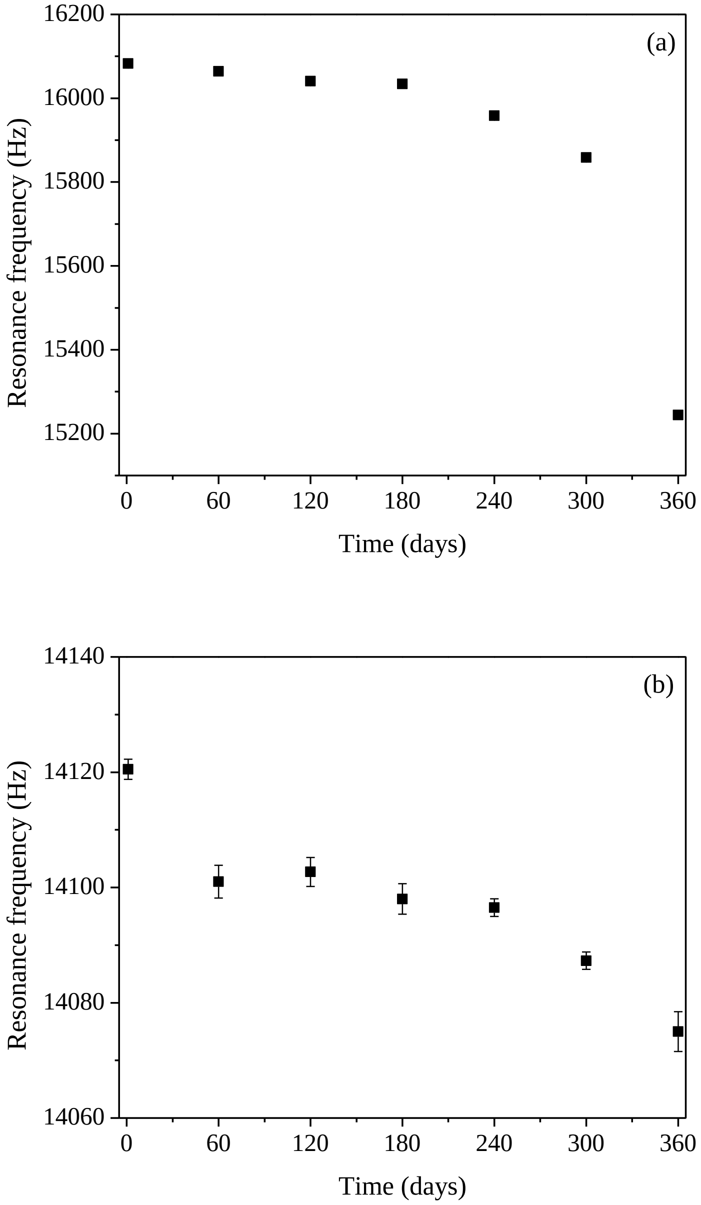


Figure 4.5: Stability response during 360 days of storage of PANI/PSS (a) and PANI.Ag (b) cantilever nanosensors.

The good stability can be correlated to the capacity of adsorption and desorption mechanism of PANI nanocomposite and nanohybrid. Only PANI films present problem of long-term stability, which has resolved change the polymer matrix properties, in this way providing good practical application of these devices. In addition, the long term stability can be relate to the form of storage, because the devices were stored in a desiccator. According to Peikertová et al. [21], PANI thin films stored in desiccator during one year no showed significant differences in the intensities of bands observed by Raman spectra in comparison of spectra measured 28th and 182nd day, while the films stored at air moisture show very significant change of color, intensity of bands, and deprotonation state.

4.4 Conclusion

The cantilever nanosensors were able to detect the 2,6,10-methyl trimethyltridecanoate compound in rubber septa observed by reduction in the resonance frequency for 60 days, demonstrating that there is interaction between the developed sensory layers and the sexual pheromone of *E. heros*. The nanosensors showed similar behavior with a relative humidity of 30 to 70%. Comparing both cantilever nanosensors was found that the functionalized with PANI.Ag showed greater stability during the storage time. Thus, the cantilever nanosensors present a great tool for smart sensing of *E. heros* in agriculture.

Acknowledgments

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Conflict of interest

The authors declare that have no competing interests.

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CAPÍTULO V

EVALUATION OF CANTILEVER NANOSENSOR IN THE DETECTION OF *Euschistus heros* (F.) PHEROMONE AND ITS INTERFERENTS COMPOUNDS *in vivo*

Abstract:

This work aim to apply PANI.Ag cantilever nanosensor to detect volatiles from environments *E. heros in vivo* and interfering compounds. The stinkbugs insects were collected in crops and kept in an environment that mimicked the soy culture. The study was carried out with male and female insects; and with distinction between male and female insects; interfering compound from stinkbug *Diceraeus furcatus*, in addition with synthetic compounds tridecane and hexenyl (Z)-3-acetate. The cantilever nanosensor exposed to the volatile from the environment containing males and females, showed a reduction in the resonance frequency, and not showed response to environment that contained only food and water (without insects), and control environment (synthetic air). The response to the volatile compound collected from the environment containing female insects was similar to the condition of the environment without insects and are very close to the control, different from the analysis that contained only males, where was observed a reduction in the resonance frequency. When exposed to the volatiles from environment with *D. furcatus*, the resonance frequency did not change considerably when compared to the control, as well as when exposed to tridecane and hexenyl (Z) - 3-acetate compounds. Thus, the cantilever nanosensor coated with PANI.Ag was able to distinguish the volatile compounds release by insects, being a promising alternative for agriculture in integrated pest management using nanotechnology.

Keywords: Volatile; Gas nanossensor; Resonance frequency; *D. furcatus*.

5.1 Introduction

The term pheromone was proposed by Karlson and Lüscher in 1959, to define substances secreted by an individual and received by a second individual of the same species (BREZOLIN et al., 2018). Pheromones are volatile organic molecules of low molecular weight and that can be used for communication between members of the same or opposite sex (JURENKA, 2004).

There are different types of pheromones, depending on the biological function presenting different chemical properties (LAW and REGNIER, 1971). Aggregation pheromones are compounds emitted to attract both males and females (BAKKE; FROYEN; SKATTABOL, 1977; KRAWIELITZKI et al. 1977). Repellent pheromones are compounds that act as warning signs to prevent sources of colonization or food (ROBINSON et al. 2008). Alarm pheromones are compounds that are initiated by aggressive behavior or can cause dispersion as a response to predators and are used mainly by insects (WILSON, 1965). Trail pheromones are compounds used by many insects to mark the way to a nest or food sources (TAI; MATSUMURA; COPPEL, 1969).

The insect's sexual pheromone is a type of volatile substance emitted by females to attract males for mating (BREZOLIN et al., 2018), considered substances capable of causing responses in very low concentrations (WEBER et al., 2017). Pheromones are used in agriculture and forestry to control and monitor pest insects, as well as to estimate the size of a population through field sampling (WONG et al. 2012; HARRIS et al. 2015).

Brown stinkbug, *Euschistus heros* is prevalent in soybean crops, but can also feed on various plants such as beans, peas, alfalfa, and cotton (PANIZZI, 1997; SORIA; DEGRANDE; PANIZZI, 2010; PANIZZI; BUENO; SILVA, 2012). This is one of the species that has caused great losses in soy in Brazil, reaching 30% (PANIZZI; BUENO; SILVA, 2012; DEGRANDE, VIVAN, 2012). Population densities can be as high as 40–80 stinkbugs per row meter in soybean fields, especially at the end of the growing season (SOSA-GÓMEZ et al., 2019). The seed-feeding complex of stink bugs (Hemiptera: Pentatomidae) was the most expensive insect pest of soybean overall during 2017 in terms of lost yield and control costs, comprising 38% of all insect costs + losses.

Corn crops can also be attacked by *E. heros*, for which populations have gradually been increasing in soybean. The potential of *E. heros* to cause injury in corn is lower compared to *Dichelop melacanthus* or *Dichelop furcatus* (SOSA-GÓMEZ et al., 2019).

The green belly stink bug, *D. furcatus* (F.) (Hemiptera: Heteroptera: Pentatomidae) is a pest in some agriculture cultures such as corn and soybean, in southern Brazil. It also occurs on wheat, but information on its damage to this crop is limited (PEREIRA et al., 2013; PANIZZI et al., 2016). Among the cultivated plants, *D. furcatus* has been reported on soybean, *Glycine max* (L.) Merrill (Fabaceae) (PANIZZI et al., 1977), sunflower, *Helianthus annuus* L. (FROTA and SANTOS, 2007), corn, *Zea mays* (L.) (Poaceae) (ROZA-GOMES et al., 2011), common oat, *Avena sativa* L. and on wheat, *Triticum aestivum* L. (Poaceae) (CHOCOROSQUI and PANIZZI, 2004; PEREIRA et al., 2013).

The management of *E. heros* is based on insecticides such as organophosphates and pyrethroids (TUELHER et al., 2018), and the intensive used causes several problems, including increased residues in food products, user poisoning and occurrence of resistant insect populations (KOPPEL et al., 2011; PAZINI et al., 2019). The practice of use foliar insecticide applications without knowing if any insects are present was common across much of the soybean production area. In some cases, the application may have controlled some insects, but in most cases, an insecticide was added to a planned fungicide or herbicide application as insurance against the risk of having insect damage (MUSSER et al., 2018). Therefore, the sustainability of cultures, in general, depends on efficient strategies, but less dangerous and aggressive to the environment (PADILHA et al., 2018).

The use of insect pheromones to monitor and control pests together with the use of nanotechnology is a valuable alternative, with technological and economic advances, reducing the use of chemical substances, which cause serious problems to environment and the health of the population.

The main sexual pheromone identified in male *E. heros* was methyl 2,6,10-trimethyltridecanoate, used to attract females (BORGES et al., 1998a, 1998b, 1999). The main methodologies that are used to extract pheromones include aeration (volatile collection) and solvent extraction (MILLAR and HAYNES, 1998). As most pheromones are volatile molecules, gas chromatography with flame ionization is the most used technique for quantitative analysis of pheromones, and gas chromatography coupled to mass spectrometry with electron impact ionization, for qualitative analysis (MILLAR et al., 2010).

Cantilever nanosensors can be used to detect synthetic pheromone from *E. heros* with extreme sensitivity, since they have the ability to change the vibrational frequency or suffer deflection due to the adsorption of molecules on the surface, making them excellent transducers and able to be used as chemical, physical or biological sensors (STEFFENS et al., 2014, BREZOLIN et al. 2019; BREZOLIN et al., 2020a; BREZOLIN et al., 2020b).

In this sense, the objective of this work is to verify and analyze the response of the PANI.Ag cantilever nanosensor exposed to volatiles collected from environments containing *E. heros* insects *in vivo* as well as interfering compounds.

5.2 Materials and Methods

5.2.1 PANI.Ag cantilever nanossensor

The sensitive layer of PANI.Ag, was obtained by the reduction of silver nitrate in the PANI powder (emeraldine base) conventional chemical synthesis as described in previous work

(BRAGA, 2012). Silicon cantilever (Budget Sensors®) with a resonance frequency of 13 ± 4 kHz and spring constant (k) of 0.2 N/m were used. The PANI.Ag nanohybrid film was deposited on the cantilever surface by dip coating technique.

5.2.2 Test with *in vivo E. heros*

The insects were collected in a soybean crop (-27° 66'58 ”S, -52° 59'50” W) in a municipality in the north state of Rio Grande do Sul, Brazil, where they were kept in an environment that mimicked the crop, in a 1800 cm^3 polypropylene chamber (20 x 10 x 9 cm), with food (soybean pods *Glycine max* and peanut grains *Arachis hypogaea*), and a cotton pad soaked in water for drinking and humidification. The food in the chamber was changed every three days. The chamber was closed in its upper part with a screen to exchange air with the environment. The insects remained in the adaptation period in the environment for 3 days before starting to collect the volatiles and evaluate by the cantilever nanosensor.

The temperature and relative humidity (RH) inside the box were monitored by a commercial sensor (Sensirium, Kit EK-H5, Brazil). The insects were maintained in a photo period, 14 h of light (7 am to 9 pm) and 10 h of dark (9 pm to 7 am) (ZHANG et al., 2003). According to Zarbin et al. (2012), the production of the main male-specific compound is dependent on light, where approximately 71% of the daily release occurred, so the collection and analysis of volatiles was carried out in the photo light period. Figure 5.1 shows one image of the environment with *E. heros* insects and with the food supplied.

Figure 5.1: Rearing environment of *E. heros* insects and with the food supplied, in the laboratory under controlled conditions of temperature and relative humidity.

The study was divided into 3 *in vivo* experiments: first, all insects (male and female) remained in a single environment for evaluation; second: the distinction between male and female insects was performed since only males of *E. heros* release sexual pheromone to attract females for the purpose of reproduction, the main compound being methyl 2,6,10-trimethyltridecanoate (ALDRICH et al., 1994; BORGES et al., 1998a, 1998b, 1999; WEBER et al., 2017). Third, with another species of bug, *Diceraeus furcatus*, and synthetic compounds: tridecane and (Z)-3- hexenyl acetate, in order to verify the distinguishing ability of the cantilever nanosensor.

5.2.3 Cantilever nanosensor responses to pheromone

After the adaptation period, the volatiles were collected once a day, in the photo light period, for 10 consecutive days and evaluate the response of PANI.Ag cantilever nanosensor by means of the resonance frequency measurement.

The cantilever was inserted into the atomic force microscopy (AFM - Nanosurf, C3000 Controller, Switzerland) using the canticlip support. Then the laser was adjusted, that is, positioned at the end of the cantilever and in the central position of the photodetector, followed by the verification of the signal strength (65-68%). To start the resonance frequency measurement procedure, the system was configured for operating in air, in dynamic mode and choose the cantilever model.

The measurement of resonance frequency was obtained in AFM, scanning the vibration frequency. In the AFM software, the option to find the vibration frequency was selected. After this command, the scan was performed; selecting the largest peak of resonance frequency and this value was recorded. In all experiments, the temperature and relative humidity were monitored with a commercial sensor (Sensirium, Kit EK-H5, resolution SHT2x, Brazil).

One hour before analysis, the boxes were closed with polyvinyl chloride (PVC) plastic film and a lid, in order to concentrate the volatile compounds. 20 µL of volatiles were collected using a gas micro syringe (Hamilton, USA), piercing the plastic film of the box, which remained next to the volatile chamber. Immediately the collected sample was inserted in the volatile chamber to measure the resonance frequency.

An identical chamber was also evaluated, but without the presence of insects, only with food (soybean pods *Glycine max,* and peanut grains *Arachis hypogaea*), cotton pad soaked in water to assess the interference of the released volatiles. In the same way; the chamber was closed one hour before collection, with polyvinyl chloride film and a lid. In addition, the measurement of the resonance frequency of the cantilever nanosensor exposed to synthetic air was also performed (control).

Subsequently an analysis with distinction of males and females in separate environments (1800 cm^3 polypropylene chamber) was performed. An environment contained only male stinkbugs, food (soybean pods and peanut grains) and cotton soaked in water for drinking and humidification. Another environment contained the same feeding and water conditions mentioned above, but only with female insects. The environment without bugs was also analyzed, containing food and cotton pad soaked in water.

The male *E. heros* has a subquadrangular pigophore, rounded posterolateral angles, a ventral margin cut out subquadrangularly and bi-sinusoidally. The female has laterotergites 8 longer than laterotergites 9, triangular posterior margin and acute apex (NATURAEDATA, 2018).

The stink bug sexual distinction was performed by the genital shape according to NaturaeData (2018), Corrêa-Ferreira and Panizzi (1999), where males have a single plate (pigophore) and females two side plates. Figure 5.2 shows the distinction of males and females *E. heros*.

After the chamber remained closed with polyvinyl chloride film and lid for 1 h, the experiment was carried out. With the aid of a micro syringe (Hamilton, USA), the volatile compounds were collected and introduced into the AFM-coupled chamber, to measure the resonance frequency of the cantilever nanosensor as previously mentioned.

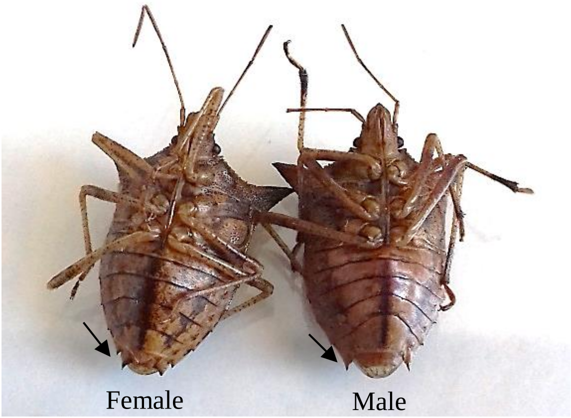

Figure 5.2: Female and male *Euschistus heros* (F.). Arrows indicate the differences in the adults genitalia used to separate the genders.

Initially, 20 µL of synthetic air was injected into the chamber, with the outlet open for cleaning. After 20 µL of synthetic air was injected again and the response of the cantilever nanosensor was monitored every 20 s, for 2 min. Then, 20 µL of the volatiles collected in the environment without the insects were injected to evaluate the resonance frequency. For the assessment of the volatiles collected from the environment that contained the insects, also 20 µL were injected into the chamber, but before, with the outlet open and then with the air outlet closed, again 20 µL were injected and measure the resonance frequency. Measurements were also taken every 20 s for 5 min. All experiments are performed in triplicate.

5.2.4 Response of the cantilever nanosensor to interference

To verify the differentiation capacity of the developed PANI.Ag cantilever nanosensor, experiments were carried out with possible interferents. An analysis of resonance frequency was carried out using the same methodology, but with another species of stink bug, *Diceraeus furcatus,* known as the green belly bug, in order to verify the distinguishing ability of the cantilever nanosensor. The experiment was carried out in the same way as the *E. heros* species, after the adaptation period, without distinction of sex, for 10 days.

Synthetic compounds were also tested: tridecane and hexenyl (Z) -3-acetate. Tridecane has a molecular weight of 184.4 g/mol and a density of 0.76 g/cm^3, and is produced as a defensive compound by males and females of the brown stink bug (BORGES et al., 2001; MORAES et al., 2008). The compound (Z)-3-hexenyl acetate is considered a volatile compound of green leaves (LI; ZHU; QIN, 2012), released by plants such as corn and soybeans, for example (MAGALHÃES et al., 2018). These were synthesized and provided by Embrapa Genetic Resources and Biotechnology of Brasília and, for the experiment, were used in the concentration of 1% (v/v). Their responses were compared to the synthetic pheromone of *E. heros*, the compound 2,6,10-trimethyltridecanoate of methyl in the concentration of 0.3 µg/mL.

5.2.5 Statistical analysis

The results of synthetic interferents were evaluated using the Principal Component Analysis (PCA) statistical method using OriginPro9.0 (© Origin Lab Corporation) software. Multivariate analysis was performed to assess the discrimination between the pheromone compound and the analyzed interferents.

5.3 Results and Discussion

5.3.1 Sensing of male and female sex pheromones of live *E. heros* insects

The resonance frequency results of the PANI.Ag cantilever nanosensor to the volatiles from field condition with male and female *E. heros* insects (15 males and 15 males) is presented in the Figure 5.3. It was also evaluated with the same Pani.Ag cantilever nanosensor the response in air (control) and in the field with food and water (without presence of insects). The temperature and relative humidity (RH) of the environment that contained the stinkbugs were monitored daily, which has remained at 32.62°C (± 0.47) and 51.30% (± 5.56), respectively.

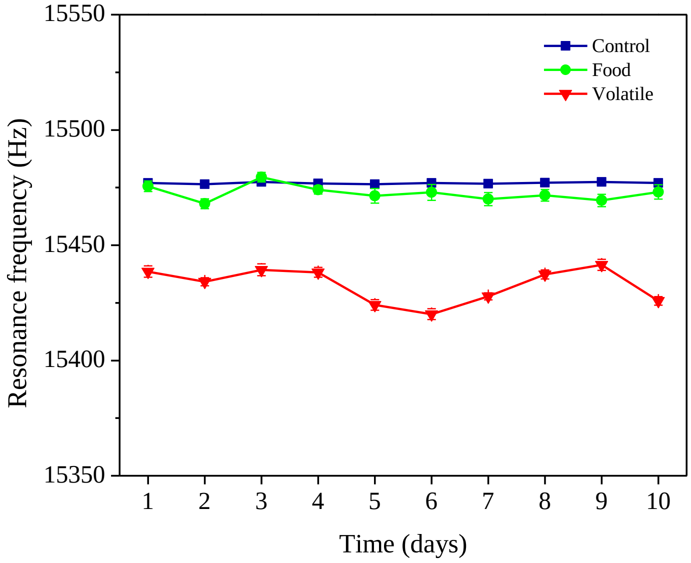

Figure 5.3: Resonance frequency response of the PANI.Ag cantilever nanosensor from the volatiles *in vivo* experiment containing male and female *E. heros* insects, food environment, and control (synthetic air).

It can be observed throughout the experiment, when the cantilever nanosensor was exposed to the volatile from *E. heros* (male and female), a reduction of the Resonance frequency when compared to the control (exposure to air) and the environment containing only food and water (without *E. heros* insects) (Figure 5.3). In first day evaluated it was observed a decrease of approximately 38 Hz, and in the last day of 51 Hz, in relation to the control environment. The total adsorbed mass was determined by standard dynamic force field equation calculating the difference in resonance frequencies before and after exposition to the volatiles, taking in account a rectangular shaped cantilever (BREZOLIN et al., 2019; STEFFENS et al., 2012) and spring constant of the cantilever. The adsorbed mass (Δm) in nanosensor exposed to the volatiles from the field condition with male and female *E. heros* was around 6 pg in each day measured. These results corroborate with the principle of detection based on the decrease of the resonance frequency with the increase of the mass of the pheromone in the device. Moitra et al. (2016) used a miniaturized microelectromechanical system of silicon and silicon dioxide functionalized with aminosilane reagent and thiosilane reagent for the optical detection of sexual pheromones from *Helicoverpa armigera* and observed a decrease in resonance frequency with the exposure to the pheromone volatiles.

The resonance frequency results of the PANI.Ag cantilever nanosensor to the volatile from field condition with distinction of males and females *E. heros* are shown in Figure 5.4.

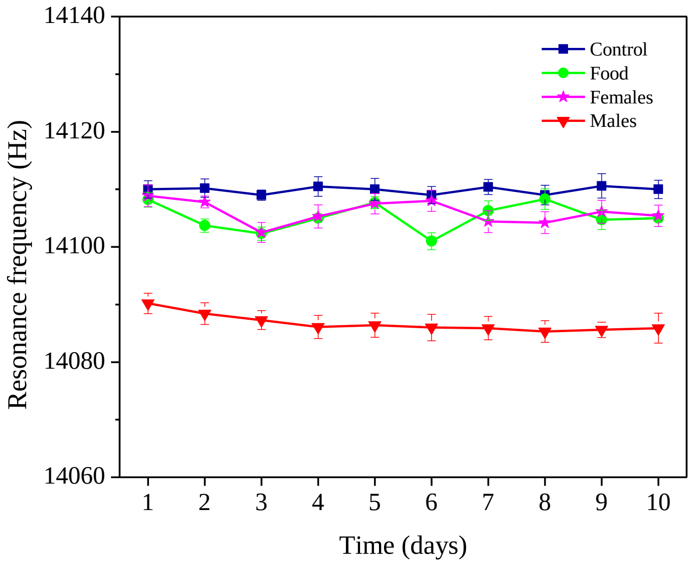

Figure 5.4: Resonance frequency response by the PANI.Ag cantilever nanosensor from the in vivo experiment with distinction of males and females sex pheromone of *E. heros* insects.

The insects were separated into two chambers, containing 25 insects in each environment. The temperature and relative humidity conditions were kept as before. The resonance frequency results for the volatile compound collected in the field condition with females insects was similar to the field condition of food (without insects), and are very close to the control. It is possible to observe a greater resonance frequency reduction when the nanosensor was exposed to the volatile compound from the field condition contained only males *E. heros* insects. The reduction was approximately 21 Hz, representing a daily adsorbed mass of approximately 10 pg.

Male's *E. heros* insects release the sexual or aggregation pheromone to attract females for breeding purposes (WEBER et al., 2017). Experiments performed in laboratory bioassays and field condition demonstrate that the females *E. heros* insects are mostly attracted by the methyl 2,6,10-trimethyltridecanoate compound. This compound is present in the sex pheromone from brown stink bug *E. heros* together with methyl (2E,4Z)-2,4-decadienoate, and methyl 2,6,10-trimethyldodecanoate (BORGES et al., 1998a; 1998b, 1999).

In this way, the results presented by the Pani.Ag cantilever nanosensor showed that the device was able to detect the sexual pheromone liberated by the male insects. Therefore, these experiments performed with vivo insects showed that the cantilever nanosensor are a powerfully tool to be applied in the agriculture. Also, the cantilever MEMS devices has advantages as inexpensive and are readily available, present fast response, high limit of detection and mass sensitivity, make them ideal for large scale application in the agriculture. In addition, it can provide alternative tactics to monitor *E. heros* population in the field.

With the results obtained (Figures 5.3 and 5.4), it is possible to understand the importance of using the cantilever nanosensor to detect semiochemicals, and later incorporation in processes of integrated pest management. According to Kovaleski and Mumford (2007), the use of the sexual pheromone from the moth *Cydia pomonella* can monitor and identify infested areas, and the early identification of areas infested contributed to the total eradication of this pest from commercial orchards. *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) is one of the main pests of apple crops in Brazil and its pheromone is used for control by means of mating disruption (PADILHA et al., 2018). Pheromones are also used for monitoring cotton weevils (*Anthonomus grandis*), banana borers (*Cosmopolites sordidus*) and coconut borers (*Rhynchophorus palmarum*) (FONTES e VALADARES-INGLIS, 2020).

5.3.2 Selectivity of the nanosensor to Interfering *D. furcatus* live insect

The selectivity of cantilever nanosensor is important due to determine whether he could detect the *E. heros* pheromone without being interfered by other ones presented in the field. The response of nanosensor to detection of stinkbug *D. furcatus* insect (30 insects in the chamber), normally present in an agricultural field, is presented in Figure 5.5. The temperature and RH of the environment that contained the bugs were monitored daily during the ten days of the experiment, where they remained around 24.75°C (± 1.01) and 48.0% (± 6.77), respectively.

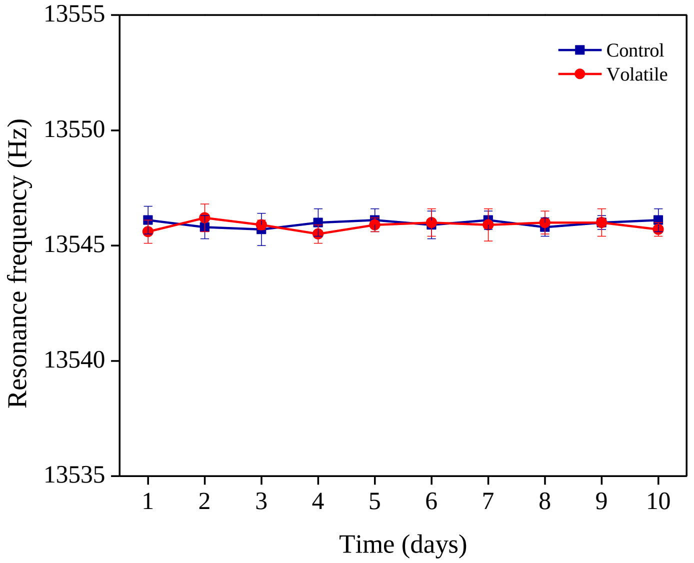

Figure 5.5: Interfering semiochemicals released from *D. furcatus* and her interaction with the cantilever nanosensor.

It is possible to observe that when the PANI.Ag cantilever nanosensor was exposed to the volatile from the field with *D. furcatus* stinkbugs, the resonance frequency did not change considerably when compared to the control (exposure to synthetic air). This indicates that not has interaction between the volatile compounds and the cantilever nanosensor, most likely due to the stinkbug not releasing methyl 2,6,10-trimethyltridecanoate, the main volatile from sexual pheromone. With this result, it is possible to affirm that the PANI.Ag cantilever nanosensor developed is capable of detect selectively the sex pheromone from *E. heros* insects.

5.3.3 Selectivity of the nanosensor to interfering semiochemicals

The selectivity of the nanosensor was determined in the presence of interfering semiochemicals (tridecane and (Z)-3- hexenyl acetate), and the synthetic pheromone of *E. heros* (methyl-2,6,10 trimethyltridecanoate). The results obtained from the volatile molecules is showed in Figure 5.6. The experimental condition was 31.64°C (± 0.75) of temperature and 49.60% (± 6.91) RH.

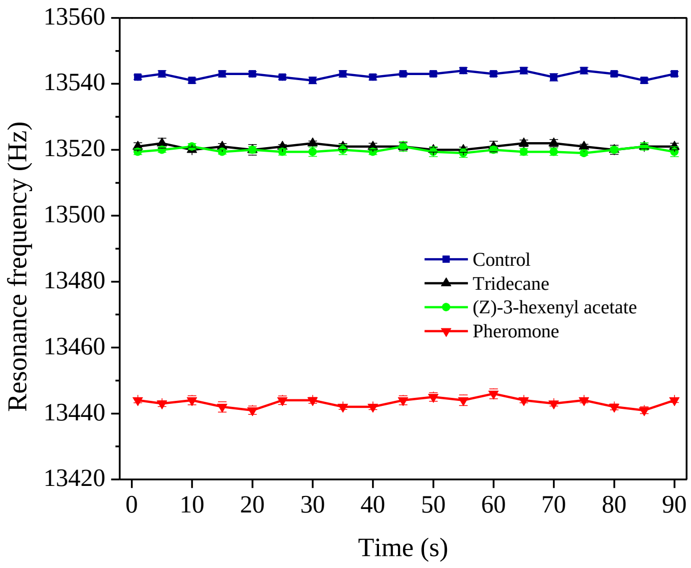

Figure 5.6: Interfering semiochemicals and synthetic pheromone response of cantilever nanosensor.

It can be seen that the resonance frequency responses for tridecane and (Z)-3- hexenyl acetate were similar, remaining around 13520 Hz during the measures (Figure 5.6). When compared to the control, it is observed a reduction in the resonance frequency in approximately 22 Hz of the interferents. The high decrease in resonant frequency was observed only in the presence of *E. heros* synthetic pheromone (methyl-2,6,10 trimethyltridecanoate) which showed a reduction around 100 Hz. This high reduction indicates the interaction of the methyl 2,6,10-trimethyltridecanoate compound which present three chiral centers (BORGES et al., 1998a) with the sensing layer of the nanosensor. When in contact with the Pani.Ag sensing layer coated on cantilever nanosensor occurred the adsorption of the compound, resulting in a decrease signal of the resonance frequency, that is, increase the mass of the device. The detection mechanism of this adsorbed volatile may be related to the structural expansion of the Pani.Ag sensor layer, changing the mass of the cantilever nanosensor (BREZOLIN et al., 2019).

PANI sensing layer have some instability of the gas sensor and sometimes present low selectivity (PANDEY, 2016). When utilized as sensing materials combined with Ag can be enhance her selectivity. As in this work, it was used cantilever nanosensor coated with PANI.Ag the selectivity results can be considered good. Also, was evaluated the selectivity coefficient (Q) (PHASUKSOM; PRISSANAROON-OUAJAI; SIRIVAT, 2020**)**, generally used to describe the selective properties of the gas sensor. The high Q value represent the great ability to differentiate *E. heros* pheromone from interfering gas molecules. It is defined as the ratio of the gas sensor responses to the target vapor and another vapor (Q = Resonance frequency_A/Resonance frequency_B) (NAVALE et al., 2014). The Resonance frequency_A and Resonance frequency_B are defined as the resonance frequency response to *E. heros* pheromone, and resonance frequency response to interfering vapor molecule, respectively. Q values of the cantilever nanosensor for the interfering semiochemicals tridecane and (Z)-3- hexenyl acetate were similar, around 0.99. This selectivity of cantilever nanosensor coated with PANI.Ag can be due characteristics of volatile molecule adsorbed on sensitive layer. Also, can be associate with the sensing mechanism of the *E. heros* pheromone adsorption and diffusion into the sensing layer.

This lower response of the device to the defensive compounds (tridecane and (Z)-3-hexenyl acetate) common to other stinkbug species and floral volatile is very appreciated indicating the detection of sexual pheromone. The tridecane compound is the most abundant aliphatic hydrocarbon in neotropical bug *Edessa meditabunda* and *Edessa rufomarginata* (MORAES et al., 2008) and has an action in both aggregation and alarm pheromone. The (Z)-3- hexenyl acetate is a green leaf volatile found in maize and cotton plants (LI; ZHU; QIN, 2012; MAGALHÃES et al., 2018).

The resonance frequency responses were analyzed by principal component analysis (PCA) at interfering semiochemicals (tridecane and (Z)-3- hexenyl acetate), and the synthetic pheromone of *E. heros* (methyl-2,6,10 trimethyltridecanoate) (Figure 5.7). The PCA showed 100% of the total information collected by the matrix in PC1 and PC2. PC1 presented the largest amount of information (97.3%); therefore, the interfering semiochemicals analysis should be based on this axis, although the small contribution of PC2 (2.7%) should be considered. The amounts of information in PC1 and PC2 were relevant and both contributed to the analysis at the Cartesian level, enabling the discrimination of the interfering semiochemicals analysis and pheromone. The samples next each to other in the plot of the main component are similar, thus being able to discriminate it in relation to interfering and control.

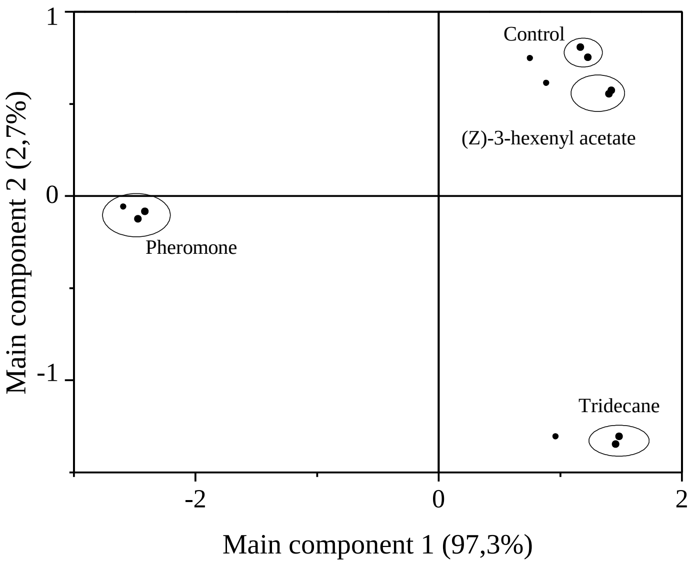

Figure 5.7: Principal Component Analysis (PCA) plot obtained from resonance frequency response of cantilever nanosensor to interfering semiochemicals and synthetic pheromone.

5.4 Conclusion

With the results carried out *in vivo* with *E. heros*, it was possible to verify that there is an interaction between the PANI.Ag nanosensor and the volatiles released by *E. heros* bugs *in vivo*, reducing the resonance frequency when compared to the control. The experiment differing males and females *E. heros* proved the efficiency of the cantilever nanosensor in distinguishing the sexual pheromone, which is only exhaled by males to attract females. The evidence of the interaction of PANI.Ag sensing layer with the volatiles was verified by the test with the *D. furcatus* bedbug species, where there was no reduction in the resonance frequency, as well as when exposed to the tridecane and (Z) -3-acetate compounds hexenyl, where there were no significant changes, thus indicating the selectivity of the cantilever nanosensor functionalized with PANI.Ag for methyl 2,6,10-trimethyltridecanoate, the main component of the sexual pheromone of *E. heros*. Therefore, cantilever nanosensor with PANI.Ag may be a promise alternative for the detection of the sexual pheromone of *E. heros*.

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Conflict of interest

The authors declare that have no competing interests.

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CAPÍTULO VI

CONSIDERAÇÕES FINAIS

De maneira geral, pode-se concluir que os nanossensores de cantilever de PANI/PSS e PANI.Ag são promissores na detecção do feromônio sexual do percevejo *E. heros*, em especial o componente 2,6,10-trimetiltridecanoato de metila.

Os dois métodos de funcionalização do cantilever, a técnica LbL e a técnica *dip coating*, foram adequados e eficientes para a construção dos nanossensores de cantilever, sendo possível observar a redução da f_{res} com a adição das camadas depositadas além da interação das camadas sensoras com os voláteis analisados por meio das diferentes respostas de f_{res} obtidas.

A caracterização das camadas sensoras pelas técnicas de Espectroscopia no Ultravioleta-Visível (UV-Vis), Difratometria de Raios X (XRD), Espectroscopia no infravermelho por transformada de Fourier com medida de refletância total atenuada (ATR-FTIR) e Microscopia de Força Atômica (AFM) comprovam a eficiente funcionalização dos cantileveres, ficando evidente a deposição dos filmes na superfície do mesmo, além de identificar a espessura, que foi de 98,36 nm (±0,04) para o nanossensor de cantilever de PANI/PSS e de 20,03 nm (±0,04) para o nanossensor de PANI.Ag, concluindo assim, que ambos podem ser chamados de nanossensores por apresentarem propriedade em escala nanométrica.

Quanto as respostas dos nanossensores de cantilever, o nanossensor de PANI/PSS apresentou tempo de resposta de 32,94 s (±0,61) e para o nanossensor de cantilever de PANI.Ag foi de 29,81 s (±0,45), ambos considerados tempos rápidos de resposta. Os nanossensores foram capazes de detectar na ordem de ppb o feromônio de *E. heros* que contém o composto 2,6,10-trimetiltridecanoato de metila. O LOD do nanossensor de cantilever de PANI/PSS foi de 0,01454 µg/mL, com uma sensitividade de 164,97 Hz.mL/µg. Já para o nanossensor de cantilever funcionalizado com o nanohíbrido de PANI.Ag, o LOD e a sensitividade foram de 0,01291 µg/mL e 185,84 Hz.mL/µg, respectivamente.

Em relação a estabilidade dos nanossensores desenvolvidos, o funcionalizado com PANI/PSS apresentou uma redução de 5,22% em relação ao seu valor inicial, com um ano de armazenamento. Com o mesmo tempo de armazenamento, o nanossensor de cantilever de PANI.Ag apresentou uma redução de apenas 0,32% ao seu valor inicial, tornando assim, os nanossensores estáveis durante o armazenamento com uma longa vida útil.

Os nanossensores de cantilever foram capazes de detectar o composto 2,6,10-trimetiltridecanoato de metila em septos de borracha por meio da redução de f_{res} durante 60 dias, demonstrando que há interação das camadas sensoras desenvolvidas e o feromônio sexual de *E. heros*.

Nos experimentos realizados com os interferentes tridecano e (Z)-3-acetato de hexenila, não houve alterações significativas na f_{res} quando expostos a ambos nanossensores de cantilever desenvolvidos, diferente do que aconteceu quando comparado ao composto feromonal. A análise estatística da PCA mostrou que o nanossensor de cantilever de PANI.Ag consegue discriminar adequadamente o feromônio de *E. heros* dos interferentes e branco.

Os testes *in vivo* confirmaram a interação da camada sensora de PANI.Ag com os voláteis liberados pelos percevejos *E. heros* durante os 10 dias avaliados, por meio da redução da f_{res}. O experimento diferindo machos de fêmeas também comprovou a eficiência do nanossensor de cantilever em distinguir o composto 2,6,10-trimetiltridecanoato de metila, que só é exalado por machos para atração de fêmeas. Ainda confirmando a resposta do nanossensor de cantilever de PANI.Ag, o teste com a espécie de percevejos *D. furcatus*, mostrou que não ocorreu redução da f_{res}.

Desta forma, os nanossensores de cantilever desenvolvidos apresentam altos valores para LOD e sensitividade, sendo promissores para a detecção em tempo real de percevejos *E. heros*, demonstrando uma alternativa no MIP com a utilização da nanotecnologia, por meio de nanossensores e feromônios. Com isso, pode-se reduzir a utilização de agroquímicos além de refletir diretamente na saúde dos seres vivos e também do meio ambiente.

SUGESTÕES PARA TRABALHOS FUTUROS

- Realizar experimentos variando a UR (%) e variando as concentrações de feromônio;
- Realizar experimentos variando UR (%) e temperaturas ao mesmo tempo;
- Realizar testes para verificar relação direta da concentração de feromônio detectável pelo nanossensor de cantilever e a quantidade de insetos;
- Realizar experimentos com diferentes números de percevejos *in vivo* para avaliar a resposta do nanossensor de cantilever e sua relação com a concentração de feromônio;
- Realizar cromatografia gasosa para observar se o volátil liberado pelos percevejos *E. heros in vivo* é o composto 2,6,10-trimetiltridecanoato de metila;
- Realizar testes a campo para verificar possível detecção de *E. heros* nas lavouras;
- Utilização do nanossensor de cantilever a campo na agricultura de precisão e aplicação de tratamento apenas onde detectar a presença dos insetos;
- Desenvolver *softwares* para informações coletadas do nanossensor de cantilever a fim de avaliar precocemente a infestação nas lavouras.