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ALISSON DA SILVA SANTANA

CHARACTERIZATION OF RESISTANCE IN COTTON GENOTYPES AND BIOACTIVITY OF ESSENTIAL OILS AND THEIR MICROEMULSIONS TO MANAGEMENT OF *Bemisia tabaci* MED (HEMIPTERA: ALEYRODIDAE)

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To my Mother, Marli Rosa da Silva, who always offered me more than she could give.

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I dedicate

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ABSTRACT

Whiteflies of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) complex stand out as severe cotton pests worldwide. These insects can cause direct damages to cotton plants through of the continuous sucking of sap as well as indirect damages by favoring sooty mold and transmitting virus. In Brazil, the introduction of *B. tabaci* Mediterranean (MED) (known previously as Q biotype) has attracted the attention of farmers and the scientific community, once this species presents high infestation capacity, besides resistance to several groups of insecticides. As an alternative to the chemical control, plant resistance and botanical derivatives can be valuable tools for integrated pest management (IPM). However, to date, in Brazil, there are no studies assessing the resistance of cotton genotypes to *B. tabaci* MED. Regarding botanical derivatives, the lack of formulations limits their use for pest control in crop fields. In this scenario, here we aimed to identify sources of resistance in cotton genotypes to *B. tabaci* MED and assess the bioactivity of formulations based on essential oils on the insect. Initially, toxicity bioassays were performed to evaluate the efficiency of essential oils (EOs) of *Piper marginatum* Jacq. (Piperaceae) and *Mansoa alliacea* Miers (Bignoniaceae) and their formulations against *B. tabaci* MED. Finally, to evaluate the possible resistance of cotton genotypes, a preliminary assay was carried out with 78 genotypes, of which 28 were selected for antixenosis and antibiosis assays. Based on the results obtained, it was possible to observe that the essential oils of *M. alliacea* and *P. marginatum* were toxic against nymphs of *B. tabaci* MED in laboratory and semifield conditions. The EOs showed ovicidal and repellent effects in addition to inhibiting oviposition and colonization on treated leaves/plants. In the same way, microemulsions based on the EOs were toxic against nymphs of *B. tabaci* MED in laboratory and greenhouse conditions, demonstrating ovicidal and repellent effects and inhibiting oviposition and colonization on treated leaves/plants. In addition, the efficacy of the emulsions was maintained when diluted in water. Regarding to host resistance assays, the genotypes IAC 23, IAC 25, FM975WS, IAC PV 010-175 were the least infested by adults of *B. tabaci* MED, while Aubum 56-7, DP 4049, Express 257, IAC-18, Empire B-4 and Reba B-50 RR showed the lowest number of eggs and IAC 97-2939 “macaco”, 101-102 B, Algodão Indiano and Paymaster 53-523 showed the lowest means of nymphs, indicating the occurrence of antixenosis. On the other hand, the genotypes IAC 13-1-76-5366, CNPA 92-1121 and Hi-Bred exhibited antibiosis, reducing the development time and causing high nymphal mortality. The density of gossypol glands was positively correlated with nymphal mortality. Based on these results, it is possible to conclude that the control strategies studied here can be tools to be used in the integrated management of pests in cotton in the future, as well as serve as a basis for new studies aiming to control whiteflies.

Keywords: plant resistance; botanical insecticides; alternative control; whitefly; antixenosis; antibiosis.

RESUMO

Moscas-brancas do complexo *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) destacam-se como pragas severas do algodoeiro em todo o mundo. Esses insetos podem ocasionar danos diretos às plantas, por meio da sucção de seiva, além de indiretos, devido ao favorecimento da ocorrência de fumagina e transmissão de vírus. No Brasil, a introdução da espécie *B. tabaci* Mediterranean (MED) (anteriormente biótipo Q), tem despertado a atenção de produtores e da comunidade científica, uma vez que essa espécie apresenta elevada capacidade de infestação, além de resistência a diversos grupos de inseticidas. Como alternativa ao controle químico, plantas resistentes e derivados botânicos destacam-se como valiosas ferramentas para o manejo integrado de pragas (MIP). No entanto, até o momento, no Brasil, não há estudos abordando a resistência de genótipos de algodoeiro a *B. tabaci* MED e no tocante a derivados botânicos, a escassez de formulações limita sua utilização no controle de pragas em grandes culturas. Nesse cenário, o objetivo deste trabalho foi identificar fontes de resistência em genótipos de algodoeiro a *B. tabaci* MED e avaliar a bioatividade de óleos essenciais e suas formulações sobre o inseto. Inicialmente, foram realizados bioensaios de toxicidade para avaliar a eficiência dos óleos essenciais (OEs) de *Piper marginatum* Jacq. (Piperaceae) e *Mansoa alliacea* Miers (Bignoniaceae) e suas formulações contra *B. tabaci* MED. Por fim, para avaliar a possível resistência de genótipos de algodoeiro, foi realizado um ensaio preliminar com 78 genótipos, dos quais 28 foram selecionados para ensaios de antixenose e antibiose. Com base nos resultados obtidos, foi possível observar que os óleos essenciais de *P. marginatum* e *M. alliacea* foram tóxicos para ninfas de *B. tabaci* MED em condições de laboratório e semicampo. Os OEs apresentaram efeitos ovicida e repelente, além de inibir a oviposição e colonização nas folhas/plantas tratadas. Da mesma forma, as microemulsões à base de OEs foram tóxicas contra *B. tabaci* MED em condições de laboratório e casa de vegetação. Além disso, a eficácia das emulsões foi mantida quando diluídas em água. Nos bioensaios de resistência, os genótipos IAC 23, IAC 25, FM975WS, IAC PV 010-175 foram os menos infestados por adultos de *B. tabaci* MED, enquanto Aubum 56-7, DP 4049, Express 257, IAC-18, Empire B-4 e Reba B- 50 RR apresentaram o menor número de ovos e IAC 97-2939 “macaco”, 101-102 B, Algodão Indiano e Paymaster 53-523 apresentaram o menor número de ninfas, indicando a ocorrência de antixenose e/ou antibiose. Por outro lado, os genótipos IAC 13-1-76-5366, CNPA 92-1121 e Hi-Bred exibiram antibiose, reduzindo o tempo de desenvolvimento e causando alta mortalidade ninfal. A densidade de glândulas de gossipol correlacionou-se positivamente com a mortalidade ninfal. Com base nesses resultados, é possível concluir que as estratégias de controle aqui estudadas podem ser ferramentas a ser utilizadas no manejo integrado de pragas do algodoeiro no futuro, bem como servir de base para novos estudos visando o controle de *B. tabaci* MED.

Palavras-chave: resistência de plantas; inseticidas botânicos; controle alternativo; mosca branca; antixenose; antibiose.

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GENERAL INTRODUCTION

Cotton is the most important natural textile fiber crop in the world (ZHAO et al., 2015). The world cotton market involves around R$ 56.76 billion every year and hire more than 350 million people directly and indirectly (planting, harvesting, logistics, ginning, processing and packaging) (ABRAPA, 2021). Brazil is among the five largest cotton producers in the world and the second largest cotton exporter; with around 1.5 million tons exported in the 2020/2021 crop season (ABRAPA, 2021; USDA, 2021). In 2021/2022, Brazilian cotton growers are expected to harvest more than 2.7 million tons of cotton lint and more than six million tons of seed (ABRAPA, 2021).

Cotton has characteristics that guarantee its wide use in Brazil and justify the productive potential of this crop in the country. Cotton plants support a wide variation in soil types and develop well in flat or gently undulating regions (BELTRÃO; ARAÚJO, 2004). The ideal temperature for cotton development comprises the range of 20ºC to 30ºC but the crop is grown in regions with temperatures below 15ºC and above 40ºC (ECHER, 2014). However, some factors limit the crop yield, especially the attack of insect pests. If not identified, monitored and controlled efficiently, pests can cause huge losses to cotton crops.

In general, losses caused by pests in Brazil reach approximately R$ 55 billion annually (OLIVEIRA et al., 2014; SINDAG, 2021). In terms of production, the attack of pests results in losses of approximately 25 million tons of food, fiber and biofuels; this is equivalent to 7.7% of the total Brazilian production (OLIVEIRA et al., 2014; SINDAG, 2021). In cotton, yield losses resulting from this attack reach 155,000 tons of lint and 250,000 tons of seed and the losses reach about R$ 2.26 billion per year (OLIVEIRA et al., 2014; SINDAG, 2021).

In addition to direct losses, the control strategies taken to manage these organisms can also cause indirect economic damage related to the purchase and application of insecticides (BUENO et al., 2011; OLIVEIRA et al., 2013, 2014). In general, around 170 thousand tons of synthetic insecticides are used annually in Brazil, an expense of more than R$ 9 billion (OLIVEIRA et al., 2014; SINDAG, 2021). Cotton is the second crop with the highest consumption of insecticides, with approximately 32 thousand tons, which represents more than R$ 2 billion (OLIVEIRA et al., 2014; ABRAPA, 2021).

The damage caused by insects in cotton is justified by the large number of pests that can attack this crop. Insects that occur in cotton can cause damage to roots, stems, leaves and fruits. Pests that attack cotton leaves are represented by sucking and chewing insects. The problems caused by these insects include direct damage (defoliation, introduction of toxins, sap suction) and indirect damage (transmission of viruses, induction of sooty mold, among others). As a consequence, the occurrence of leaf feeding pests decrease the crop yield, since it reduces the photosynthetic capacity of the plants, in addition to promoting the occurrence of viruses.

Among the pests that damage cotton, the whiteflies of the *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) complex stand out as one of the most severe worldwide (PRADO et al., 2016). The damage caused by whiteflies can be direct or indirect. Direct damage is related to the continuous suction of phloem sap, resulting in physiological disorders related to a significant reduction in production (NARANJO et al., 2010). Among the disorders, it is possible to observe leaf fall, the appearance of chlorotic spots, silvering, yellowing and/or whitening of vegetative structures and irregular maturation or other abnormalities in fruiting structures (BUNTIN; GILBERTZ; OETTING, 1993; ISLAM; SHUNXIANG, 2009). Additionally, direct nutrient sucking results in leaf chlorosis, growth retardation and reduced plant vigor (ISLAM; SHUNXIANG, 2009; SUEKANE et al., 2013).

Indirectly, whiteflies can cause damage related to the appearance of sooty mold and the transmission of viruses to different crops. The occurrence of sooty mold is related to the excretion of 'honeydew' that serves as a growth substrate for fungi of the genus *Capnodium* (ISLAM; SHUNXIANG, 2009; MILENOVIC et al., 2019). As a consequence, there is a reduction of the photosynthetic capacity of the plant and crop yield, in addition to the fiber contamination, which reduces the potential for commercialization (ISLAM; SHUNXIANG, 2009; STANSLY; NARANJO, 2010). In addition, whiteflies can transmit more than 300 viruses that affect different crops worldwide, causing yield losses of up to 100% (GILBERTSON et al., 2015; HUSSAIN et al., 2019). Most whitefly-transmitted viruses belong to the *Begomovirus* genus (Geminiviridae family) (GILBERTSON et al., 2015; QUADROS et al., 2019; THOMPSON, 2011). Additionally, whiteflies are also vectors of *Crinivirus*, *Ipomovirus*, *Torradovirus* and some *Carlaviruses* (GILBERTSON et al., 2015; NAVAS-CASTILLO; FIALLO-OLIVÉ; SÁNCHEZ-CAMPOS, 2011).

Until 2013, the damage caused by *B. tabaci* reached approximately R$ 3 billion annually in Brazil (OLIVEIRA et al., 2013). These damages were probably potentiated by the introduction of the species *B. tabaci* Mediterranean (MED), also known as biotype Q, which was recorded for the first time in 2014 in Rio Grande do Sul (BARBOSA et al., 2015). The species *B. tabaci* MED shares many of the same general biological characteristics of the species *B. tabaci* Middle East-Asia Minor 1 (MEAM1), widely distributed in the national territory and has lower susceptibility to some insecticides classes and high competitive ability (HOROWITZ; ISHAAYA, 2014). Additionally, *B. tabaci* MED is considered an important cotton pest in the countries where it was introduced (ZHANG et al., 2014; TOCKO-MARABENA et al., 2017) and, therefore, represents a high risk for this crop in Brazil.

Chemical control has been widely used for the management of whiteflies. However, this practice has gradually become less efficient due to the selection of populations resistant to several synthetic insecticides (SOHRABI et al., 2011). In addition, the excessive use of these products contributes to the elimination of natural enemies, environmental degradation, increased production costs and increased toxicological risks for farmers and consumers. As an alternative to chemical control, resistant genotypes and botanical derivatives have been studied for the management of whiteflies (BALDIN et al., 2015, 2017; FANELA et al., 2016; DOMINGOS et al., 2018) and have potential for use in integrated management programs for *B. tabaci* MED.

Plant resistance refers to the integrated management tactic in which a resistant plant genotype is intentionally employed, alone or in combination with other tactics, to reduce the impact of herbivorous arthropods on crop yield or quality (STOUT, 2014). A resistant genotype is defined as one that, due to its genotypic composition, is less damaged compared to other genotypes under similar conditions of arthropod infestation (PAINTER, 1951; BALDIN et al., 2019). The use of resistant plants is a key method of regulating insect pests in agricultural crops (SCHOONHOVEN et al., 2005; STOUT, 2014). This is because this tactic has high efficiency in pest control and at the same time is less aggressive to the environment and farmers due to the ease of adoption, specificity, persistence, cumulative effect, low cost and compatibility with other control methods (BACCI et al., 2007; DOMINGOS et al., 2018; BALDIN et al., 2019).

There are a few ways in which plants resist attack by insect pests. In most cases, the plant directly affects the behavior or biology of the insect and, in some cases, the plant continues its development without causing any effect on the insect (LARA, 1991, BALDIN et al., 2019). In light of this, (PAINTER, 1951) classified resistance into three types: antibiosis, antixenosis and tolerance.

A plant resistant by antixenosis has chemical, physical or morphological factors that make it less used by the insect for food, oviposition or shelter (LARA, 1991; SCHOONHOVEN et al., 2005; STOUT, 2014). As an example, it was found that some cabbage, soybean and bean genotypes caused reduction in oviposition and colonization by *B. tabaci* MEAM1*, Aphis glycines* Matsumura (Hemiptera: Aphididae) and *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae), respectively (BALDIN et al., 2017; DOMINGOS et al., 2018; MORANDO et al., 2015).

Antibiosis refers to plant properties that adversely affect the physiology of an herbivore, which can cause reduced fecundity, size or longevity, and increased insect mortality (LARA, 1991; SCHOONHOVEN et al., 2005; BACCI et al., 2007). Antibiosis resistance was observed in cowpea and soybean genotypes for *B. tabaci* MEAM1 and *A. glycines*, respectively (CRUZ et al., 2014; BALDIN et al., 2019).

Tolerance is characterized by the ability of the plant to resist or recover from an injury caused by insects, without affecting its biology and behavior (LARA, 1991; SCHOONHOVEN et al., 2005). Resistance by tolerance was observed in soybean genotypes against the attack of *B. tabaci* MEAM1 and *A. glycines* in rice genotypes exposed to *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) infestation (CRUZ et al., 2016; SARAO; BENTUR, 2016; BALDIN et al., 2018).

The types of resistance are conditioned by chemical, physical or morphological factors (=causes) (LARA, 1991). It should be noted that, although the classification into three factors is useful, resistance to insect attack is often coordinated by a combination of two or even all three causes of resistance (SCHOONHOVEN et al., 2005).

The physical causes of resistance are related to variations in the light radiation spectrum by plants (LARA, 1991; SCHOONHOVEN et al., 2005). As an example, the color of the leaves altered the reproductive behavior of *Pieris brassicae* in cabbage plants (MASKATO et al., 2014), the preference for oviposition of *Chrysodeixis includens* in bean genotypes (MORANDO et al., 2015) and the rate of colonization by *B. tabaci* in cabbage genotypes (DOMINGOS et al., 2018).

The morphological factors of resistance mechanically interfere with host selection, feeding, digestion and oviposition of the arthropod (BACCI et al., 2007; LARA, 1991). The occurrence of glandular or non-glandular trichomes (BALDIN et al., 2017; STOUT et al., 2018), cuticle thickness (SCHOONHOVEN et al., 2005; SILVA et al., 2014), epidermis hardness and texture (MIYAZAKI et al., 2017; ENTLING et al., 2019) and the presence of wax on the leaf surface (ZHANG et al., 2018; KARIYAT et al., 2019) are some of the morphological barriers that reduce the attack potential of insect pests to the host plant.

Finally, chemical factors of resistance can influence the behavior of insects (eg attractants, arrestants, stimulants and deterrents) (LARA, 1991; SCHOONHOVEN et al., 2005) or inhibit their physiological processes (LARA, 1991; BACCI et al., 2007). In fact, chemical compounds present in cabbage, soybean and rice plants inhibited feeding and caused physiological changes in *Delia radicum* (Linnaeus) (Diptera: Anthomyiidae), *A. glycines* and *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), respectively (SHUHANG et al., 2016; BALDIN et al., 2017; TABARI et al., 2017; BALDIN et al., 2018).

Plants are recognized as a rich source of chemical compounds. Most of the compounds originating from the secondary metabolism of these plants probably evolved as defense agents against insect and other herbivore attacks (ISMAN, 2017). Such compounds can be applied in the development of alternative strategies for pest management and are called botanical insecticides (BENELLI et al., 2017; ISMAN, 2017).

Botanical insecticides are increasingly gaining the preference of farmers and consumers, and studies predict strong growth in sales of botanical products (ISMAN, 2015; PAVELA; BENELLI, 2016). This could make botanicals grow from 1-2% of the global pesticide market to 7% of the market by 2025 (ISMAN, 2020).

Essential oils (EOs) are one of the most important botanical insecticides (CAMPOS et al., 2018), which, in the form of isolated substances or complex mixtures, exhibit a wide range of biological activities (BENELLI et al., 2017; ISMAN, 2017). These substances can alter behavior (repellence, food deterrence, oviposition deterrence), promote physiological changes (acute toxicity, developmental disruption, growth inhibition) or cause mortality in insect (ISMAN, 2017). In addition, essential oils have low toxicity to mammals and other non-target organisms, reduced environmental impact and low persistence in the environment (BENELLI et al., 2017).

The use of EOs as alternative insecticides has gained the attention of the scientific community and the plant protection industry probably due to two main factors (ISMAN, 2017): i) yields of essential oils from aromatic plants are typically in the range of 0.5 to 2.0%, which means they represent a highly concentrated extract from their plant of origin- this tends to make them much more potent than many other plant extracts; ii) the compounds present in essential oils can be analyzed using gas chromatography-mass spectrophotometry, a technique that has become cheaper over the years. Additionally, many EOs are produced in large quantities for other uses (drinks, foods, fragrances for skin products and household cleaning agents, among others) and are therefore available at a reasonable cost (ISMAN, 2017; PAVELA; BENELLI, 2016).

The bioactivity EOs of various species has been studied in the last years. The essential oil of *Piper marginatum* Jacq. (Piperaceae) had insecticidal activity on some species of arthropods, such as *Tetranychus urticae* Koch (Acari: Tetranychidae) (Ribeiro et al., 2016), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Guedes et al., 2020), *Solenopsis saevissima* (F Smith) (Hymenoptera: Formicidae) (Souto et al., 2012) and *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) (Souza et al., 2020). The essential oil of *Mansoa alliaceae* Miers (Bignoniaceae) does not have extensive documented bioactivity against agricultural pests, but its insecticidal activity has already demonstrated against nymphs and adults of *B. tabaci* MEAM1 (Biotype B) by means of fumigation exposure (Fanela et al., 2016).

Despite this potential, the insecticidal activity of EOs may be reduced when these substances are released in the environment due to their rapid degradation. In this sense, emulsions containing essential oils appear as strategies to overcome this drawback (PAVONI et al. 2020). The advantages of EO-based emulsions for the development of eco-friendly insecticides include (i) reduction in EO particle size; (ii) delayed release of active ingredients in the environment; and (iii) increased EO penetration in the insect body (ATHANASSIOU et al. 2018; PAVONI et al. 2020).

Considering that the species *B. tabaci* MED has high dispersal potential, high competitive ability and high tolerance to conventional insecticides, it is necessary to study alternative tactics to control this pest in cotton crops. Thus, this research aims to characterize the possible resistance of 78 cotton genotypes and evaluate the bioactivity of essential oils from *P. marginatum* and *M. alliaceae* Miers (Bignoniaceae) and their formulations for the control of *B. tabaci* MED.

The thesis was divided in three chapters in order to reach the objectives. The first chapter was entitled “New challenges demand new solutions: Selected essential oils as an alternative to control *Bemisia tabaci* MED in Brazil” written according to of Crop Protection’s guidelines; the second was entitled “Bringing botanical insecticides to the field: assessment of the efficacy of microemulsions based on essential oils against *Bemisia tabaci* MED", written according to Industrial Crops and Products ’s guidelines and the third chapter entitled “Looking for sources of resistance to *Bemisia tabaci* MED in different cotton genotypes” written according to Journal of Pest Science’s guidelines.

FINAL CONSIDERATIONS

Considering the risks of the exaggerated use of insecticides on cotton, there is a demand for the use of control alternatives that are less harmful to the environment, applicators, consumers and the environment. In this context, the use of resistant genotypes and botanical derivatives stands out, as these tools aim to reduce the population level of the pest and can be used in association with other IPM tools, such as chemical, biological, cultural control, among others.

Based on the results obtained, it was possible to observe that the essential oils of *M. alliacea* and *P. marginatum* were toxic against nymphs of *B. tabaci* MED in laboratory and semifield conditions. The EOs showed ovicidal and repellent effects in addition to inhibiting oviposition and colonization on treated leaves /plants. In the same way, microemulsions based on the EOs were toxic against nymphs of *B. tabaci* MED in laboratory and greenhouse conditions, demonstrated ovicidal and repellent effects and inhibiting oviposition and colonization on treated leaves/plants. In addition, the efficacy of the emulsions was maintained when diluted in water.

It was possible to observe differences on the preference and biology of different biotypes of *B. tabaci* on different cotton genotypes. The genotypes IAC 23, IAC 25, FM975WS, IAC PV 010-175 were the least infested by adults of *B. tabaci* MED while Aubum 56-7, DP 4049, Express 257, IAC-18, Empire B-4 and Reba B-50 RR showed the lowest number of eggs and IAC 97-2939 “macaco”, 101-102 B, Algodão Indiano and Paymaster 53-523 showed the lowest means of nymphs, indicating the occurrence of antixenosis. On the other hand, the genotypes IAC 13-1-76-5366, CNPA 92-1121 and Hi-Bred exhibited antibiosis, reducing the development time and causing high nymphal mortality. The density of gossypol glands was positively correlated with nymphal mortality.

This is the first study evaluating the effect of microemulsions against *B. tabaci* MED in Brazil. This is also the first time that the resistance of cotton genotypes has been studied to this pest in the country. This is important, considering that, to date, there are no insecticidal molecules registered for the control of this pest in Brazil. The strategies studied here can be tools to be used in the integrated management of pests in cotton in the future, as well as serve as a basis for new studies aiming to control whiteflies.

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