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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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Orientador: Prof. Dr. Edson Luiz Lopes Baldin

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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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SUMMARY

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.

CHAPTER 1

New challenges demand new solutions: Selected essential oils as an alternative to control *Bemisia tabaci* MED in Brazil

Alisson da Silva Santana, Edson Luiz Lopes Baldin, Ana Paula Santana Lima, Thais Lohaine Braga dos Santos, Maria Clezia dos Santos, Tatiana Manzini Vieira, Antônio Eduardo Miller Crotti and Renata Takeara

Abstract

The introduction of *Bemisia tabaci* Mediterranean (MED) (Gennadius) (Hemiptera: Aleyrodidae) (Biotype Q) into Brazil has attracted the attention of farmers and the scientific community since this species has a high capacity for infestation and high tolerance to conventional insecticides. As an alternative to chemical control, botanical derivatives stand out as a valuable tool for integrated pest management (IPM). In this context, this work evaluated for the first time the bioactivity of essential oils of *Piper marginatum* Jacq. (Piperaceae) (PM-EO) and *Mansoa alliaceae* (Lam.) A.ww H. Gentry (Bignoniaceae) (MA-EO) against *B. tabaci* MED. First, concentration-mortality bioassays were performed to estimate the lethal concentrations (LCs) on nymphs of *B. tabaci* MED. The LC_{50}s and LC_{90}s were tested in multi-choice assays (repellency and oviposition deterrence) and no-choice assays (ovicidal effects and infestation ability). Finally, assays were carried out in a greenhouse to check the efficiency of the essential oils under semifield conditions. The major compounds identified in PM-EO were (*E*)-methyl eugenol (34.7%) and (*Z*)-methyl eugenol (27.5%), while diallyl trisulfide (52.8%) and diallyl disulfide (33.9%) were the major compounds in MA-EO. The EOs were toxic against nymphs in the laboratory and semifield and showed ovicidal effect and repellent action. The EOs also reduced oviposition and inhibited the colonization by *B. tabaci* MED. Our results reveal two promising sources of botanical pesticides to control *B. tabaci* MED. These compounds can cause lethal and sublethal effects in all insect life stages, increasing the control efficiency.

Keywords: alternative control, *Bemisia tabaci* MED, *Mansoa alliaceae, Piper marginatum*, whitefly

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1.1 Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) comprises a complex of cryptic species with at least 44 species, among which the species Middle East-Asia Minor 1 (MEAM1), also known as biotype B, and Mediterranean (MED), also known as biotype Q, have greater importance worldwide (Kanakala and Ghanim, 2019). Mediterranean species were introduced into Brazil in 2014, which increased the damage caused by whiteflies in this country. In most countries where *B. tabaci* MED has been introduced, this insect has become an important pest of crops (Zhang et al., 2014; Tocko-Marabena et al., 2017) and therefore represents a high risk for major crops in Brazil, such as cotton and soybeans.

Chemical control has been widely used for the management of whiteflies. However, the excessive use of synthetic insecticides contributes to the reduction of populations of natural enemies, pollinators and other non-target insects, environmental degradation and an increase in toxicological risks for farmers and consumers (Abati et al., 2021; Goulson et al., 2015; Oliveira et al., 2018; Sánchez-Bayo and Wyckhuys, 2019; Wagner, 2020). Additionally, this practice is gradually becoming less efficient due to the selection of resistant insect populations (Sohrabi et al., 2011). However, the lack of environmentally safe molecules contributes to maintaining the dominance of synthetic insecticides in the control of agricultural pests worldwide (Isman, 2017).

The application of essential oils (EOs) in the management of insect pests is promising. EOs can change insect behavior and promote physiological changes, in addition to causing mortality in insect pest populations (Isman, 2017). In addition, EOs have low toxicity to nontarget organisms, high biodegradability and limited impact on the environment (Benelli et al., 2017; Castilhos et al., 2018; Miura et al., 2021; Stenger et al., 2021).

The *B. tabaci* MED species has a high dispersion potential, high competitive ability and high tolerance to conventional insecticides (Luo et al., 2010; Guo et al., 2012) Therefore, it is necessary to study alternative tactics to control this pest. The essential oil of *Piper marginatum* Jacq. (Piperaceae) has insecticidal activity against various insects (Ribeiro et al., 2016; Guedes et al., 2020); however, its potential for controlling *B. tabaci* MED is unknown. The effects of the essential oil of *Mansoa alliaceae* Miers (Bignoniaceae) has already been studied for *B. tabaci* MEAM1 (Fanela

et al., 2016) but not for *B. tabaci* MED. In light of this, this study aimed to assess the bioactivity of these essential oils for the control of *B. tabaci* MED.

1.2 Materials and Methods

1.2.1 *Bemisia tabaci* MED rearing

The initial population of *B. tabaci* MED was provided by Dr. André Luiz Lourenção (IAC, Campinas-SP) and its identification was performed periodically (De Barro et al., 2003). The insects were kept in metal cages (3 x 3 x 2.5 m). The lateral and frontal sides of the cage were protected with white anti-aphid screens. Plants of tomato (*Solanum lycopersicum* L.) and green pepper (*Capsicum* annuum L.) were used as hosts.

1.2.2 Plant material essential oil extraction and chemical analysis

Piper marginatum and *M. alliaceae* were collected near Itacoatiara (3°8′S, 58°26′W; 6 m asl), Amazonas state, Brazil, and identified by Dr. Ari de Freitas Hidalgo. Voucher specimens of these species were deposited at the Herbarium of Federal Univerity of Amazonas (HUAM/UFAM) (voucher numbers UFAM 8.651 and 8.266, respectively).

Fresh leaves of each plant studied were subjected to hydrodistillation in a Clevenger-type apparatus for 4 h using 500 g of fresh leaves and 500 mL of distilled water; hydrodistillation was conducted after boiling. Then, the EOs obtained from *P. marginatum* (PM-EO) and *M. alliaceae* (MA-EO) were centrifuged for 10 min at 3500 rpm to separate and remove the water with micropipettes. The EOs were stored in amber bottles in a refrigerator at 4 °C until further analysis.

The composition of EO was analyzed using gas chromatography (GC-FID) and gas chromatography–mass spectrometry (GC–MS). Gas chromatography (GC) was performed by a Shimadzu GC2010 Plus gas chromatograph equipped with an AOC-20s autosampler and fitted with FID and a data-handling processor. An Rtx-5 (Restek Co., Bellefonte, PA, USA) fused silica capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness) was employed. The column temperature was programmed to rise from 60 to 240 °C at 3 °C/min and then to hold at 240 °C for 5 min; carrier gas = He (99.999%), at 1.0 mL/min; injection mode; injection volume, 0.1 µL (split ratio of 1:10);

and injector and detector temperatures were 240 and 280 °C, respectively. Relative concentrations of the components were obtained by peak area normalization (%). Relative areas were the average of triplicate GC-FID analyses.

GC–MS analyses were carried out by a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i autosampler. The column was an RTX-5MS (Restek Co., Bellefonte, PA, USA) fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). Electron ionization mode occurred at 70 eV. Helium (99.999%) was employed as the carrier gas at a constant flow of 1.0 mL/min. The injection volume was 0.1 µL (split ratio of 1:10). Injector and ion-source temperatures were set at 240 and 280 °C, respectively. The oven temperature program was the same as that used for GC. Mass spectra were taken at scan intervals of 0.5 s in the mass range from 40 to 600 Da.

The identification of constituents was based on their retention indices on an Rtx-5MS capillary column under the same operating conditions as those in the case of GC relative to a homologous series of *n*-alkanes (C_8-C_{20}). Structures were computer-matched with the Wiley 7, NIST 08 and FFNSC 1.2 spectra libraries, and their fragmentation patterns were compared with literature data (Adams, 2007). The retention indices of *M. alliaceae* essential oil were also compared to reference literature for sulfur-containing compounds (Iranshahi, 2012; Satyal et al., 2017).

1.2.3 Bioassays

Initially, concentration-mortality bioassays were performed to estimate the lethal concentrations (LCs) of essential oils on *B. tabaci* MED nymphs. The LC_{50}s and LC_{90}s were tested in multi-choice assays (repellency and oviposition deterrence) and no-choice assays (infestation ability and ovicidal effect). In the last phase, assays were carried out in a greenhouse to check the efficiency of essential oils under greenhouse conditions.

In the concentration-mortality assay, only essential oils were tested at varying concentrations. For the other assays, treatments consisted of the LC_{50}s and LC_{90}s of the essential oils. In addition, cyantraniliprole (3.75 ml L^{-1}) (Benevia®, FMC Química of Brasil Ltda.) and water + acetone were added as positive and negative controls, respectively. There are no registered insecticides for the control of *B. tabaci* MED to

date in Brazil. Cyantraniliprole was used as positive control here because it is a registered insecticide for the control of *B. tabaci* MEAM1 in Brazil (AGROFIT, 2021). The essential oils were diluted in water and acetone (1:1; v v^{-1}), while the commercial insecticide was diluted in water.

Cotton plants (Cv. Epamig Liça- susceptible to *B. tabaci* MEAM1) were used in the bioassays. The plants were cultivated in plastic pots (1 L) containing soil, sand, manure and substrate at a proportion of 1:1:1:1 (v:v:v:v). The plants were conditioned in plastic trays and kept inside a greenhouse [T= 26 ± 2 °C, RH= 65 ± 10%, 14:10 h (L:D) photoperiod].

1.2.3.1 Determination of the lethal concentration

To obtain nymphs, cotton plants in the V4 stage (Marur and Ruano 2001) were placed in the *Bemisia tabaci* MED rearing cages for 24 hours. After this period, the adults were removed, and the plants were transported to the laboratory for oviposition assessment under a microscope stereoscope (up to 40x). When the individuals reached the second instar, an area with 20 nymphs per leaf was marked using glitter glue (Baldin et al., 2021; Soares et al., 2021).

Initially, three random concentrations (5, 10 and 20 µL of the essential oils per mL of solution) were applied to cotton leaves containing the nymphs. Based on the obtained mortality, intermediate concentrations were used to plot the concentration-mortality curves. The solutions were sprayed on the abaxial face of the leaves by means of a gravity-type pistol (Model 5A Arprex). These leaves were inserted inside plastic straws and placed in glass vials (9 x 2.5 cm). After that, the vials were filled with distilled water to maintain the turgescence of the leaves. Treated leaves were kept under controlled laboratory conditions (26 ± 3 ºC, 70% ± 10% RH, and 12-h photoperiod). A completely randomized design was adopted with five replications per concentration. Each replicate consisted of a cotton leaf containing 20 nymphs of *B. tabaci* MED (n = 100), and the mortality was assessed after five days of spraying. The individuals were considered dead when they had a change in color and turgidity.

1.2.3.2 Ovicidal effect

In this assay, the infestation was carried out similarly to Item 2.3.1; however, an area containing 20 eggs of *B. tabaci* MED was demarcated. After five days of oviposition, the treatments were applied and the leaves were kept under controlled laboratory conditions in a similar way to Item 2.3.1. The number of emerged nymphs was quantified daily up to 15 days after application.

1.2.3.3 Multi-choice assays

To evaluate the repellency and oviposition deterrence of the treatments to adults of *B. tabaci* MED, insects were given the chance to choose between treated (leaves sprayed with cyantraniliprole, LC_{50}s and LC_{90}s of the essential oils) and untreated leaves (control). Spraying was carried out, and the leaves were placed in glass vials, as described in 2.3.1. Two vials containing treated and untreated leaves were placed 20 cm apart inside cages (30 x 30 x 30 cm). Then, 20 couples of *B. tabaci* MED were released in the center of each cage. After 24 hours, the number of adults on each leaf was quantified, as well as the number of eggs deposited. A completely randomized design with eight replicates was used, and each cage containing the treated and untreated leaves was considered a replicate. The cages were kept in the laboratory under controlled conditions (26 ± 3 ºC, 70% ± 10% RH, and 12-h photoperiod with artificial light).

1.2.3.4 No-choice assay

In this assay, cotton plants in stage V4 were used. Initially, the treatments were applied to the plants similar to the previous assays. Then, the leaves were marked with string. The plants were individually placed in tubular cages made of PVC (11.5 cm in diameter by 43 cm in height) and covered with organdy cloth. Then, 20 whitefly couples were released inside each tubular cage. The cages were kept in the laboratory under controlled conditions (26 ± 3 ºC, 70% ± 10% RH, and 12-h photoperiod with artificial light). After 21 days, the number of nymphs in the marked leaves was quantified. A completely randomized design with five replicates was used, and each cage containing one plant was considered a replicate.

1.2.3.5 Semifield assay

The cotton plants were infested, the arenas were demarcated and the treatments were sprayed on the plants similarly to Item 2.3.1. The plants were kept in a greenhouse (average temperature of 25.6 °C, with a maximum of 35.9 °C and minimum of 15.4 °C; 60 ± 10% RH; and natural light). This bioassay consisted of five replicates, and each plant was considered a replicate. The number of dead nymphs was quantified in the marked area five days after spraying

1.2.4 Statistical analysis

Mortality results from lethal concentration bioassays were corrected based on mortality observed in the control using Abbott's formula (Abbott, 1925). Probit analyses were performed to determine the concentration-mortality curves using Statistical Analysis System (SAS) University Edition, SAS® Studio (SAS Institute, NC, USA). The curves with a probability higher than 0.05 of acceptance of the null hypothesis by a χ^2 test were accepted. The LCs were compared by the criterion of non-overlapping confidence intervals (IC95) with the origin of the interval.

The number of hatched eggs (ovicidal effect) in each treatment were subjected to analysis of variance followed by the Tukey test ($P < 0.05$) using the PROC GLM (SAS University Edition, SAS® Studio).

To verify differences between the number of adults and eggs in the treated and untreated leaves within the treatments in the multi-choice bioassay, the data were subjected to the paired t-test (PROC TTEST, SAS). The same procedure was performed to verify differences between the number of nymphs in the treated and untreated plants within the treatments in the no-choice bioassay. The oviposition deterrence index (ODI) was calculated using the equation $ODI = [(T - C)/(T + C)] \times 100$, where T = number of eggs on the treated leaf and C = number of eggs on the untreated leaf (control). When the ODI has a negative and significant value (based on the chi-square test), it indicates an anti-oviposition effect of the treatment, since the insects prefer to oviposit on the untreated leaf. When the ODI has a positive and significant value, it indicates that the treatment had an oviposition-stimulating effect, since insects prefer to oviposit on the treated leaf (Baldin et al., 2015; Ponnusamy et al., 2017). The

chi-square test was performed using the PROC FREQ procedure with Chisq on SAS. The calculation and analysis for the colonization index (CI) were performed in a similar way, but considering the number of nymphs.

1.3 Results

1.3.1 Chemical characterization of the essential oils

The major compounds identified in PM-EO were (*E*)-methyl eugenol (34.7%) and (*Z*)-methyl eugenol (27.5%) (Table 1). On the other hand, diallyl trisulfide (52.8%) and diallyl disulfide (33.9%) were the major compounds in MA-EO (Table 1).

1.3.2 Lethal concentrations

Based on the confidence interval, it was not possible to observe a significant difference between the LCs of the essential oils. The concentrations of PM-EO required to cause 50 and 90% mortality of *B. tabaci* MED nymphs were 9.39 µl mL^{-1} (CI 95= 8.61 - 10.20) and 20.79 µl mL^{-1} (CI 95= 18.51 - 24.09), respectively (Table 2). For MA-EO, the LC_{50} was 10.99 µl mL^{-1} (CI 95= 10.18 - 11.79), and the LC_{90} was 22.25 µl mL^{-1} (CI 95= 20.06 - 25.46) (Table 2).

1.3.3 Ovicidal effect

There was a significant reduction in the hatching of eggs exposed to the treatments (df= 5,24; F= 8.66; $P < 0.001$). The highest concentrations of essential oils (LCs_{90}) differed from the positive and negative controls and reduced egg hatching by approximately 50% (Fig. 1). The LCs_{50} of the essential oils significantly reduced egg hatching compared to the negative control.

1.3.4 Multi-choice assay

Significant differences were observed between the number of adults of *B. tabaci* MED in the treated and not treated cotton leaves for all treatments ($P < 0.001$). In general,

adults of *B. tabaci* MED preferred untreated leaves regardless of the treatment and concentration of essential oils used (Fig. 2).

The number of eggs in treated and untreated cotton leaves was significantly different for all treatments ($P < 0.001$). Females laid 2.6 to 5.1 times more eggs on untreated leaves. The LC_{90} of PM-EO caused the largest reduction in oviposition (ODI = -74.65) (Fig. 3).

1.3.5 No-choice assay

All treatments significantly affect the colonization by *B. tabaci* MED in cotton plants (df = 5,29; F = 24.28; $P < 0.001$). The LC_{90} of PM-EO reduced the colonization by approximately 75% compared to the negative control, and did not differ statistically from the positive control (Fig. 4). The LC_{90} of MA-EO and the LCs_{50} of both essential oils reduced the colonization compared to the negative control, but did not differ between them. In addition, all treatments showed a negative and significant colonization index ($\chi^2 \neq 0$; $P < 0.001$), indicating colonization inhibition.

1.3.6 Semifield assay

The treatments caused significant mortality in nymphs of *B. tabaci* MED under greenhouse conditions (df = 5,29; F = 166.08; $P < 0.001$) (Table 3). The LCs_{90} of the essential oils showed control efficacy higher than 90% and did not differ significantly from the positive control (Table 3).

1.4 Discussion

The present study demonstrated for the first time the lethal and sublethal effects of PM-EO and MA-EO against *B. tabaci* MED. These compounds showed repellency and oviposition deterrence effects and reduced colonization by *B. tabaci* MED on cotton plants. In addition, the essential oils presented high efficiency under semifield conditions.

EOs are known to have insecticidal activity, present fast degradation times, lower rates of insect resistance, lower risks of environmental contamination and increased safety to applicators (Benelli et al., 2017; Isman, 2017). These

characteristics make EOs a potential tool for the management of agricultural pests. Indeed, studies have shown that EOs can be an alternative to manage insect pests, including *B. tabaci* (Baldin et al., 2015; Fanela et al., 2016). Despite this, studies addressing the use of these compounds to control *B. tabaci* MED, one of the most important cryptic species of *B. tabaci* in the world, are not extensive. Investigations such as these can be very useful, considering that conventional insecticides have failed to control *B. tabaci* since this insect species has a high tendency to develop resistance to insecticides, including the neonicotinoids and insect growth regulators commonly used for its chemical management (Kim et al., 2011).

The insecticidal activity of PM-EO has already been proven for 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, 1931) (Diptera: Drosophilidae) (Souza et al., 2020). On the other hand, despite not having wide documented bioactivity against agricultural pests, MA-EO has already demonstrated insecticidal activities against nymphs and adults of *B. tabaci* MEAM1 (Biotype B) by means of fumigation exposure (Fanela et al., 2016).

The insecticidal activity observed in our study may initially be related to the compounds in the oils. MA-EO is comprised of allyl polysulfides, compounds recognized as having potential as biopesticides (Anwar et al., 2017). These compounds demonstrated insecticidal activity against *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) (Plata-Rueda et al., 2018), *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) (Yang et al., 2012), *Cacopsylla chinensis* (Yang et Li) (Hemiptera: Psyllidae) (Zhao et al., 2013) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Huang et al., 2000). Despite not having a fully elucidated mode of action, many studies indicate that sulfide compounds can act by inhibiting the enzyme acetylcholinesterase (Plata-Rueda et al., 2018), causing effects on the octopamine pathway, disturbing the insect cuticle, and inhibiting molting and respiration (Shahriari and Sahebzadeh, 2017).

(*E*)-methyl eugenol (34.7%) and (*Z*)-methyl eugenol), the major compounds in PM-EO also have potential as insecticides and, therefore, might be responsible for the toxicity of this EO against *B. tabaci* MED. Even with no studied mode of action, the

effects of these compounds have already been verified in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *S. zeamais* (Huang et al., 2002), *Nilarparvata lugens* Stal (Hemiptera: Delphacidae), *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Pyralidae), *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) (Xu et al., 2015) and *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (Wu et al., 2021).

In this work, we verified for the first time an ovicidal effect of MA-EO. This opens up a huge range of possibilities for insect control and studies to determine how EOs harm insect eggs. A similar study with *S. frugiperda* was carried out recently to verify the ovicidal effect of PM-EO (Guedes et al., 2020). According to the authors, PM-EO affected embryonic development, changing the morphology of the eggs and modifying the structure of the chorion and making the eggs of the fall armyworm nonviable. This ovicidal effect is highly desirable for pest management, as it allows for a reduction of the insect population before damage to crops occurs.

In addition to the ovicidal effect, we observed lower rates of oviposition on leaves treated with both EOs in the multi-choice assay. Most likely, this was a consequence of the repellent activity of these EOs against adults of the whitefly, which preferred to oviposit on leaves not treated with the essential oils. These results are highly valuable, as a reduction in oviposition can represent a significant reduction in the whitefly population, and the repellent effects can lead to a substantial reduction in the transmission of viruses to plants (Fanela et al., 2016).

The effects of repellency and oviposition deterrence can also be related to the major constituents of EOs. As an example, the majority of MA-EO compounds (diallyl disulfide and diallyl trisulfide) were found to be highly repellent and cause a reduction in the oviposition rate of adults from *S. cerealella* (Yang et al., 2012). Similarly, the compound diallyl disulfide was also highly repellent against *T. molitor* (Plata-Rueda et al., 2018). In addition, the major compounds in PM-EO ((E-) and (Z-) methyl eugenol) had a substantial oviposition deterrent effect against *P. operculella* (Wu et al., 2021).

The reduction in the number of nymphs observed in cotton plants treated with the LCs of EOs in relation to the control can also be explained by the repellent and/or deterrent actions of the compounds present in the EOs, which may have restricted oviposition in the treated leaves. In addition, mortality of hatched nymphs may have occurred, which suggests residual effects of the tested compounds.

The nymphicidal activity observed in the laboratory assays was also observed under greenhouse conditions. This is important, as the high volatility of EOs can impair their use in field and semifield conditions. In addition, considering that there are no insecticides registered to manage *B. tabaci* MED in Brazil (AGROFIT, 2021), the results found here become even more relevant as they can be explored in research and development of new products for the management of this pest.

The results observed here demonstrate the strong potential of these essential oils for the management of *B. tabaci* MED. Additional studies are necessary to understand how these oils inhibit the colonization of cotton plants by whiteflies, to develop strategies for their use under field conditions and to verify the possibility of using their formulations to control this pest.

1.5 Conclusions

The EOs of *M. alliacea* and *P. marginatum* were toxic against nymphs of *B. tabaci* MED in laboratory and semifield conditions. These EOs had ovicidal and repellent effects in addition to inhibiting oviposition and colonization on treated leaves /plants. This supports the potential of these EOs as alternative strategies for the management of *B. tabaci* MED.

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Table 1. Chemical composition of the essential oils of *Piper marginatum* (PM-EO) and *Mansoa alliacea* (MA-EO).

Compound	RT (min)	RI_{exp}	RI_{lit}	%RA	
				PM-EO	MA-EO
1-Octen-3-ol	12.25	970	979		6.0
diallyl disulphide	18.81	1076	1079		33.9
cis-anethole	30.02	1266	1269	4.8	
trans-anethole	30.63	1280	1283	2.8	
diallyl trisulphide	31.53	1301	1301		52.8
α-cubebene	33.52	1347	1351	0.7	
β-elemene	34.25	1364	1375	1.2	
β-caryophyllene	36.54	1418	1418	4.5	
(*Z*)-methyl isoeugenol	37.66	1447	1451	27.5	
β-selinene	39.06	1483	1485	3.5	
(*E*)-methyl isoeugenol	39.44	1492	1495	34.7	
diallyl tetrasulphide	41.24	1539	1540		7.3
trans-nerolidol	41.31	1541	1565	0.6	
α-elemol	41.45	1545	1549	4.4	
elemicin	41.73	1552	1554	0.8	
germacrene D-4-ol	42.47	1572	1574	0.3	
caryophyllene oxide	42.80	1580	1581	0.6	
β-asarone	43.79	1607	1629	6.2	
β-Eudesmol	45.32	1649	1649	1.3	
Eudesm-7(11)-en-4-ol	47.08	1698	1700	1.3	
Sesquiterpene hydrocarbons				9.9	
Oxygenated sesquiterpene				8.5	
Phenylpropanoids				76.8	
Sulfur compounds					94
Others					6
Not identified				4.8	

RT: Retention time. RI_{exp}: Retention index relative to $_n$-alkanes (C_8–C_{20}) on the Rtx-5MS column. RI_{lit}: Retention Index from the literature. RA%: relative area.

Table 2 Lethal concentrations of the essential oils of *Piper marginatum* and *Mansoa alliacea* against *Bemisia tabaci* MED, after five days of exposure.

Treatment	N	LC_{50} (µL mL^{-1}) (95% CI)	LC_{90} (µL mL^{-1}) (95% CI)	Slope	χ^2	*P*
P. marginatum	600	9.39 (8.61 - 10.20)	20.79 (18.51 - 24.09)	3.71±0.28	5.09	0.16
M. alliacea	600	10.99 (10.18 - 11.79)	22.25 (20.06 - 25.46)	4.18±0.34	3.38	0.33

χ^2=Chi-square; *P*=Probability

Table 3 Mortality of *B. tabaci* MED nymphs exposed to the essential oils of *P. marginatum* and *M. alliacea*, after five days of exposure under semifield conditions.

Treatment	Number of dead nymphs (± SE)	Efficacy (%)[1]
P. marginatum (LC_{90})	18.80 ± 0.26 a	91.55
P. marginatum (LC_{50})	9.40 ± 0.11 b	44.26
M. alliacea (LC_{90})	18.60 ± 0.11 a	90.54
M. alliacea (LC_{50})	9.00 ± 0.32 b	42.25
Benevia ®	19.40 ± 0.18 a	94.56
Control	0 ± 0.00	-
F	166.08	-
df	4,24	-
P-value	<0.001	-

Means followed by the same letter do not differ by Tukey's test (P <0.001);

[1]: The control efficacy was obtained through the Abbott formula.

Fig. 1 Number of hatched *Bemisia tabaci* MED eggs exposed to the essential oils of *Piper marginatum* and *Mansoa alliaceae*. Means followed by the same letter do not differ by Tukey's test (P <0.001).

Fig. 2 Repellency of the essential oils of *Piper marginatum* and *Mansoa alliacea* against adults of *Bemisia tabaci* MED. * represents significant differences between treated and untreated leafs with t-test (P < 0.001).

Fig. 3 Oviposition deterrent index (ODI) and number of *Bemisia* tabaci MED eggs on leaves exposed to essential oils of *Piper marginatum* and *Mansoa alliaceae*. * represents significant differences between treated and untreated leaves in each treatment by the t-test (P <0.001). Index = 0, indicates that ODI does not differ from zero and index ≠ 0 indicates that ODI differ from zero, by the χ^2 test.

Fig. 4 Colonization index (CI) and number of *Bemisia tabaci* MED nymphs on plants exposed to essential oils of *Piper marginatum* and *Mansoa alliaceae*. Means followed by the same letter do not differ by Tukey's test (P <0.001); Index = 0, CI does not differ from zero and index ≠ 0 CI differ from zero, by the χ^2 test.

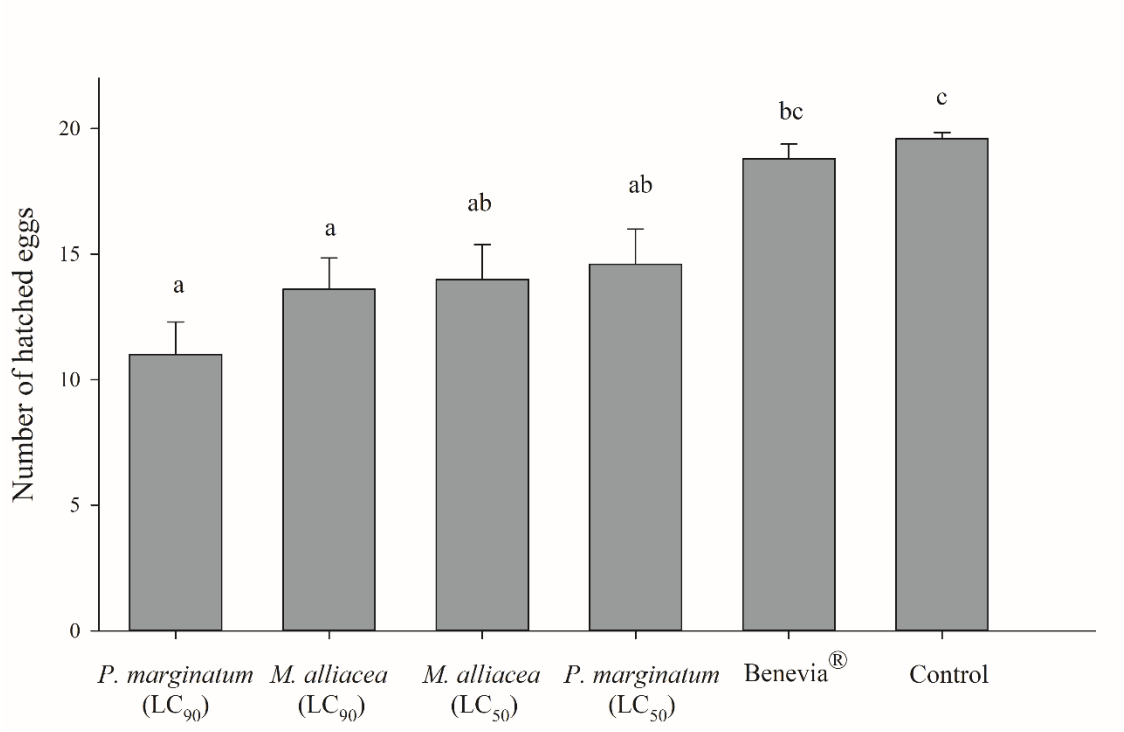
Number of hatched eggs
0
5
10
15
20
a
a
ab
ab
bc
c
P. marginatum (LC90)
M. alliacea (LC90)
M. alliacea (LC50)
P. marginatum (LC50)
Benevia®
Control

Fig. 1

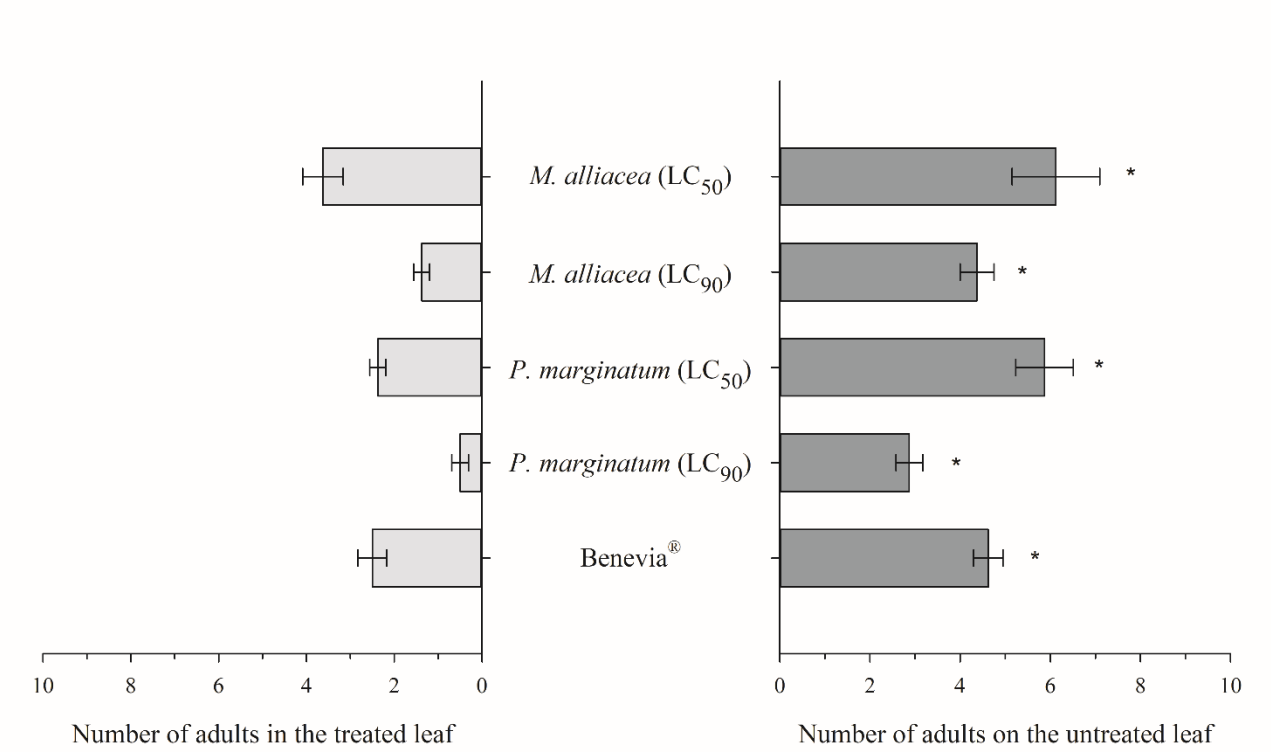
M. alliacea (LC50)
M. alliacea (LC90)
P. marginatum (LC50)
P. marginatum (LC90)
Benevia®
*
10 8 6 4 2 0
0 2 4 6 8 10
Number of adults in the treated leaf
Number of adults on the untreated leaf

Fig. 2

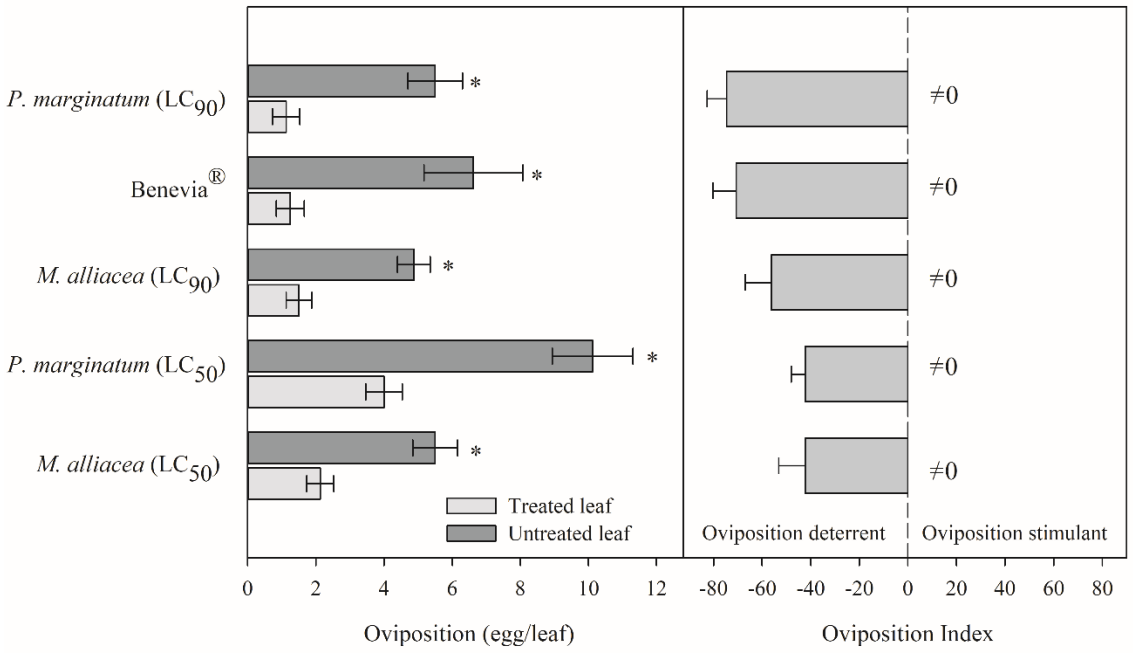
P. marginatum (LC90)
Benevia®
M. alliacea (LC90)
P. marginatum (LC50)
M. alliacea (LC50)
*
≠0
Treated leaf
Untreated leaf
Oviposition deterrent
Oviposition stimulant
0 2 4 6 8 10 12
-80 -60 -40 -20 0 20 40 60 80
Oviposition (egg/leaf)
Oviposition Index

Fig. 3

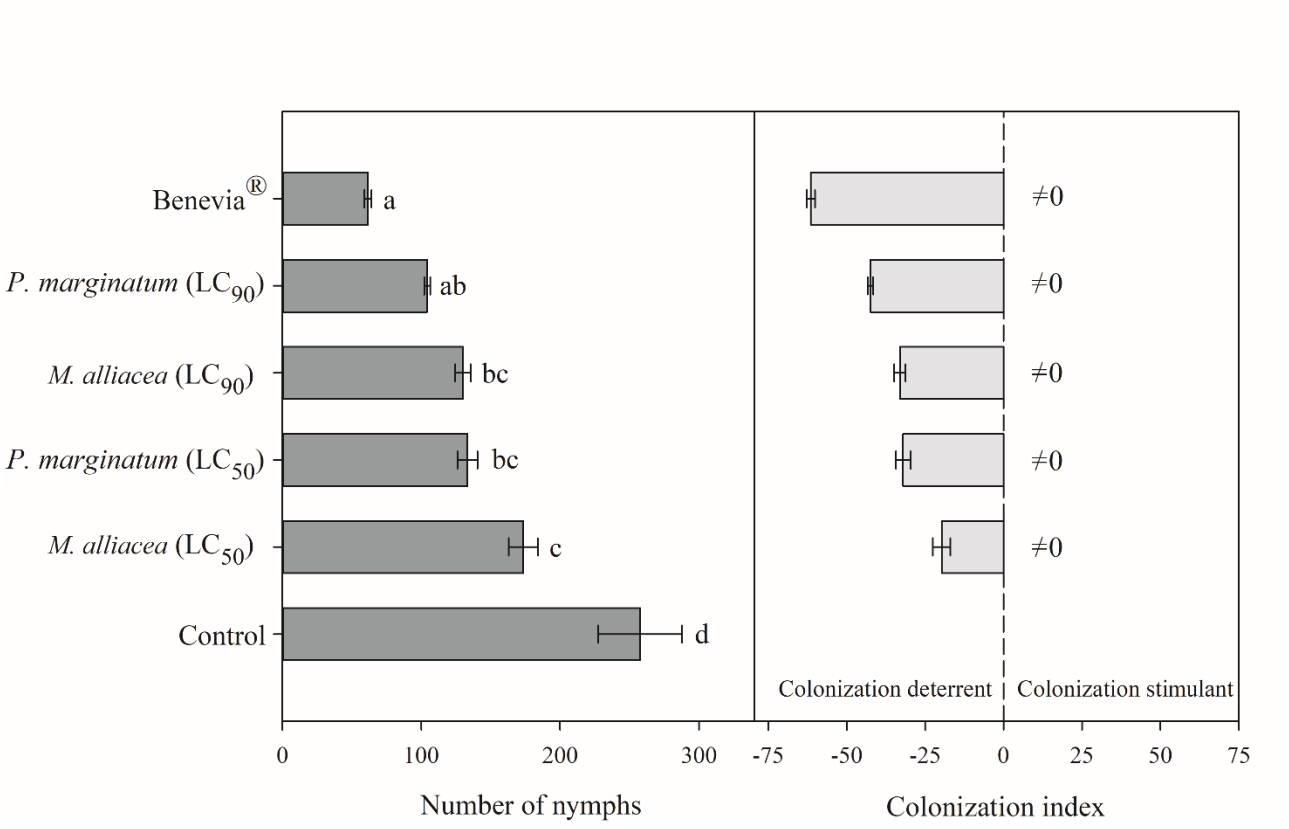
Benevia®
P. marginatum (LC90)
M. alliacea (LC90)
P. marginatum (LC50)
M. alliacea (LC50)
Control
a
ab
bc
bc
c
d
≠0
≠0
≠0
≠0
≠0
Colonization deterrent
Colonization stimulant
0
100
200
300
-75
-50
-25
0
25
50
75
Number of nymphs
Colonization index

Fig. 4

CHAPTER 2

Bringing botanical insecticides to the field: assessment of the efficacy of microemulsions based on essential oils against *Bemisia tabaci* MED

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Abstract

Essential oils are promising alternatives that can be applied on the management of insect pests, however its rapid degradation can reduce the activity of these substances in natural conditions. Microemulsions based on essential oils can be applied to overcome this drawback. Thus, this work aimed to assess for the first time the bioactivity of microemulsions based on the essential oils of *Piper marginatum* Jacq. (Piperaceae) (PM-EO) and *Mansoa alliaceae* (Lam.) A.ww H. Gentry (Bignoniaceae) (MA-EO) against *Bemisia tabaci* Mediterranean (MED) (Gennadius) (Hemiptera: Aleyrodidae). The chemical constitution of the essential oils was assessed. Concentration-mortality bioassays were performed to estimate the lethal concentrations (LCs) on nymphs of *B. tabaci* MED. The lethal concentrations (LC_{50}s and LC_{90}s) were applied in multi-choice assays to verify the repellent and oviposition deterrence effects. In addition, no-choice assays were performed to assess the ovicidal effects and infestation ability. Finally, the microemulsions were diluted in different solvents (water and water + acetone) and applied to verify their efficacy under semifield conditions. The microemulsions were toxic against nymphs under laboratory and greenhouse conditions and showed ovicidal and repellent effects. The microemulsions also reduced oviposition and inhibited the colonization by *B. tabaci* MED. Our results reveal the potential of microemulsions based on essential oils of *P. marginatum* and *M. alliaceae* as a new product to be applied in the management of *B. tabaci* MED.

Keywords: Microemulsion, alternative control, *Mansoa alliaceae, Piper marginatum*, whitefly

*** This chapter was submitted to the journal 'Industrial Crops and Products' and the text formatting follows its rules.**

2.1 Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a complex of cryptic species, among which Mediterranean (MED), also known as biotype Q, has worldwide importance (Kanakala and Ghanim, 2019). This species was introduced into Brazil in 2014 and represents a high risk for major crops in this country, such as cotton and soybeans (Barbosa et al., 2015). *Bemisia tabaci* MED can cause directly damage to plants by feeding on phloem sap and indirectly by excreting honeydew on leaves and fruits, which forms a substrate for the growth of black sooty mold that stains the leaves, impairs photosynthesis and reduces fruit quality (Horowitz et al., 2020). In cotton, the honeydew may cause fiber stickiness that interferes with the spinning process in the textile mills and reduces significantly the product's value (Horowitz et al., 2020; Zhang et al., 2020). This insect is also an important vector diverse plant viruses (Gilbertson et al., 2015).

Chemical control has been widely used for the management of whiteflies, however this practice is becoming less efficient due to the selection of resistant insect populations (Sohrabi et al., 2011). In fact, different levels of resistance to conventional insecticides such as organophosphates, pyrethroids, pyriproxyfen, neonicotinoids and diamides were observed in populations of *B. tabaci* MED (Horowitz et al., 2020). In addition, the excessive use of synthetic insecticides contributes to the reduction of populations non-target organisms, environmental degradation and increase the toxicological risks for farmers and consumers (Abati et al., 2021; Wagner, 2020).

Essential oils of plants (EOs) stand out as an alternative for pest management since they are known for causing mortality, alter behavior and promote physiological changes in insect species (Isman, 2017). EOs have low toxicity to natural enemies and pollinators (Lima et al., 2020; Melo et al., 2018; Santos et al., 2018), reduced impact on the environment and low risk to farmers and consumers (Benelli et al., 2017; Miura et al., 2021). In addition, another interesting characteristic of EOs is the high number of components in their chemical constitution, which can decrease the probability of insect resistance development (Santana et al., 2022).

Despite this high potential, bringing EOs to the field is still a challenge due to their handling and storage issues. EOs are subject to conversion or degradation reactions (e.g.: oxidation, isomerization, polymerization and rearrangement) that are

dependent on temperature, light and atmospheric oxygen (Turek and Stintzing, 2013). These instability issues may result in a reduction or loss of effectiveness. EOs also have physico-chemical properties that make it difficult to handle and use, such as water insolubility, high volatility and quick half-life (Pavoni et al., 2019). The low permanence of insecticidal substances on the fields can cause control failures, reducing the management efficacy and increasing the risk of selecting resistant populations.

Emulsions containing EOs appear as strategies to overcome these drawbacks and can be promising alternatives for EOs vehiculation (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) controlled release of active ingredients on the target; iii) improvement of the physico-chemical properties and stability of EOs, by enabling their water dispersability, reducing their volatility and by protect them from the interaction with the environment and and; iv) increased EO penetration in the insect body (Athanassiou et al. 2018; Pavoni et al. 2020).

In our previous research, we observed that the essential oils of *Piper marginatum* Jacq. (Piperaceae) (PM-EO) and *Mansoa alliaceae* Miers (Bignoniaceae) (MA-EO) have potential to control *B. tabaci* MED. These essential oils showed lethal and sublethal effects against eggs, nymphs and adults of this insect. Based on this, here we aimed to assess the bioactivity of emulsions based on EOs of *P. marginatum* Jacq. and *M. alliaceae* against *B. tabaci* MED.

2.2 Materials and Methods

2.2.1 *Bemisia tabaci* MED rearing

The initial population of *B. tabaci* MED was provided by Dr. André Luiz Lourenção (IAC, Campinas-SP) and its identification was performed periodically (De Barro et al., 2003). The insects were kept in metal cages (3 x 3 x 2.5 m). The lateral and frontal sides of the cage were protected with white anti-aphid screens. Plants of tomato (*Solanum lycopersicum* L.) (cv. Santa Clara) and green peppers (*Capsicum* annuum L.) (cv. Cascadura Ikeda) were used as hosts.

2.2.2 Plant material, essential oil extraction and chemical analysis

The collection of *P. marginatum* and *M. alliaceae* was performed near Itacoatiara (3°8′S, 58°26′W; 6 m asl), Amazonas state, Brazil. The identification was performed by Dr. Ari de Freitas Hidalgo and voucher specimens of these species were deposited at the Herbarium of Federal University of Amazonas (HUAM/UFAM) (voucher numbers UFAM 8.651 and 8.266, respectively). Fresh leaves (500 g) from each plant were subjected to hydrodistillation in a Clevenger-type apparatus for 4 h using 500 mL of distilled water. The essential oils (EOs) obtained from *P. marginatum* (PM-EO) and *M. alliaceae* (MA-EO) were centrifuged for 10 min at 3500 rpm to separate the traces of water; then the water was removed using micropipettes. The EOs were stored in amber bottles in a refrigerator at 4 °C until further analysis.

The composition of EO was analyzed using gas chromatography (GC-FID) and gas chromatography–mass spectrometry (GC–MS). Gas chromatography (GC) was performed by a Shimadzu GC2010 Plus gas chromatograph equipped with an AOC-20s autosampler and fitted with FID and a data-handling processor. An Rtx-5 (Restek Co., Bellefonte, PA, USA) fused silica capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness) was employed. The column temperature was programmed to rise from 60 to 240 °C at 3 °C/min and then to hold at 240 °C for 5 min; carrier gas = He (99.999%), at 1.0 mL/min; injection mode; injection volume, 0.1 µL (split ratio of 1:10); and injector and detector temperatures were 240 and 280 °C, respectively. Relative concentrations of the components were obtained by peak area normalization (%). Relative areas were the average of triplicate GC-FID analyses.

GC–MS analyses were carried out by a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i autosampler. The column was an RTX-5MS (Restek Co., Bellefonte, PA, USA) fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). Electron ionization mode occurred at 70 eV. Helium (99.999%) was employed as the carrier gas at a constant flow of 1.0 mL/min. The injection volume was 0.1 µL (split ratio of 1:10). Injector and ion-source temperatures were set at 240 and 280 °C, respectively. The oven temperature program was the same as that used for GC. Mass spectra were taken at scan intervals of 0.5 s in the mass range from 40 to 600 Da.

The identification of constituents was based on their retention indices on an Rtx-5MS capillary column under the same operating conditions as those in the case of GC

relative to a homologous series of *n*-alkanes (C_8-C_{20}). Structures were computer-matched with the Wiley 7, NIST 08 and FFNSC 1.2 spectra libraries, and their fragmentation patterns were compared with literature data (Adams, 2007). The retention indices of *M. alliaceae* essential oil were also compared to reference literature for sulfur-containing compounds (Iranshahi, 2012; Satyal et al., 2017).

2.2.3 Emulsion preparation and physical properties

The emulsions were composed of 36% Tween 80 (Dinâmica Química Contemporânea Ltda., São Paulo, Brazil; surfactant), 36% ethanol (Neon Comercial Ltda., São Paulo, Brazil, 95% PA; co-surfactant), 18% EO of essential oil (oil phase), and 10% ultra-pure water (aqueous phase). The emulsions were kept at 25 °C in darkness for 24 h for complete homogenization in amber vials.

Physical characterization of the emulsions was performed considering the Zeta potential, microparticle size, pH and polydispersity index. The Zeta potential is related to the repulsive forces existing between the microparticles in an emulsified system, where the increase of these forces gives the emulsions greater stability avoiding the agglomeration. The polydispersity index indicates how much the particle size has deviated from the mean. The microparticle size and polydispersity indexes were determined by photon correlation spectrometry after dilution of the sample with 1: 500 (v: v) ultrapurified water in Zetasizer Nanoseries Nono-Zs (Malvern Instruments, Worcestershire, RU). The electrical conductivity of isotropic systems (IONLAB CON-500) was measured at scale up to 2000 uΩ/cm with standard KCl solution of 1412 uΩ/cm at room temperature. The pH was measured through microprocessed bench pH meter, with Pt100 sensor in stainless steel electrode. All characterizations were performed at 25 °C and in triplicate.

2.2.4 Bioassays

2.2.4.1 Treatments

In the first assay (lethal concentration), emulsions were tested at varying concentrations. For subsequent assays, treatments consisted of the LC_{50}s and LC_{90}s

of the emulsions, cyantraniliprole (3.75 ml L^{-1}) (Benevia®, FMC Química of Brasil Ltda.) as positive control and water + acetone as negative control. The commercial insecticide was diluted in water and the emulsions were diluted in water + acetone (1:1; v v^{-1}), except for the greenhouse assay, where the emulsions were also diluted in water alone.

2.2.4.2 Lethal concentration assay

To obtain nymphs, cotton plants in the V4 stage (Marur and Ruano 2001) were placed in the whitefly rearing cage for 24 h for oviposition by adults of *B. tabaci* MED. After this period, the adults were removed, and the plants were kept in the laboratory and when the individuals reached the second instar, an area with 20 nymphs per leaf was marked using glitter glue (Soares et al., 2021). The plants were cultivated in plastic pots (1 L) containing soil, sand, manure and substrate at a proportion of 1:1:1:1 (v:v:v:v). The plants were conditioned in plastic trays and kept inside a greenhouse [T= 26 ± 2 °C, RH= 65 ± 10%, 14:10 h (L:D) photoperiod].

Initially, three random concentrations (5, 10 and 20 µL of the emulsion per mL of solution- µL mL^{-1}) were applied on cotton leaves containing nymphs. Based on the obtained mortality, intermediate concentrations were applied in order to obtain the concentration-mortality curves. The solutions were sprayed on the abaxial face of the leaves by means of a gravity-type pistol (Model 5A Arprex). These leaves were inserted inside plastic straws and placed in glass vials (9 x 2.5 cm). After that, the vials were filled with distilled water to maintain the turgescence of the leaves. Treated leaves were kept under controlled laboratory conditions (26 ± 3 ºC, 70% ± 10% RH, and 12-h photoperiod). A completely randomized design was adopted with five replications per concentration. Each replicate consisted of a cotton leaf containing 20 nymphs of *B. tabaci* MED (n = 100), and the mortality was assessed after five days of spraying. The individuals were considered dead when they had a change in color and loss of turgidity.

2.2.4.3 Ovicidal activity assay

Initially cotton plants were infested similarly to item 2.2.4.2. After this, an area containing 20 eggs of *B. tabaci* MED was demarcated. After five days, the treatments were applied and the leaves were kept under controlled laboratory conditions in a

similar way to item 2.2.4.2. The number of emerged nymphs was quantified daily up to 15 days after application.

2.2.4.4 Multi-choice assay

In this assay, insects were exposed to treated (leaves sprayed with cyantraniliprole, LC_{50}s and LC_{90}s of the emulsions) and untreated leaves in order to evaluate the repellent effects and oviposition deterrence. After spraying, the leaves were placed in glass vials, as described in 2.2.4.2 and two vials containing treated and untreated leaves were placed 20 cm apart inside cages (30 x 30 x 30 cm). Then, 20 couples of adults of *B. tabaci* MED were released in the center of each cage. After 24 hours, the number of adults and the number of eggs on each leaf were quantified. A completely randomized design with eight replicates was used, and each cage containing the treated and untreated leaves was considered a replicate. The cages were kept in the laboratory under controlled conditions (26 ± 3 ºC, 70% ± 10% RH, and 12-h photoperiod with artificial light).

2.2.4.5 No-choice assay

In this assay, the treatments were applied to the cotton plants in stage V4. The plants were individually placed in tubular cages made of PVC (11.5 cm in diameter by 43 cm in height) and covered with organdy cloth. Then, 20 whitefly couples were released inside each tubular cage. The cages were kept in the laboratory under controlled conditions (26 ± 3 ºC, 70% ± 10% RH, and 12-h photoperiod with artificial light). After 21 days, the number of nymphs was quantified. A completely randomized design with five replicates was used, and each cage containing one plant was considered a replicate.

2.2.4.6 Semifield assay

In this assay, we assessed the efficacy of the emulsions using water and water + acetone solvents. For this, cotton plants were infested and sprayed similarly to 2.3.1. The plants were kept in a greenhouse (average temperature of 25.6 °C, with a maximum of 35.9 °C and minimum of 15.4 °C; 60 ± 10% RH; and natural light). This

bioassay consisted of five replicates, and each plant was considered a replicate. The number of dead nymphs was quantified in the marked area five days after spraying.

2.2.5 Statistical analysis

Mortality results from lethal concentration bioassays were corrected based on mortality observed in the control using Abbott's formula (Abbott, 1925). Probit analyses were performed to determine the concentration-mortality curves using Statistical Analysis System (SAS) University Edition, SAS® Studio (SAS Institute, NC, USA). The curves with a probability higher than 0.05 of acceptance of the null hypothesis by a χ^2 test were accepted. The LCs were compared by the criterion of non-overlapping confidence intervals (IC95) with the origin of the interval.

The number of hatched eggs (ovicidal effect) in each treatment were subjected to analysis of variance followed by the Tukey test ($P < 0.05$) using the PROC GLM (SAS University Edition, SAS® Studio).

To verify differences between the number of adults and eggs in the treated and untreated leaves within the treatments in the multi-choice bioassay, data were subjected to the paired t-test (PROC TTEST, SAS). The same procedure was performed to verify differences between the number of nymphs in the treated and untreated plants within the treatments in the no-choice bioassay. The oviposition deterrence index (ODI) was calculated using the equation $ODI = [(T - C)/(T + C)] \times 100$, where T = number of eggs on the treated leaf and C = number of eggs on the untreated leaf (control). When the ODI has a negative and significant value (based on the chi-square test), it indicates an anti-oviposition effect of the treatment, since the insects prefer to oviposit on the untreated leaf. When the ODI has a positive and significant value, it indicates that the treatment had an oviposition-stimulating effect, since insects prefer to oviposit on the treated leaf (Baldin et al., 2015; Ponnusamy et al., 2017). The chi-square test was performed using the PROC FREQ procedure with Chisq on SAS. The calculation and analysis for the colonization index (CI) were performed in a similar way, but considering the number of nymphs.

2.3 Results

2.3.1 Chemical characterization of the essential oils

The major compounds of PM-EO were (E)-methyl eugenol (34.7%) and (Z)-methyl eugenol (27.5%) (Table 1). Diallyl trisulfide (52.8%) and diallyl disulfide (33.9%) were the major compounds in MAEO (Table 1).

2.3.2 Physical properties of the emulsions

The emulsions of the PM-EO and MA-EO showed particle size of 320.80 and 620 nm, respectively, indicating a microparticle size. The microemulsions (MEs) had suitable stability as indicated by conductivity (0.05 and 0.06 mS cm^{-1}, for PM-EO and MA-EO, respectively) and zeta potencial (-3.29 and -5.60 mV for PM-EO and MA-EO, respectively (Table 2). The microemulsion of *P. marginatum* (PM-ME) showed polydispersion index of 0.25 and pH of 7.66, while the microemulsion of *M. alliacea* (MA-ME) presented polydispersion index of 0.39 and pH of 7.84 (Table 2).

2.3.3 Lethal concentrations

Based on the confidence interval, the LCs of the essential oils (Santana et al., 2022) and MEs were not statistically different. The concentrations of PM-ME required to cause 50 and 90% mortality of *B. tabaci* MED nymphs were 8.17 µl mL^{-1} (CI 95= 7.11 - 9.32) and 18.81 µl mL^{-1} (CI 95= 16.73 - 21.18), respectively (Table 3). For MA-ME, the LC_{50} was 9.65 µl mL^{-1} (CI 95= 8.33 - 11.06), and the LC_{90} was 20.01 µl mL^{-1} (CI 95= 18.33 - 22.63) (Table 2).

2.3.4 Ovicidal effect

There was a significant reduction in the number of nymphs hatched in the eggs exposed to the treatments compared to the untreated control, except for Benevia ($df= 5,24$; $F= 8.66$; $P < 0.001$). The highest concentrations of MEs (LC_{90}s) reduced egg hatching by more than 50% and differed from the positive and negative controls (Fig. 1). The LC_{50}s of MEs significantly reduced egg hatching compared to the negative control.

2.3.5 Multi-choice assay

Significant differences were observed between the number of adults of *B. tabaci* MED in the treated and not treated cotton leaves for all treatments ($P < 0.001$). Adults of *B. tabaci* MED preferred untreated leaves regardless of the treatment and concentration of MEs used (Fig. 2). In addition, there are significant differences in the number of eggs on treated and untreated leaves for all treatments ($P < 0.001$) and *B. tabaci* MED adults laid more eggs on the untreated leaves regardless of the treatment and concentration (Fig. 3).

2.3.6 No-choice assay

The LC_{90} of PM-ME did not differ statistically from the positive control and reduced the colonization by approximately 80% compared to the negative control (Fig. 4). All MEs reduced the colonization compared to the negative control, but the colonization did not differ among them. In addition, all treatments showed a negative and significant colonization index ($\chi^2 \neq 0$; $P < 0.001$), indicating colonization inhibition.

2.3.7 Greenhouse assay

The treatments caused significant mortality in nymphs of *B. tabaci* MED under greenhouse conditions (Table 4). The LC_{90}s of the MEs showed control efficacy higher than 90% regardless of solvent and did not differ significantly from the positive control (Table 3).

2.4 Discussion

Essential oils can be a potential tool for the management of agricultural pests in the future as they are known for having insecticidal activity, lower risks for applicators and increased safety to the environment compared to synthetic insecticides (Benelli et al., 2017; Isman, 2017). Despite of this potential, some characteristics of EOs (e.g. water insolubility, chemical instability, high volatility, short residual activity due to

degradation by temperature and light) may be obstacles to their efficacy under field situations. EO-based emulsions have been developed to overcome EO's rapid degradation and particle size, resulting in similar or higher insecticidal activity than its pure form (Santos et al., 2022).

In our previous study, we assessed the bioactivity of essential oils of *P. marginatum* and *M. alliaceae* against *B. tabaci* MED (Santana et al., 2022). The essential oils caused lethal and sublethal effects against eggs, nymphs and adults of *B. tabaci* MED. In light of this potential, the present study aimed to assess the particle size of emulsions based on these essential oils and verify their toxicity against *B. tabaci* MED. The emulsions were classified as microemulsions, and had toxic effects, showed repellency, oviposition deterrence effects and reduced colonization by *B. tabaci* MED on cotton plants. Notably, the MEs diluted in water presented high efficiency under greenhouse conditions. This is the first time that the lethal and sublethal effects of MEs of essential oils are assessed against *B. tabaci* MED.

Despite the lower EO content (18%) in the MEs, the estimated lethal concentrations obtained here are equivalent between the essential oils and their MEs (Santana et al., 2022), indicating the potential of the MEs for the control of *B. tabaci* MED. Probably the main reasons for such success include the reduction in the EO particle size associated with suitable conductivity, pH, and zeta potential (i.e., greater particle repulsion), which can increase the efficiency and uniformity of a substance deposition on a surface; the slower release of active ingredient resulting in prolonged insecticidal activity, which has been found for other EOs (González et al., 2015; Oliveira et al. 2017; Rocha et al. 2018); and the proprieties of (co) surfactants that can increase the penetration of EOs in the insect cuticle (Kogan and Garti 2006; Hashem et al. 2018).

Previous studies have shown the potential of essential oils emulsions as insecticides. Oliveira et al. (2017) showed that emulsions based on the EO of *L. sidoides* and its major compound (thymol) are promising for the management of *S. zeamais* populations. Santos et al., (2022) verified that the emulsion of the essential oil of *Pogostemon cablin* (Lamiaceae) was toxic, reduced reproduction and feeding, increased walking activity, and caused histopathological changes in the midgut of the coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae). Also, the emulsion of the *P. cablin* essential oil demonstrated efficient

insecticidal activity and irritability to *Atta opaciceps* (Borgmeier, 1939), *Atta sexdens* (Linnaeus, 1758), and *Atta sexdens rubropilosa* Forel, 1908 (Formicidae: Myrmicinae) (Rocha et al., 2018).

MEs can be defined as the nanometric dispersion of one liquid phase into another one in which it is not soluble (Pavoni et al., 2020). They are generally composed of a water and an oil phase. The dispersion of one in the other is allowed by the presence of amphiphilic molecules able to decrease the interfacial tension between the two immiscible phases, called surfactants (Yamashita et al., 2016). In our study, we used the polysorbate Tween 80 as a surfactant, which provide suitable oil particle size stability (Liu et al. 2011; Rocha et al. 2018). Other surfactants such as Procetyl® AWS (Oliveira et al., 2017) and polyethylene glycol (González et al., 2015) or also encapsulating agents such as β-cyclodextrin (Galvão et al., 2015), poly-β-hydroxybutyrate and poly-ε-caprolactone (Carvalho et al., 2015) are also used in studies involving essential oil formulations to control insect pests.

The use of co-surfactants is common in ME formulation. A co-surfactant is generally a surface active amphiphilic molecule that supports synergistically the surfactant action (Pavoni et al., 2020). A co-surfactant is able to reduce the interfacial tension, increasing the fluidity and entropy of the system, in addition to allow a higher penetration of the oil between the surfactant tails (Flanagan and Singh, 2007). Here, we used ethanol as a co-surfactant. This is the most used co-surfactant in the formulation of EOs-based MEs because it induces the formation of systems able to be diluted in the aqueous phase (Spernath and Aserin, 2006).

MEs can facilitate the vehiculation of essential oils, but the insecticidal activity of these substances is mostly related to the compounds present in their chemical constitutions. MA-EO is composed of allyl polysulfides, and these compounds had insecticidal activity against *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) (Plata-Rueda et al., 2018), *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) (Yang et al., 2012), *Cacopsylla chinensis* (Yang et Li) (Hemiptera: Psyllidae) (Zhao et al., 2013) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) (Huang et al., 2000). We also observed that (*E*)-methyl eugenol (34.7%) and (*Z*)-methyl eugenol), the major compounds in PM-EO, were toxic against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *S. zeamais* (Huang et al., 2002), *Nilarparvata lugens* Stal (Hemiptera: Delphacidae), *Cnaphalocrocis medinalis* Guenee (Lepidoptera:

Pyralidae), *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) (Xu et al., 2015) and *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) (Wu et al., 2021).

The major constituents of EOs can also be responsible for the repellency and oviposition deterrence effects. In fact, diallyl disulfide and diallyl trisulfide were highly repellent and caused significant reduction in the oviposition rate of adults of *S. cerealella* (Yang et al., 2012). Similarly, the compound diallyl disulfide was also highly repellent against *T. molitor* (Plata-Rueda et al., 2018). In addition, the major compounds in PM-EO ((E-) and (Z-) methyl eugenol) had a substantial oviposition deterrent effect against *P. operculella* (Wu et al., 2021).

In the multi-choice assay it was possible to observe lower rates of oviposition on leaves treated with both MEs. In addition, the physical characteristics of the MEs can increase the penetration of the EOs components in the egg. This seems to be true, as a strong ovicidal effect was observed here for both MEs. This antioviposition and ovicidal effects are highly desirable for pest management, as it allows for a reduction of the insect population before damage to crops occurs. In addition, mortality of hatched nymphs may have occurred, which suggests residual effects of the tested compounds.

The nymphicidal activity observed in the laboratory assays was also observed under greenhouse conditions using water as a solvent. This is important, as the high volatility and water insolubility of EOs can impair their use in field and semifield conditions. In addition, considering that there are no insecticides registered to manage *B. tabaci* MED in Brazil (AGROFIT, 2021), the results found here become even more relevant as they can be explored in research and development of new products for the management of this pest.

2.5 Conclusions

The microemulsions based on EOs of *M. alliacea* and *P. marginatum* were toxic against nymphs of *B. tabaci* MED in laboratory and greenhouse conditions. These MEs demonstrated ovicidal and repellent effects in addition to inhibiting oviposition and colonization on treated leaves/plants. Notably, the efficacy of the emulsions was maintained when diluted in water. This supports the potential of these emulsions as alternative strategies for the management of *B. tabaci* MED.

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Table 1. Chemical composition of the essential oils of *Piper marginatum* (PM-EO) and *Mansoa alliacea* (MA-EO).

Compound	RT (min)	RI_{exp}	RI_{lit}	%RA	
				PM-EO	MA-EO
1-Octen-3-ol	12.25	970	979		6.0
diallyl disulphide	18.81	1076	1079		33.9
cis-anethole	30.02	1266	1269	4.8	
trans-anethole	30.63	1280	1283	2.8	
diallyl trisulphide	31.53	1301	1301		52.8
α-cubebene	33.52	1347	1351	0.7	
β-elemene	34.25	1364	1375	1.2	
β-caryophyllene	36.54	1418	1418	4.5	
(*Z*)-methyl isoeugenol	37.66	1447	1451	27.5	
β-selinene	39.06	1483	1485	3.5	
(*E*)-methyl isoeugenol	39.44	1492	1495	34.7	
diallyl tetrasulphide	41.24	1539	1540		7.3
trans-nerolidol	41.31	1541	1565	0.6	
α-elemol	41.45	1545	1549	4.4	
elemicin	41.73	1552	1554	0.8	
germacrene D-4-ol	42.47	1572	1574	0.3	
caryophyllene oxide	42.80	1580	1581	0.6	
β-asarone	43.79	1607	1629	6.2	
β-Eudesmol	45.32	1649	1649	1.3	
Eudesm-7(11)-en-4-ol	47.08	1698	1700	1.3	
Sesquiterpene hydrocarbons				9.9	
Oxygenated sesquiterpene				8.5	
Phenylpropanoids				76.8	
Sulfur compounds					94
Others					6
Not identified				4.8	

RT: Retention time. RI_{exp}: Retention index relative to $_n$-alkanes (C_8–C_{20}) on the Rtx-5MS column. RI_{lit}: Retention Index from the literature. RA%: relative area.

Table 2 Physical properties of emulsions containing essential oils of *Piper marginatum* (PM-EO) and *Mansoa alliaceae* (MA-EO).

Emulsion	Particle size (nm)	Conductivity (mS cm^{-1})	Zeta potential (mV)	Polydispersity	pH
PM-EO	320.80 ± 41.26	0.05	-3.29 ± 1.23	0.25 ± 0.04	7.66 ± 0.02
MA-EO	620.00 ± 59.68	0.06	-5.60 ± 0.22	0.39 ± 0.07	7.84 ± 0.03

Table 3 Lethal concentrations of the essential oils of *Piper marginatum* (PM-EO) and *Mansoa alliacea* (MA-EO) and their microemulsions against *Bemisia tabaci* MED, after five days of exposure.

Treatment	N	LC_{50} (µL mL^{-1}) (95% CI)	LC_{90} (µL mL^{-1}) (95% CI)	Slope	χ^2	*P*
PM-EO*	600	9.39 (8.61 - 10.20)	20.79 (18.51 - 24.09)	3.71 ± 0.28	5.09	0.16
MA-EO*	600	10.99 (10.18 - 11.79)	22.25 (20.06 - 25.46)	4.18 ± 0.34	3.38	0.33
PM-EO emulsion	600	8.17 (7.11 - 9.32)	18.81 (16.73 - 21.18)	3.12 ± 0.28	5.09	0.16
MA-EO emulsion	600	9.65 (8.33 - 11.06)	20.01 (18.33 - 22.63)	4.18 ± 0.34	3.38	0.33

* Data from previous study (Santana et al., 2022a); χ^2=Chi-square; *P*=Probability

Table 4 Mortality of *B. tabaci* MED nymphs exposed to the microemulsions based on essential oil of *Piper marginatum* and *Mansoa alliacea*, after five days of exposure under semifield conditions.

Solvent	Treatment	Number of dead nymphs (± SE)
Water + Acetone	PM-EO emulsion (LC_{90})	19.20 ± 0.26 a
	PM-EO emulsion (LC_{50})	11.31 ± 0.11 b
	MA-EO emulsion (LC_{90})	18.60 ± 0.15 a
	MA-EO emulsion (LC_{50})	10.32 ± 0.41 b
Water	PM-EO emulsion (LC_{90})	19.41 ± 0.26 a
	PM-EO emulsion (LC_{50})	12.40 ± 0.24 b
	MA-EO emulsion (LC_{90})	18.98 ± 0.19 a
	MA-EO emulsion (LC_{50})	11.41 ± 0.32 b
-	Benevia ®	19.40 ± 0.18 a
-	Control	0 ± 0.00
F	-	69.34
df	-	8,44
P-value	-	<0.001

Means followed by the same letter do not differ by Tukey's test (P <0.001);

[1]: The control efficacy was obtained through the Abbott formula.

Fig. 1 Number of hatched *Bemisia tabaci* MED eggs exposed to the microemulsions based on essential oils of *Piper marginatum* (PM-ME) and *Mansoa alliaceae* (MA-ME) Means followed by the same letter do not differ by Tukey's test (*P* <0.001).

Fig. 2 Repellency of the micremulsions based on essential oils *Piper marginatum* (PM-ME) and *Mansoa alliaceae* (MA-ME) against adults of *Bemisia tabaci* MED. * represents significant differences between treated and untreated leafs with t-test (P < 0.001).

Fig. 3 Oviposition deterrent index (ODI) and number of *Bemisia* tabaci MED eggs on leaves exposed to microemulsions based on essential oils of *Piper marginatum* (PM-ME) and *Mansoa alliaceae* (MA-ME). * represents significant differences between treated and untreated leaves in each treatment by the t-test (*P* <0.001). Index = 0, indicates that ODI does not differ from zero and index ≠ 0 indicates that ODI differ from zero, by the χ^2 test.

Fig. 4 Colonization index (CI) and number of *Bemisia tabaci* MED nymphs on plants exposed to microemulsions based on essential oils of *Piper marginatum* (PM-ME) and *Mansoa alliaceae* (MA-ME). Means followed by the same letter do not differ by Tukey's test (*P* <0.001); Index = 0, CI does not differ from zero and index ≠ 0 CI differ from zero, by the χ^2 test.

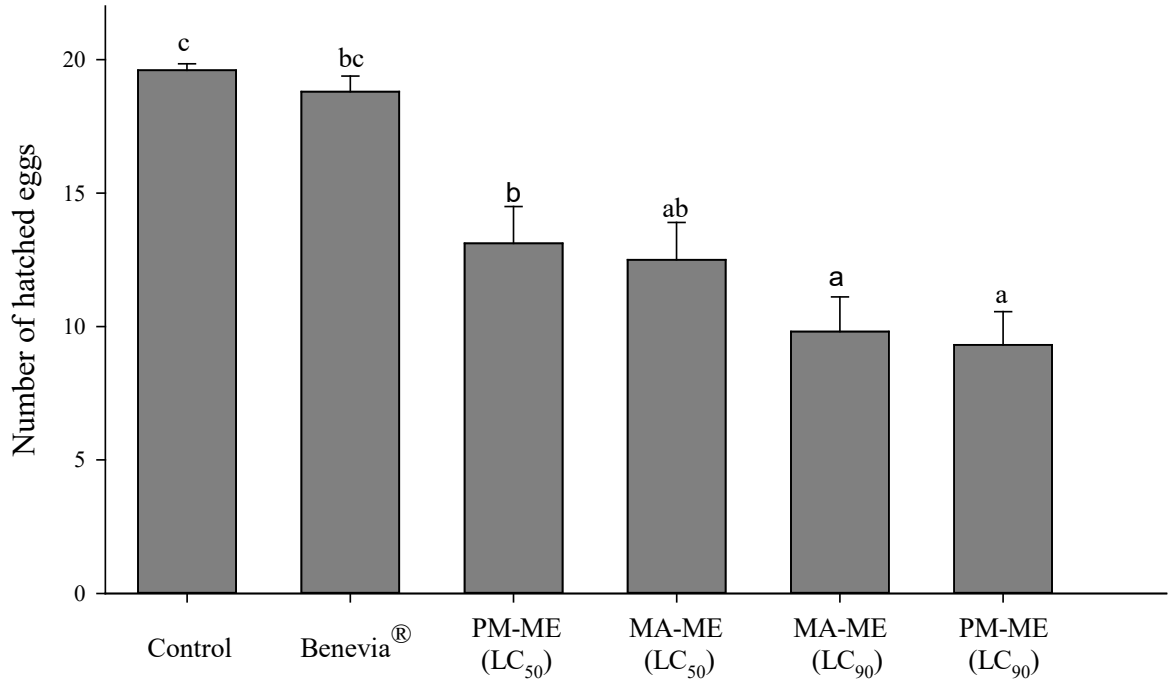
Number of hatched eggs
0
5
10
15
20
c
bc
b
ab
a
a
Control
Benevia®
PM-ME (LC50)
MA-ME (LC50)
MA-ME (LC90)
PM-ME (LC90)

Fig 1

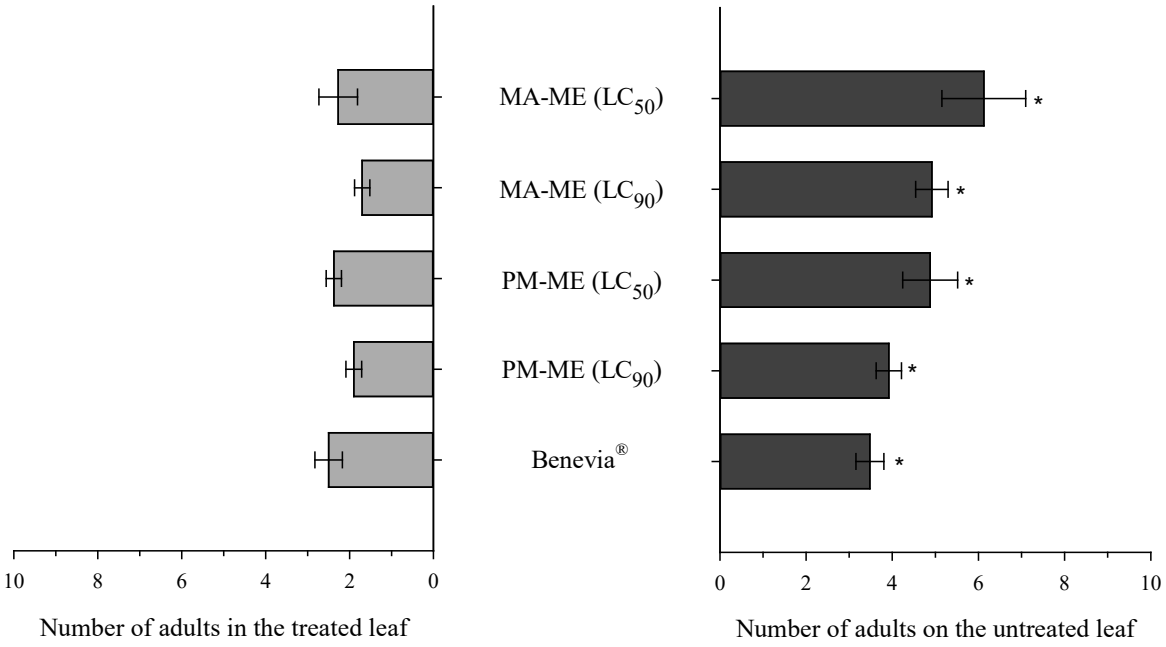
MA-ME (LC50)
MA-ME (LC90)
PM-ME (LC50)
PM-ME (LC90)
Benevia®
10
8
6
4
2
0
0
2
4
6
8
10
*
Number of adults in the treated leaf
Number of adults on the untreated leaf

Fig 2

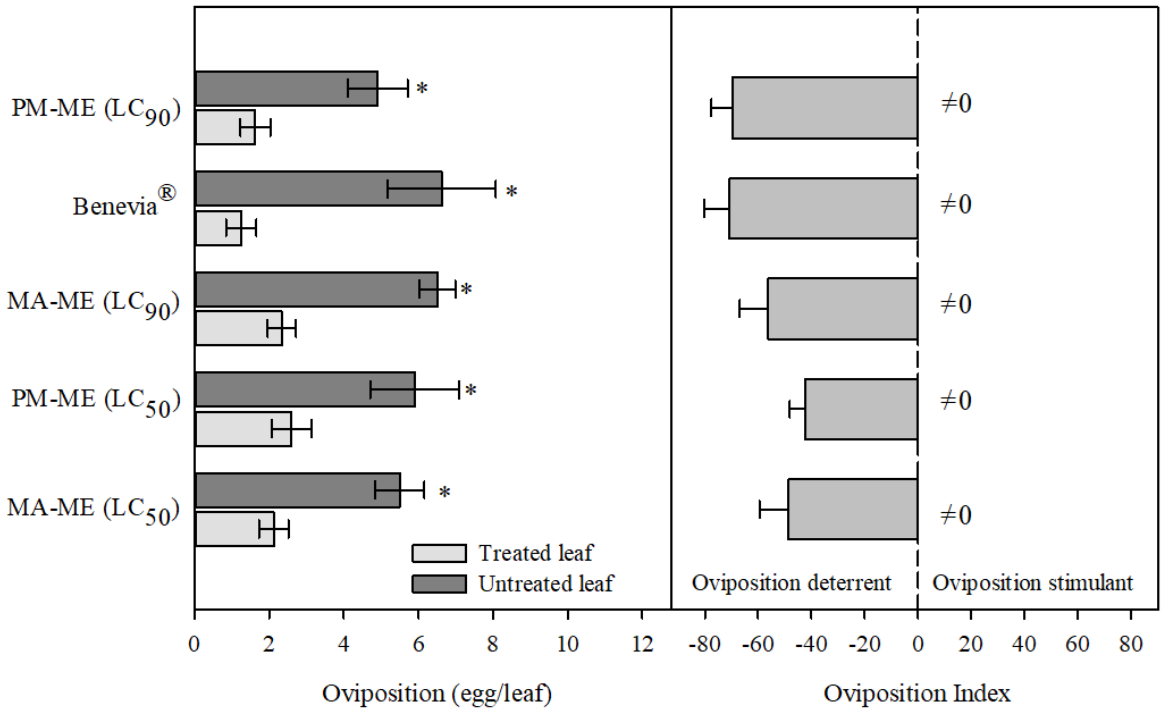
PM-ME (LC90)
Benevia®
MA-ME (LC90)
PM-ME (LC50)
MA-ME (LC50)
*
Treated leaf
Untreated leaf
0
2
4
6
8
10
12
Oviposition (egg/leaf)
≠0
Oviposition deterrent
Oviposition stimulant
-80
-60
-40
-20
0
20
40
60
80
Oviposition Index

Fig 3

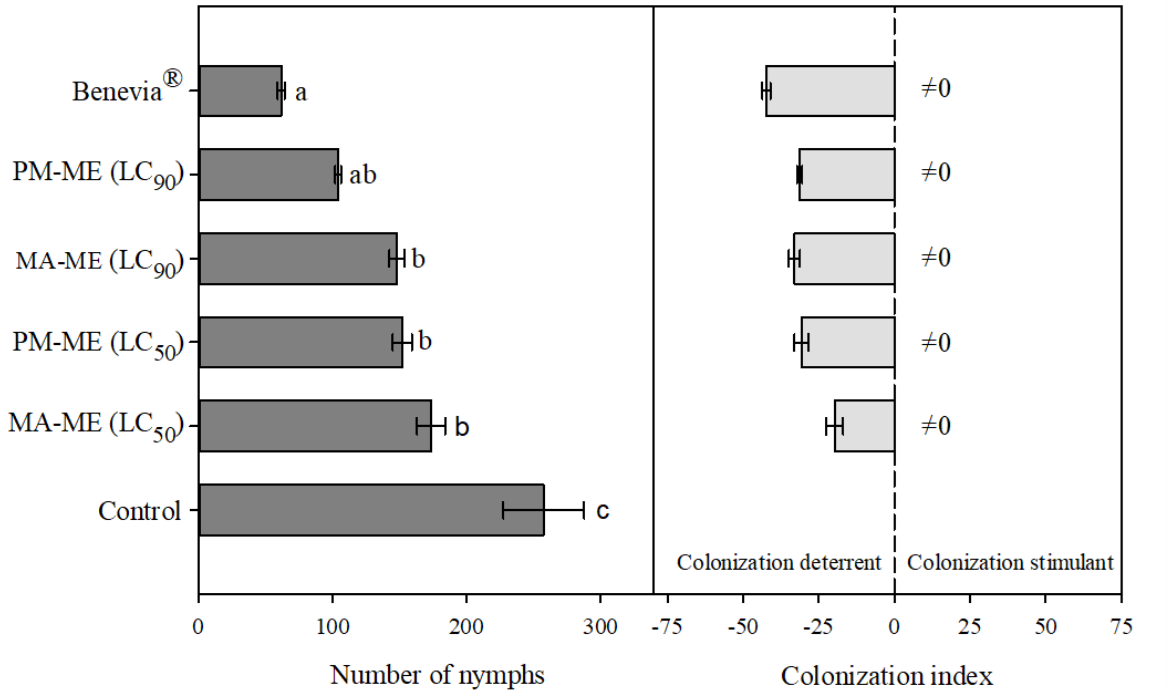
Benevia®
PM-ME (LC90)
MA-ME (LC90)
PM-ME (LC50)
MA-ME (LC50)
Control
a
ab
b
b
b
c
≠0
≠0
≠0
≠0
≠0
Colonization deterrent
Colonization stimulant
0
100
200
300
-75
-50
-25
0
25
50
75
Number of nymphs
Colonization index

Fig 4

CHAPTER 3

Looking for sources of resistance to *Bemisia tabaci* MED in different cotton genotypes.

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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) (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 resistant can be a valuable tool for integrated pest management (IPM). However, to date, in Brazil, there are no studies assessing the resistance of cotton genotypes to *B. tabaci* MED. In this scenario, this study aimed to identify sources of resistance in cotton genotypes to *B. tabaci* MED. For this, a preliminary assay was carried out with 78 genotypes, 28 of which were selected for multiple-choice and biological performance assays. The genotypes 101-102 B, 3 -E313 and IAC 13-1-76/5366 differed from most genotypes and were less infested by adults of *B. tabaci* MED while Aubum 56-7, DP 4049, Express 257, 3 -E313 and CNPA 92-1121 differed from most genotypes and showed lower number of eggs and IAC 97-2939 “macaco”, 101-102 B, and Algodão Indiano presented less than one nymph, indicating the occurrence of antixenosis and/or antibiosis. 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 gossypol gland density can be associated to those effects, since, in general, genotypes with a high number of glands caused higher mortality and prolonged the nymphal period of *B. tabaci*. These results are unprecedented and may help in breeding programmes to develop cotton lines with resistance to *B. tabaci* MED.

Key words: host plant resistance, whitefly, antixenosis, antibiosis

*** This chapter was submitted to the journal ‘Journal of Pest Science' and the text formatting follows its rules.**

3.1 Introduction

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most important, invasive and destructive pests worldwide (Easson et al., 2021; Rehman et al., 2021; Zhou et al., 2022). This insect is described as a complex of morphologically indistinguishable cryptic species that differ in biological, physiological, and genetic structure (Rehman et al., 2021). These cryptic species are polyphagous phloem-feeding insects capable of feeding on over 600 species of various agricultural and horticultural plant families (Zhou et al., 2022). They suck sap from leaves and tender plant parts in addition to secrete honeydew that favors the growth of sooty mold fungus. Infested plants develop chlorotic spots, wither, or drop leaves. In addition, the cryptic species of *B. tabaci* are also an important worldwide crop pest due to their ability to vector over 300 plant viruses belonging to the genera *Begomovirus*, *Crinivirus*, *Carlavirus*, *Closterovirus* and *Ipomovirus* (Easson et al., 2021; Perez-Fons et al., 2019).

The species *B. tabaci* Middle East-Asia Minor 1 (MEAM1) (biótipo B) is widely distributed in Brazil, causing damage to several crops of high agricultural importance, such as soybean, cotton and tomato. These damages can be increased by the introduction of *B. tabaci* Mediterranean (MED), also known as Q biotype, which was recorded for the first time in 2014 (Barbosa et al., 2015). The species *B. tabaci* MED has low susceptibility to some insecticides classes, high competitive ability and is considered an important pest of cotton in countries where it was introduced (Horowitz and Ishaaya, 2014; Tocko-Marabena et al., 2017; Zhang et al., 2014) and, therefore, represents a high risk for this crop in Brazil. This is very important, since the attack of pests on cotton already causes losses amount to about R$ 2.26 billion per year in Brazil (Oliveira et al., 2014).

Chemical control has been widely used for whiteflies management, 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 reduction of populations of non-target organisms, environmental degradation, increased production costs and increased toxicological risks for farmers and consumers (Goulson et al., 2015; Oliveira et al., 2018; Wagner, 2020). Some strategies have been studied as alternatives to chemical control and have potential for use in integrated management programs for *B. tabaci* MED. Between them, resistant genotypes can be an interesting tool for the

management of whiteflies (Baldin et al., 2017, 2021; Domingos et al., 2018) and other pest managemant programs (Baldin et al., 2019).

Plant resistance can be classified into three categories: antibiosis, antixenosis, and tolerance (Painter, 1951). A plant resistant by antixenosis has chemical, physical or morphological factors that make it less used by the insect for food, oviposition or shelter. Antibiosis is related to plant properties that adversely affect the physiology of an herbivore. Tolerance, on the other hand, is characterized by the ability of the plant to resist or recover from an injury caused by the insect, with no effects on its biology and behavior (Baldin et al., 2019; Smith, 2005; Painter, 1951).

The resistance of a large number of cotton genotypes has already been studied for *B. tabaci* MEAM1 in Brazil (Prado et al., 2016). However, it is known that a genotype can be resistant to one insect species and susceptible to another (Lara, 1991). In addition, different biotypes/species of the *B. tabaci* complex exhibit differences in preference and colonization over host plants (Hadjistylli et al., 2016; Yates and Michel, 2018).

Thus, there is a hypothesis that there may be differences in the levels of resistance of cotton genotypes between the species *B. tabaci* MEAM1 and *B. tabaci* MED. However, the absence of studies addressing the resistance of cotton genotypes to *B. tabaci* MED makes it impossible to reject or accept this hypothesis and, consequently, limits the use of this tool for the management of this pest. In this context, here we aimed to characterize the possible resistance categories of 78 cotton genotypes to *B. tabaci* MED.

3.2 Materials and methods

Bemisia tabaci MED rearing

The initial population of *B. tabaci* MED was provided by Dr. André Luiz Lourenção (IAC, Campinas, SP, Brazil) and and its identification was performed periodically (De Barro et al., 2011). The insects were kept in metal cages (3 x 3 x 2.5 m). The lateral and frontal sides of the cage were protected with white anti-aphid screens. Plants of tomato (*Solanum lycopersicum* L.) (cv. Santa Clara) and green peppers (*Capsicum annuum* L.) (cv. Cascadura Ikeda) were used as hosts.

Germplasm

78 cotton genotypes were obtained from the Germplasm Active Bank of the Agronomic Institute of Campinas (IAC) Campinas, Brazil (Table 1). These 78 genotypes belong to the Program of Cotton Genetic Breeding at the IAC and were used to represent a wide genetic variation, including sources of resistance against fungi, bacteria and nematodes.

Obtaining the plants

In a greenhouse, cotton genotypes were sown in 2.5 L plastic pots containing soil, sand, manure and, substrate at a proportion of 1:1:1:1 (v:v:v:v). The pots received the fertilization commonly recommended for this crop.

Preliminary assay

Initially, a no-choice assay was performed with 78 cotton genotypes (Table 1). Each plant (V2 stage) was infested with 100 adults of *B. tabaci* MED. The genotypes were individualized in metallic cages (35 cm in diameter × 55 cm high) and covered with voile fabric and placed in a greenhouse (average temperature of 24.6 °C, with a maximum of 36.4 °C and minimum of 16.8 °C; 60 ± 10% RH; and natural light). Eighteen days after infestation (DAI), the plants were evaluated by counting the total number of eggs and nymphs on each plant. A completely randomized design was established, with 78 treatments (genotypes) and four replicates (pots isolated by metallic cages). Based on the preliminary bioassay, we selected 28 genotypes for subsequent assays. For the selection, the materials with low oviposition (less than 200 eggs) and low colonization (less than 100 nymphs) or high oviposition (more than 1000 eggs) and high colonization (with more than 400 nymphs) were chosen.

Multiple-Choice bioassay

To evaluate the setling preference of the whitefly, pots containing plants of the tested genotypes were randomly arranged in a circle inside metal cages (2.5 × 3 × 2.5 m), with a coated ceiling of plastic and monofilament fabric (mesh with 30% shading (Sombrite®, Campo Belo, SP, Brazil)), and the sides were coated with anti-aphid

mesh. The plants were spaced 20 cm apart, avoiding contact between their leaves. Whitefly adults were released from the ground and center of each cage in a proportion of 50 couples per genotype, which were collected from the stock colony with the aid of an insect aspirator. The experimental design was completely randomized, with four replications. Each cage was considered a replication (total of four), with one plant of each genotype per cage. The number of adults on the abaxial leaf surface of three leaves of the plant was assessed 24, 48 and 72 h after infestation with the aid of a mirror. The oviposition was evaluated in the same leaves, which were detached from the respective genotypes, and the number of eggs counted with the aid of a stereoscopic microscope (× 40 magnification). Eighteen days after infestation, two leaves of each genotype were detached of the middle third, and the number of eggs and nymphs was determined. In order to determine the number of eggs and nymphs.cm^{-2} the leaf area was determined with the aid of a LI 3000A leaf area meter (L-Cor Inc., Lincoln, NE, USA).

Biological performance assay

Leaves (middle third) of the genotypes were individualized with cages made of “fabric voile” tissue (15 × 15 cm), which were fixed to the petioles of the leaves with a satin ribbon. With the aid of a buccal aspirator, 100 pairs of whiteflies were collected from the rearing plants and released inside the cages, where they remained for 24 h, in order to obtain the eggs on the previously selected leaves. After this period, the cages were removed as well as the insects from the plants. Under a stereoscopic microscope (40× magnification), the abaxial face of the leaves was examined and an area containing 30 viable eggs per leaf was delimited with Glitter (Acrilex, São Bernardo do Campo, SP, Brazil). Six pots per genotype were used and each leaf (1 per plant) represented one replicate, totaling six replicates per genotype (n = 180), arranged under a completely randomized design. The insects were daily observed to evaluate the following biological parameters: duration of instars, total nymphal period, mortality of nymphal instars, and nymphal viability.

Density of gossypol glands and trichomes

To quantify the trichome and gossypol glands densities, the 28 genotypes were cultivated following the same method described to obtain the genotypes. The assessment was performed in plants at the same developmental stages used in the bioassays. The densities were obtained by counting the number of trichomes and gossypol glands in four distinct areas of 1 cm^2 on the abaxial leaf surface from four leaves of four different plants per genotype using a stereoscopic microscope. A completely randomized design was used, with four repetitions per genotype.

Leaf hardness analysis

Hardness analysis was performed using a CT3 Texture Analyzer (Brookfield, Middleboro, MA, USA), calibrated for a penetration depth of 3 mm at a speed of 2.0 mm s-1 with a TA 9/1000 point. The measurement was performed in four distinct points on the abaxial leaf surface from four leaves of four different plants per genotype. The results are expressed in gram-force per centimeter (gf cm^{-1}) and represent the maximum force required for the point to enter cotton leaves, simulating the process by which insects insert their mouthparts into a leaf. A completely randomized design was used, with four repetitions per genotype.

Leaf colorimetric analysis

Leaves of the 28 selected genotypes were evaluated using colorimetry. The colour evaluation of the genotypes was performed in four distinct regions surface from four leaves of four different plants per genotype through reflectance in the color space CIELab (CR300, Minolta Colorimeter, Osaka, Japan), which determines the parameters L * (luminosity), a * (green intensity), and b * (yellow intensity). A completely randomized design was used, with four repetitions per genotype. The value of L * can range from 0 to 100, where 0 is black and 100 is white. The value of a * is represented by positive numbers when the object is red and by negative numbers when the object is green. The value of b * is positive when the object is yellow and negative when it is blue (Takatsui 2012).

Statistical analysis

For all variables assessed, we first verified the normality of residues with the Shapiro-Wilk test (Shapiro & Wilk 1965), as well as the homogeneity of variances with the Bartlett test (Bartlett 1937). The screening data were submitted to analysis of variance, and the means were compared by Scott-Knott test ($p < 0.05$). Tukey–Kramer test was used to adjust the data from the multiple choice assay, biological performance assay, density of gossypol and trichomes, leaf hardness and colorimetric analysis, for multiple comparisons. The density of thricomes, gossypol glands, the colour parameters and leaf hardness were submitted to Pearson correlation analysis between the corresponding variables. Analyses were performed using the statistical software SAS Studio and Sigmaplot.

2.3 Results

Preliminary bioassay

There were significant differences between the treatments regarding to the total number of eggs and total number of nymphs after 18 days of infestation (Table 1). The genotype RR-0489 presented low number of eggs (16.5), differing from the most susceptible genotypes CNPA 92-1121 (1395.5), BRS Acácia Acala (1266) and Deltapine Smooth Leaf (1258). Regarding the number of nymphs, the genotypes Algodão Indiano (31.25), Nu-16 – 78/664 (31.75), Aubum 56-7 (32.75), IAC 13-1-76-5366 (34.25), IAC 18 (36.50), CNPA 92-1121 (37.50), 3 -E313 (42.25), IAC 19-158 (50.50), Fundação MT 95-743 (54.25), RR-0489 (58.25), SIOKRA L-22 (60.75), MOCÓ (68.75), IAC PV 010-163 (71.50), Deltapine 4049 (72.00) and Reba B-50 PR (73.75) differed from the genotype FM975WS (524.75).

Based on the total number of eggs, the genotypes RR-0489 (16.50), Paymaster 53-523 (63.75), Rowden-40 (68.75), IAC 18 (104.75), Express 257 (167.50), IAC 97-2939 “macaco” (168), IACRR-97/86 (IAC 23) (179.50), IAC PV 010-175 (184.25), IAC 13-1-76-5366 (1003), Empire B-4 (1027.50), Hi-Bred (1063.25), FMT 707 (1185), Deltapine Smooth Leaf (1258), BRS Acácia Acala (1266) and CNPA 92-1121 (1395.50) were selected for having or less than 200 or more than 1000 eggs. Considering the number of nymphs, Algodão Indiano (31.25), Nu-16 – 78/664 (31.75), Aubum 56-7 (32.75), IAC 13-1-76-5366 (34.25), IAC 18 (36.50), CNPA 92-1121 (37.50), 3 -E313 (42.25), IAC 19-158 (50.50), Fundação MT 95-743 (54.25), RR-0489

(58.25), SIOKRA L-22 (60.75), Mocó (68.75), IAC PV 010-163 (71.50), Deltapine 4049 (72), Reba B-50 PR (73.75), Oc-96-252 (87.19), 101-102 B (95), Nu-16 (B2 B3 B6) – 78/658 (402.25), IAC 25 (425.25), IAC FC 01 (496), FM975WS (524.75) presented or less than 100 or more than 400 nymphs and were then selected.

Multiple-Choice assay

Overall, *B. tabaci* MED adults were found in lower number in 101-102 B (2.92), 3 -E313 (6.67), IAC 13-1-76/5366 (7.58) and IAC PV 010-175 (7.75) compared to most of genotypes (Table 2). The genotypes Aubum 56-7 (50.95), DP 4049 (57.42), Express 257 (57.81), 3 -E313 (59.34) and CNPA 92-1121 (60.40) showed low mean of eggs.cm^{-2}, differing from most of the genotypes. Considering the number of nymphs.cm^{-2}, the genotypes IAC 97-2939 “macaco” (0.43), 101-102 B (0.47) and Algodão Indiano (0.75) differed from the most susceptible genotypes IAC PV 010-163 (10.83), Siokra L-22 (10.20), Oc-96-252 (5.04), FMT 707 (4.36), Mocó (3.74), Empire B-4 (3.59) and IAC 18 (3.42).

Biological performance assay

Regarding the 1st instar duration, the genotypes Hi-Bred (2.31 days), IAC 13-1-76-5366 (2.26) and CNPA 92-1121 (2.17) differed from the most of genotypes (1.26-1.84) (Table 3). For the second instar, the longest duration was observed in nymphs confined in 3-E313 (3.63), 101-102 B (3.48), CNPA 92-1121 (3.42) and IAC RR – 97/86 (IAC 23) (3.33), differing from the most of genotypes. The highest durations for the third instar were verified in genotypes IAC 13-1-76-5366 (6.39) and FMT 707 (6.12). The nymphs confined to the genotypes Hi-Bred (10.10) and IAC 97-2939 “macaco” (9.24) presented a significant prolongation of the duration of the fourth instar. The nymphal period varied from 15.27 to 22.71 days. The longest duration was observed in nymphs confined in genotypes Hi-Bred (22.71), IAC 13-1-76-5366 (20.12), Aubum 56-7 (19.28) and CNPA 92-1121 (19.14). The shortest duration was observed for FM975WS (15.27) and Oc-96-252 (15.42).

Regarding to the nymphal mortality, only the genotypes FMT 707, Siokra L-22, Nu-16 – 78/664, IAC PV 010-163, Mocó, Fundação MT 95-743, Paymaster 53-523 and IAC-13-1-5366 caused mortality during the 1st instar (Fig. 1). The highest mortality rates for

the 2nd instar were observed for IAC-13-1-5366 (24.19%), Hi-Bred (23.26%) and Siokra L-22 (21.12%). The same pattern was observed for the 3rd instar (Fig. 1). Regarding the 4th instar, the highest mortality rates were observed with CNPA 92-1121 (21.42%), Hi-Bred (19.31%), Aubum 56-7 (18.46%) and Paymaster 53-523 (17.11%) (Fig. 1).

Density of trichomes and gossypol glands

High number of trichomes was found on the genotype Algodão Indiano (26.2 trichomes.cm^{-2}) which differed from all genotypes, except 101-102B (21.4 trichomes.cm^{-2}) (Fig.2). The genotype Hi-Bred showed the highest number of gossypol glands (121.5 glands.cm^{-2}), differing for all treatments except Aubum 56-7 (104.6 glands.cm^{-2}) (Fig.2).

Leaf hardness

There was no difference between the genotypes regarding the leaf hardness.

Leaf colorimetric analysis

The genotypes IAC PV 010-175 and IAC PV 010-163 differed from most of the genotypes and showed the lowest values for luminosity (L *) (32.79 and 34.07, respectively), intensity of green (a *) (-1.79 and -1.83, respectively) and yellow intensity (b *) (12.57 and 14.03, respectively) (Fig.3).

Correlation analysis

Based on the calculated coefficients (r), the correlations were significant among three studied interactions (Table 4). There was a negative correlation between the number of trichomes and the number of whitefly nymphs ($r = -0.21$; $P = 0.0212$) (Table 4). There were also significant positive correlations between the density of gossypol glands and nymphal period ($r = 0.41$; $P < 0.0001$) and nymphal mortality ($r = 0.39$; $P < 0.0001$) (Table 4) (Fig. 4).

3.4 Discussion

Plant resistance is an important tool for pest management. The selection of resistant genotypes from a wide germplasm increases the viability in the use of this tool. Here, we identified resistant plant materials to *B. tabaci* MED in a germplasm with 78 cotton genotypes. In the preliminary assay, 28 genotypes were selected for further studies (multiple-choice and biology assays) based on the total number of eggs and nymphs per pant.

Based on the multiple-choice test, we observed that the genotypes 101-102 B, 3 -E313, IAC 13-1-76/5366 and IAC PV 010-175 showed the lowest number of adults (less than 7.8 adults per plant); the genotypes Aubum 56-7, DP 4049, Express 257, 3 -E313 and CNPA 92-1121 were less preferred for oviposition (less than 61 eggs per cm^2) and; the genotypes IAC 97-2939 “macaco”, 101-102 B and Algodão Indiano were less preferred regarding to establishment of whitefly nymphs. These results indicate the expression of antixenosis and/or antibiosis resistance.

Interestingly, our results differ from those found by Prado et al. (2015) regarding the most preferred genotypes in a similar germplasm of cotton. Those authors observed that only the genotypes Aubum 56-7 and Paymaster 53-816 were also less preferred by adults of *B. tabaci* MEAM1. Those results reinforce the hypothesis that there are differences in host preference between *B. tabaci* MED and *B. tabaci* MEAM1. This is important, since the development of arthropod pest biotypes poses a continuous threat to the stability of resistant varieties and the sustainability of breeding programs focused on insect resistance. In fact, the utilization resistant varieties may be seriously limited in time and space by the occurrence of new biotypes of the target pest (Sharma 2014). Thus, continuous and systematic evaluation of new germplasm must be explored to identify new genes for resistance.

Insect pests that feed on plants that express high levels of resistance generally have reduced colonization (Smith and Clement, 2012). This can be related to the morphological and physical characteristics of the host (Bernays and Chapman, 1994). As an example, a negative correlation between the density of trichomes and the number of adults and eggs of *B. tabaci* MEAM1 was observed on common beans genotypes (Santos et al., 2021). On the other hand, studies with genotypes of tomato melon and eggplant, showed that genotypes with high density of trichomes are more attractive and oviposited by *B. tabaci* MEAM1 (Coelho et al., 2009; Oriani & Vendramim, 2010; Hasanuzzaman et al., 2016). Regarding to cotton, it was observed a positive relation between the density of trichomes and infestation by *B. tabaci*

MEAM1 (Prado et al., 2015). Differently, here we noticed that there is no correlation between the trichome density and number of adults and eggs of *B. tabaci* MED.

Physical characteristics (e.g. leaf color) can also affect host-plant preference by insect pests. It seems like yellow-reflecting surfaces stimulate the establishment behavior of whiteflies on the host plant and the green intensity influences the landing of *B. tabaci* and its orientation (Moreau & Isman 2011). As an example, *B. tabaci* MEAM1 was significantly more attracted to cotton genotypes with higher intensity of green (Prado et al., 2015). Also, Santos et al. (2021) observed that the genotypes of common beans that presented the largest number of *B. tabaci* MEAM1 adults also showed a high intensity of luminosity (L *), green (a *), and yellow (b *). However, in the present study, it was not possible to observe correlations between the cotton leaf color parameters and infestation by *B. tabaci* MED. These results suggest that other factors may be associated to the colonization by *B. tabaci* MED on cotton genotypes, such as chemical volatiles.

Insects that attempt to colonize plants with the capacity to affect their biology (antibiosis resistance) present reduction in size and weight, diverse deformities, and prolongations in the lifecycle phases and, consequently, high mortality rates (Painter 1951). Here, we noticed that the genotypes CNPA 92-1121, IAC 13-1-76/5366, Hi-Bred and Aubum 56-7 significantly prolonged the nymphal period of *B. tabaci* MED. In addition, the genotypes IAC 13-1-76/5366 and Hi-Bred caused high mortality of nymphs of 2nd and 3rd instars. The genotypes Hi-Bred and CNPA 92-1121 also caused significant mortality on 4th instar nymphs.

In general, the negative effects of different plant genotypes on the insect biology may be associated to the presence of morphological factors (e.g. tissue hardness) or by chemical factors (e.g. gossypol) associated with resistance (Bernays and Chapman 1994). In fact, in a previous study it was observed a negative and signifcant correlation between nymphal viability of *Dalbulus maidis* (Hemiptera: Cicadellidae) and rib hardness of corn genotypes (Faria et al., 2021). However, we noticed that the leaf hardness does not influence on the biology of *B. tabaci* MED. Similarly, the leaf hardness of cotton genotypes had no influence on the damage caused by *Thrips tabaci* (Lindeman), *Frankliniella occidentalis* (Pergande) and *Frankliniella schultzei* (Trybom) (Thysanoptera: Thripidae) (Miyazaki et al., 2017).

The mortality and prolongation of the nymphal phase was significant negatively correlated with the density of gossypol glands, which suggests that this factor is evolved with the resistance of cotton genotypes against *B. tabaci* MED (Fig. 4) Here, the genotypes IAC 13-1-76/5366 and Hi-Bred caused high mortality on nymphs of *B. tabaci* MED and showed high number of gossypol glands. Similarly, whitefly adult population exhibited negative response to gossypol glands (Kong et al., 2010).

Gossypol glands are specialized cavity structures of *Gossypium* spp. These cavity structures appear as small dark dots on different tissues of cotton plants and store high concentrations of a wide variety of secondary metabolites (Kong et al., 2010). The large amount of secondary metabolites stored in the gossypol glands protect plants against pathogens, insects and herbivores (Gao et al., 2019). Gossypol is one of the most important secondary metabolites found on these structures (Khalil et al., 2017). This compound is mainly synthesized in cotton roots before its transport and storage in mature gossypol glands (Gao et al., 2019). Gossypol is a polyphenolic binaphthyl dialdehyde known for present toxic, anti-nutritive, and feeding deterrent effects on various species of insects (Kong et al., 2010). The toxicity of gossypol has already been studied for several species of insects, including *Helicoverpa armigera* (Hübner, 1809) (Lepidoptera: Noctuidae), *Helicoverpa zea* (Boddie, 1850) (Lepidoptera, Noctuidae), *Heliothis virescens* (Fabricius, 1781) (Lepidoptera: Noctuidae), *A. gossypii* and *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidade) (Stipanovic et al.,2006; Kong et al., 2010; Krempl et al., 2016; Peng et al., 2016).

Conclusion

Based on all the results obtained here, we observed that there are differences on the preference and biology of different biotypes of *B. tabaci* on different cotton genotypes. The genotypes 101-102 B, 3-E313, IAC 13-1-76-5366, Algodão Indiano, Deltapine 4049, Hi-Bred, IAC 97-2939 “macaco”, Paymaster 53-523, CNPA 92-1121, and Aubum 56-7 stand out for presenting resistance by antixenosis and/or antibiosis against *B. tabaci* MED. We also observed that the gossypol glands can be an important factor of resistance of cotton genotypes against *B. tabaci* MED. Thus, these genotypes may constitute important sources of resistance to *B. tabaci* MED for breeding programs to obtain resistant cultivars.

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Table 1 Total number (mean ±SE) of eggs and nymphs of *Bemisia tabaci* MED on 78 cotton genotypes after 18 days of infestation, in preliminary assay.

Genotype	Species/subspecies	Seed origin	Number of eggs	Number of nymphs
RR-0489	*G. barbadense*	EMBRAPA/CNPA-BR	16.5 ± 2.60 a	58.25 ± 14.20 ab
Paymaster 53-523	*G. hirsutum*	USA	63.75 ± 7.30 ab	330.25 ± 17.37 abc
Rowden-40	*G. hirsutum*	USA	68.75 ± 21.70 ab	163.75 ± 69.12 abc
IAC 18	*G. hirsutum*	IAC, Brazil	104.75 ± 22.55 ab	36.5 ± 8.82 a
Express 257	*G. hirsutum*	USA	167.5 ± 85.25 abc	215.5 ± 79.40 abc
IAC 97-2939 “macaco”	*G. hirsutum*	IAC, Brazil	168 ± 12.47 abc	133 ± 50.66 abc
IAC RR – 97/86 (IAC 23)	*G. hirsutum*	IAC, Brazil	179.5 ± 12.74 abc	272.75 ± 66.42 abc
IAC PV 010-175	*G. hirsutum*	IAC, Brazil	184.25 ± 18.19 abc	189.75 ± 71.85 abc
Var. REBA = BTK-12	*G. hirsutum*	IRCT – France	226.5 ± 147.74 abc	227.75 ± 48.29 abc
101-102 B	*G. hirsutum*	USA	291.25 ± 120.50 abc	95 ± 27.77 abc
Acala 44	*G. hirsutum*	USA	317.25 ± 189.23 abc	181.25 ± 66.41 abc
Sicala V-1	*G. hirsutum*	USA	324 ± 224.49 abc	120 ± 67.78 abc
Nu-16 – 78/664	*G. hirsutum*	USA	326.25 ± 97.47 abc	31.75 ± 1.49 a
Algodão Indiano	*G. hirsutum*	India	328.25 ± 33.04 abc	31.25 ± 13.49 a
3 -E313	*G. hirsutum punctatum*	USA	353 ± 114.77 abc	42.25 ± 15.44 a
IAC 96/280	*G. hirsutum*	IAC, Brazil	368 ± 180.07 abc	141.25 ± 68.68 abc
Deltapine 4049	*G. hirsutum*	USA	374.75 ± 57.53 abc	72 ± 6.86 ab
Mocó	*G. hirsutum* var. *maria galante*	Northeast, Brazil	377.25 ± 23.10 abc	68.75 ± 23.37 ab
Arkansas green	*G. hirsutum*	USA	377.75 ± 93.21 abc	154 ± 97.93 abc
Empire WR	*G. hirsutum*	USA	399 ± 70.54 abc	156 ± 54.93 abc

Siokra L-22	*G. hirsutum*	USA	407.25 ± 391.96 abc	60.75 ± 27.82 ab
Hancok	*G. hirsutum*	USA	416.25 ± 178.05 abc	112.25 ± 42.15 abc
Hybee 100 A	*G. hirsutum*	USA	417.25 ± 60.19 abc	288 ± 165.57 abc
IAC FC 01	*G. hirsutum*	IAC, Brazil	421.25 ± 48.66 abc	396 ± 121.69 bc
MA-0430	*G. hirsutum*	Northeast, Brazil	430.25 ± 301.77 abc	250.75 ± 81.63 abc
IAC PV 010-163	*G. hirsutum*	IAC, Brazil	435.5 ± 184.28 abc	71.5 ± 19.45 ab
P.R. 701/77	*G. hirsutum*	IAPAR, Brazil	452.75 ± 138.00 abc	272.25 ± 12.50 abc
IB 1 (IAC 12 – RM1)	*G. hirsutum*	IB, Brazil	474 ± 30.99 abc	164.75 ± 66.39 abc
M 57-205	*G. hirsutum*	USA	476.75 ± 179.49 abc	117 ± 49.95 abc
Lockett 140	*G. hirsutum*	USA	488.25 ± 176.68 abc	229 ± 84.89 abc
IAC PV010-1664	*G. hirsutum*	IAC, Brazil	535.75 ± 201.27 abc	147 ± 53.44 abc
CNPA 87/33	*G. hirsutum*	EMBRAPA/CNPA, Brazil	537.75 ± 271.88 abc	211.25 ± 84.19 abc
Aubum 56-7	*G. hirsutum*	USA	548.25 ± 122.03 abc	32.75 ± 12.48 a
Nuopal RR deltapine	*G. hirsutum*	Monsanto, Brazil	548.75 ± 217.74 abc	128.75 ± 17.73 abc
AMZ-1435	*G. barbadense*	EMBRAPA/CNPA, Brazil	554 ± 298.00 abc	162.75 ± 73.58 abc
Reba-B-50-78/668	*G. hirsutum*	IRCT, France	558.5 ± 143.44 abc	188 ± 46.80 abc
FM 966 LL	*G. hirsutum*	Bayer, Brazil	580.25 ± 234.50 abc	227.75 ± 51.49 abc
Texas – str 1514	*G. hirsutum*	USA	601.75 ± 145.07 abc	166 ± 88.74 abc
Fundação MT 95-743	*G. hirsutum*	FMT, Brazil	603.25 ± 63.19 abc	54.25 ± 15.34 ab
BRS Aroeira	*G. hirsutum*	EMBRAPA ALGODÃO, Brazil	623 ± 39.44 abc	134.75 ± 51.76 abc
Nu-15-79/117	*G. hirsutum*	USA	639.75 ± 218.67 abc	156.75 ± 50.39 abc
FM975WS	*G. hirsutum*	Bayer, Brazil	674.75 ± 270.54 abc	524.75 ± 298.98 c
95 – 96a, Bu 61 (B2B3B6)	*G. hirsutum*	USA	682.25 ± 207.20 abc	290.5 ± 165.42 abc
Paymaster 53-816	*G. hirsutum*	USA	682.5 ± 141.30 abc	197.5 ± 74.86 abc
Acala 4-42	*G. hirsutum*	USA	695.5 ± 130.97 abc	305 ± 140.43 abc

046-981-00	*G. hirsutum*		706 ± 112.31 abc	189 ± 17.30 abc
Tamcot SP-21 S	*G. hirsutum*	USA	706.25 ± 249.70 abc	326 ± 63.32 abc
FM 910	*G. hirsutum*	Bayer, Brazil	719.25 ± 72.50 abc	265 ± 20.00 abc
AMZ-1442	*G. hirsutum*	EMBRAPA/CNPA, Brazil	731 ± 37.98 abc	211 ± 103.74 abc
BJA	*G. hirsutum*	IRCT, France	737.5 ± 188.31 abc	116 ± 47.02 abc
IAC 19-158	*G. hirsutum*	IAC, Brazil	738.75 ± 123.85 abc	50.5 ± 20.83 a
Inta La Banda 56	*G. hirsutum*	Argentina	745.38 ± 69.70 abc	113.5 ± 8.74 abc
Deltapine acala 90	*G. hirsutum*	USA	748.75 ± 339.17 abc	194.75 ± 31.71 abc
Oc-96-252	*G. hirsutum*	OCEPAR, Brazil	755 ± 180.08 abc	87.19 ± 33.58 abc
Oro Blanco	*G. hirsutum*	Argentina	773.75 ± 200.28 abc	391.75 ± 97.23 abc
Reba B-50 PR	*G. hirsutum*	IRCT, France	774.25 ± 159.28 abc	73.75 ± 5.50 ab
IAC 12-2	*G. hirsutum*	IAC, Brazil	786.5 ± 219.18 abc	131.25 ± 35.90 abc
IAC FC 02	*G. hirsutum*	IAC, Brazil	793 ± 115.34 abc	163.75 ± 11.82 abc
IAC-12 – 58/348	*G. hirsutum*	IAC, Brazil	794 ± 280.93 abc	131.5 ± 60.44 abc
Deltapine	*G. hirsutum*	USA	828 ± 94.84 abc	104.5 ± 7.77 abc
IAC 25	*G. hirsutum*	IAC, Brazil	849.25 ± 246.95 abc	325.25 ± 48.00 abc
Inta Saenz Pena Guaicuru	*G. hirsutum*	Argentina	849.5 ± 211.18 abc	105.75 ± 73.46 abc
FMT 709	*G. hirsutum*	TMG	857.25 ± 374.19 abc	160 ± 55.67 abc
Big-Boll	*G. hirsutum*	USA	863.25 ± 79.74 abc	145.5 ± 47.46 abc
RedTexas	*G. hirsutum*	USA	900.75 ± 65.88 abc	199.5 ± 145.79 abc
Ipeaco SL 26-64142	*G. hirsutum*	IPEACO, Brazil	911.75 ± 177.84 abc	330 ± 100.27 abc
Deltapine DP555 BGRR	*G. hirsutum*	Bayer, Brazil	921.25 ± 341.78 abc	358.5 ± 41.99 abc
AMZ-1412	*G. hirsutum*	EMBRAPA/CNPA, Brazil	927.75 ± 40.44 abc	130.25 ± 22.76 abc
Gregg	*G. hirsutum*	USA	961.75 ± 413.84 abc	163.5 ± 70.40 abc
IMA-031318	*G. hirsutum*	IMAmt	971.5 ± 87.69 abc	290.5 ± 23.06 abc

Ferguson's	*G. hirsutum*	USA	993.5 ± 380.75 abc	262.5 ± 103.94 abc
IAC 13-1-76/5366	*G. hirsutum*	IAC, Brazil	1003 ± 219.35 abc	34.25 ± 11.21 a
Empire B-4 (diferencial bact.)	*G. hirsutum*	USA	1027.5 ± 337.01 abc	330.5 ± 54.27 abc
Hi-Bred	*G. hirsutum*	USA	1063.25 ± 143.00 abc	103.5 ± 37.39 abc
FMT 707	*G. hirsutum*	TMG	1185 ± 572.26 abc	147.75 ± 76.20 abc
Deltapine Smooth Leaf	*G. hirsutum*	USA	1258 ± 318.82 bc	181.5 ± 30.88 abc
BRS Acácia Acala	*G. hirsutum*	EMBRAPA/CNPA, Brazil	1266 ± 261.80 bc	367.5 ± 28.51 abc
CNPA 92-1121	*G. hirsutum*	EMBRAPA/CNPA, Brazil	1395.5 ± 351.57 c	37.5 ± 19.16 a
p			<0.0001	<0.0001

*Means followed by the same letter in the column are not significantly different by Scottt-Knott test ($p > 0,05$).

Table 2 Colonization of *Bemisia tabaci* MED on 28 cotton genotypes, in multiple-choice assay.

Genotype	Infestation level*		
	Number of adults[a]	Number of eggs	Number of nymphs[c]
101-102 B	2.92 ± 0.21 a	64.11 ± 9.87 ab	0.47 ± 0.09 a
3 -E313	6.67 ± 0.47 a	59.34 ± 6.89 ab	1.05 ± 0.11 ab
IAC 13-1-76/5366	7.58 ± 0.97 a	69.27 ± 7.65 ab	1.19 ± 0.09 ab
Algodão Indiano	7.75 ± 0.99 ab	80.57 ± 10.29 bc	0.75 ± 0.07 a
BRS Acácia Acala	8.83 ± 0.86 ab	74.53 ± 8.96 bc	1.49 ± 0.12 ab
CNPA 92-1121	9.25 ± 0.91 ab	60.40 ± 6.24 ab	2.73 ± 0.18 ab
Deltapine Smooth Leaf	9.58 ± 1.22 ab	66.87 ± 5.75 ab	1.5 ± 0.13 ab
Deltapine 4049	9.92 ± 1.89 abc	57.42 ± 6.32 ab	1.03 ± 0.09 ab
Express 257	10.42 ± 1.33 abc	57.81 ± 5.29 ab	2.82 ± 0.14 ab
FM975WS	12.58 ± 2.12 bc	93.92 ± 11.85 bc	2.01 ± 0.28 ab
FMT 707	13.08 ± 2.65 bc	78.83 ± 6.78 bc	4.36 ± 0.46 bc
Fundação MT 95-743	15.58 ± 2.13 bc	77.07 ± 8.36 bc	2.15 ± 0.19 ab
Hi-Bred	17.17 ± 4.61 bc	119.13 ± 12.96 c	1.17 ± 0.12 ab
IAC 18	18.08 ± 3.11 bc	66.00 ± 6.25 ab	3.42 ± 0.23 bc
IAC 19-158	18.17 ± 2.41 bc	82.18 ± 7.89 bc	1.76 ± 0.15 ab
Aubum 56-7	18.67 ± 3.66 bc	50.95 ± 5.49 a	1.58 ± 0.16 ab
IAC 97-2939 “macaco”	19.08 ± 1.27 bc	89.71 ± 10.86 bc	0.43 ± 0.07 a
Empire B-4	19.67 ± 3.74 bc	69.17 ± 6.98 ab	3.59 ± 0.31 bc

IAC PV 010-163	20.33 ± 2.21 bc	74.70 ± 8.23 bc	10.83 ± 1.16 c
IAC PV 010-175	22.41 ± 3.89 bc	84.75 ± 10.21 bc	2.08 ± 0.17 ab
IAC RR – 97/86 (IAC 23)	23.33 ± 2.43 bc	100.64 ± 11.96 c	3.05 ± 0.23 ab
Nu-16 – 78/664	24.58 ± 2.89 bcd	93.36 ± 8.35 bc	2.48 ± 0.16 ab
Mocó	27.17 ± 2.35 bcd	108.88 ± 12.63 c	3.74 ± 0.28 bc
Oc-96-252	31.58 ± 4.25 bcd	90.19 ± 12.84 bc	5.04 ± 0.65 bc
Paymaster 53-523	35.23 ± 4.91 cd	77.52 ± 9.25 bc	1.26 ± 0.11 ab
Reba B-50 PR	39.83 ± 5.76 cd	70.20 ± 11.69 ab	3.07 ± 0.31 ab
Rowden-40	41.17 ± 7.63 cd	90.69 ± 10.35 bc	1.88 ± 0.12 ab
Siokra L-22	51.58 ± 7.75 d	80.65 ± 11.84 bc	10.2 ± 1.13 c
p	<0.0001	<0.0001	<0.0001

[*]Means followed by the same lowercase letters per column or capital letter in the line do not differ by the Tukey–Kramer's test ($p > 0.05$);

[a]Means of the number of adults found on the abaxial leaf surface after 24, 48 and 72 hours;

[b] Number of eggs cm^{-2} after 3 days of infestation;

[c] Number of nymphs cm^{-2} after 18 days of infestation.

Table 3 Means (± SE) of duration of nymphal instar, and nymphal period of *Bemisia tabaci* MED in 28 cotton genotypes, in a greenhouse assay.

Genotype	Duration (Days)				
	1st instar	2nd instar	3rd instar	4th instar	Nymphal period
FM975WS	1.26 ± 0.06 d	2.38 ± 0.08 d	5.31 ± 0.07 bc	6.71 ± 0.21 ef	15.27 ± 0.09 e
BRS Acácia Acala	1.35 ± 0.11 d	2.83 ± 0.15 bc	5.44 ± 0.42 abc	7.14 ± 0.14 cd	16.48 ± 0.62 de
Empire B-4	1.42 ± 0.09 cd	3.25 ± 0.15 abc	5.39 ± 0.27 abc	7.56 ± 0.09 cd	17.62 ± 0.47 cd
Oc-96-252	1.58 ± 0.12 cd	3.42 ± 0.18 abc	3.82 ± 0.33 cd	6.13 ± 0.26 f	15.42 ± 0.15 e
FMT 707	1.58 ± 0.08 cd	2.61 ± 0.13 cd	6.12 ± 0.42 a	7.31 ± 0.39 cd	17.68 ± 0.21bc
Deltapine 4049	1.59 ± 0.14 cd	3.04 ± 0.09 bc	3.41 ± 0.26 d	6.89 ± 0.18 def	16.92 ± 0.23 cde
3 -E313	1.61 ± 0.11 cd	3.63 ± 0.33 a	4.53 ± 0.19 cd	7.08 ± 0.13 cd	17.01 ± 0.14 cde
IAC PV 010-175	1.62 ± 0.13 cd	2.48 ± 0.14 cd	5.99 ± 0.14 ab	6.71 ± 0.09 ef	16.85 ± 0.26 cde
Algodão Indiano	1.65 ± 0.08 bcd	2.75 ± 0.15 cd	5.02 ± 0.12 bc	7.49 ± 0.14 cd	17.03 ± 0.39 cde
Siokra L-22	1.66 ± 0.15 bcd	2.94 ± 0.31 cd	4.12 ± 0.73 cd	8.07 ± 0.24 bc	16.87 ± 0.12 cde
IAC 97-2939 “macaco”	1.67 ± 0.11 bcd	3.27 ± 0.26 abc	4.96 ± 0.08 bcd	9.24 ± 0.46 a	16.67 ± 0.28 cde
Express 257	1.69 ± 0.21 bcd	3.02 ± 0.19 bc	5.18 ± 0.15 bc	7.11 ± 0.19 cd	17.11 ± 0.18 bcd
Rowden-40	1.73 ± 0.19 bc	2.78 ± 0.14 cd	5.24 ± 0.18 bc	6.97 ± 0.12 def	16.72 ± 0.42 cde
Nu-16 – 78/664	1.73 ± 0.13 bc	2.89 ± 0.12 bc	4.09 ± 0.13 cd	7.54 ± 0.23 cd	16.13 ± 0.64 de
IAC 18	1.74 ± 0.07 bc	3.27 ± 0.46 abc	4.22 ± 0.09 cd	7.35 ± 0.15 cd	16.25 ± 0.19 cde
IAC PV 010-163	1.75 ± 0.13 bc	3.12 ± 0.23 abc	4.36 ± 0.18 cd	8.02 ± 0.31 bc	17.25 ± 0.08 bc
IAC RR – 97/86 (IAC 23)	1.75 ± 0.21 bc	3.33 ± 0.08 ab	5.52 ± 0.13 abc	6.87 ± 0.07 ef	17.47 ± 0.28 bc
101-102 B	1.81 ± 0.19 bc	3.48 ± 0.15 ab	5.01 ± 0.09 bc	6.59 ± 0.42 ef	17.01 ± 0.27 cde
Mocó	1.83 ± 0.32 bc	2.69 ± 0.09 cd	5.62 ± 0.14 ab	6.57 ± 0.27 ef	16.93 ± 0.26 cde

IAC 19-158	1.84 ± 0.19 bc	2.57 ± 0.26 cd	5.19 ± 0.24 bc	7.11 ± 0.33 cd	16.81 ± 0.33 cde
Reba B-50 PR	1.87 ± 0.14 abc	3.27 ± 0.19 abc	5.32 ± 0.46 bc	8.43 ± 0.42 ab	19.03 ± 0.75 ab
Fundação MT 95-743	1.91 ± 0.08 abc	3.01 ± 0.13 bc	5.29 ± 0.23 bc	8.01 ± 0.26 bc	18.41 ± 0.82 ab
Deltapine Smooth Leaf	1.92 ± 0.07 ab	2.98 ± 0.09 bc	4.91 ± 0.15 bcd	7.61 ± 0.19 cd	17.37 ± 0.42 bc
Aubum 56-7	2.11 ± 0.11 ab	3.16 ± 0.23 abc	5.79 ± 0.31 ab	8.19 ± 0.14 ab	19.28 ± 1.16 a
Paymaster 53-523	2.12 ± 0.08 ab	3.07 ± 0.31 abc	5.67 ± 0.23 ab	7.89 ± 0.12 ab	18.91 ± 0.73 ab
CNPA 92-1121	2.17 ± 0.07 a	3.42 ± 0.12 ab	5.44 ± 0.31abc	7.88 ± 0.73 ab	19.14 ± 0.64 a
IAC 13-1-76-5366	2.26 ± 0.13 a	2.77 ± 0.16 cd	6.39 ± 0.12 a	8.63 ± 0.08 ab	20.12 ± 0.91 a
Hi-Bred	2.31 ± 0.08 a	2.98 ± 0.09 bc	4.87 ± 1.13 bcd	10.10 ± 0.15 a	22.71 ± 1.13 a
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a]Means followed by the same lowercase letters per column or capital letter in the line do not differ by Tukey–Kramer's test ($p > 0.05$).

Table 4 Pearson correlation coefficients (r) and probabilities (P) among the parameters of *Bemisia tabaci* MED and characteristics of cotton genotypes

Parameters	Coefficients	Gossypol gland	Trichome	Leaf hardness	L	a	b
Number of adults	r	0.23	0.108	-0.024	0.13	0.108	0.12
	P	0.667	0.413	0.858	0.413	0.413	0.231
Number of eggs	r	0.013	-0.035	0.033	-0.19	-0.035	-0.14
	P	0.920	0.794	0.802	0.794	0.123	0.692
Number of nymphs	r	-0.14	-0.21	0.078	0.108	-0.027	0.161
	P	0.792	0.021	0.577	0.413	0.845	0.223
Nymphal period	r	0.41	0.19	-0.141	-	-	-
	P	0.001	0.166	0.283	-	-	-
Nymphal Mortality	r	0.39	0.161	-0.146	-	-	-
	P	0.001	0.223	0.269	-	-	-

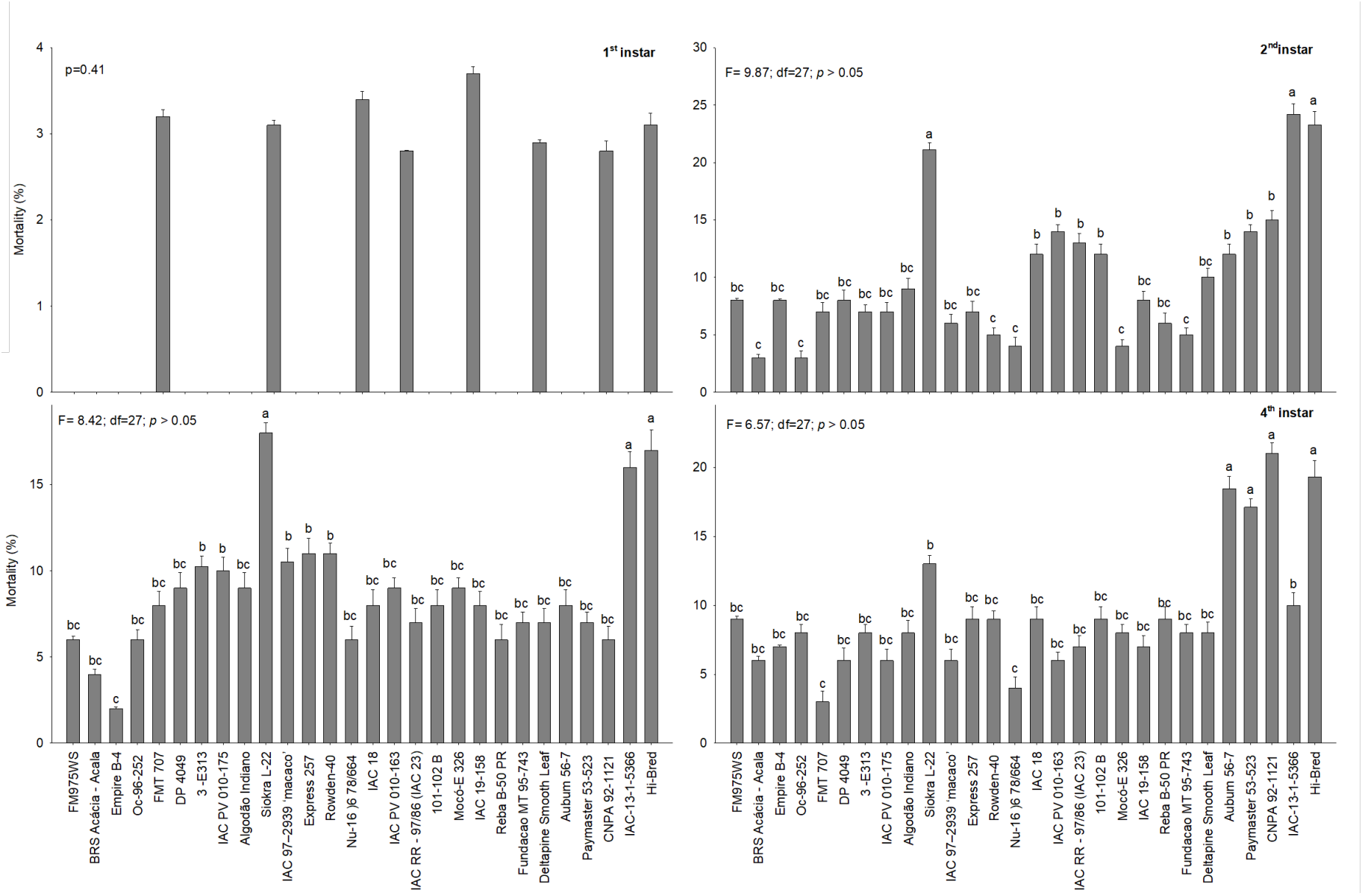

Figure 1. Means (± SE) of mortality per nymphal instar of *Bemisia tabaci* MED confined in 28 cotton genotypes. Means followed by the same letter do not differ statistically from each other by Tukey–Kramer's test (p > 0.05).

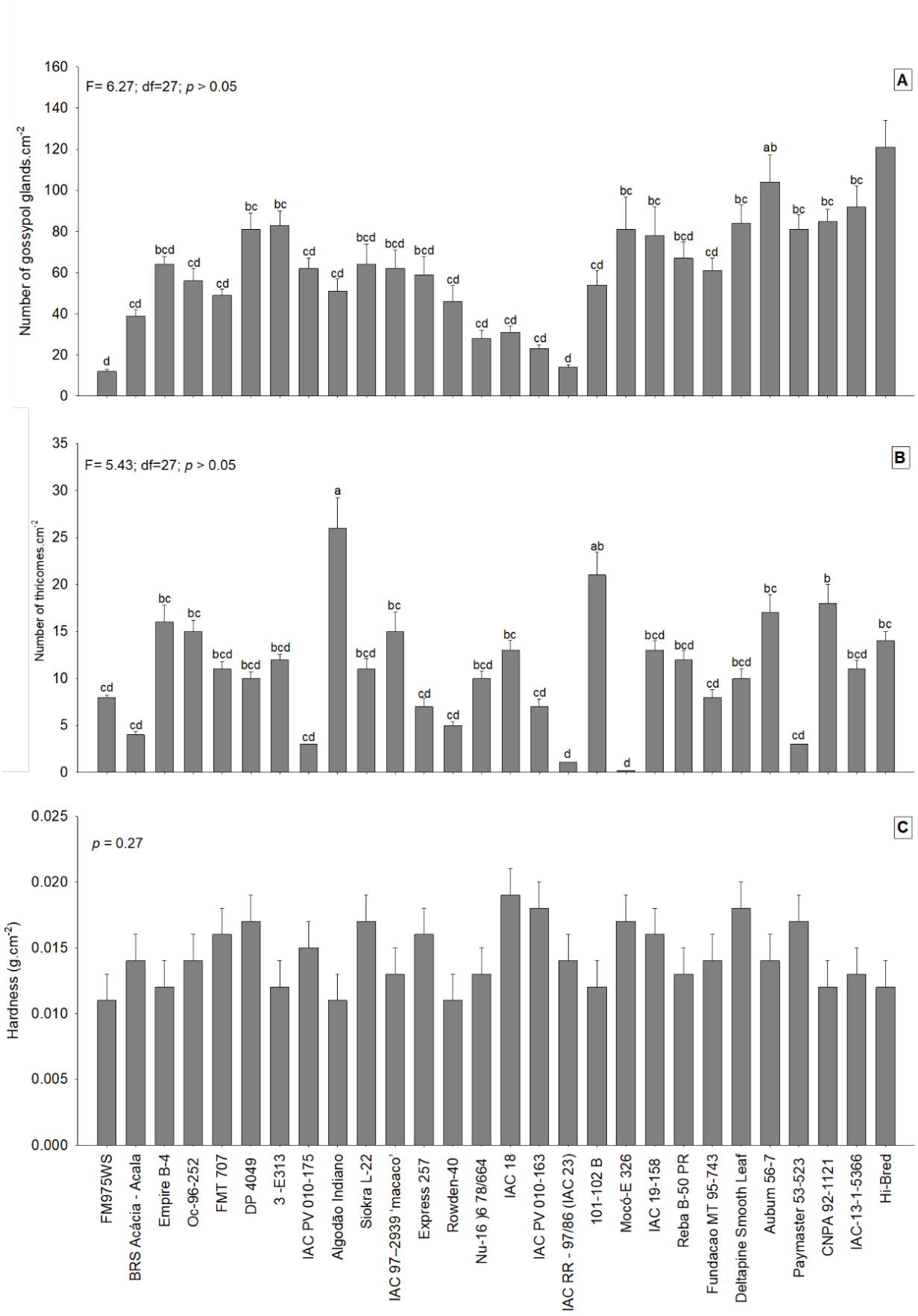

Figure 2. Means (± SE) of the densities of trichomes (A) and gossypol glands (B) and leaf hardness (C) of 28 cotton genotypes. Means followed by the same letter do not differ statistically from each other by Tukey–Kramer's test ($p > 0.05$).

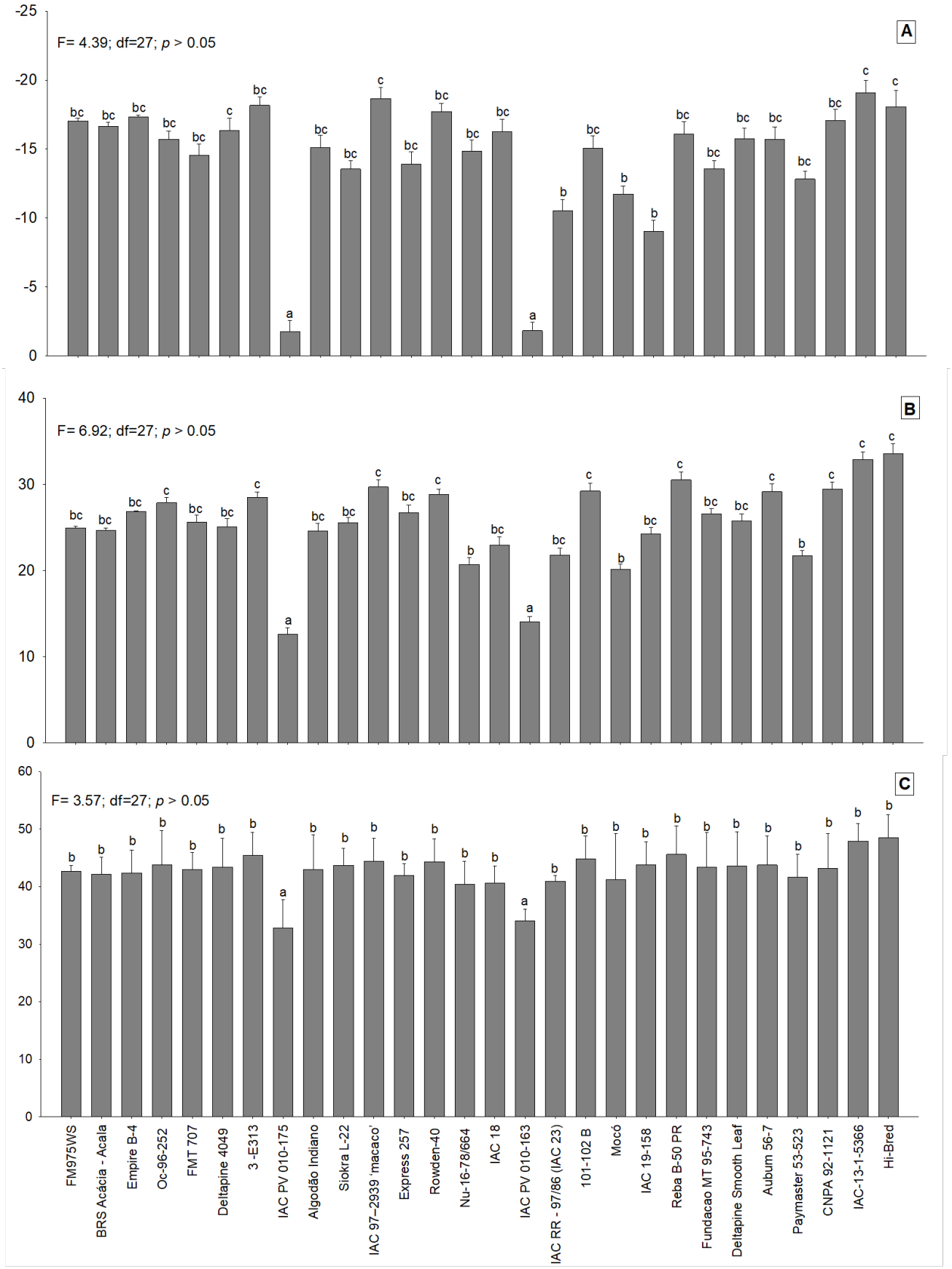

Figure 3. Means (± SE) of the parameters a * (green intensity) (A), b * (yellow intensity) (B) and L * (luminosity) (C), in the colorimetric analysis of the adaxial surface of the leaves of the different cotton genotypes. Means followed by the same letter do not differ statistically from each other by Tukey–Kramer's test (p > 0.05).

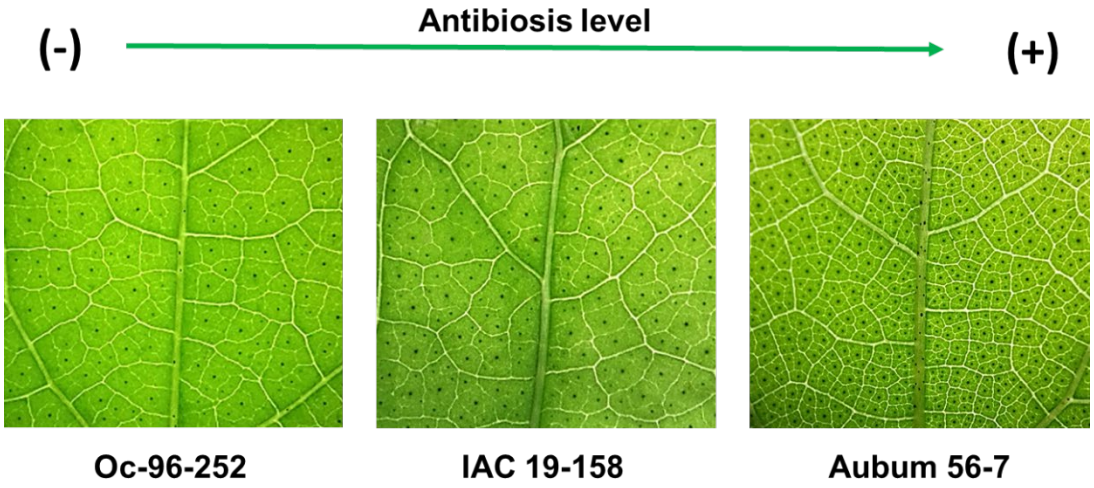

Figure 4. Gossypol glands in leaves cotton genotypes with low, medium and high resistance by antibiosis to *Bemisia tabaci* MED

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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