Essential oils on the control of melon leafminer

Efeito de óleos essenciais de plantas no controle da mosca-minadora do meloeiro

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ABSTRACT
Studies on the effect of essential oils for the control of melon leaf miner adults (*Liriomyza trifolii* Burgess) and the phytoxicity on melon plants (*Cucumis melo* L.) were carried out under green house and laboratory experiments with the aim to find the effectivity of essential oils to control this pest. Essential oils (treatments) used in the tests belonged to the following plant species: *Ocimum basilicum, Ocimum kilimandshanicum, Lippia alba, Ocimum gratissimum, Cymbopogon citratus, Ocimum micrantum, Lippia sidoides, Pipper aduncum, Citrus sinensis, Ocimum selloi* and water as the control. In addition, two mixtures were evaluated: Mix 1 (*Ocimum selloi* + *Pipper aduncum*) and Mix 2 (*Ocimum kilimandshanicum* + *Ocimum gratissimum*). Each essential oil evaluated had the following dilutions: 0.125%, 0.25%, 0.5%, 1.0%, 3.0%, 5.0%, 7.0%, and 10.0%. The dilution 0.125% of *Ocimum basilicum, Ocimum kilimandshanicum, Ocimum seloi, Cimbopogon citratus, Lippia alba, Peper aduncum* and Mix 2 presented the best results by not causing any phytotoxicity damage to melon leaves. The other dilutions were significantly different from the pattern water. As result, the essential oil of the species *Ocimum kilimandshanicum* with 0.125% caused 100.0% of mortality to melon leaf miner adults and it did not cause any phytotocixity damage to melon leaves. Therefore, these results suggest that this essential oil presents a high potential to be a future commercially friendly product against melon leaf miner pest.

Keywords: damage, mortality, control, dose, evaluation.

RESUMO
Estudos sobre o efeito de óleos essenciais no controle de adultos da mosca-minadora do meloeiro (*Liriomyza trifolii Burgess*) e sua fitotoxicidade em folhas do meloeiro (*Cucumis melo* L.) foram desenvolvidos em casa de vegetação e laboratório com o objetivo de encontrar um óleo essencial que fosse efetivo no controle dessa praga. Os óleos essenciais usados (tratamentos) pertencem às seguintes espécies: *Ocimum basilicum, Ocimum kilimandshanicum, Lippia Alba, Ocimum gratissimum, Cymbopogon citratus, Ocimum micrantum, Lippia sidoides, Pipper aduncum, Citrus sinensis, Ocimum selloi* e agua como o controle. Foi também avaliado o efeito de duas misturas: Mix 1 (*Ocimum selloi* + *Pipper aduncum*) e Mix 2 (*Ocimum kilimandshanicum* + *Ocimum*

gratissimum) . Cada óleo essencial foi avaliado seguindo as concentrações (diluições): 0,125%; 0,25%; 0,5%; 1,0%; 3,0%; 5,0%; 7,0%; e 10,0%. A concentração 0,125 de *Ocimum basilicum*, *Ocimum kilimandshanicum*, *Ocimu seloi*, *Cimbopogon citratus*, *Lippia alba*, *Pipper aduncum* e Mix 2 apresentaram os melhores resultados por não causarem danos às folhas do meloeiro. As outras concentrações foram significativamente diferentes do padrão água. Como resultado final, o óleo essencial da espécie, *Ocimum kilimandshanicum*, com 0,125 causou 100,0% de mortalidade em adultos da mosca-minadora do meloeiro. Portanto, estes resultados sugerem que este óleo essencial apresenta um alto potencial, após testes de campo, para ser um futuro produto natural contra a praga da mosca minadora do meloeiro.

Palavras-chave: dano, mortalidade, controle, dose, avaliação.

1 INTRODUCTION

The melon leaf miner, *Liriomyza trifolii Burgess*, is a major pest of melon (*Cucumis melo* L), in northeast Brazil. This insect has been causing severe reductions in melon production and quality in the region. This species is considered highly aggressive, causing major losses in different plant species (MURPHY; LASALLE, 1999; BUENO et al., 2007; CELIN et al., 2017). In the last twenty years, this pest has been reducing melon production and quality and increasing the production cost in the semiarid growing areas of the states of Rio Grande do Norte and Ceará, Brazil, main melon producer and exporter states in Brazil (ARAUJO et al., 2007; BRAGA SOBRINHO et al., 2007; 2008; 2011).

The use of chemical pesticides has been the main alternative to control this pest. These pesticides result in a high cost-effective system of controlling pests and are highly toxic to other species in the environment. The environmental problems caused by overuse of pesticides have been always the matter of concern for scientists, public authorities, official agencies and consumers around the world (RAO et al., 2003). The use of plant essential oils is other environmentally friendly control method. This alternative can be less toxic to humans, readily biodegradable, safer for the environment, suitable for use by small scale farmers, and yet capable of protecting crops from attack by wide range of insect pests (ROSENTHAL, 1986; Ma et al., 2020). Several researchers have emphasized the importance of bioactive extracts from plants as a strategy for the control of agricultural pests. These products are usually composed by terpenoid, sesquiterpenes and detones (HIKAL et al., 2017; COSTA et al., 2018; IKBAL and PAVELA, 2019; KLEIN et al., 2020; FOLKOWSKI et al. 2020; ISMAN, 2020).

Plant essential oils are in general composed of complex mixtures of monoterpenes, biogenetically related phenols, sesquiterpenes, phenylpropanoides and metabolites that confer the mixtures with organoleptic characteristics of biological activities (ISMAN, 2006; STEFANELLO et al., 2011). Essential oil is any volatile oil that has strong aromatic components with distinctive

odor, flavor and scent. It is the by-product of plant metabolism and is well known as volatile plant secondary metabolism. They are a promising new class of ecological products for the management of pest insects (LOPES; PASCUAL VILLALOBOS, 2010; COSTA et al., 2018). There are several examples of essential oils like *Cymbopogon citratus*, *Ocimum* spp., *Mentha* spp., *Lippia alba* that have been proved to be efficient to control many agricultural pests (ZARIDAH et al., 2003; KORDALI et al., 2005; TRIPATHI et al., 2009). Therefore, this study has the aim of testing different plant essential oils against the melon leaf miner pest and also, their phytotoxicity effects on melon leaves.

2 MATERIAL AND METHODS

All tests and procedures were undertaken in the Laboratory of Entomology and green house belonged to Embrapa Agroindustry Tropical Research Center - in Fortaleza, Ceará Brazil.

Melon seeds used in the tests were the hybrid SEMINIS (Goldmine) ^{R}. Melon seed was individually planted in tray cells. Melon seedlings were transplanted to plastic pots for posterior tests.

Plants for oil extraction were taken from the collection of aromatic plants of EMBRAPA. Essential oils were extracted in the Multiuse Laboratory of Chemistry Natural Products of EMBRAPA by using hydrodestilation method (MELECCHI, 2005; BARBOSA and BARBOSA, 2006). Essential oil dilutions were individually sprayed on melon plant leaves. The mixtures (Mix 1 and Mix 2) were also used to verify the synergistic effects against the melon leaf miner pest.

Data were subjected to statistical analysis using a completely random design. The experiments consisted of thirteen treatments (ten essential oils, mix 1, mix 2 and the control), eight oil dilutions for each treatment and four replicates. The significance of the results was performed using ANOVA and Turkey's test based on Stats Direct Statistical Software, version 2.2.7. Differences between treated and control were considered not statistically significant when $p > 0.05$. Probability levels are specified within the text and graphics/figures.

Essential oil/treatments used in the tests belonged to the following plant species: *Ocimum basilicum*, *Ocimum kilimandshanicum*, *Lippia alba*, *Ocimum gratissimum*, *Cymbopogon citratus*, *Ocimum micrantum*, *Lippia sidoides*, *Pipper aduncum*, *Citrus sinensis*, *Ocimum selloi* and water as the control. The effect of two mixtures was also evaluated: Mix 1 (*Ocimum selloi* + *Pipper aduncum*) e Mix 2 (*Ocimum kilimandshanicum* + *Ocimum gratissimum*). Each essential oil above was tested following the concentrations (dilutions): 0.125%, 0.25%, 0.5%, 1.0%, 3.0%, 5.0%, 7.0%, and 10.0%.

Phytotoxicity effects of essential oils on melon leaves

The first step of the work was to test each essential oil in order to know the phytotoxicity effect on melon leaves. Therefore, the objective of these tests was to know the limit of the dilution that cause zero fit toxicity effect on melon leaves. A visual damage scale based on values from zero to three was established based on Braga Sobrinho et al., 2012 as shown below.

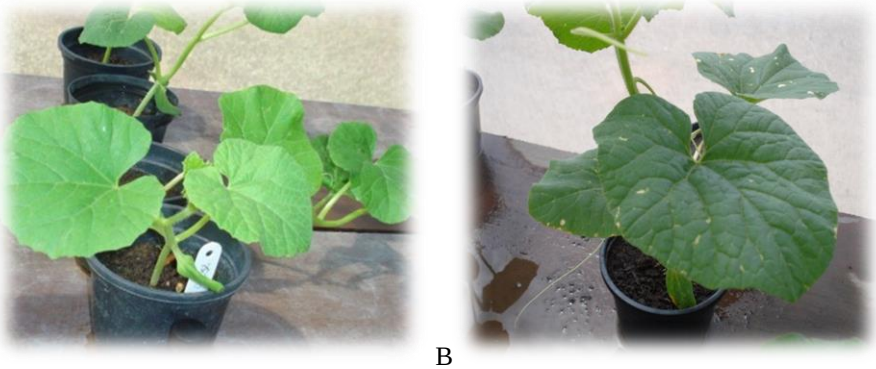
Table 1. Scale for evaluation of phytotoxicity damage on melon leaves by different dilutions f oessential plant oils.

Degree	Percentage of damage on melon leaves
00 - A	Without symptoms - No damage or yellow spot
01 - B	1 to 10% of yellow spot and burn on leaf edges
02 - C	11 to 50% of severe yellow spots and burn on leaf edges
03 - D	51 to 100% of severe yellow spots and burn on leaf edges

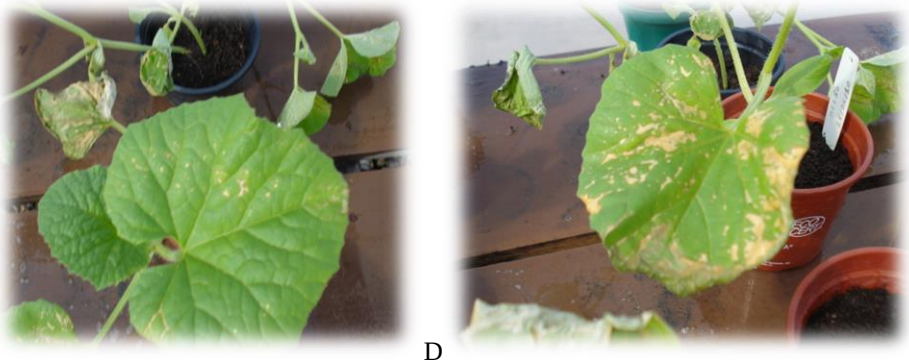
This test was carried out at screen green house. For each essential oil (treatment) were used eight dilutions. All treatments had a replicate of four plants with age of 20 days. The dilution was sprayed on each plant, as shown in Figure 1. The symptoms on leaves were observed one, two and three day after the application. The plants were examined, evaluated and accounted for leaf damage and plant death (Figure 2 - A, B, C and D), based on the scale presented above (Table 1). Dilutions that did not caused any damage to leaves (grade zero - A) were selected for the tests on the effects on leaf miner adults.

Figure 1 – Demonstration of essential oil application on melon plants

Figure 2 – Evaluation of plants with phytotoxicity symptoms based on the degree of damage/scale from 0.0 (A), B (1,0), C (2,0), D (3,0)



A B



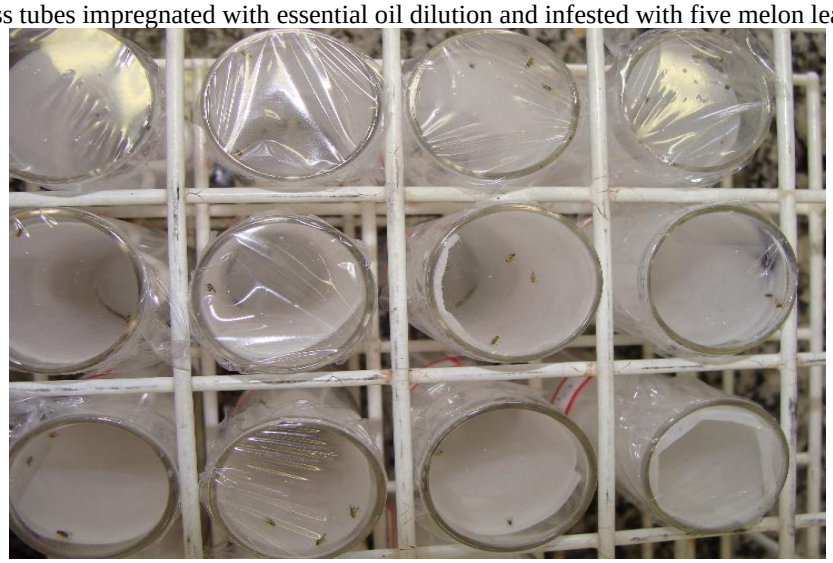
C D

Effects of essential oils on melon leaf miner

This test was carried out with the objective to evaluate the effect of several dilutions of essential oils on adults of leaf miner under laboratory experiments. Only essential oil dilutions that did not cause any phytotoxicity effect on melon leaves were selected in these tests.

Leaf miner adults used in these tests came from a colony maintained in the Laboratory of Entomology of Embrapa Tropical Agroindustry Research Center in Fortaleza (ARAÚJO et al., 2007b; BRAGA SOBRINHO et al., 2011). Glass tubes with 10.0 cm long and 2.0 cm of diameter were used for this experiment. Each glass tube internally coated with a filter paper in order to absolve the essential oil. On each filter paper was sprayed 0.5 ml of the treatment (oil dilution). Afterward, five leaf miner adults of the same age were released into the glass tube. For each essential oil dilution was used five replicates (glass tube) with 5 melon leaf miner adults. The top of the tube sealed with a fine plastic film full of several pin holes for oxygen exchange (Figure 3). The evaluations were performed after one hour, two hours and four hours after the introduction of insects into the glass tube. Dead and alive insects were counted and registered during the observation period.

Figure 3 – Glass tubes impregnated with essential oil dilution and infested with five melon leaf miner adults.

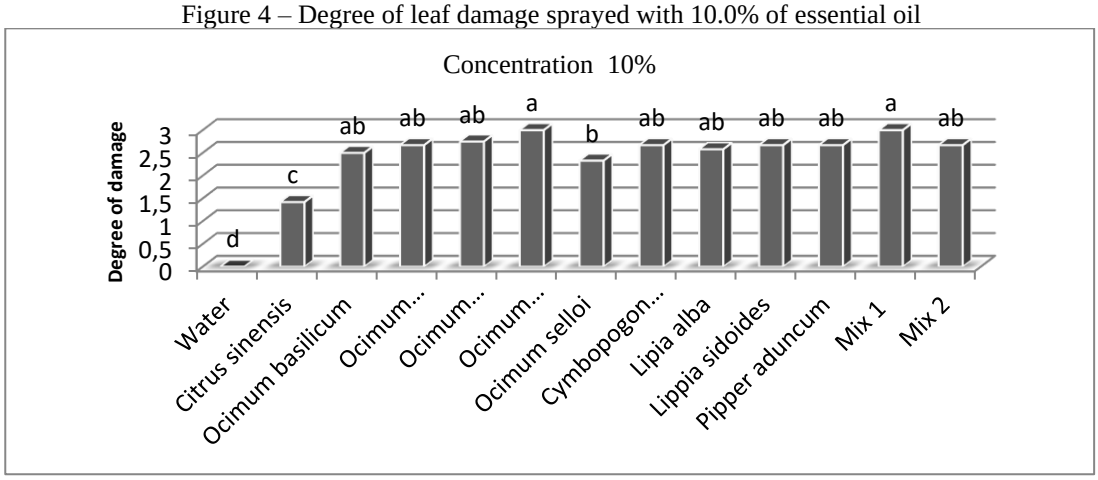


3 RESULTS AND DISCUSSION

Phytotoxicity tests on melon leaves

The results in Figure 01 infer that twelve treatments with 10.0% of concentration of each essential oil caused median to severe damages on melon leaves. For eleven treatments the degree of severity was two (C), which means high level of damages on melon leaves. Only the *Citrus sinensis*, had a degree of damage on leaves above one (B) considered very low damage.

Figure 4 – Degree of leaf damage sprayed with 10.0% of essential oil



Other essential oils dilutions shown in Figures 5, 6 e 7 presented also high damage to melon leaves indicating that these essential oils under dilution of 7.0, 5.0 and 3.0% caused severe phytotoxicity effects to melon leaves. The essential oil *Ocimum micrantum* with 10.0%, 7,0, and 5.0% of dilution caused the highest damages to leaves. Comunale (2010) found similar results by using essential oils extracted from *Cymbopogon citratus*, *Ocimum gratissimum*, *Ocimum micrantum*, *Piper aduncum*, *Lippia sidoides* and *Ocimun seloi* on melon plants.

Figure 5 - Degree of damage on melon leaves sprayed with 7.0% of essential oil

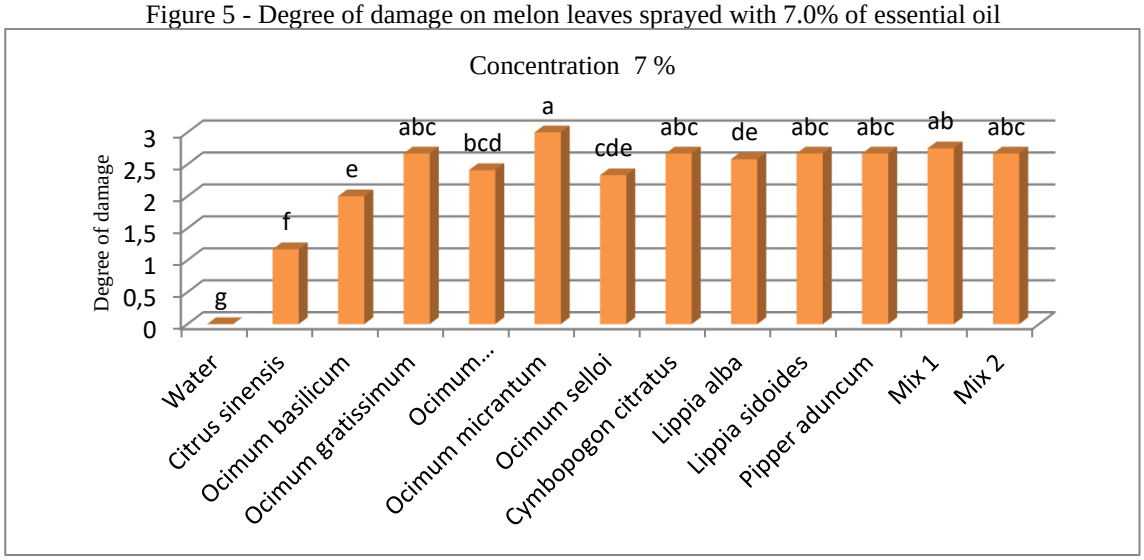
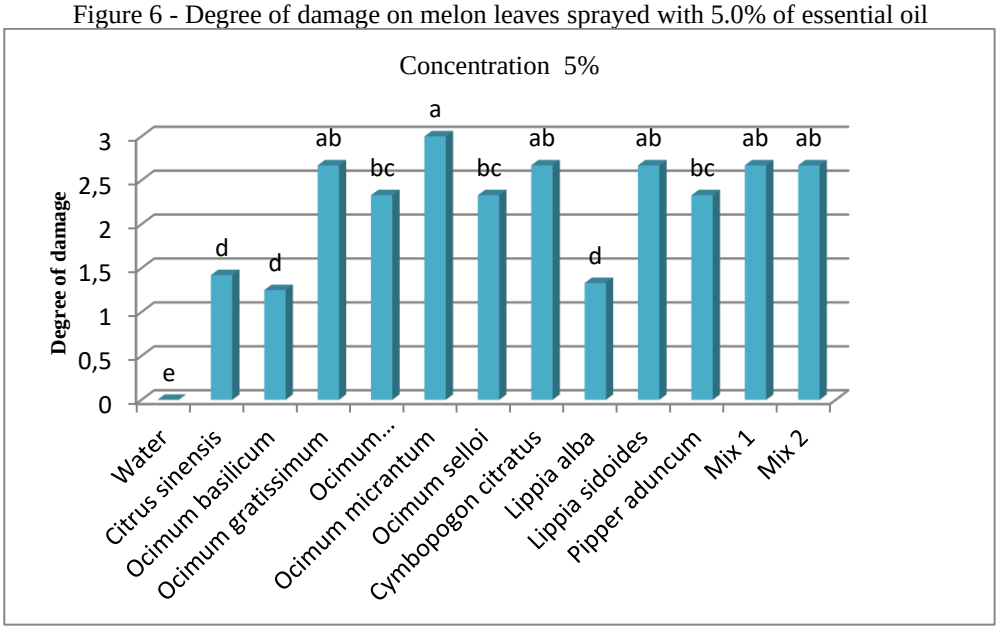
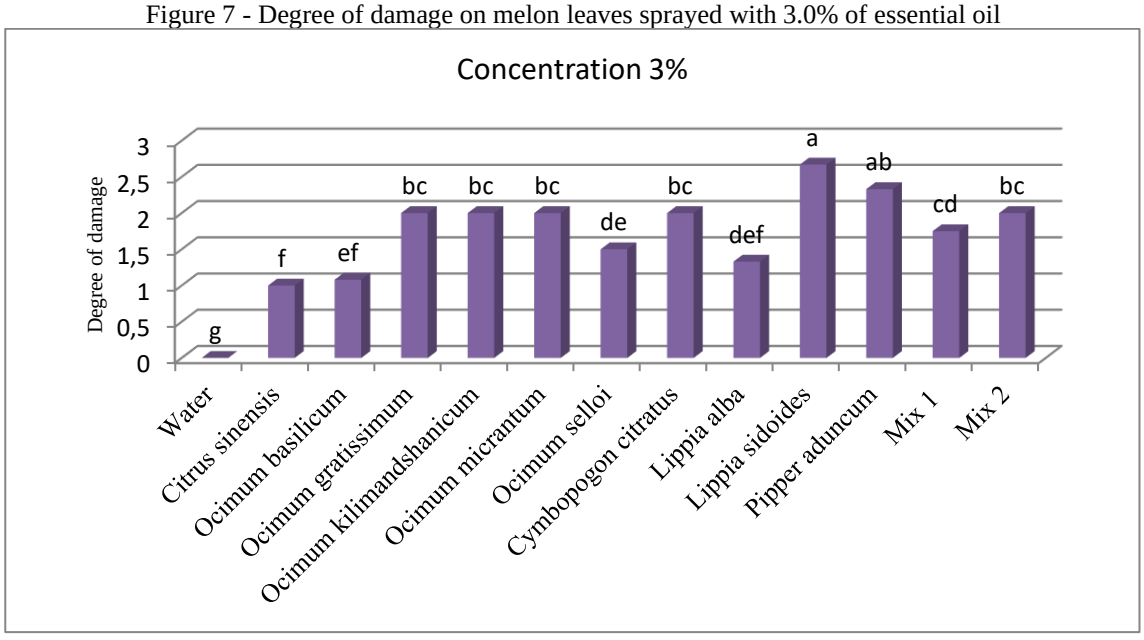


Figure 6 - Degree of damage on melon leaves sprayed with 5.0% of essential oil



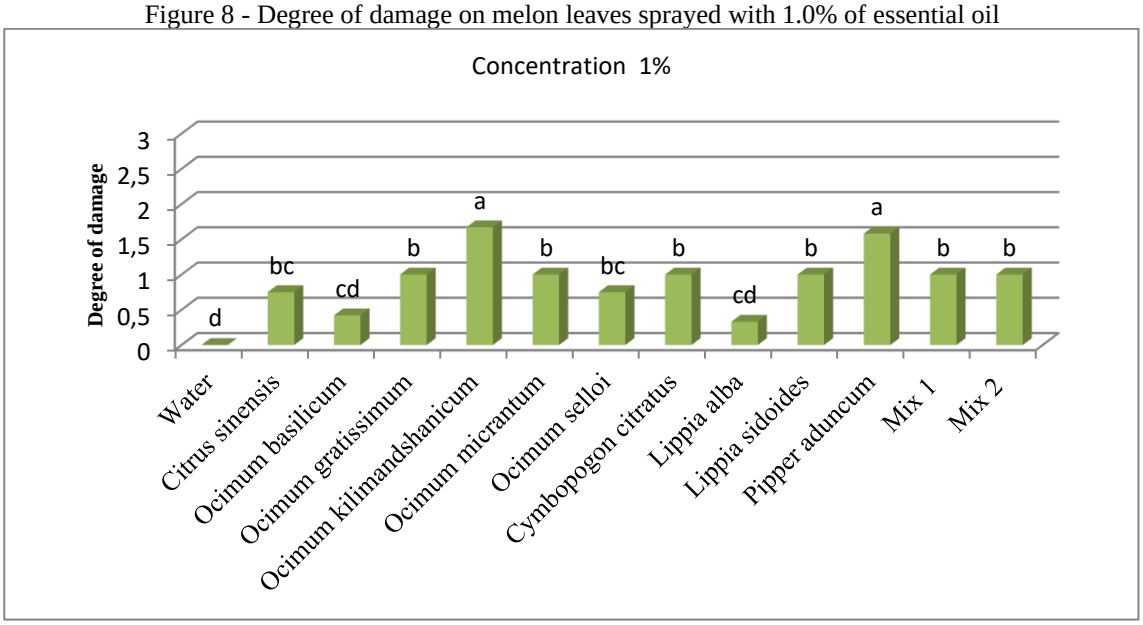
Tests with essential oils extracted from the species *Lippia sidoides* and *Pipper aduncum* with 3.0% of dilution (Figure 7) presented a high significant degree of damage to melon leaves when compared to other species.

Figure 7 - Degree of damage on melon leaves sprayed with 3.0% of essential oil



The essential oil with a concentration of 1.0% extracted from species *Ocimum kilimandshanicum* and *Pipper aduncum* presented significantly high the degree of severity by causing damages on melon leaves. Only the treatments *Ocimum basilicum* and e *Lippia alba* were not significantly different ($p \geq 0.05$) from the treatment water, Figure 8.

Figure 8 - Degree of damage on melon leaves sprayed with 1.0% of essential oil



Concentrations of essencial oils ranging from 0.5 to 0.25 also presented damage to melon leaves. Only four treatments with 0.5% concentration, *Ocimum basilicum*, *O. seloi*, *L. alba* and Mix 2 were not significantly different from the pattern water (Figure 9). On the other hand, essential oils with concentration 0.25% of *O. micrantum*, *L. sidoides* and Mix 1 presented significant level of damage when compared to pattern water (Figure 10).

Figure 9 - Degree of damage on melon leaves sprayed with 0.5% of essential oil

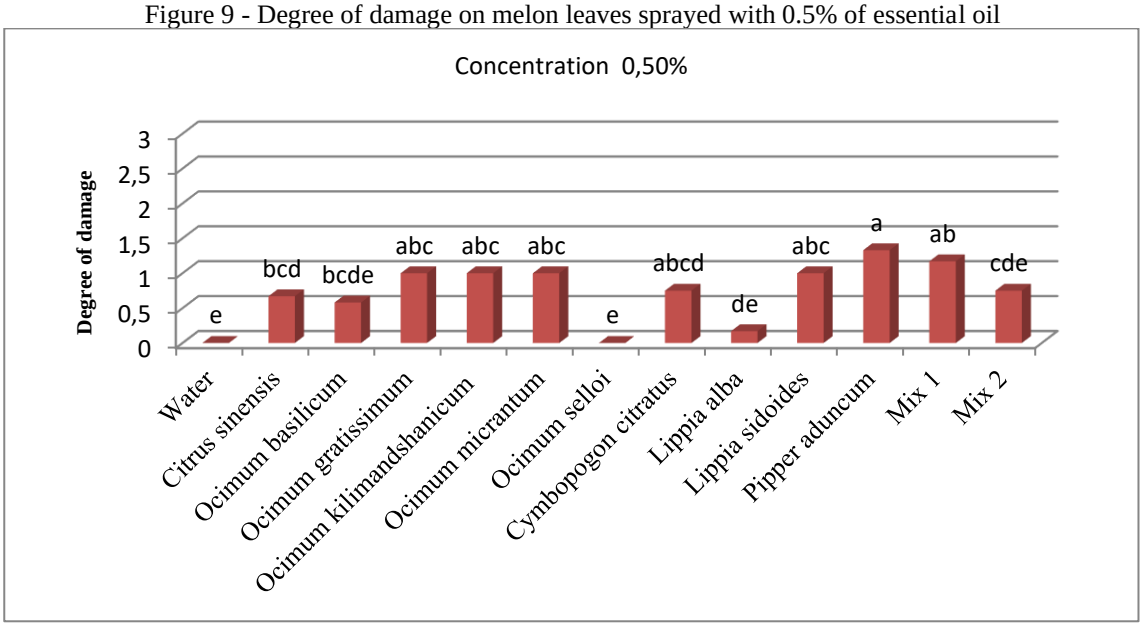
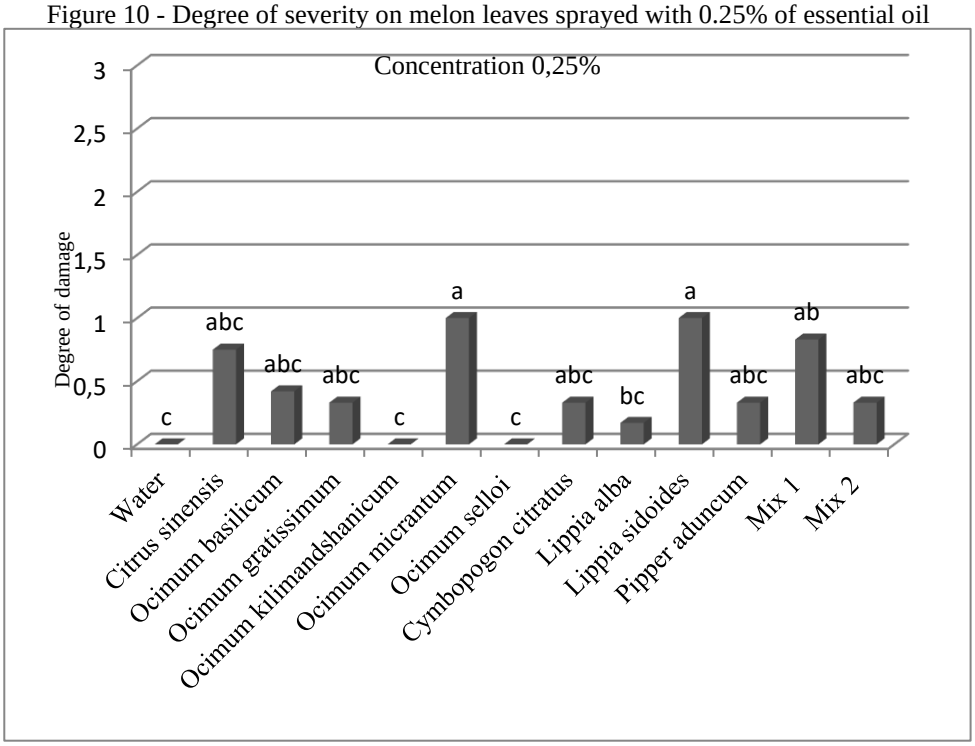
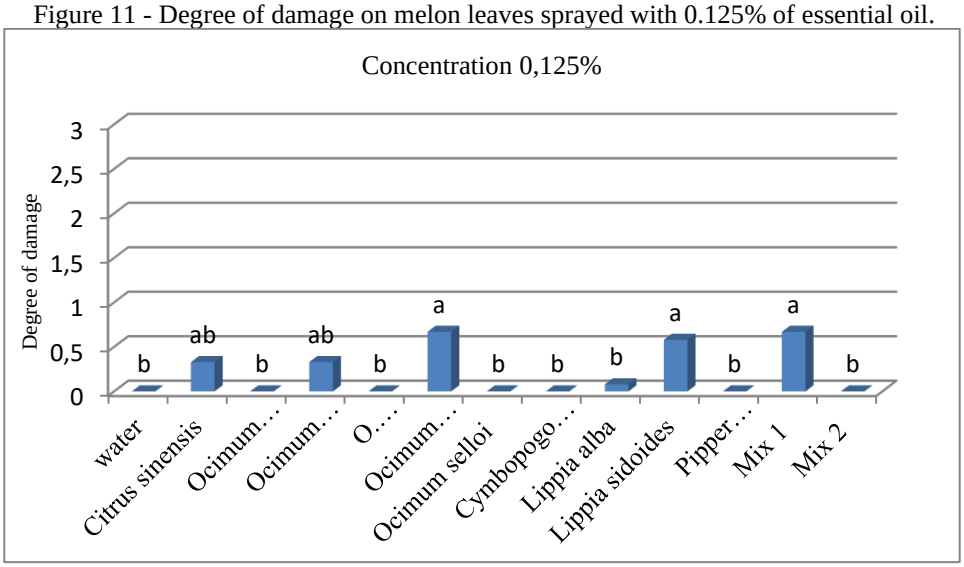


Figure 10 - Degree of severity on melon leaves sprayed with 0.25% of essential oil



The lowest concentration 0,125% (Figure 11) presented only three treatments *O. micrantum*, *L. sidoides* and Mix 1 (*O. selloi* + *P. aduncum*) with small degree of damage to leaves, being significantly different from the treatment *water*.

Figure 11 - Degree of damage on melon leaves sprayed with 0.125% of essential oil.

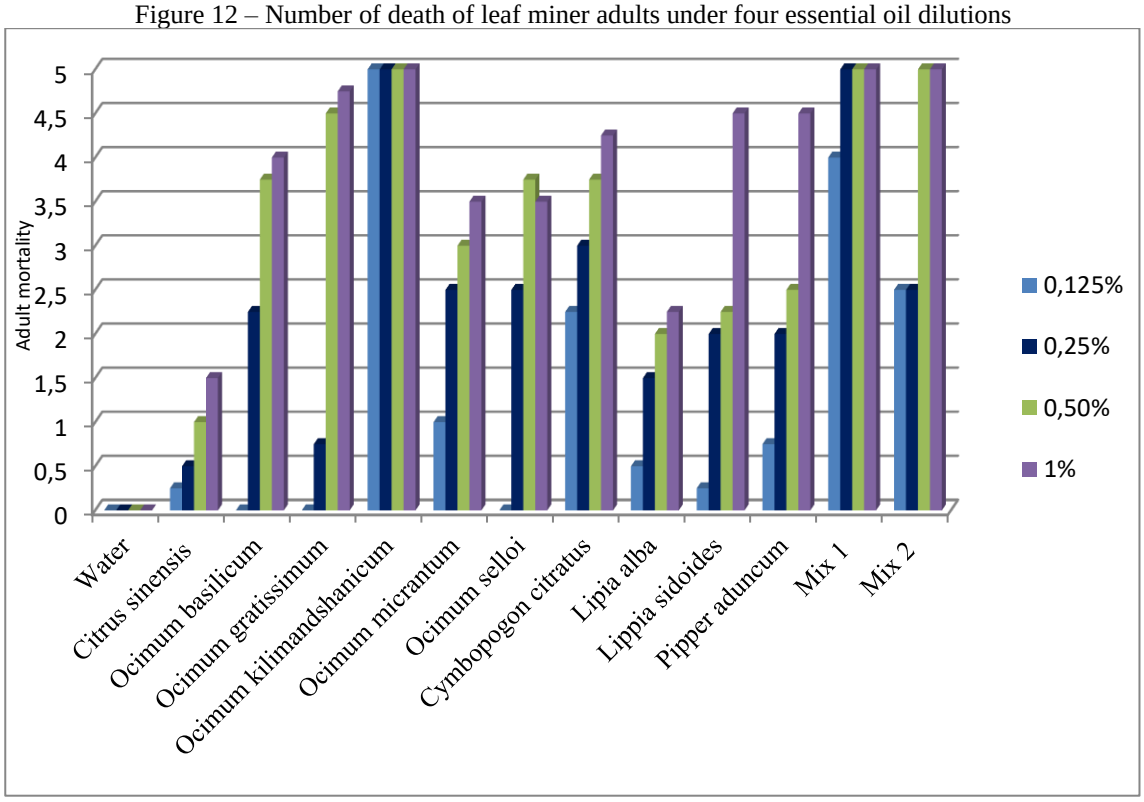


Toxicity tests against melon leaf miner pest

Based on phytotoxicity tests discussed above, essential oil dilutions that did not cause any damage to melon leaves were selected for the essays against melon leaf miner pest. Therefore, the dilutions selected were 1.0%, 0.5%, 0.25% and 0.125%. According to the methodology, these

concentrations were sprayed against adults of melon leaf miner. The essential oils extracted from the species *Ocimum kilimandshanicum* with 0.125% caused 100.0% of mortality in adults of melon leaf miner, Figure 12. Other results obtained by Lima et al., 2012, working with essential oil from leaves of *Eugenia sulcata* with a a dilution of 500mg/ml sprayed against *Oncopeltus fasciatus* and *Dysdercus peruvianus* shown 100% of adult mortality. From the results shown in Figure 12, seems that the expected synergistic effect of Mix 1 and Mix 2 did occur with very important results. The Mix 1 with a dilution of 0,125 caused an adult mortality around 76.0%, but the other dilutions 0.25; 0.50; and 1.0% caused a 100.0% of mortality. On the other hand, Mix 2, with dilutions 0.125 and 0.25 caused low adult mortality of 47.0%, but the other dilutions caused 100.0% of adult mortality (Figure 12).

Figure 12 – Number of death of leaf miner adults under four essential oil dilutions



Previous works about effect of the essential oil from *Eugenia sulcata* leaves on *Dysdercus peruvianus* indicated that its chemical components are promising candidates for insecticidal activity studies and for ecological control against pest populations in agriculture. The current view of the mode of action of plant metabolites is still rather linear, whereas in reality, it is likely that there are many compounds present in the oil and the mechanisms involved may forming a network in which pathway molecules interact in a flexible and dynamic way (RATTAN, 2010; GARCIA et al., 2012; FERNANDES et al., 2013; ISMAN, 2017; 2020).

It is well known that conventional pesticides can cause severe effects on the environment and harmful problem on human health. For this reason, there is a worldwide growing interest in botanical insecticides, like essential oils, due to their minimal costs and lack of ecological side effects, which make them desirable alternatives to synthetic chemical insecticides for controlling insect pests. The use of these alternatives are best suited for use in organic food production in industrialized countries and also can play a much greater role in developing countries like Brazil as a new class of ecofriendly products for pests (KHATER, 2012). Some essential oils have specific modes of action that make them good synergists. Terpenes, for example, present in some essential oils are neurotoxic to insects (ENAN, 2005; REGNAULT-ROGER, 1997).

The results of the synergistic effect of Mix 1 (*Ocimum selloi* + *Pipper aduncum*) did occur 100% of mortality for three dilutions (1.0; 0.5; 0.25) but not for 0.125% one. However, for Mix 2 (*Ocimum kilimandshanicum* + *Ocimum gratissimum*) presented high degree of synergistic effect for dilutions 1.0% and 0.5% (100% of adult mortality), but not for the dilutions 0.125% and 0.25% which caused only about 50.0% of mortality on adults of melon leaf miner, Figure 12,

Considering the threshold level for melon leaf miner adult recommended by Integrated Pest Management sampling method (BRAGA SOBRINHO et al., 2007) is 10.0%; the essential oil dilution *Ocimum kilimandshanicum* with 0.125 of dilution did not cause any damage to melon leaves; and with this dilution, it resulted in a 100.0% of adult mortality. Therefore, this find suggests that this essential oil fulfilled the requirements for other future tests under field experiments. This also indicates that this essential oil has a great potential to be a commercial product for the control of melon leafminer pest.

4 CONCLUSIONS

The results of this work indicate that essential oil extracts from plants are promising candidates for insecticidal activity studies and for ecological control use of plague insect populations in agriculture.

An important and valuable task, before to start testing any essential oil against a pest, is to screen each essential oil and its dilution to verify what percentage of dilution causes phytotoxicity damage on leaves of the plant. In this work was found that for the dilution 0.5% only the essential oil *Ocimum selloi* did not cause damage to melon leaves. For dilution 0.25%, *Ocimum kilimandshanicum*, *O. seloi* and *Lippia alba* did not damage to melon leaves. However, the dilution 0.125% of *Ocimum basilicum*, *Ocimum kilimandshanicum*, *Ocimum seloi*, *Cimbopogon citratus*, *Lippia alba*, *Peper aduncum* and Mix 2 (*Ocimum kilimandshanicum* + *Ocimum gratissimum*)

presented the best results by not causing any damage on melon leaves. The other concentrations were significantly different from the pattern water.

As final results, the essential oil of the species *Osmium kilimandshanicum* with 0.125% caused 100.0% of mortality of melon leaf miner adults and also, did not cause any damage to melon leaves. Therefore, this result suggests that this essential oil presents a high potential to be a future commercial friendly product against melon leaf miner pest.

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