Volatile and Phenolic Compounds In The Resistance of The Melon to The Vegetable Leafminer, *Liriomyza Sativae* Blanchard *(Diptera: Agromyzidae)*

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Abstract

The aim of this study was to evaluate whether volatile compounds released by melon genotypes interfere in the attractiveness and repellency of *Liriomyza sativae* Blanchard (Diptera: Agromyzidae), and to characterise the phenolic compounds of melon genotypes, as well as the antibiotic action of these compounds on *L. sativae*. Through experiments, it was shown that the volatiles of the melon genotypes CNPH 06-1047-343, CNPH 06-1047-333 and CNPH 06-1047-341 showed little attractiveness to *L. sativae*, while the volatiles of the Goldex commercial hybrid were more attractive. By analysing the volatile profiles, it was possible to identify the compounds acetic acid, (Z)-3-hexen-1-ol, α-pinene, (Z)-β-ocimene, (E)-β-ocimene, linalool, allo-ocimene and neo-allo-ocimene. The CNPH 06-1047-343, CNPH 06-1047-333 and CNPH 06-1047-341 melon genotypes show resistant characteristics due to the non-preference of *L. sativae* because of the smaller number of volatiles and the higher concentration of acetic acid in their volatile composition. The phenolic compounds characterised were hydroxybenzoic-hexoside acid, ferulic acid and trihydroxy-octadecadienoic acid. The CNPH 06-1047-333 and CNPH 06-1047-341 melon genotypes presented, respectively, the highest and lowest levels of total phenolics, but there was no difference in the larval or pupal viability of *L. sativae*, indicating a lack of any relationship between total phenols and antibiotic resistance of the melon genotypes to *L. sativae*. In general, based on the analysis of volatile and phenolic compounds, the CNPH 06-1047-343, CNPH 06-1047-333 and CNPH 06-1047-341 genotypes are less attractive to *L. sativae* than the commercial hybrid genotypes (Goldex).

Introduction

The leafminer, *Liriomyza sativae* Blanchard (Diptera: Agromyzidae), has been recorded as a serious agricultural pest, attacking many crops all over the world. Its pest status may vary by location over the years, but damage is on the increase due to the extensive use of pesticides. The leafminer is a key pest in the melon, and has caused serious problems to crops. Due to the feeding habits of the larvae, mines are formed in the leaves, reducing their photosynthetic capacity and resulting in fruit with low levels of total soluble solids (ºbrix) (Araújo et al. 2007; Gullan and Cranston 2012). The loss of leaves exposes the fruit to the sun, generating marks on the skin that reduce the external quality of the fruit and often make it impossible to sell (Rauf et al. 2000).

The leafminer is mainly controlled by spraying with synthetic insecticides, but indiscriminate use has caused population outbreaks of the pest (Araujo et al. 2012, Hernández et al. 2011). The indiscriminate use of insecticides increases production costs and can reduce the populations of natural enemies (Campos et al. 2012, Fernandes, 2004). In order to minimise these problems, and keep the leafminer population below the level of economic damage, new management approaches have been investigated, such as the selection of resistant melon genotypes (Celin et al. 2017, Celin et al. 2018, Oliveira et al., 2021).

Many factors may be related to the non-preference of Liriomyza adults for the melon, such as the release of chemical compounds by the plant, the thickness of the epidermis, the stiffness and thickness of the leaf cuticle, the density of the palisade and spongy tissue, and the number of trichomes, among others (Theobald et al. 1979, Wei et al. 2000, Oliveira et al., 2021). According to Kang et al. (2009), the chemical compounds synthesised by plants may be fundamental to plant selection in leafminer species. In tomato genotypes (Solanum lycopersicum L.), the non-preference of *Liriomyza trifolii* for feeding is related to high levels of allelochemicals (acylsugars, 2-tridecanone) (Silva et al. 2017). In the melon, *L. sativae* is associated with the smaller number of trichomes and greater thickness of the leaf epidermis (de Oliveira et al., 2021).

Volatile compounds released by plants can be used as a way for insect pests to locate the plants in their environment (Rajabaskar et al. 2013), with the amount of these released compounds controlled by the genetic condition of the plants (Splivallo et al. 2012, Wason and Hunter 2014). Therefore, studies that identify plant volatiles and test their attractiveness to pest species are very important for creating new control strategies, and can even help to identify resistant cultivars (Li et al. 2014; Robbins et al. 2012).

One of the factors that involves plant resistance to pests is the presence of phenolic compounds in the composition of the plant (Kulbat 2016, Nutt et al. 2004). These compounds play a fundamental role in plant resistance against insect pests, blocking consumption of the plant tissue and inhibiting digestion (Boerjan et al. 2003, Kulbat 2016). Quantifying the types of phenolic compound is important for selecting genotypes that are resistant to insect pests, as phenolic compounds affect the characteristics of plant susceptibility and resistance (Kumar et al. 2014).

In the melon, investigations aimed at characterising volatile and phenolic compounds produced by different genotypes can be very useful in selecting genotypes resistant to *L. sativae.* As such, the aim of this study was (1) to evaluate whether volatile compounds released by melon genotypes interfere in the attractiveness and repellency of *L. sativae*, and (2) to characterise the phenolic compounds of melon genotypes, as well as the antibiotic action of these compounds on *L. sativae*.

Materials And Methods

Plant Material

The genotypes were selected based on preliminary tests of resistance of the melon plants to *L. sativae*, with the Goldex commercial hybrid used as the standard for susceptibility, and the CNPH 06-1047-343, CNPH 06-1047-333 and CNPH 06-1047-341 genotypes resistant through antibiosis to *L. sativae* (de Oliveira et al. 2021). These accessions were provided by the germplasm bank of Embrapa Hortaliças, in Brasília. Seeds from each of the melon progeny were placed in 200-cell polystyrene trays containing HS Florestal substrate (Holambra Substrates, Artur Nogueira, São Paulo, Brazil) and coconut fibre (1:1 w/w), and grown in a greenhouse (27 ± 3°C, 70 ± 10% relative humidity, and 12:12 h L:D photoperiod). Seven days after sowing, the emerging seedlings were transferred to plastic pots (7.5 cm in height × 10.5 cm in diameter; 0.4 kg capacity) containing HS Florestal substrate and sand (1:1 w/w) and kept under greenhouse conditions (27 ± 3°C, 70 ± 10% RH and a 12:12h L:D photoperiod) until two to three permanent leaves had fully developed (21 days after sowing). The plants were irrigated based on their water requirement (twice a day).

Rearing the Insects.

L. sativae larvae were collected from leaves of infested melon plants in plantations located in the district of Mossoró, Rio Grande do Norte, Brazil (04°03'53.7” S 40°53'34.0" W, altitude 32 m). A leafminer population was reared in the laboratory from adults that emerged from the collected larvae. The jack bean [*Canavalia ensiformis* (L.) (Fabaceae)] was selected as the host plant species to prevent pre-imaginal conditioning. Adult leafminers (at a sex ratio of 1:1) were transferred to wooden cages (100 × 100 × 100 cm) that were covered with voile and contained jack bean plants grown from seeds sown in 200-cell polystyrene trays and kept in a greenhouse at ambient temperature (27 ± 3°C, 70 ± 10% RH and a 12:12h L:D photoperiod) until showing sufficient leaf area to allow the leafminer to develop (10 days after sowing). The cages were kept in an insect rearing room (IRR) under controlled conditions (27 ± 2°C, 75 ± 10% RH and a 12:12h L:D photoperiod).

Olfactometry Bioassays.

The behavioural response of females of *L. sativae* to the volatiles of melon genotypes was obtained using a four-arm acrylic olfactometer (12 cm x 12 cm) (PETTERSSON, 1970) under controlled conditions (25 ± 1°C and 70 ± 5% RH). In order to guarantee the impartiality of the bioassay, a blank test was initially carried out with the four arms of the olfactometer containing only constitutive volatiles of the Goldex commercial hybrid.

Two experiments were carried out. In the first, an olfactometer arm with constitutive melon volatiles (treatment) was compared to three of the arms containing air (control); four bioassays were conducted: (1) constitutive volatiles of the Goldex commercial hybrid versus air; (2) constitutive volatiles of the CNPH 06-1047-343 genotype versus air; (3) constitutive volatiles of the CNPH 06-1047-333 genotype versus air, and (4) constitutive volatiles of the CNPH 06-1047-341 genotype versus air.

In the second experiment, constitutive volatiles of the Goldex commercial hybrid were compared with each of the genotypes under study (CNPH 06-1047-343, CNPH 06-1047-333 and CNPH 06-1047-341). For these tests, two arms of the olfactometer with Goldex were compared to two arms containing one of the other genotypes. Three bioassays were conducted: (1) Goldex commercial hybrid versus the CNPH 06-1047-343 genotype, (2) Goldex commercial hybrid versus the CNPH 06-1047-333 genotype, and (3) Goldex commercial hybrid versus the CNPH 06-1047-341 genotype.

To supply the leafminer with the constitutive volatiles, the pots with the melon seedlings containing substrate were completely covered with aluminium foil to block the release of volatiles from the substrate. The seedlings were then wrapped in polyester bags (100%, 27 cm x 41 cm) that were completely sealed with Teflon tape. The bags were connected to the olfactometer using silicone hoses. In the olfactometry system, air is passed through an air pump with flow meters set at 250 mL/min for each arm of the olfactometer. Twenty replications were conducted for each bioassay. For each replication, one adult female was tested after fasting for 24h. The female was released through an orifice of the olfactometer in a neutral area, and exposed to the volatiles for 10 minutes. With each replication, the olfactometer was rotated 90° to reduce any positional effect. The response of the female was considered when it went beyond the neutral area and opted for one of the arms of the olfactometer. The replication was cancelled whenever the female remained in the neutral area for the first five minutes.

The frequency and length of stay of *L. sativae* in the different arms of the olfactometer were recorded using the SOLF software (Result Management System for Olfatometry Bioassays) v7.0 (Fancelli et al. 2017). At the end of five replications, the olfactometer was cleaned (with neutral detergent, distilled water and 70% alcohol) and a new melon seedling was offered as a source of odour .

Data on the frequency or number of times the *L. sativae* entered the different arms of the olfactometer (treatment and control) were compared using a χ^2 test ($\alpha = 0.05$). In the first experiment, the frequency of each individual in the arms containing air (three) was compared with the expected fractions of 25% for the arm offering melon volatiles (one). In the second experiment, the frequency of each individual in the arms containing the constitutive volatiles of the Goldex commercial hybrid (two) was compared with the expected fractions of 50% for the arms offering constitutive volatiles of the other melon genotypes (two).

Data on the time *L. sativae* remained in the arms containing the constitutive melon volatiles were submitted to the T-test ($\alpha = 0.05$), as described by Hegde et al. (2011) and Sobhy et al. (2017), for the arms containing air, mean values were obtained for the tests (Togni et al. 2010; Hegde et al. 2011). All the analyses were performed using the SAS statistical software (SAS Institute, 2019).

Extracting Volatiles from Melon Leaves.

The extraction of volatiles was performed by solidphase microextraction using the HS-SPME (Headspace Solid Phase Microextraction) method using the 1cm DVB/Car/PDMS (Divinylbenzene/Carboxen/ Polydimethylsiloxane) 50/30 fber. The fber was exposed to the vial headspace containing approximately 1g of leaf sample at 30°C for 15 min after the stabilization period conducted at 30°C for 30 min. For each melon genotypes (CNPH 06-1047-343, CNPH 06-1047-333, CNPH 06-1047-341 and Goldex), four replicates (plants) were used for sample withdrawal. Coupled GC-MS (Gas chromatography–mass spectrometry) analysis was performed on an Agilent model GC-7890B/MSD-5977 A (quadrupole) instrument with electron impact at 70 eV, HP-5MS methylpolysiloxane column (30 m x 0.25 mm x 0.25 µm, Agilent), helium carrier gas with 1.00 mL.min-1 (7.1 psi) fow and constant linear velocity of 36.3 cm.s-1, injector temperature 260°C, detector temperature 150°C, transfer line temperature 280°C. Chromatographic oven programming: initial temperature of 40°C, with a heating ramp from 7°C.min-1 to 260°C for 5 min at the end of the run. The identifcation of compounds was performed by analyzing the fragmentation patterns displayed in the mass specterwith those present in the database provided by the equipment (NIST version 2.0 of 2012-243,893 compounds), and from literature data.

Survival of Larvae and Pupae.

Six plants from the same genotype, each with three fully developed leaves (21 days after sowing), were transferred to a separate wooden cage (100 × 100 × 100 cm) covered with voile. Each cage received 24 L. sativae female adults (8 insects/plant), of up to 48 h in age (Celin et al. 2017), starved for 24 h, and previously maintained under controlled conditions for 24 h (27 ± 2°C .75 ± 10% RH and 12:12 h L:D photoperiod). The plants were kept under controlled conditions (27 ± 2ºC, 75 ± 10% RH and 12-hour photophase) until the leafminers emerged, and the number of emerged leafminers, pupae and adults was then quantified. Survival of the larvae and pupae was calculated as per Equations 1 and 2, respectively.

Larval survival (%) =100 (number of pupae/number of larvae) Eq. 1

Pupal survival (%) = 100 (number of emerged adults/number of pupae) Eq. 2

The larval and pupal viability data did not show a normal distribution, and were therefore submitted to the non-parametric Kruskal-Wallis test, followed by the post hoc DSCF (Dwass-Steel-Critchlow-Fligner) test at a significance level of 0.05 using the NPAR1WAY procedure of the SAS software (SAS Institute Inc, 2019).

Extracting Chemical Substances from the Ethanol Extract of the Melon Leaves.

Seedlings of the genotypes were grown in a greenhouse for 21 days, the time necessary for the leaves to completely expand. The extracts were prepared by liquid-liquid partition in an ultrasonic bath; 50 mg of plant material, weighed after drying and ground in triplicate, was weighed in test tubes, 4Ml hexane was added to eliminate interference. The material was homogenised in a vortex for 1 minute and then placed in an ultrasonic bath for 20 minutes at a fixed power of 135W with 4 Ml of an ethanol/water solution (70:30), the tubes were then added and remained in the ultrasonic bath for 20 minutes to extract the compounds of interest. The tubes were centrifuged for 10 minutes at 3000 rpm to facilitate separation of the partition. A 1 Ml aliquot of the lower (ethanolic) phase was removed using a Pasteur pipette, filtered in a 0.20 µm PTFE filter, collected in vials and stored in an ultra-freezer (-80°C) for further analysis by ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UPLC-QTOF-MS^E).

Phenolic Analysis.

To quantify the total phenolics in the ethanol extracts, the Folin-Ciocalteu method was used, with modifications. Each extract was solubilised in an ethanol/water mixture and added to 10-ml flasks in triplicate. A test tube containing 0.5 ml of each solubilised extract was then used.

For the blank, 0.5 ml of 10% ethanol was used. Then, 0.5 ml of Folin-Ciocalteu reagent was added to each tube, shaken in a vortex, and, after 3 minutes, 0.5 ml 20% anhydrous sodium carbonate solution (Na2CO3) was added, topping up the volume to 5 ml with 3.5 ml distilled water. Solutions of the extracts were left to react for 90 min in the absence of light.

Absorbance readings were taken using a Carry spectrophotometer at a wavelength of 725 nm, comparing the sample with the blank. The calibration curve was calculated, and for each concentration point, the calibration curve was constructed, and the straight-line equation obtained. The mean absorbance value for each extract was then determined from the mean value of the triplicates.

The data for total phenolic concentration were tested for normality by the Shapiro-Wilk test and for homoscedasticity by Levene's test. The difference between the genotypes for total phenolic concentration was verified by analysis of variance, with multiple comparison of the mean values by Tukey's test at 0.05, using the GLM procedure of the SAS software (SAS Institute Inc, 2013).

The chemical profiles found using the UPLC-QTOF-MSE system were analysed with the MassLynx software (v4.1); chemical compounds in the melon leaves, particularly the phenolic compounds, were then identified. The analysis was carried out using an Acquity UPLC system (Waters, USA) coupled to a Xevo Quadrupole and Time-of-Flight mass spectrometer (Q-TOF, Waters). The separations were performed in a C18 column (Waters Acquity® UPLC C18 - 150 mm × 2.1 mm, 1.7 µm). A 2 µL aliquot of phenolic extract was submitted to an exploratory gradient with the mobile phase comprising deionised water (A) and acetonitrile (B), both containing formic acid (0.1% v/v) under the following conditions: 2-95% for 15 min, flow 400 µL min-1.

The spectrometric analysis was performed in negative and positive electrospray ionisation (ESI) mode, acquired in the 110 to 1200 Da range. In negative mode, the capillary voltage was set to 2800 V, cone voltage to 50 V, source temperature to 120ºC, desolvation temperature to 350ºC, gas-cone flow to 20 Lh-1 and desolvation gas flow to 500 Lh-1. In positive mode, the parameters were as follows: capillary voltage, 3200 V; cone voltage, 35 V; source temperature, 120°C; desolvation temperature, 350°C and desolvation gas flow, 500 Lh-1. The acquisition mode was MSE, and the system was controlled using the MassLynx 4.1 software (Waters Corporation).

To analyse the various compounds identified in the genotypes under study, hierarchical cluster analysis was carried out, resulting in the visualisation of a heat map. The area of the 10 leaf compounds was visualised using the GENE-E software for extract recognition, with classification in rows and columns. The Pearson correlation distance method using full linkage was applied to cluster the retention times (rows) and measure the proximity of the samples (columns). The result illustrates a 3D dendrogram (heat map), in which the red colour represents the highest relative concentrations, light blue the intermediate concentrations, and dark blue the lowest relative concentrations.

Results

Olfactometry Bioassays.

The females of *L. sativae*, when exposed to volatiles of the melon genotypes compared to air, showed significant differences in the frequency of entry to the olfactometer arms ($\chi^2 > 4.08$; df=1; $P < 0.04$), showing a higher frequency in

arms containing volatiles of the genotypes (Figure 1a). For residence time, females of *L. sativae* remained longer in the arm containing volatiles of the Goldex commercial hybrid (t= 3.47; df= 23.8; P= 0.002) than in the arms containing air (Figure 1b). In contrast, the residence time of the insects was not significantly different for the CNPH 06-1047-343, CNPH 06-1047-333 and CNPH 06-1047-341 genotypes compared to air (P>0.23) (Figure 1b).

The adults of *L. sativae* showed a higher frequency for the arm containing volatiles of the Goldex commercial hybrid than of the CNPH 06-1047-333 and CNPH 06-1047-341 genotypes (χ^2 > 5.8; df=1.0; P< 0. 01) (Figure 2a). The insects remained longer in the odour of the Goldex commercial hybrid than in plants of the four CNPH 06-1047-343 (t = 2.38; df=38.0; P=0.02) and CNPH 06-1047-341 (t = 5.26; df=40.0; P<0.01) genotypes (Figure 2b). The other treatments showed no difference (P> 0.06) (Figure 2a,b).

Figure 1. (Insert Figure 1 here)

Figure 2. (Insert Figure 2 here)

Volatile Compounds.

By analysing the volatile profiles of the melon genotypes, it was possible to identify eight volatile organic compounds (Table 1). In the Goldex commercial hybrid, the major compounds with a volatile percentage greater than 1% were: (E)-β-ocimene, acetic acid, (Z)-β-ocimene, allo-ocimene, neo-allo-ocimene and linalool.

Table 1
Characterised compounds and volatile composition (%) from the leaves of melon genotypes using SPME-GC/MS.

RI_{calc} [a]	RI_{lit} [b]	Compound	Genotypes			
			GOLDEX (%)	CNPH 06-1047-343(%)	CNPH 06-1047-333(%)	CNPH 06-1047-341 (%)
>800	536	Acetic Acid	5.59	31.56	39.43	22.02
854	859	(Z)-3-hexen-1-ol	0.22	-	-	-
933	932	α-pinene	0.23	-	-	-
1036	1032	(Z) -β-ocimene	3.14	-	-	5.11
1046	1044	(E)-β-ocimene	46.29	-	-	6.82
1115	1088	linalool	1.36	-	-	-

Only acetic acid was identified in the volatile composition of the CNPH 06-1047-343 and CNPH 06-1047-333 melon genotypes. In the CNPH 06-1047-341 genotype, acetic acid, (E)-β-ocimene and (Z)-β-ocimene were identified as major compounds.

Table 1. (Insert Table 1 here)

Survival of Larvae and Pupae.

The larval and pupal viability of *L. sativae* in melon genotypes differed significantly (Figure 3). The greatest larval and pupal viability was seen in the Goldex commercial hybrid followed by the CNPH 06-1047-341 and CNPH 06-1047-343

genotypes (Figure 3a,b). The lowest viability was found in CNPH 06-1047-333 (Figure 3a,b).

Figure 3. (Insert Figure 3 here)

Phenolics.

The amount of total phenolic compounds varied significantly between the melon genotypes. The highest amount of phenolic compounds was found in the CNPH 06-1047-333 genotype (18.68 mg/g). A significant amount was found in the CNPH 06-1047-343 genotype (13.16 mg/g) and in the Goldex commercial hybrid (susceptible) (11.97 mg/g), while CNPH 06-1047-341 showed a low amount of phenolic compounds (8.27 mg/g) (Figure 4).

Figure 4. (Insert Figure 4 here)

Three phenolic compounds were identified: hydroxybenzoic-hexoside acid, ferulic acid and trihydroxy-octadecadienoic acid. The compounds and structures identified in the samples of the melon-leaf extracts are shown in Table 2.

Table 2
Chemical compounds in the melon-leaf extract by UPLC-QTOF-MSE using negative mode, identified using the MassLynx 4.1 software (Waters Corporation) and data from the literature.

Peaks	Rt (min	$[M-H]^-$ Observed	$[M-H]^-$ Calculated	Ion Fragments (MS/MS)	Empirical Formula	Error (ppm)	Name	References
1	0.88	195.0499	195.0505	-	$C_6H_{11}O_7$	-3.1	Gluconic acid	Ozarowski et al. 2018
2	0.99	133.0135	133.0137	-	$C_4H_5O_5$	-1.5	Malic acid	Ozarowski et al. 2018
3	1.01	191.0185	191.0192	111	$C_6H_7O_7$	-3.7	Citric acid	Rodríguez-pérez et al. 2013
4	2.29	380.1566	380.1577	146	$C_{16}H_{22}N_5O_6$	-1.1	Zeatin hexoside	Rodríguez-pérez et al. 2013
5	2.71	203.0826	203.0821	116	$C_{11}H_{11}N_2O_2$	2.5	Tryptophan	Ozarowski et al. 2018
6	2.94	299.0759	299.0759	137	$C_{13}H_{15}O_8$	-2.7	Hexosideo-hydroxybenzoic acid	Ozarowski et al. 2018
7	3.36	193.051	193.0501	134	$C_{10}H_9O_4$	4.7	Ferlic acid	Rodríguez-pérez et al. 2013
8	3.9	593.1506	593.1506	473; 413; 293	$C_{27}H_{29}O_{15}$	0	Vitexin 2"-O-glucoside	Ozarowski et al. 2018
9	4.18	467.2084	467.207	205; 163	$C_{27}H_{31}O_7$	3	Not identified	-
10	5.26	577.2291	577.2285	471; 442	$C_{29}H_{37}O_{12}$	1	Not identified	-
11	5.35	361.1367	361.1359	145; 127	$C_{13}H_{21}N_4O_8$	2.2	Glutamine derivative	Ozarowski et al. 2018
12	6.28	693.3204	693.3204	205; 161	$C_{28}H_{53}O_{19}$	3.3	Not identified	-
13	6.83	327.2163	327.2171	229; 211	$C_{18}H_{31}O_5$	-2.4	Trihydroxy octadecadienoic acid	Rodríguez-pérez et al. 2013
14	8.4	177.067	177.0664	116	$C_9H_9N_2O_2$	3.4	Not identified	-

Table 2. (Insert Table 2 here)

From the cluster analysis that resulted in visualisation of the heat map, it could be seen that the hydroxybenzoic-hexoside acid concentration was greater in the Goldex commercial hybrid, while the ferulic acid concentration was greater in both the Goldex commercial hybrid and the CNPH 06-1047-343 genotype. Trihydroxy-octadecadienoic acid was highly concentrated in the CNPH 06-1047-343 genotype, compared to each of the genotypes under study (Figure 5).

Figure 5. (Insert Figure 5 here)

Discussion

It is essential to understand how plant volatile compounds act in the host-selection of insect pests, as such knowledge can be of help in sustainable pest management (Li et al. 2014), and be useful in plant breeding programs, helping to identify cultivars the are resistant or tolerant to insect pests (Robbins et al. 2012, Uvah and Coaker 1984). From the first bioassay, it became clear that constitutive volatiles of both the Goldex commercial hybrid and the four melon genotypes under test are highly attractive to *L. sativae* females. Furthermore, it was possible to confirm the greater susceptibility of the Goldex commercial hybrid compared to the other hybrids, since the residence time of *L. sativae* females was greater for the volatiles of these plants than for air. As such, the volatile compounds of the Goldex commercial hybrid showed an arresting effect, i.e. they stimulated the insects to remain longer in the arms of the olfactometer containing the volatiles.

Although the CNPH 06-1047-333 genotype stimulated fewer visits, and CNPH 06-1047-343 a shorter residence time than the Goldex commercial hybrid, CNPH 06-1047-341 was the only one among the three genotypes under test that showed fewer visits and less residence time than the Goldex hybrid. In fact, *L. sativae* can distinguish and select specific hosts by recognising the volatile compounds (Zhao and Kang 2002). This information, together with the results of the present study, suggest not only that the Goldex hybrid is more susceptible to *L. sativae* than the other genotypes, but also that CNPH 06-1047-341 is the least susceptible genotype among all those tested. Despite a large number of volatile compounds from host and non-host plants of the leafminer having been identified (Wei et al. 2007), the behavioural responses of *Liriomyza* species apparently relate to 'key mixtures' of volatile compounds (Bruce et al. 2005).

Of the eight identified volatile organic compounds, only linalool, allo-ocimene, and neo-allo-ocimene were present in the most attractive genotype (Goldex hybrid) while they were absent from the less attractive genotypes (CNPH 06-1047-333, CNPH 06-1047-341, and CNPH 06-1047-343). These results suggest that the presence of linalool, allo-ocimene, and neo-allo-ocimene, whether alone or in double or triple combinations, is positively associated with attractiveness to *L. sativae*. This possibility is supported by Egonyu et al. (2013) and Wanjiku et al. (2014), who also identified the compounds allo-ocimene and neo-allo-ocimene in cashew leaves, and showed, using behavioural bioassays, that as mixtures these compounds are attractive to the insect-pest *Pseudotheraptus wayi* (Heteroptera: Coreidae) and their natural enemy *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae). In addition, olfactometry studies Guohui et al. (2005) have found that the isolated linalool attracted adult females of *L. sativae*.

The chemical profiles of the volatiles identified as unattractive for a particular insect pest are an indicator of resistant or tolerant plants (Robbins et al. 2012, Saraiva et al. 2022). It was found that despite responding to the volatiles of the CNPH 06-1047-343, CNPH 06-1047-333 and CNPH 06-1047-341 genotypes compared to air, *L. sativae* preferred and/or spent more time in the volatiles of the Goldex commercial hybrid compared to these genotypes. The low residence time of the insects in plant volatiles may suggest less oviposition (Fancelli et al. 2018). In fact, the CNPH 06-1047-343 and CNPH 06-1047-341 genotypes express antixenotic characteristics to the feeding and oviposition of *L. sativae*. Furthermore, CNPH 06-1047-341 and CNPH 06-1047-333 are antibiotics to the insect, as they support low rates of larval viability (Oliveira et al. 2021).

Differences in the chemical profiles of the melon genotypes under evaluation may explain the lower preference of *L. sativae* for the volatiles of CNPH 06-1047-343, CNPH 06-1047-333 and CNPH 06-1047-341. These genotypes have a lower amount of volatile compounds than the Goldex commercial hybrid; in addition, they have higher concentrations of acetic acid, which is very possibly related to the repellency of *L. sativae* for these genotypes. The main form of action of acetic acid is to repel insect pests, such as in the control of whitefly *Bemisia tabaci* (Gennadius, 1889)

(Hemiptera: Aleyrodidae) in melon plants (Azevedo et al. 2005). Additionally, Ren et al. (2008) determined the oviposition-repellent effects of bitter-melon oil (*Momordica charantia*) in adults of *L. sativae*, and attributed its deterrence to the chemical content, including acetic, formic and benzoic acid. It should be noted that although (Z)-β-ocimene has been identified in the attractive genotype (the Goldex commercial hybrid), the compound has been found in higher concentration in CNPH 06-1047-341. It is known that in insects, (Z) -β-ocimene can cause stimuli that vary between attractiveness and repellency (Andrade et al. 2016, Egonyu et al., 2013, Wanjiku et al. 2014), and is present in considerable quantities in the genotypes of *Citrus* sp. less preferred by the psyllid *Diaphorina citri*, Kuwayama (Hemiptera: Psylidae) (Andrade et al. 2016). There is therefore evidence that selections of genetic material obtained from CNPH 06-1047-343, CNPH 06-1047-333 and CNPH 06-1047-341 can contribute to melon breeding programs that offer resistance to *L. sativae*.

In accordance with the olfactometry tests and our previous results (Oliveira et al., 2021), in this study with no-choice tests, we found that the genotypes CNPH 06-1047-343, CNPH 06-1047-333, and CNPH 06-1047-341 showed resistance against *L. sativae*, as they supported reduced rates of larval and pupal viability of the insect. The main effects of antibiosis are immature mortality, reduced growth and weight, deformation, and an increase in the life cycle of the insect (Boiça Junior and Jesus 2009). When plant resistance is characterised by antibiosis, this in turn is related to chemical factors produced by the secondary metabolism of the plant, which negatively affect insect biology, interfering with its cycle of development, reproduction and survival (Smith, 2005; Boiça Junior and Jesus 2009). Studies with mango genotypes (*Mangifera indica* L.) have shown that plants with a lower total phenol content are susceptible to attack by the fruit fly, *Bactrocera dorsalis* Hendel (Tephritidae: Diptera). Those with a higher total phenol content are resistant to attack by the insect (Verghese et al. 2012).

The same authors observed in the field that susceptible plants suffered infestations of between 22% and 64%, while no infestation was seen in resistant plants. A higher amount of phenols was found in eggplant genotypes (*Solanum melongena* L.) resistant to whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Hasanuzzaman et al. 2018). In these genotypes there was less oviposition, fecundity or viability of nymphs and adults compared to susceptible genotypes, which is probably related to the higher amount of total phenols in the chemical composition of the genotype profiles (Hasanuzzaman et al. 2018). In the present study, the CNPH 06-1047-333 and CNPH 06-1047-341 melon genotypes presented, respectively, the highest and lowest total phenolic content among the genotypes and hybrids under study. However, no significant difference was seen between these two genotypes regarding larval and pupal viability in *L. sativae*. This lack of consistency between the results for phenolic content and pest viability indicates a lack of relationship between the presence of phenols and the antibiotic resistance of melon genotypes to *L. sativae*. For the avocado, *Persea americana* Mill., genotypes with higher levels of total phenolics generally had a positive effect on the resistance of the armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) and a negative effect on *Copaxa multifenestrata* (Herrich-Schäffer) (Lepidoptera: Saturniidae) (Cumplido et al. 2021). Each species of herbivorous insect can therefore present different responses to the chemical composition of plants, requiring an analysis of each species of herbivorous insect for the genotypes of interest.

Evaluation of the phenolic profile of leaves of the melon genotypes revealed that the samples have very similar chemical profiles, with small quantitative differences, i.e. each of the genotypes under study has the same phenolic compounds in different concentrations. The production of compounds by the secondary metabolism of plants, is activated by biotic or abiotic stimuli (Tais and Zeiger 2015, Tremacoldi 2009), and as with identifying chemical substances in the present study, took place in leaves with no biotic or abiotic damage; possibly for this reason, the chemical profiles of the genotypes are similar, with small variations in concentration.

The p-hydroxybenzoic compound is a phenolic glycoside derived from salicylates. Several benzenoids derived from salicylic acid, such as benzoic acid, have been linked to constitutive plant resistance to insect pests (Wang et al. 2017). Trihydroxy octadecadienoic acid, which is highly concentrated in the CNPH 06-1047-343 genotype compared to the genotypes under study, may be aiding the resistance of this genotype. Octadecadienoic acid is a conjugated linoleic acid (Yurawecz et al. 1995) and may be an antioxidant (Park et al. 1997). Phenolic compounds are activated by oxidation, preventing the consumption of plant tissue and inhibiting digestion in insect pests (Boerjan et al. 2003, Kulbat 2016).

The compounds, gallic acid, ferulic acid and cinnamic acid, were identified in leaves of the sugarcane cultivar (*Saccharum* sp.) susceptible to the sugarcane borer Diatraea saccharalis (Lepidoptera: Crambidae), but were not found in the composition of the resistant cultivar (Tavares 2016). In the resistant plant, ferulic acid was produced only under herbivory, while for the susceptible cultivar, ferulic acid was already present in the plant composition, with no compound being induced in the susceptible plant after herbivory (Tavares 2016). These results demonstrate the importance of studies on both constitutive and induced phenolic plant profiles.

Hydroxycinnamic acids, such as p-coumaric acid and ferulic acid, can act as reservoirs of phenylpropanoids for the biosynthesis of lignin and suberin. The increased biosynthesis of lignin in the root tissue after herbivory is possibly related to a strengthening of the cell wall, which increases the protective barrier of the plant (Tavares 2016), and may also occur in the leaf tissue. Such acids are precursors of other classes of plant defence compounds, such as flavonoids and tannins, among others, which can act as astringents and/or deterrents (Solecka 1997).

Ferulic acid binds to the cell wall of plants via a coupling reaction catalysed by peroxidase, resulting in the production of diferulic acid (Markwalder and Neukom 1976). This compound was identified in the present study at the highest concentration in the Goldex commercial hybrid and in the CNPH 06-1047-343 genotype. Ferulic acid and its derivatives act by protecting plants from insect pests, viruses and fungi (Phelps and Young 1996). Diferulic acid, on the other hand, reinforces and intertwines the primary cell wall, aiding plant defence against insect pests and pathogens (Bartolome et al. 1997, Bervingson; et al. 1997). Diferulic acid increases resistance to insect pests in maize by making the cell wall of the leaves more rigid (Bergvinson et al. 1997). Ferulic acid can be linked to lignin, proteins and polysaccharides, interconnecting them covalently, with these cross-links having a negative effect on the digestibility of the cell wall (Du et al. 2009).

In this study, we concluded through olfactometry assays that all melon genotypes tested are attractive to females of *L. sativae*. However, the genotypes CNPH 06-1047-343, CNPH 06-1047-333, and especially CNPH 06-1047-341 are less attractive than the commercial hybrid Goldex. This suggests lower susceptibility of genotypes CNPH 06-1047-343, CNPH 06-1047-333, and CNPH 06-1047-341 to *L. sativae*. We also verified that the lower attractiveness of the genotypes is associated with the absence of linalool, allo-ocimene, and neo-allo-ocimene compounds, and possibly also a higher concentration of acetic acid in its volatile composition.

In plant resistance tests, we verified that the melon genotypes CNPH 06-1047-343, CNPH 06-1047-333, and CNPH 06-1047-341, showed reduced viability of pupae and larvae, characterized by antibiosis-like resistance to *L. sativae*. Nevertheless, we found that the total phenolic content present in the leaves of the genotypes did not influence this resistance. The phenolic compounds identified in all studied genotypes (in different concentrations) were hydroxybenzoic-hexoside acid, ferulic acid, and trihydroxy octadecadienoic acid. Although, further research must be carried out to identify the contribution of these compounds to the resistance or susceptibility of melon genotypes to *L. sativae*.

Declarations

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Conflict of Interest

The authors declare that they have no conflict of interest.

Availability of Data and Material

Not applicable.

Code Availability

Not applicable.

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Figures

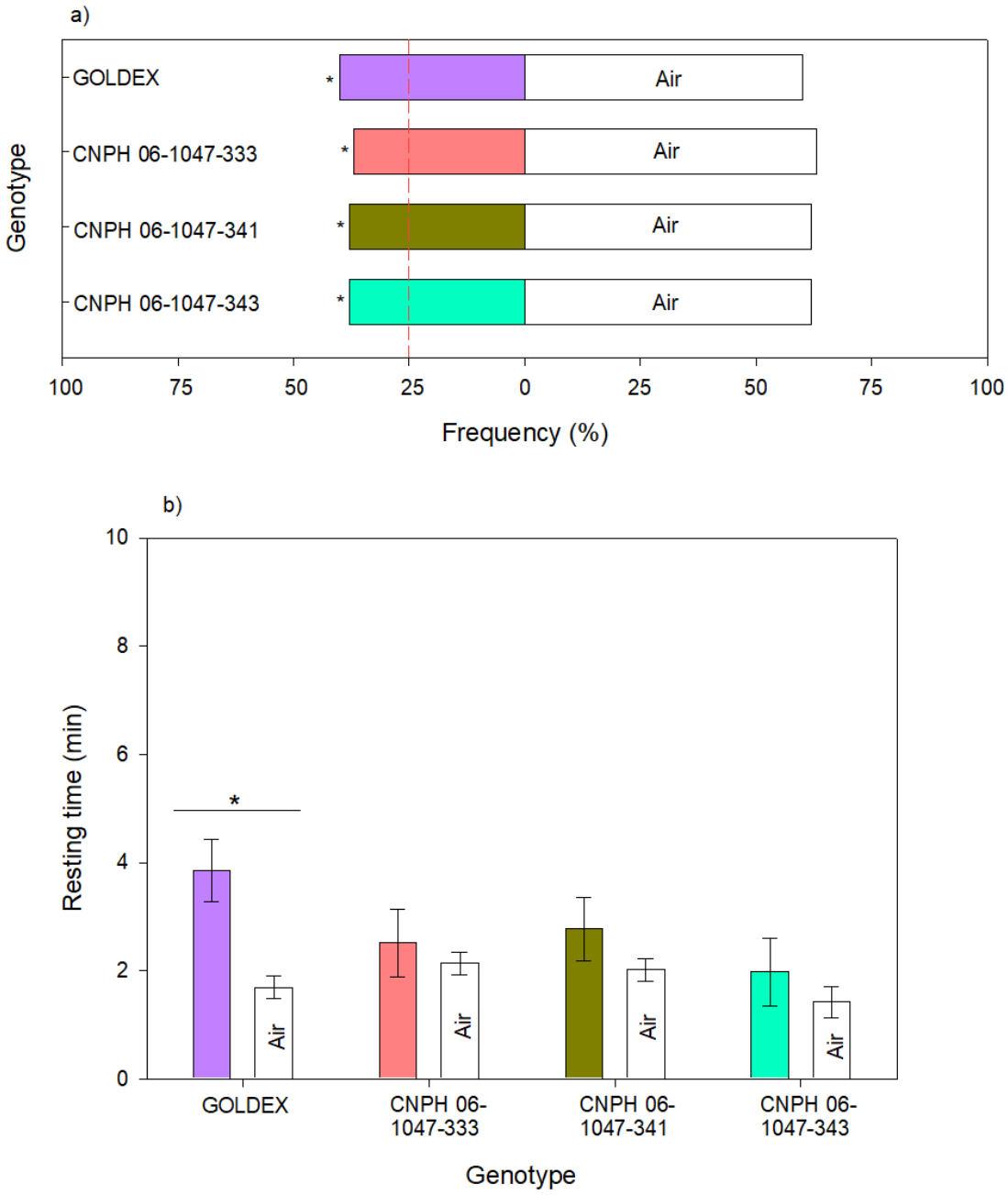

Figure 1

Frequency (number of entries in percentual) (a) and residence time (± standard error) (b) for *Liriomyza sativae* females in treated olfactometer arms (volatile compounds from melon plants) and the control (air) (line: 25% of the proportion expected for statistical significance (a); *: significant difference).

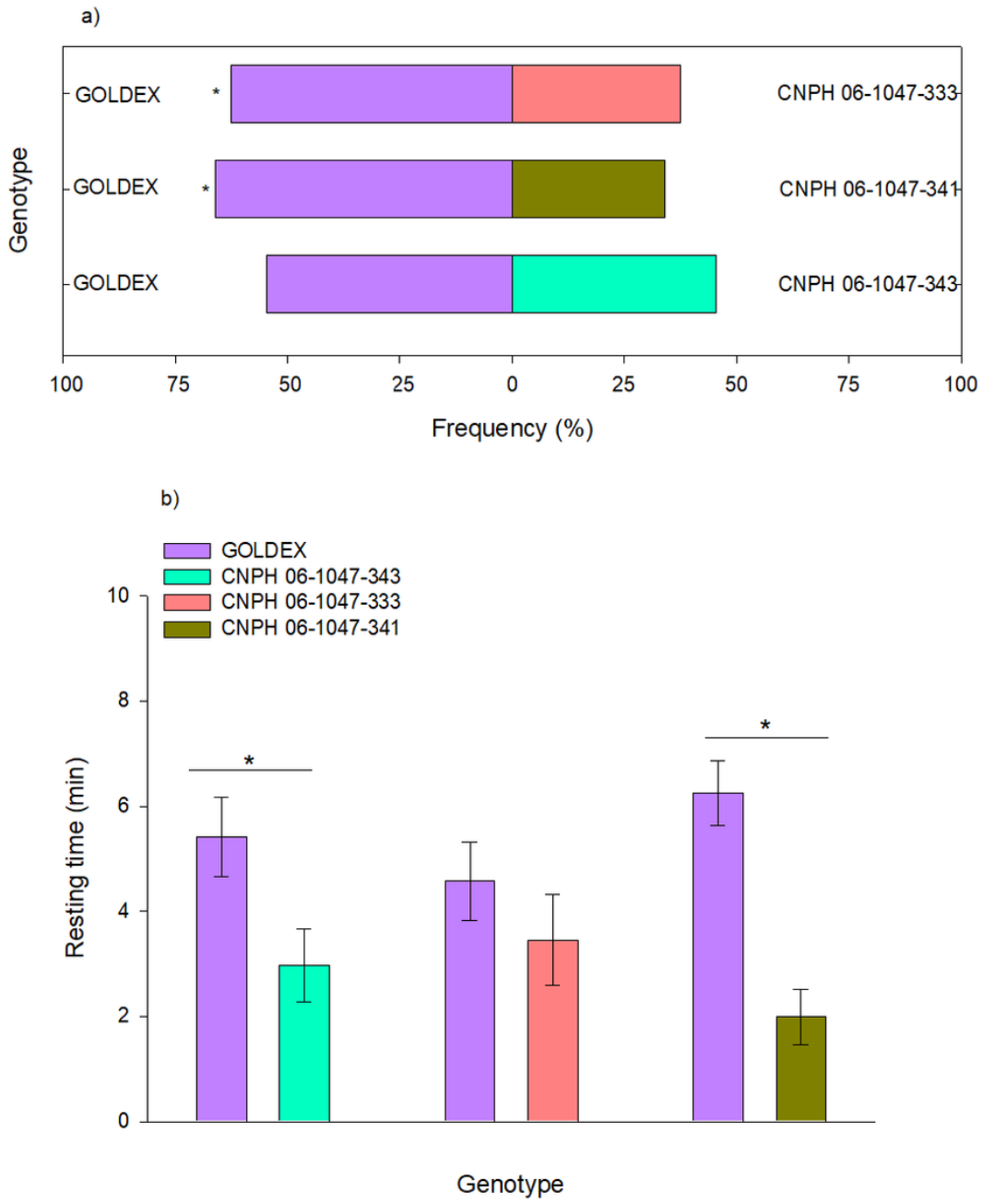

Figure 2

Frequency (number of entries in percentual) (a) and residence time (± standard error) (b) for *Liriomyza sativae* females in treated olfactometer arms treated with constitutive volatile compounds of melon genotypes versus constitutive volatiles of the Goldex commercial hybrid (*: significant difference).

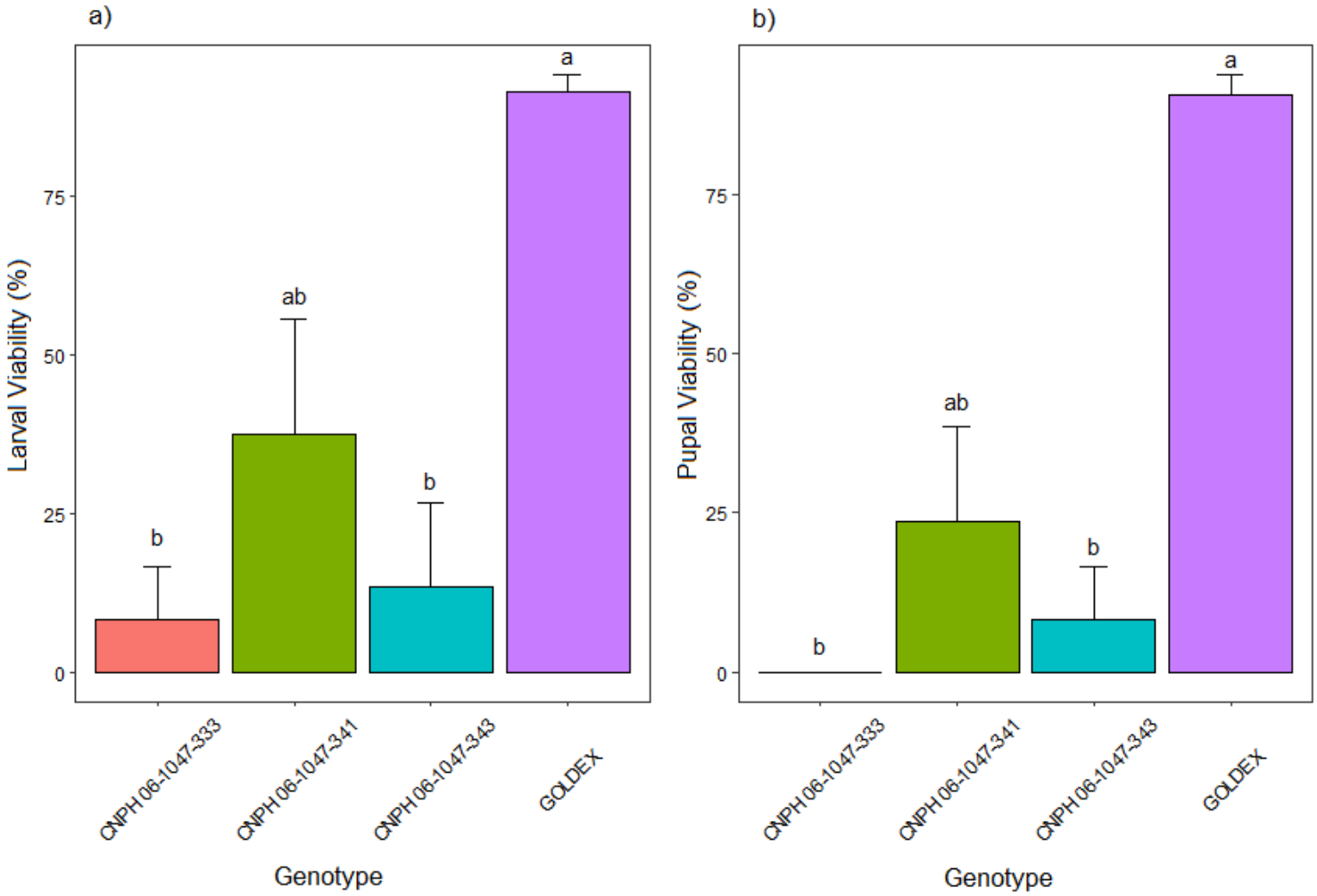

Figure 3

Larval (a) and pupal (b) viability of *Liriomyza* sativae in melon genotypes, using the Shapiro-Wilk test for normality and Levene's test for homoscedasticity of the variances, submitted to the non-parametric Kruskal-Wallis test, followed by the post hoc DSCF test (Dwass-Steel-Critchlow-Fligner), at a significance level of 0.05 using the NPAR1WAY procedure of the SAS software (SAS Institute Inc, 2019).

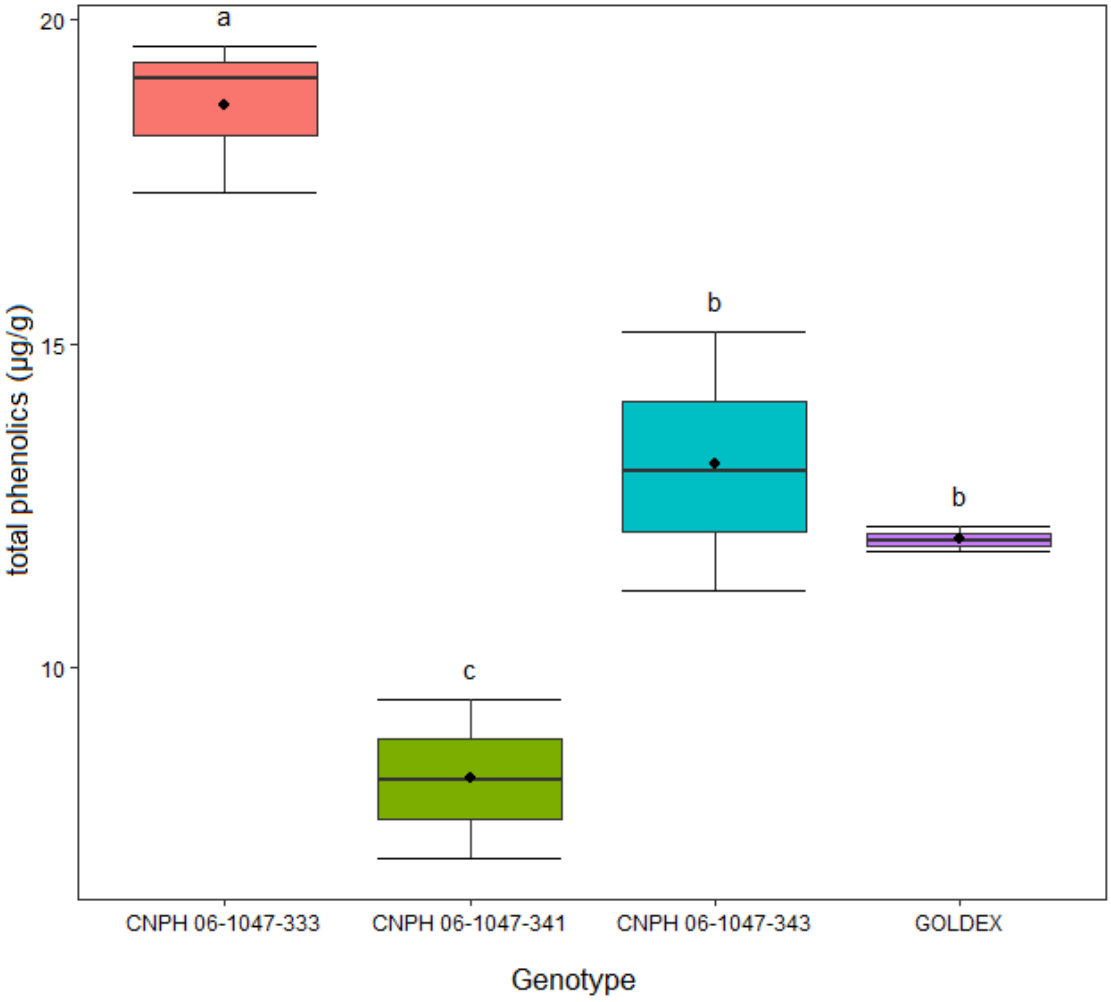

Figure 4

Levels of total phenolic compounds in melon genotypes (*Cucumis melo* L.) using the Shapiro-Wilk test for normality and Levene's test for homoscedasticity of the variances. The differences between genotypes for the total phenolic concentration weree verified by analysis of variance and multiple comparison of the mean values by Tukey's test at 0.05, using the SAS software (SAS Institute Inc, 2013).

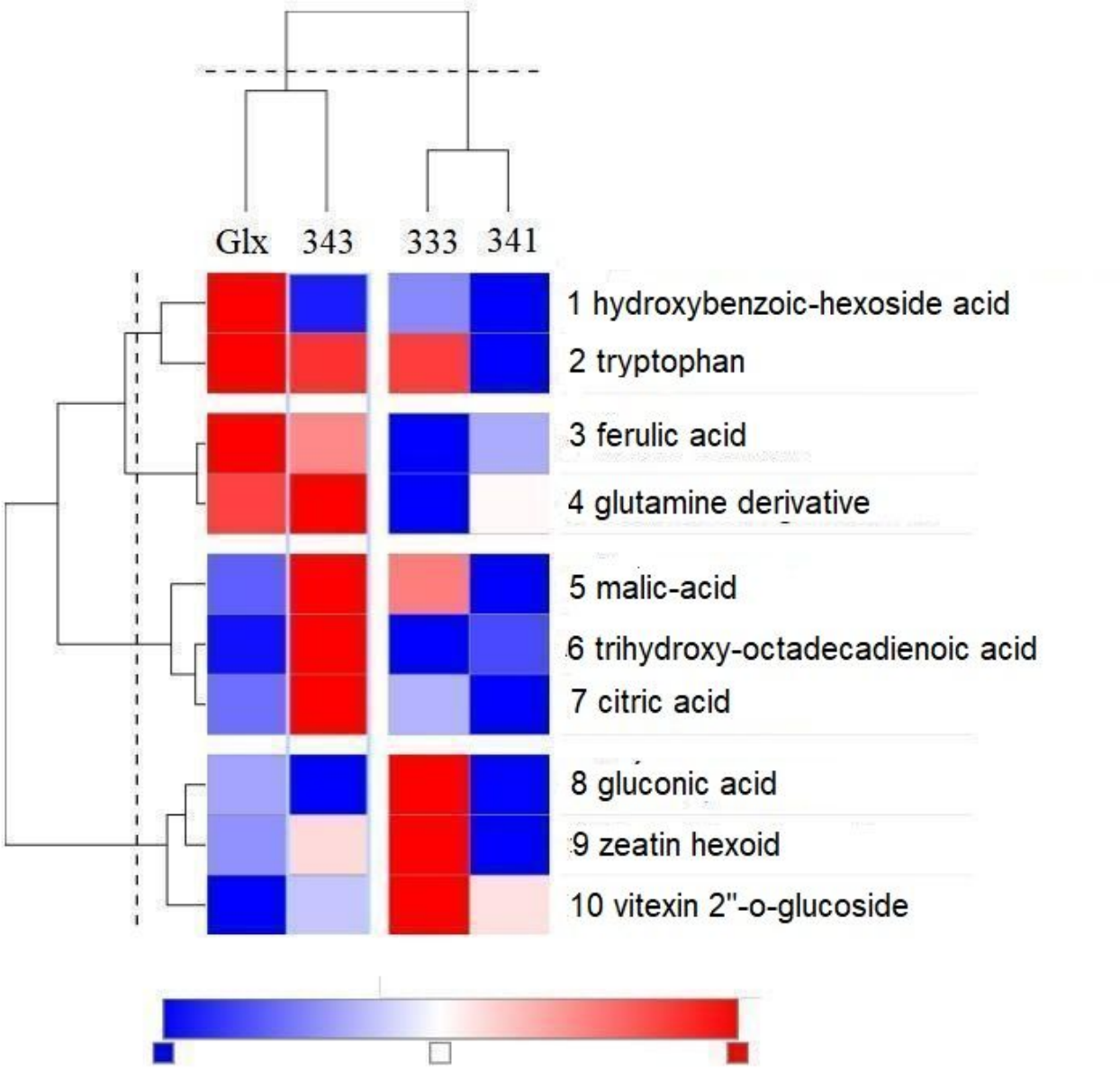

Figure 5

Dendrogram (3D) of the concentrations of the chemical compounds identified in the melon genotypes by means of the cluster method, resulting in the visualisation of a heat map. The red colour represents the highest concentrations, light blue the intermediate concentrations and dark blue the lowest concentrations.