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# Resistance of chickpea cultivars to *Chloridea virescens* (Lepidoptera: Noctuidae)

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**ABSTRACT.** Chickpea is a legume with nutrient-rich grains important for human feeding. Tobacco budworm, *Chloridea virescens* (Lepidoptera: Noctuidae), is one of the most major pests of chickpea (*Cicer arietinum*) in Brazil. This pest damages leaves, flowers, pods, and grains. Plant resistance to insects is an important tactic of pest management, which usually facilitates and reduce costs of implementing an Integrated Pest Management for farmers. Thus, this study aimed to evaluate the resistance in chickpea cultivars to *C. virescens*. Six chickpea cultivars were evaluated for antixenosis, initial antibiotic parameters, and behavior under field conditions. The cultivars BRS Kalifa and BRS Cícero were less attractive in a free-choice test, while Jamu 96 and BRS Kalifa were less attractive in a no-choice test. BRS Kalifa and BRS Toro leaves had a higher trichome density. Jamu 96 and BRS Toro had higher contents of oxalic and malic acids. *C. virescens* larvae in BRS Cícero, BRS Toro, and BRS Kalifa showed the lowest weights. Jamu 96 pods were the least damaged, and BRS Aleppo and Jamu 96 had the highest yields. The chickpea cultivars Jamu 96 and BRS Aleppo, which had resistance levels and mechanisms, can be used in integrated pest management programs to control *C. virescens*.

**Keywords:** *Cicer arietinum*; plant resistance to insects; pulse crop; tobacco budworm.

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## Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown worldwide and widely used in human nutrition due to its protein value (Jukanti, Gaur, Gowda, & Chibbar, 2012). Its annual cultivation adapted to dry and mild climate and its low production cost make it an excellent option for crop rotation systems. Thus, chickpea has shown great potential for cultivation in Brazil (Artiaga, Spehar, Silva Boiteux, & Nascimento, 2015; Nascimento, Silva, Artiaga, & Suinaga, 2016).

Insect pests have been responsible for chickpea low yields. Larvae, which damage plants at vegetative and reproductive stages, comprise the most destructive stage. Chickpea average losses vary from 30 to 40% and have the potential for full yield loss (Patanker et al., 2001; Sarwar, Ahmad, & Toufiq, 2009; Wakil, Ashfaq, Ghazanfar, Afzal, & Riasat, 2009).

*Chloridea virescens* (Fabricius 1781) (Lepidoptera: Noctuidae) is a key pest in cotton crops but considered a polyphagous species, attacking 37 other plant families (Fitt, 1989; Blanco, Vargas, Lopez, & Kaufmann, 2007). In South America, *C. virescens* damage has been reported in chickpea crops, causing severe problems when they are grown in succession cropping systems (Murua et al., 2016). Females lay eggs on leaves and flowers. When neonate larvae hatch from eggs, they feed on leaves during their first stages. Afterwards, later instars migrate to pods, damaging grains (Bajia & Bairwa, 2015; Golla, Rajasekhar, Sharma, Hari Prasad, & Sharma, 2018).

The main tactic used for *C. virescens* control has been spraying chemical insecticides. However, no insecticides have been registered for its control in chickpeas (Agrofit, 2020). Moreover, excessive use of chemical insecticides could lead to the selection of resistant insects (Roe et al., 2010). In this sense, Integrated Pest Management (IPM) strategies may reduce the need for insecticides (Viteri, Sarmiento, Linares, & Caberna, 2019).

Plant resistance to insects (PRI) is an important management tactic, which usually facilitates and reduce costs of implementing IPMs for farmers. Plant resistance effects are often constant and cumulative on target insects, negatively affecting pest biological parameters without harming the environment. In general, PRI is compatible with other control means such as chemical and biological controls (Boiça Júnior, Bottega, Souza, Rodrigues, & Michelin, 2015; Baldin, Venframim, & Lorenção, 2019).

Three PRI categories have been proposed: antixenosis, antibiosis, and tolerance. Antixenosis is usually associated with trichomes, leaf color changes, or volatile compounds, which affect insect oviposition, feeding, and sheltering (Smith, 2005; Seife, Visser, & Yuling, 2013). Whereas antibiosis is manifested by plant chemical constituents and their effect on insect biology and/or physiology such as life cycle prolongation, body deformation, sexual proportion changes, and larva and pupa weight reductions (Sharma, Pampapathy, Lanka, & Ridsdill-Smith, 2005; Souza et al., 2014; Almeida, Silva, Paiva, Araujo, & Jesus, 2017). Likewise, tolerance is the ability of plants to resist or recover production of new vegetative or reproductive structures after insect damages (Smith, 2005; Seife et al., 2013; Paiva et al., 2018).

Larvae have different feeding patterns in chickpeas due to their morphological traits such as trichome (Ascensão, Marques, & Pais, 1995; Hossain, Haque, & Prodhan, 2008; Golla et al., 2018) and exudates with organic acids, e.g., malic and oxalic acids (Narayanamma, Sharma, Vijay, Gowda, & Sriramulu, 2013; Rachappa, Teggelli, Yelshetty, & Amaresh, 2019). *Helicoverpa armigera* (Hübner 1808) (Lepidoptera: Noctuidae) females laid fewer eggs on the wild relative chickpeas IG 70012 and IG 72933 under multi-choice and non-choice conditions, respectively (Golla et al., 2018). Also, the genotypes IG 70012, IG 70022, IG 70018, IG 70006, PI 599046, PI 599066 (*Cicer bijugum*), IG 69979 (*Cicer cuneatum*), PI 568217, PI 599077 (*Cicer judaicum*), and ICCW 17148 (*Cicer microphyllum*) showed antibiosis to *H. armigera* (Golla et al., 2018). However, studies on resistance of chickpea cultivars to insects are limited in Brazil. Therefore, our objective was to evaluate such resistance (antixenosis and antibiosis) in six chickpea cultivars to *C. virescens*.

## Material and methods

### Plant material

The chickpea cultivars used (BRS Aleppo, BRS Cícero, BRS Cristalino, BRS Toro, BRS Kalifa, and Jamu 96) were obtained from the Embrapa Hortaliças (Brasília, Distrito Federal, Brazil). These are adapted to cultivation in the Brazilian Cerrado conditions (savanna). Seeds were sown in 5-L pots containing substrate (3:1:1 - soil, sand, organic compost) corrected and fertilized according to recommendations for chickpeas (Nascimento et al. 2016). The plants were kept in a greenhouse under natural light and temperature conditions, and daily irrigation. Experiments were carried out at the Instituto Federal Goiano, Campus Urutaí, Urutaí, Goiás State, Brazil, under laboratory and field conditions.

### Insect rearing

*Chloridea virescens* colony was established in the laboratory from larvae obtained from the Laboratory of Plant Resistance to Insects, Universidade Estadual Paulista (FCAV), Campus Jaboticabal, São Paulo State, Brazil.

Pupae were sexed and separated in couples (15 males and 15 females), maintained in polyvinyl chloride (PVC) cages (15 cm diameter × 20 cm height) for emergence and mating. Adults were fed 10% honey solution, methylparaben, and vitamin solution (Armes, Bond, & Cooter, 1992) and kept in the same PVC cages covered internally with a sheet of paper (oviposition substrate).

Eggs of *C. virescens* were collected and transferred to plastic pots (14 cm diameter and 9 cm height) containing an artificial diet. Second instar larvae were individualized in B16 PET trays (CM&CM Comercio de Plásticos, São Paulo, São Paulo State, Brazil) and fed an artificial diet until the pupal stage (Greene, Leppla, & Dickerson, 1976).

The insects were maintained under controlled conditions ( $25 \pm 2^\circ\text{C}$ ,  $70 \pm 10\%$  relative humidity, and a 12:12h light/dark photoperiod) during all development stages.

### Antixenosis and trichome density

In a non-choice test, leaflets of each cultivar (40 days after plant emergence [DAE]) were given to second-instar larvae of *C. virescens* in the B16 PET trays (CM&CM Comercio de Plásticos, São Paulo, São Paulo State, Brazil), with a moistened filter paper. After larvae release, the average number of insects feeding on each

cultivar was evaluated at 1, 3, 5, 10, 15, 30, 60, 120, 180, 360, 720, and 1,440 min. This experiment was conducted as a fully randomized design, with six treatments and 10 replicates.

In a free-choice test, leaflets of each cultivar were distributed equidistantly within a circular arena (14 cm diameter and 2 cm high) on a moistened filter paper. Only one second-instar larva of *C. virescens* was released per cultivar. A randomized block design with six treatments and 10 replicates were used for the same evaluation times described previously.

In both tests, all leaflets were photocopied (Xerox work center 3220, São Paulo, São Paulo State, Brazil) to determine the leaf areas consumed by caterpillars (CLA), using the ImageJ version 1.46R software.

Glandular and non-glandular trichome numbers were determined at 40 DAE, in two 9-mm<sup>2</sup> points on the adaxial part of each leaflet, parallel to the central vein. Counting was performed using a stereomicroscope (Bel Photonics, Model Bio SSI, Italy) at 40x magnification. This study was organized in a fully randomized design, with six treatments and 10 replicates.

### Antibiosis and oxalic and malic acid contents

Second-instar *C. virescens* larvae were set in groups of five and placed into 4-L glass jars, with 2 chickpea branches (30 DAE). These were put into 50-mL plastic containers filled with water for leaf turgor maintenance. The branches were changed twice a week to avoid contamination. Larval weight and mortality were recorded ten days after infestation. This experiment was arranged in a randomized block design, with six treatments and 10 replicates.

Leaves of chickpea cultivars were collected at 30 DAE for determination of malic and oxalic acid contents by titratable acidity method, with 0.1-N NaOH and phenolphthalein (AOAC, 2010).

### Field experiment

The experimental area has been cultivated for over ten years. The local soil is a Red Latosol (Oxisol). Soil analysis at the 0.0-0.2 m depth layer showed the following physical-chemical properties: pH in water of 5.7; K, Ca, Mg, H + Al of 0.0, 30.0, 2.0, 7.0, 0.0, 4.0, and 2.6 cmol<sub>c</sub> dm<sup>-3</sup>, respectively; P of 53 mg dm<sup>-3</sup>; organic matter of 1.2 dag kg<sup>-1</sup>; S, Zn, B, Cu, Fe, Mn, and Mo of 5.6, 5.6, 0.12, 1.8, 47.3, 27.0, and 0.07 mg dm<sup>-3</sup>, respectively; and grain-size distribution of 45, 16, and 39 dag kg<sup>-1</sup> clay, silt, and sand, respectively.

The soil was previously plowed and harrowed and then sown from August to November 2018. Seeds were distributed in rows 0.5 m apart at ten seeds per meter. During the experiment, air temperatures ranged from 16.2 to 36°C (25.7°C, on average) and relative humidity from 13 to 95.2% (60.5%, on average). Each plot had five rows (4-m long), totaling 10 m<sup>2</sup> total area and 06 m<sup>2</sup> useful area. Plants were fertilized with 300 kg ha<sup>-1</sup> 04-30-16 NPK formulation, and at 30 DAE with 150 kg ha<sup>-1</sup> N in urea form. Neither chemical insecticides nor fungicides were sprayed for pest control. Weeds were controlled by applying fomesafen (0.8 L ha<sup>-1</sup>) and fluazifop-p-butyl (0.5 L ha<sup>-1</sup>) at 20 DAE. The plots were irrigated whenever needed and cultural practices performed according to recommendations for chickpeas (Nascimento et al., 2016).

Evaluations started at 30 DAE after the first natural caterpillar infestation. Thereafter, they were made weekly at vegetative, flowering, and podding stages. Larvae were sampled using an adaptation of the cloth sampling method by Corrêa-Ferreira (2012). A sampling cloth was inserted between the two central rows in each plot, wherein 10 plants were evaluated by counting the average number of larvae. At the reproductive stage, damaged pod rates (% DP) were estimated for 10 plants per plot, using the following equation: % DP = (number of damaged pods/numbers of total pods) x 100. When plants reached physiological maturity, plants within 2 m of the two central rows were harvested to determine grain yield (kg ha<sup>-1</sup>). The experiment was arranged in a randomized block design, with six treatments and five replicates.

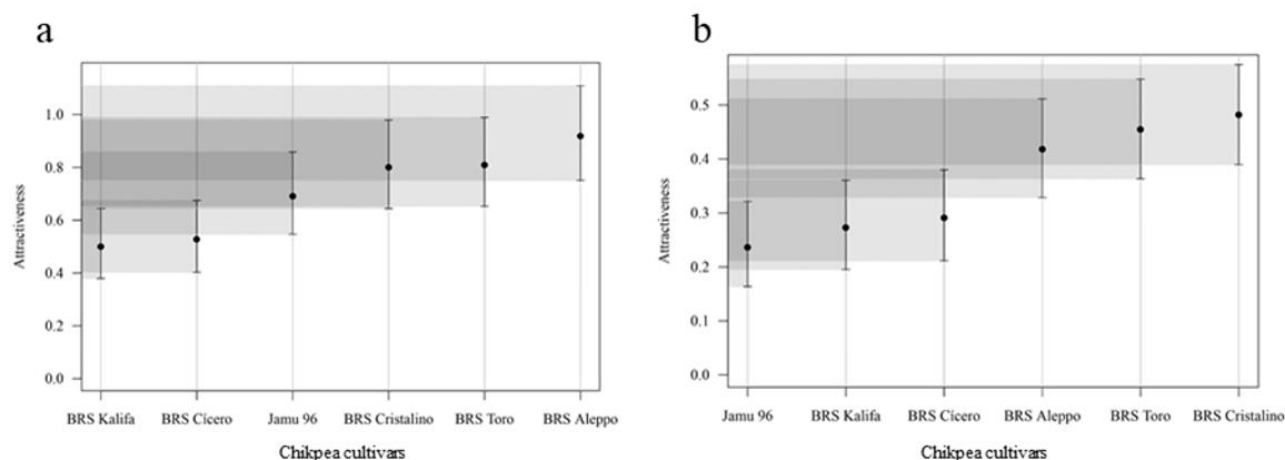
### Statistical analysis

An analysis-of-variance model was fit to experimental data. Residual normality and homoscedasticity were tested by Shapiro-Wilk and Bartlett tests. Averages of all cultivars were compared by Tukey's test ( $\alpha = 0.05$ ). All statistical analyses were processed using the R software, version 3.6.0 (R Core Team, 2019).

## Results and discussion

Chickpea cultivars influenced *C. virescens* attractiveness both in free-choice and no-choice tests (Figure 1A and B). In the free-choice test, BRS Aleppo (0.92) was the most attractive to *C. virescens* larvae, while BRS

Kalifa (0.50) and BRS Cícero (0.53) were less attractive (Figure 1A). In the no-choice test, BRS Cristalino (0.48) and BRS Toro (0.45) were the most attractive, and BRS Kalifa (0.27) and Jamu 96 (0.29) the least attractive to *C. virescens*.



**Figure 1.** 95% confidence intervals for *C. virescens* attractiveness to different chickpea cultivars in free-choice (A) and no-choice (B) tests.

Host attractiveness to insects can be mainly mediated by plant physical traits such as coloration or morphological features, namely, trichome presence or absence, plant tissue hardness or chemical compounds, which act on insect behavior or metabolism (Silva, Baldin, Canassa, Souza, & Lourenção, 2014). In this way, the interaction between insects and injured host plants can stimulate or prevent feeding (Boiça Junior et al., 2015).

Chickpea cultivars did not influence consumed leaf area (CLA) by *C. virescens* (Table 1). The genotypes had statistically different numbers of glandular trichomes. BRS Toro (34.65) and BRS Kalifa (36.75) showed the highest trichome densities, while BRS Cícero (9.85) and Jamu 96 (14.25) the lowest ones.

**Table 1.** Consumed leaf area (CLA, in cm<sup>2</sup>) by *C. virescens* of leaves from six chickpea cultivars in no-choice test for attractivity and glandular and non-glandular trichome densities (counts within 9 mm<sup>2</sup>; mean  $\pm$  standard error).

| Cultivar       | CLA              | Glandular trichome | Non-glandular trichome |
|----------------|------------------|--------------------|------------------------|
| BRS Cícero     | 0.408 $\pm$ 0.07 | 9.85 $\pm$ 0.70 c  | 92.00 $\pm$ 4.31       |
| BRS Aleppo     | 0.475 $\pm$ 0.04 | 24.75 $\pm$ 0.67 b | 100.15 $\pm$ 4.28      |
| BRS Cristalino | 0.440 $\pm$ 0.09 | 20.40 $\pm$ 2.02 b | 99.65 $\pm$ 1.84       |
| BRS Toro       | 0.503 $\pm$ 0.05 | 34.65 $\pm$ 1.11 a | 95.05 $\pm$ 3.73       |
| BRS Kalifa     | 0.406 $\pm$ 0.10 | 36.75 $\pm$ 0.75 a | 91.35 $\pm$ 3.91       |
| Jamu 96        | 0.345 $\pm$ 0.12 | 14.25 $\pm$ 0.73 c | 89.80 $\pm$ 3.38       |
| F              | 0.45             | 91.33              | 14.38                  |
| p-value        | 0.8090           | < 0.0001           | 0.2257                 |

Means followed by the same letter do not differ significantly from each other by the Tukey's test at 5% probability.

The lower preference of *C. virescens* for BRS Kalifa may be associated with its high leaf hairiness. Trichome presence has been studied in several crops and is considered an important plant-resistance mechanism against insects (Rachappa et al., 2019). The high trichome density in chickpea plants acts as a morphological barrier, making it hard for caterpillars to feed. Conversely, Ascensão et al. (1995) noted that *H. armigera* fed less on plants with high pubescence. However, such morphological trait of chickpea had no effect on pod damages by larvae (Hossain et al. 2008). Glandular and non-glandular trichome numbers have been a mechanism of antixenosis against *C. virescens* oviposition on chickpeas (Golla et al., 2018).

This morphological characteristic in isolation may not be the only mechanism of resistance of chickpeas to *C. virescens*. The cultivar BRS Cícero, which was also less attractive to *C. virescens*, had fewer glandular trichomes. Thus, other causes of chickpea resistance to *C. virescens* should be studied.

Chickpea cultivars influenced *C. virescens* larval weight (Table 2). The lowest mean was found in Jamu 96 (0.75), without statistical difference from BRS Aleppo (1.10) and BRS Cristalino (1.05). Yet the largest means were obtained in BRS Toro (1.34), BRS Cícero (1.36), and BRS Kalifa (1.38). Similarly, the cultivars had no influence on *C. virescens* larval survival.

**Table 2.** Larval weight (mg), larval survival (%) (mean  $\pm$  standard error), malic and oxalic acid contents (in mg of acid per 100 g of sample) in six chickpea cultivars.

| Cultivar       | Larval weight      | Larval survival  | Malic acid | Oxalic acid |
|----------------|--------------------|------------------|------------|-------------|
| BRS Cícero     | 1.36 $\pm$ 0.03 a  | 93.75 $\pm$ 7.22 | 0.480 bc   | 0.322 bc    |
| BRS Aleppo     | 1.10 $\pm$ 0.02 ab | 87.50 $\pm$ 7.22 | 0.475 c    | 0.320 c     |
| BRS Cristalino | 1.05 $\pm$ 0.03 ab | 87.50 $\pm$ 7.22 | 0.530 bc   | 0.355 bc    |
| BRS Toro       | 1.34 $\pm$ 0.03 a  | 87.50 $\pm$ 6.25 | 0.605 ab   | 0.408 ab    |
| BRS Kalifa     | 1.38 $\pm$ 0.02 a  | 93.75 $\pm$ 6.25 | 0.570 bc   | 0.355 bc    |
| Jamu 96        | 0.75 $\pm$ 0.03 b  | 81.25 $\pm$ 6.25 | 0.710 a    | 0.478 a     |
| F              | 7.31               | 0.76             | 9.62       | 9.36        |
| p-value        | 0.0012             | 0.5152           | 0.0001     | 0.0001      |

Means followed by the same letter do not differ significantly from each other by the Tukey's test at 5% probability.

The lower larval weights in Jamu 96, BRS Aleppo, and BRS Cristalino may be due to substances produced by these plants that cause antibiosis. This resistance type occurs due to a negative effect on pests feeding on such plants (Smith, 2005; Seifi et al., 2013). Such negative effects on insect biology are because of plant morphological characteristics such as trichome density or secondary compounds (e.g., glucosinolates, isoflavonoids, terpenoids, alkaloids (War et al., 2012). A high density of non-glandular trichomes in chickpea has been negatively correlated to *H. armigera* larval survival (Golla et al., 2018). The same authors have already reported the least damage and the lowest *H. armigera* larval survival for the resistant chickpea genotype ICC 506EB, which has high glandular and non-glandular trichome densities, as well as high oxalic and malic acid contents (Golla et al., 2018).

The cultivars Jamu 96 (0.710 and 0.478 mg) and BRS Toro (0.605 mg and 0.408 mg) obtained the highest malic and oxalic acid contents, respectively (Table 2). Whereas the lowest ones were observed in BRS Aleppo (0.475 mg and 0.320 mg, respectively).

Chickpea plants produce organic acids as exudates in leaves and pods, including malic and oxalic acids, which have been associated to antibiosis (Narayanamma et al., 2013; Golla et al., 2018). When fed chickpeas with increased production of malic and oxalic acids, *H. armigera* caterpillars had reductions in weight and larval survival (Yoshida, Cowgill, & Wightman, 1995; Simmonds & Stevenson 2001; Rachappa et al., 2019). Therefore, the lower *C. virescens* larval weight in Jamu 96 may be associated with its higher malic and oxalic acid contents.

The cultivars also did not influence *C. virescens* counts during vegetative stage (Table 3). But at flowering, there was a high infestation of *C. virescens* in BRS Aleppo (10.0 caterpillars per plant). At podding, the highest infestations were observed not only in BRS Aleppo (7.5) but also in BRS Cícero (6.25). Considering the entire plant cycle, the largest infestations of *C. virescens* were registered for BRS Aleppo (7.21), without statistical difference from BRS Kalifa (4.96), and the other cultivars showed the lowest infestations.

**Table 3.** Average numbers of *C. virescens* (mean  $\pm$  standard error) larvae infesting chickpea cultivars at different development stages.

| Cultivar       | Vegetative      | Flowering          | Podding            | Total cycle        |
|----------------|-----------------|--------------------|--------------------|--------------------|
| BRS Cícero     | 3.00 $\pm$ 0.54 | 4.88 $\pm$ 0.62 b  | 6.25 $\pm$ 0.72 ab | 4.7 $\pm$ 0.54 b   |
| BRS Aleppo     | 4.13 $\pm$ 0.43 | 10.00 $\pm$ 0.54 a | 7.50 $\pm$ 0.87 a  | 7.21 $\pm$ 0.43 a  |
| BRS Cristalino | 2.75 $\pm$ 0.32 | 6.75 $\pm$ 0.60 b  | 4.25 $\pm$ 0.63 b  | 4.58 $\pm$ 0.32b   |
| BRS Toro       | 3.13 $\pm$ 0.47 | 6.13 $\pm$ 1.11 b  | 4.13 $\pm$ 0.66 b  | 4.46 $\pm$ 0.47b   |
| BRS Kalifa     | 4.38 $\pm$ 0.90 | 5.63 $\pm$ 1.21 b  | 4.88 $\pm$ 0.59 b  | 4.96 $\pm$ 0.90 ab |
| Jamu 96        | 4.63 $\pm$ 0.24 | 5.00 $\pm$ 0.46 b  | 4.25 $\pm$ 0.43 b  | 4.63 $\pm$ 0.24 b  |
| F              | 2.15            | 7.72               | 4.32               | 3.54               |
| p-value        | 0.1153          | 0.0009             | 0.0123             | 0.0069             |

Means followed by the same letter do not differ significantly from each other by the Tukey's test at 5% probability.

*Chloridea virescens* population increased during the reproductive stage of chickpeas. In other crops, such as soybeans and cotton, this species also had a major preference for reproductive stages (Tomquelsky & Maruyama, 2009; Bueno, Hirose, & Sosa-Gómez, 2013).

The cultivar Jamu 96 (28.47%) had the lowest percentage of damaged pods, differing statistically from BRS Kalifa (46.62%) with the highest value. The cultivars BRS Aleppo (491.54 kg ha<sup>-1</sup>) and Jamu 96, (497.61 kg ha<sup>-1</sup>) showed the highest grain yields, while BRS Cícero (258.04 kg ha<sup>-1</sup>) had the lowest one (Table 4).

**Table 4.** Damaged pods (%) and grain yields (Kg ha<sup>-1</sup>) (mean ± standard error) of six chickpea cultivars infested by *C. virescens*.

| Cultivar       | Damaged pods  | Yield           |
|----------------|---------------|-----------------|
| BRS Cícero     | 44.62±1.39 ab | 258.04±27.34 b  |
| BRS Aleppo     | 39.53±2.74 ab | 491.54±77.07 a  |
| BRS Cristalino | 35.65±5.59 ab | 312.5±62.27 ab  |
| BRS Toro       | 37.90±4.94 ab | 307.8±33.15 ab  |
| BRS Kalifa     | 46.62±2.05 a  | 351.37±40.09 ab |
| Jamu 96        | 28.47±2.89 b  | 497.66±43.970 a |
| F              | 3.263         | 4.0401          |
| p-value        | 0.0343        | 0.0160          |

Means followed by the same letter do not differ significantly from each other by the Tukey's test at 5% probability.

Morphological characteristics such as pod hardness and wall thickness may be related to plant resistance and hence result in less damage to plants (Clement, El-din, Weigand, & Lateef, 1994; Sreelatha, Sharma, & Gowda, 2018). The highest grain yield in BRS Aleppo and Jamu 96 may be associated with their productive potentials. Although the genotype BRS Aleppo had high productivity, it also showed the highest pest incidence during the flowering, which is a critical period. The highest yield in Jamu 96 may be associated with antibiosis. This cultivar also showed the lowest larval weight and percentages of damaged pods.

## Conclusion

The chickpea cultivars Jamu 96 and BRS Aleppo, which had different resistance levels and mechanisms, could be used as a complementary tactic in integrated pest management programs against *C. virescens*. Other field trials interacting resistant and tolerant cultivars, as well as other different control tactics, need to be performed to increase IPM effectiveness.

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