

Homeopathic solutions induce production of winged forms in aphid

Nilbe Carla Mapeli¹, Gustavo Ferreira da Silva^{2*}, Ricardo Henrique Silva Santos³,
Cassiano Cremon¹, Larissa Chamma², Bruno Cesar Ottoboni Luperini²

¹State University of Mato Grosso, Cáceres, Brazil

²São Paulo State University, Botucatu, Brazil

³Federal University of Viçosa, Viçosa, Brazil

*Corresponding author, e-mail: gustavo.ferreira@unesp.br

Abstract

The aphids control (*Brevicoryne brassicae* L.) in kale (*Brassica oleracea* L. var. *acephala*) is one of the main challenges for its production. This obstacle becomes greater in agroecological production systems. Thus, the objective of this study was to verify the formation of winged individuals in colonies of *B. brassicae* aphids, when exposed to kale treated with homeopathic solutions. The treatments were resistant 5CH, susceptible attacked 5CH, aphid nosode 30CH, and the controls that are water (without dynamization) and distilled water + 70% ethanol 5CH, being applied every day for 15 days. Kale plants were inoculated with aphids and irrigated using homeopathic solutions. After 15 days of inoculation, the plants were removed from the bag and the number of winged forms and nymphs of *B. brassicae* was counted. Homeopathic preparation Resistant 5CH provided a smaller number of winged individuals and a larger number of nymphs per colony. The plants treated with the susceptible attacked 5CH homeopathy showed a higher number of winged aphids, but a reduced number of nymphs. Aphid nosode 30CH treatment was promising to *Brevicoryne brassicae* control in kale plants, as it induced polymorphism, presenting a greater number of winged aphids and a reduced number of nymphs per colony

Keywords: *Brassica oleracea*, *Brevicoryne brassicae*, ecological control, homeopathy, kale

Introduction

Kale (*Brassica oleracea* L. var. *acephala*) has an important economic and nutritional value, and its worldwide consumption has become increasingly larger (Di Noia, 2014; Šamec et al., 2019). However, the attack of pests significantly affects the quality and productivity of this vegetable, being aphid (*Brevicoryne brassicae*) the main pest (Shonga & Getu, 2021).

In Brazilian climatic conditions, these aphids are viviparous that reproduces by thelytokous parthenogenesis, developing quickly numerous colonies. In unfavorable conditions, such as low food quality and high density, stimulate the emergence of winged individuals, responsible for the migration/dispersion of the species (Sharma et al., 2017; Sampaio et al., 2017). Females that reproduce by parthenogenesis, forming clones, have high prolificity, provide the generation of about 50 to 100 nymphs (Bacci et al., 2009; Ogawa &

Miura, 2014).

This aphid defense process of decreasing the number of apterous forms in the colony and increasing the number of winged forms, is characterized as polymorphism. Responses of this nature can influence the behavior of the insect, which will migrate to new hosts, making it an easy target in this route of natural enemies and also in the physiology due to the energy cost that the polymorphism generates, manifesting a drop in the fecundity of females (Dixon, 1977; Riessen, 1992; Zhang et al., 2019).

Aphids cause direct and indirect damage to plants. Damage comes from their feeding habits causing loss of secondary compounds and nutritional deficiency of the plants. Besides that, the injection of toxins can induce malformation of leaf tissues and in more severe cases galls formation. There is also a reduction in photosynthesis due to the presence of sooty mold and, in

the most severe cases, virus transmission, causing a loss in productivity. Thus, it is essential to monitor the activity of these insects, anticipating their occurrence and deciding the best control strategy to be adopted (Dedryver et al., 2010; Soufbaïf et al., 2018; Bhattacharya, 2019).

Homeopathic products, in turn, are alternative substances that can be used to control insects, promoting tolerance in plants and increasing secondary metabolites, which can repel or even kill certain agricultural pests (Boff et al., 2016; Schmidt, 2021), being an important resource for ecological pest management (Betti et al., 2009; Toledo et al., 2016).

However, there are few researches related to the management this aphid through homeopathic preparations (Mapeli et al., 2018), thus, further studies are needed. Once there are studies that report the efficiency of these preparations in the control of other insects in the Brassica crops (Mapeli et al., 2010; Mapeli et al., 2015; Ücker et al., 2018).

Therefore, the objective of this study was to evaluate the formation of winged individuals in colonies of aphids *B. brassicae* (L.) when exposed to kale treated by homeopathic solutions.

Material and Methods

Site description

The experiment was carried out under field conditions, situated at latitude 20°45'14" S, longitude 42°52'53" and altitude of 651 m. According to Köppen classification, the region climate is humid subtropical with dry winters and hot summers, with an average annual temperature of 21 °C and annual rainfall of 1200 mm.

Kale seedlings obtaining

Kale seedlings were obtained from *Brassica oleracea* L. var. *acephala* DC., clone Manteiga cv. Santo Antonio, which is considered susceptible to aphids (*Brevicoryne brassicae* L.). They were collected 60 days before the experiment beginning and transplanted into rigid polyethylene pots with a capacity of 10L, containing sieved ravine soil mixed with organic compost, in the proportion of 1:1(w/w). Chemical characteristics of the soil used were: pH (CaCl₂): 6,4; organic matter.: 5 g dm⁻³; P (Mehlich): 2 mg dm⁻³; H+Al: 26 mmol_c dm⁻³; K: 2,1 mmol_c dm⁻³; Ca: 43 mmol_c dm⁻³; Mg: 4 mmol_c dm⁻³; sum of bases: 49.1 mmol_c dm⁻³; capacity of exchange cation: 75.1 mmol_c dm⁻³ and base saturation: 65.4%.

Experimental design and treatments

The experimental design was completely

randomized with twelve replications and five treatments. Each plot consisted of three pots, containing one plant in each.

Treatments tested were kale resistant 5CH, kale susceptible attacked 5CH, aphids (nosodium) 30CH, and the controls were distilled water (without dynamization) and distilled water + 70% ethanol 5CH.

Treatments choice was based on "transfer of information". If kale cultivar is susceptible or resistant to the attack of these pests, it is expected that the solutions made with this cultivar will pass the information of susceptibility and resistance to the other cultivar, stimulating the reaction to attacks, through responses of plant's secondary metabolism, such as an increase in the production of defense metabolites, thus triggering some adverse effect in the insect (Rossi et al., 2004; Mapeli et al., 2015).

The 5CH dynamization was chosen by the influence on the plant's primary metabolism (Andrade et al., 2012), favoring the attractiveness of insects to homeopathized plants. And 30CH potentiation was chosen because it has an influence on the plant's secondary metabolism and thus favors insect repellency (Almeida et al., 2003).

Homeopathic solutions preparation

The 5CH resistant homeopathic solution was prepared with kale leaves 'Roxa cv. Crista de Galo', considered resistant to the aphids attack (*B. brassicae*). The 5CH attacked susceptible solution was prepared with kale leaves 'cv. Santo Antonio' attacked by *B. brassicae*.

Susceptible and resistant criteria were based on the plant's infestation on experimental field by aphids and the absence of aphids on the plants, respectively. Daily inspections were carried out in the field for 15 consecutive days, according to the methodology described by Webster et al., 1991; Reese et al., 1994.

In laboratory, kale leaves collected were washed with water and, subsequently, the excess moisture was removed using paper towels. From each kale cultivar (Santo Antonio and Crista de Galo), 100 g of leaves were weighed and crushed in a blender (turbolysis) with 1000 mL of distilled water for approximately 1 minute. After, they were placed separately in amber glass vials covered with aluminum foil and for 15 days they were shaken daily for 20 seconds, a procedure that originates the mother tincture (Farmacopeia Homeopática Brasileira, 2011). After this period, the mother tinctures were strained, filtered and placed in new amber glasses.

Aphids nosode 30CH was prepared with nymphs

and adults of *B. brassicae* collected on kale leaves from the plant collection. Aphids collected on field were placed in a rigid polyethylene box with a lid (7 cm in diameter). In laboratory, aphids were removed from the box and weighed approximately 2g. Then, they were placed in an amber glass with 20 mL of distilled water covered by aluminum foil for 15 days, with daily agitation for 20 seconds to obtain the mother tincture (Farmacopeia Homeopática Brasileira, 2011). The mother tincture material after this period was strained, filtered and placed in a new amber glass.

The dynamizations were performed using amber glasses with a capacity of 30 mL, with 20 mL of 70% ethanol and 0.2 mL of the mother tincture (measured with an automatic pipette) being placed. It was shaken in ascending and descending movements, in a succussor device (mechanical arm" model Denise 10-50) for 100 times and the 1CH (Hahnemannian Centesimal in the first power) was obtained. Then, 0.2 mL of 1CH was removed and added to a glass with 20 mL of 70% ethanol, shaken 100 times, thus obtaining 2CH. The process was repeated until 30CH.

The water control treatment was prepared with distilled water only. As for the control distilled water + 70% ethanol 5CH, 20 mL of distilled water were placed in the glass and 0.2 mL of 70% ethanol was added, shaken 100 times and done at 1CH, and so on, up to 5CH.

The vials containing the treatments and the controls were labeled and coded so that the applicator was not aware of the solutions, a procedure called double-blind.

Homeopathic solutions application

Homeopathic solutions were applied to kale plants 'cv. Santo Antonio', which grown in pots.

From each solution, 0.2 ml were taken, added to 200 ml of distilled water and homogenized. Of this amount, 30 mL were sprayed on kale leaves, with a hand sprayer individualized per solution, and 70 mL were applied via soil. Applications were made at 8:00 am, on alternate days.

Experiment conduction

Before transplanting, seedlings were cleaned, removing aphids and other insects, leaving only four to five leaves per seedling. Irrigations during this period were performed daily. After transplanting, the pots were placed in a greenhouse, irrigation with water was stopped and the plants began to receive only the solutions (treatments).

Winged aphids were collected in the kale experimental field and placed in glasses. Immediately after collecting the total number of insects needed (250 winged aphids), the plants were inoculated.

One winged aphid was inoculated per kale plant, and they were housed in the upper part of the apical leaf. The leaf was covered by a thin fabric like "voil", in the form of a bag, tied with string. After this procedure, the applications of homeopathic solutions in inoculated kale plants began.

After a period of 15 days of application, the inoculated leaf of each plant was removed from the bag and the total number of winged aphids was counted, to verify the morphological defense (polymorphism) and the number of nymphs per colony, to estimate the fecundity of females.

The characteristics analyzed were the total number of winged aphids per plant and the total number of nymphs.

Data analysis

Data were submitted to normality tests (Lilliefors) and homogeneity of variances (Cochran and Bartlett), when significant, analysis of variance was performed. Mean differences were discriminated by Duncan's test at the level of 5% error probability.

Results

Aphid colonies that developed on kale plants treated with homeopathic solutions had similar numbers of winged individuals to plants treated with control solutions. However, there was a difference between homeopathies, whereas the number of winged in colonies exposed to susceptible attacked 5CH (8.83 winged) and aphid 30CH (8.08 winged) solutions was significantly higher than the number of winged in colonies exposed to the solution resistant 5CH (2.42 winged). Indicating that some colonies responded to the presence of homeopathic solutions with greater production of winged forms than others (Figure 1).

Aphids fecundity in the colony was analyzed through the total number of nymphs. It was found that there was no difference between treatments and controls, but between treatments the aphids 30CH solution showed fewer nymphs per colony when compared to the resistant 5CH solutions (Figure 2).

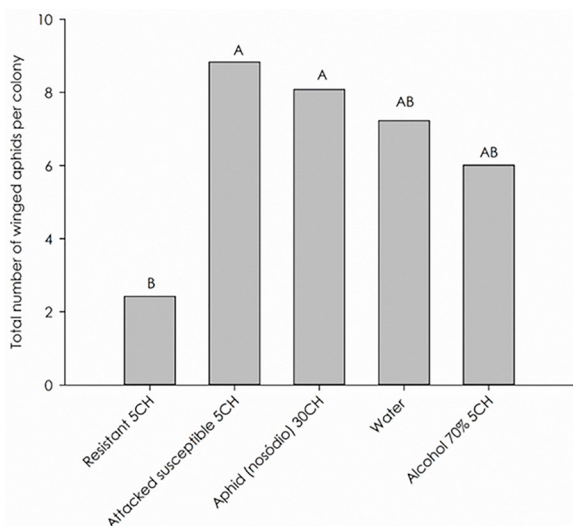


Figure 1. Production of winged forms in *Brevicoryne brassicae* colonies, in kale plants treated with homeopathic solutions. Means followed by the same letter did not differ from each other, by Duncan's test, at the level of 5% error probability.

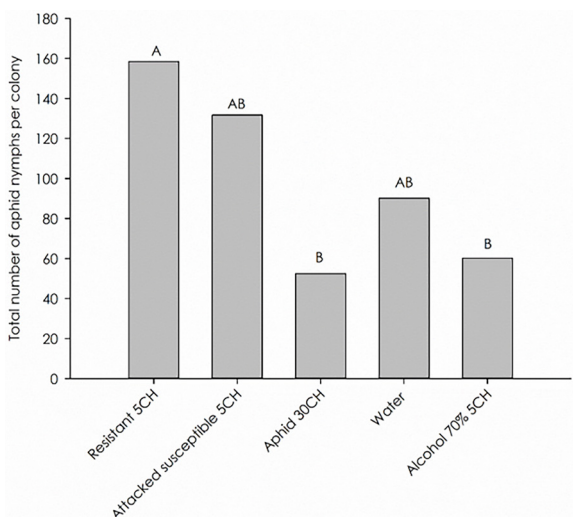


Figure 2. Number of nymphs in *Brevicoryne brassicae* colonies, in kale plants treated with homeopathic solutions. Means followed by the same letter did not differ from each other, by Duncan's test, at the level of 5% error probability.

Aphid 30CH treatment, which produced the highest number of winged individuals (Figure 1), resulted in fewer nymphs per colony (Figure 2). On the other hand, the resistant 5CH solution, which presented the lowest number of winged individuals, had the highest number of nymphs (Figure 1 and 2).

Discussion

The response of aphid *B. brassicae* to the presence of homeopathic solutions in the host plant, producing winged forms (Figure 1), can be considered a defense mechanism. Stimulating the production of winged aphids would be a strategy to maintain the clone's survival, escaping by flight and colonizing other host

plants. This fact becomes relevant for the management of the population of this insect in the kale crop, since homeopathies stimulated the production of winged forms. Thus, the greater number of wingless forms tends to decrease due to the emergence of winged aphids, which is an important factor for controlling this insect (Cividanes, 2002; Dedryver, et al. 2010; Bhattacharya, 2019).

Pereira & Lomônaco (2003) studied the production of winged forms in *B. brassicae* colonies by induction of the parasitoid *Diaeretiella rapae*, and found that in aphids colonies exposed to the parasitoid, the number of winged (five winged per colony) was higher than in the control without parasitoid (one winged per colony). In terms of average, the same occurred in this study, where the homeopathies susceptible attacked 5CH and aphid 30CH presented a greater number of winged individuals (approximately ten winged per colony) in relation to the controls (from five to six winged per colony).

The homeopathic solutions susceptible attacked 5CH and aphid 30CH may promote a stress condition to the insect, through direct contact with the homeopathized kale plants, inducing the production of winged forms, as a food rejection mechanism (Soufbafe et al., 2018).

It is proven that homeopathic solutions are inducing sources of defense mechanism in plants, mainly with the increase of active principles (Andrade et al., 2012; Lorenzo et al., 2021). So, the plants may have increased the concentration and levels of active defense principles, so the insects perceived some adverse information (Pereira & Lomônaco, 2003; Viegas Júnior, 2003; Zhang, et al. 2019), and knowing that aphids can perceive the presence of unfavorable conditions through chemical receptors, these were able to induce polymorphism (Bhatia et al., 2011; Bhattacharya, 2019).

The higher number of winged individuals and lower number of nymphs in the colony in the treatment aphid 30 CH, as well as the lowest number of winged aphids and the highest number of nymphs in the resistant 5CH treatment (Figure 1 and 2), confirm the strategy used by insects in unfavorable conditions, stimulating the emergence of winged individuals, responsible for the migration/dispersion of the species (Bacci et al., 2009; Bhattacharya, 2019).

Energy expenditure with the polymorphism influenced the number of nymphs per *B. brassicae* colony, considering the fecundity of aphids tended to decrease with the appearance of winged forms (Simon & Peccoud, 2018; Chen et al., 2019).

In this way, the lower number of winged individuals present in the susceptible attacked 5CH treatment explains the higher number of nymphs found in this same treatment. Likewise, the lower number of nymphs in the aphid 30CH treatment can be explained.

It was expected that, through the principle of similarity, the resistant 5CH homeopathy, prepared with a cultivar considered genetically resistant to the aphid (kale 'Roxa cv. Crista de Galo'), would pass some type of chemical information to the tolerant plants and, thus, stimulate tolerance to aphid attack in them. However, there was no such exchange of information. Perhaps the greater dynamization of this homeopathy could stimulate polymorphism in *B. brassicae*, considering the aphid 30CH treatment was able to reduce the number of nymphs and increase the winged forms in the colonies by the same principle of cure by similar. Once the homeopathic solutions can promote in plants the increase of secondary metabolites capable of conferring unfavorable conditions to the insect, either by low nutritional quality or by passing information to high-density aphids (Mapeli et al., 2015; Lorenzo et al., 2021). According to Bacci et al. (2009) low food quality conditions and high population density are factors that stimulate the production of winged forms in *B. brassicae*.

The susceptible attacked 5CH treatment there was the transfer of information to the homeopathized plants, but in an incomplete way, because despite the number of winged individuals having decreased, the number of nymphs was expressive, thus giving the plant a greater tolerance to the attack of the aphid when compared to aphid 30CH treatment.

Conclusions

Homeopathic solutions application in kale plants affected the reproduction of *Brevicoryne brassicae*. The homeopathic solutions susceptible attacked 5CH and aphid 30CH promoted an increase in the number of winged aphids in kale plants. Meanwhile, the aphid nosode 30CH induced the polymorphism in *B. brassicae*.

Aphid 30CH homeopathy showed promise for the control of *Brevicoryne brassicae* in kale plants, proving to be an excellent tool for the management of this pest in agroecological production systems.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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