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MINOR REVIEW

Control of *Brevicoryne brassicae* (Hemiptera: Aphididae) with extracts of *Agave americana* var. *Marginata* Trel. in *Brassica oleracea* crops

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Alternative methods of pest control can and should be encouraged, especially those that consider the reality of smallholder family farmers. Here, we evaluated the potential of *Agave americana* (agave) extracts for the control of the aphid *Brevicoryne brassicae* in cabbage (*Brassica oleracea* L. var acephala) in the field and laboratory. The field experiments consisted of the evaluation of the proportion of dead aphids on cabbage plants after application of agave extracts. In the field, agave mixed with cow milk caused mortality above 80% and was the most effective extract. Agave mixed with water and agave mixed with ethanol elicited mortality above 60%. In the laboratory, we evaluated the mortality of aphids after the application of different concentrations of aqueous agave extracts; the commercial insecticide deltamethrin was included as positive control. Evaluation took place at 3, 6, 12, 24, 48 and 72 hr after applying the treatment. As expected, deltamethrin was the most effective treatment. However, agave extract at concentrations of 0.750 and 0.500 g/mL caused >70% mortality 3 hr after application. We conclude that *A. americana* extracts decreased aphid populations and is a promising alternative to the commercial insecticide against aphids in cabbage.

KEYWORDS
agave, aphids, biopesticides, horticulture, plant extracts

1 | INTRODUCTION

The cabbage aphid, *Brevicoryne brassicae* (Hemiptera: Aphididae), is widely distributed in agricultural regions and is one of the principal pests of vegetables, especially Brassicae (Hao, Hou, Hu, Huang, & Dang, 2017; Sharma et al., 2017). Turnip, Brussels sprout and mustard are strongly affected by aphids, which makes wrinkles and chlorotic marks on the leaves (Hughes, 1963; Kroes et al., 2017; Luria, Reingold, Lachman, Sela, & Dombrovsky, 2016). In addition to consuming plant phloem, the aphid may also pave the way for pathogens to enter the plant during feeding (Guimarães, Michereff, & Liz, 2011; Imenes & Ide, 2002). Currently, a number of control methods are used against the cabbage aphid (Cesar et al., 2016). Physical methods include the use of traps; biological control is based on the use of natural enemies such as predators and parasitoids, to reduce aphid densities (Holland et al., 2012; Sarwar, 2015); and chemical methods involve the use of pesticides.

Chemical control of aphids is often carried out by farmers by applying the pyrethroid insecticide deltamethrin (Bai, Zhou, & Wang, 2006), a product considered of high toxicity (Rehman et al., 2014) for various insect orders, such as Orthoptera, Thysanoptera, Lepidoptera, Coleoptera, Diptera, Hemiptera, among others (Baptista, Carvalho, Carvalho, Carvalho, & de, Souza Bueno Filho, 2009; Hughes et al., 2008; Stecca et al., 2017). Deltamethrin is considered to be the pyrethroid most toxic to vertebrates (Santos, Areas, & Reyes, 2007). In water, it presents a half-life ranging from 17 to 110 days, tends to be stable at acidic and neutral pH and becomes susceptible to hydrolysis under alkaline conditions (Santos et al., 2007). The application of chemical insecticides in vegetable crops presents many problems, such as the contamination of food, water and soil (Bai et al., 2006; Sarwar, 2015). Moreover, these products are capable of eliminating entire populations of non-target organisms (Sarwar, 2015) that are part of a complex and diverse agroecosystem (Balvanera et al., 2006; Benton, Vickery, & Wilson, 2003; Bomfim, Azevedo, de Araujo Viana, Manzano, & Vasconcelos, 2015; Rusch et al., 2016). Hence, the insecticides in horticulture must be used responsibly, and efforts are needed to make feasible control methods without risking the health of farmers and the environment (Bomfim et al., 2015; Enserink, Hines, Vignieri, Wigginton, & Yeston, 2013; Gangwar, Singh, Tyagi, Kumar, & Sharma, 2014).

Instead of using chemical insecticides, biopesticides can be used to prevent loss of biodiversity and to enable economically sustainable cropping systems without causing significant damage to the people and environment (Carvalho, 2017). The use of biopesticides allows reducing pest insect populations to a level below that producing economic damage (Botti et al., 2015; Chaplin-Kramer, O'Rourke, Blitzer, & Kremen, 2011).

Several plants and plant-based products can be used as biopesticides. Essential oils of cumin (*Cuminum cyminum*), anise (*Pimpinella anisum*), oregano (*Origanum syriacum* var. bevanii) and eucalyptus (*Eucalyptus camaldulensis*) have been shown to be effective against the cotton aphid *Aphis gossypii* and the carmine spider mite *Tetranychus cinnabarinus* (Isman, 2000). While biopesticides may also negatively affect populations of natural enemies and predators, in many cases they can be used without eliminating interactions that occur naturally between the organisms (Botti et al., 2015; Paes, Santana, Barbosa de Azevêdo, De Morais, & Calixto, 2010; Scott et al., 2002). The extract of *Agave americana* var. *marginata* Trel. (agave) is a plant-based product that has the potential to be used for the control of insects (Abrahem & Awad, 2015). The use and study of agave-extracts as a pesticide is relatively recent, but has gained increasing attention over time (Barrêto et al., 2003; Barrêto, Araújo, & Bonifácio, 2010). Farmers have used *A. americana* extracts as a biopesticide against the aphid *B. brassicae* in the cabbage *Brassica oleracea* (Personal communication; A.J. Pereira), but there is little scientific evidence about its efficiency. Here, we aimed to evaluate the insecticidal effectiveness of *A. americana* extracts on the aphid *B. brassicae* on *B. oleracea*.

2 | MATERIALS AND METHODS

This study was divided into three parts: two on-farm experiments and one laboratory trial, as follows:

Part I. *A. americana* for the control of *B. brassicae* in *B. oleracea*. On-farm research I.

To evaluate the effect of agave extracts on the mortality of aphids on *B. oleracea*, field experiments were performed on a smallholder farm in the city of Viçosa, Minas Gerais State, Brazil, from May to November 2013, the growing season of cabbage in this region. The farmer had been using extracts of agave to control aphids and the formulations used in our experiments were based on those that he had previously used. The extracts were prepared with fresh leaves of a plant that was estimated to be approximately 4 years old, with no signs of maturity. The agave leaves were cut with the aid of scissors in the direction of its growth and transverse to the fibre of the leaf and then weighed. The extract of agave leaves was prepared following the farmer's recommendation. For a full mixture, 100 g of fresh leaves were incorporated into 100 mL of water, milk and ethanol each. For the other extracts, 200 g of leaves and 200 mL of one of the other ingredients were used (Table 1). The milk and the tap water were obtained from the farm on which the experiment was conducted. The hydrated ethanol (96° GL, Quimidrol Ltd, Joinville, Brazil) was purchased locally. This product is allowed and approved for domestic use by Brazilian legislation.

To test the effect of the extracts, an experimental area was divided into five blocks 1 m apart. Each block contained 10 adult plants of *B. oleracea* planted in the soil, arranged in two rows of five plants. The rows were placed 1 m apart, and the plants were 50 cm apart within each row. Within each block, each treatment specified in Table 1 was randomly applied to two plants. Within each block, the two plants with the same treatment were considered one replicate, hence, the experiment had a randomised block design with five treatments and five replicates per treatment.

The extracts were applied to the middle leaf, in the centre of the adult plant and to the abaxial surface of the leaf, with constant pressure, using compression sprayer equipment (Guarany PCP-1P). Each plant received 30 mL of extract. The first application was made when the aphids had already infested the plants. After 7 days, a second application was made. Three days later, the infested leaves were removed from the plants and the number of dead and live aphids of all phenological stages was assessed. To ascertain whether aphids were alive or dead, individuals were touched with a fine brush to check for a reaction. Individuals with no apparent response were considered dead. Because population fluctuations cannot be controlled and because of the impossibility of counting the insects without damaging them, counting was performed only after the end of the experiment.

The mean proportions of dead insects were analysed using Generalised Linear Models with a binomial error distribution and a logit link function adjusted with the likelihood ratio function using SAS software (version 9.2, McCullagh, 1984). A contrast analysis was performed to determine differences among means.

Part II. *A. americana* for the control of *B. brassicae* in *B. oleracea*. On-farm research II.

The second experiment was conducted in the same way as the first one and at the same site. However, the treatment with Agave + water + milk + ethanol was excluded (AX_1, Table 1) and two new control treatments were added, that is, milk + water and ethanol (Table 2). The experiment also had a randomised block design with six treatments (Table 2) and five replicates per treatment. The application of the treatments, the counting of the aphids and the statistical analysis was conducted as described above.

TABLE 1 Description of the treatments used in on-farm research I

Identification	Mixture	Ratio
1—AX_1	Agave + water + milk + ethanol	1:1:0.8
2—AM_1	Agave + milk	1:1
3—AW_1	Agave + water	1:1
4—AH_1	Agave + ethanol	1:0.8
5—W_1	Water	1

TABLE 2 Description of the treatments used in on-farm research II

Identification	Mixture components	Ratio
1—AM_2	Agave + milk	1:1
2—AW_2	Agave + water	1:1
3—AH_2	Agave + ethanol	1:0.8
4—MW_2	Milk + water	1:1
5—W_2	Water	1
6—H_2	Ethanol	1

Part III. *A. americana* for the control of *B. brassicae* in *B. oleracea*. Laboratory research.

Because the solutions containing agave extracts gave the most efficient, simplest and cheapest results on farm, they were evaluated under laboratory conditions. A comparison was made between different concentrations of agave in aqueous solution with only water as a negative control and an insecticide as a positive control. This experiment was conducted at the Ecotoxicology Laboratory of the Department of Entomology of the Federal University of Viçosa.

First, the aqueous extracts of the agave were obtained from the fresh aerial part of the plant, which was homogenised with water in a blender for 3 min and filtered through cotton for 5 min. The solutions were: 0.125 g agave mL^{-1} water (T25), 0.250 g/mL (T50), 0.375 g/mL (T75), 0.500 g/mL (T100), 0.750 g/mL (T150), water (W) and the insecticide deltamethrin (DECIS® 25 EC; Bayer S.A., São Paulo, Brazil) at a concentration of 3 mL/L in distilled water.

Cabbage leaf discs (diameter 6 cm) were placed on moistened filter paper, each in a separate Petri dish and dried at $25 \pm 2^{\circ}$ for 1 hr. Aphids were taken from a single cabbage plant collected in the field. Three infested leaves were removed from the plant, and five apterous adult aphids were randomly placed on the leaf discs in all Petri dishes within 15 min after collecting. Aphids were placed on each disc with a fine brush, taking care not to damage their stylets. The Petri dishes with aphids were randomised, and then the solutions were sprayed onto the discs with a Potter Tower (Burkard Agronomics) with an automatic load system (1,041.1 kPa pressure 1 mL volume of each treatment [Potter, 1941, 1952]). Each solution was sprayed in four different Petri dishes, yielding a total of 20 aphids per treatment.

The sequence of the application was the control (water), followed by treatments at increasing concentrations. After washing and sterilising the Potter Tower, deltamethrin was applied. The Petri dishes were kept at a temperature of $25 \pm 2^{\circ}$C, and evaluation of aphid mortality was carried out at 3, 6, 12, 24, 48 and 72 hr after spraying. Aphid mortality was assessed with a brush as above.

The filter papers in the Petri dishes were moistened every 3 hr to keep the cabbage discs hydrated. The experiment was conducted using a completely randomised design with seven treatments and four Petri dishes per treatment.

To assess the overall effect of treatments on aphid survival, we used a Cox Proportional Hazards model with treatment as fixed factor. Differences among treatments were assessed with general linear hypothesis testing (function glht of the package multcomp; Hothorn, Bretz, & Westfall, 2008). For representation of the data (number of dead aphids in each observation), a Kaplan–Meier survivorship function was fit. These tests were performed using R (R Development Core Team, 2014).

3 | RESULTS

Part I. *A. americana* for the control of *B. brassicae* in *B. oleracea*. On-farm research I.

In the first on-farm experiment, there was a significant effect of the extract used on the mean proportion of dead aphids (χ^2 = 482.5; df = 4; p < .0001). The agave plus milk mixture (AM_1) killed more aphids than the other extracts (Figure 1), the full mixture (AX_1) was the second most efficient extract, AW_1 and AH_1 did not differ in their efficiency and caused more mortality of aphids than W_1 (Figure 1).

Part II. *A. americana* for the control of *B. brassicae* in *B. oleracea*. On-farm research II.

There was a significant effect of the extract used on the mean proportion of dead aphids (χ^2 = 298.21, df = 5; p < .0001). The agave and milk mixture (AM_2) was also the most efficient here, killing more aphids than the other extracts. AW_2 and MW_2 did not differ in their efficiency and were the second most efficient extracts, killing more aphids than the remaining extracts. AH_2 caused more mortality of aphids than W_2 and H_2, which did not differ in their efficiency (Figure 2).

Part III. *A. americana* for the control of *B. brassicae* in *B. oleracea*. Laboratory research.

The survival curves of aphids exposed to different concentrations of aqueous extracts of agave, water and deltamethrin are shown in Figure 3. The mortality rate differed significantly among treatments (Likelihood ratio test = 77.95; df = 6; p < .0001). There was no significant difference among replicates (χ^2 = 0.78; df = 3; p = .85).

The insecticide deltamethrin was the most efficient treatment, killing aphids faster than the other extracts. Then the most efficient was T150, followed by T100. T75, T50, T25 and water did not differ in efficiency and were less efficient than the others.

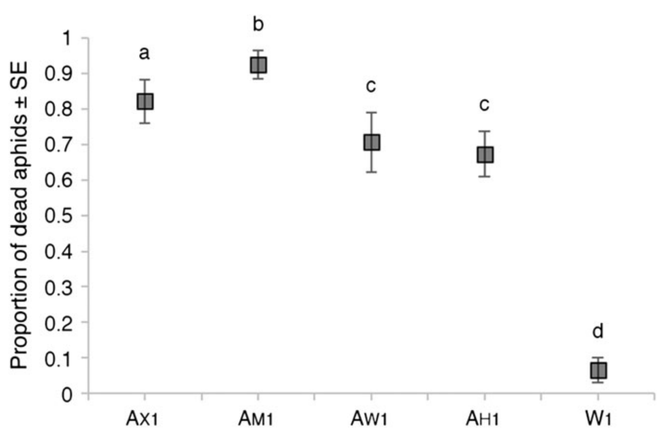

FIGURE 1 Mean proportion (±*SE*) of dead aphids exposed to different agave extracts and water (farm research I). See Table 1 for explanation of the labels of the horizontal axis. Means followed by different letters are significantly different. *SE* = Standard Error

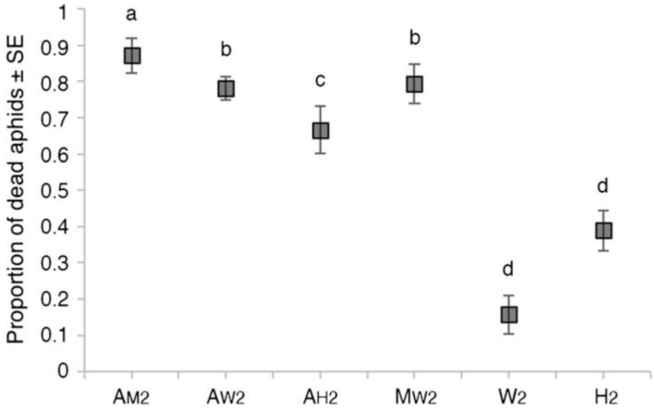

FIGURE 2 Mean proportion (±*SE*) of dead aphids exposed to different agave extracts, water and ethanol (farm research II). See Table 2 for explanation of the labels of the horizontal axis. Means followed by different letters are significantly different. *SE* = Standard Error

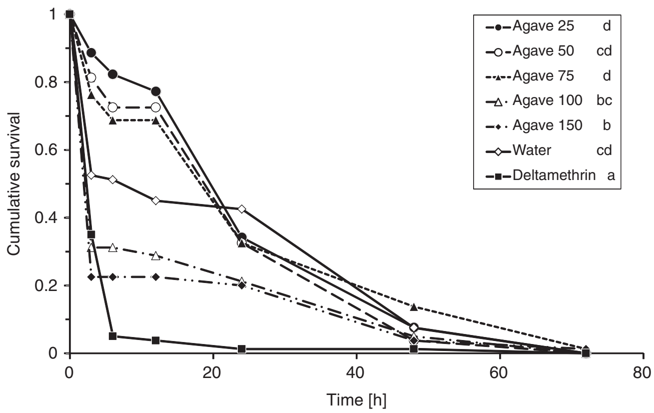

FIGURE 3 Cumulative aphid survival (number of live aphids) over time when exposed to different concentrations of aqueous extracts of agave, water and deltamethrin. Letters in the legend indicate significance of difference among treatments

4 | DISCUSSION

The field experiments showed that the treatment with a mixture of agave and milk was the one that caused the highest insect mortality. The insecticidal effect of diluted milk is already recognised (Paes, 2015; Weingärtner, Aldrighi, & Pereira, 2006) and is a practice used by rural farms. However, we emphasise that the milk does not need necessarily to be used as an insecticide when there is a plant that controls the aphids with similar effectiveness (Figure 2). Even in small quantities, milk would be better used as food than in agricultural practices. In this case, one can choose to use agave only.

The mortality of aphids when using the agave and ethanol extract was higher than 60%, considerably better than using water or ethanol alone. However, the use of ethanol in the extract, also used and tested by the farmer, did not add much to the control of aphids. Hence, alcohol can also be excluded from the formulation without affecting its insecticidal properties. All in all, the aqueous agave extract seems to be the best and cheapest option. In contrast, some studies have reported that ethanol extracts of other plants (for example, the black pepper *Piper nigrum*) contributed to the control of *B. brassicae*, causing repellency and food deterrence (Salerno, Sobrinho, & Cocarelli, 2002).

This study confirms the potential of *A. americana* for aphid control. The insecticidal effect of agave plants has also been observed for other organisms. Pizarro et al. (1999) used an extract of *A. americana* for the control of the tick *Boophilus microplus*, with a 95% mortality after 13 days of testing. *A. americana* also controlled *Alternaria brassicae*, a fungus that infests the mustard *Brassica juncea* (Guleria & Kumar, 2009). Another species of agave, *Agave sisalana*, has been shown to control ants (Barbosa, Silva, & Carvalho, 2006; Pérez López, 2012), mites and caterpillars such as *Spodoptera frugiperda* in maize (Botti et al., 2015; Cerqueira, Osuna, & Costa, 2011; Luria et al., 2016). *Agave tequilana* extracts were efficient to kill the whitefly (*Bemisia tabaci*), also a Hemiptera, and the nematode *Panagrellus redivivus* (Herbert-Doctor, Saavedra-Aguilar, Villarreal, Cardoso-Taketa, & Vite-Vallejo, 2016).

Extracts from plants of the Agavaceae family contain compounds such as tannins, steroids, alkaloids and different saponins. Saponins act against insects and microorganisms (Barrêto et al., 2003; Cerqueira et al., 2011). However, Barrêto et al. (2010) have attributed the insecticidal effect of the agave extract to its slightly acid pH.

Research of plant extracts for aphid control is common. Atanasova (2017) found insecticidal properties in plant extracts as coriander (*Coriandrum sativum*) oil, lavender (*Lavandula spica*) oil, tobacco (*Nicotiana tabacum*) oil and fenugreek (*Trigonella foenum-graecum*) oil. Ganchev & Atanasova (2016) evaluated the insecticide potential of four natural products based on soaps prepared from sunflower (*Helianthus annuus*) and olive (*Olea europaea*) oils against *Aphis nerii* and *Macrosiphum rosae*. They found a high insecticidal activity, mainly against *M. rosae*.

Espírito Santo, Queiroz, and Souza (2011) compared the insecticidal effect of *A. sisalana* and deltamethrin on *S. frugiperda* infesting cowpea plants (*Vigna unguiculata*). The extract of *A. sisalana* (in concentrations of 20 and 25%) caused much less mortality than deltamethrin (93% mortality of the insects). In our study, 3 hr after application, the extract of *A. americana* caused approximately 80% mortality, lower than when using deltamethrin (96%), although higher than the results of Espírito Santo et al. (2011).

Usually, for pest and disease control, the goal is to reduce pest populations below the economic threshold (Sarwar, 2015). The use of synthetic insecticides can potentially contaminate humans and the environment, as well as lead to the development of resistance, reducing their efficacy (Liu, Xu, & Luo, 2006). Therefore, even with the higher number of live aphids treated with agave extract compared to deltamethrin, as well as considering results of the field experiments and the reports of the farmer, agave extract may be regarded as efficient in the control of aphids.

The use of the *A. americana* extract provides a potential alternative to the use of commercial insecticides to control the aphid *B. brassicae* in cabbage. Agave with milk and agave with water elicited a highly insecticidal effect against the aphids. The latter may have a better cost–benefit because milk can be used as food. We suggest that future research should study the chemical compounds present in the extract, as well as the effect of the extract on other insect pests and natural enemies and on longer-term population dynamics.

Moreover, the effects of milk as a potential natural insecticide deserve further study.

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