SHORT COMMUNICATION

Bioactivity of Meliaceae, Amaryllidaceae, Solanaceae and Amaranthaceae plant aqueous extracts against the cattle tick *Rhipicephalus microplus*

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**ABSTRACT**

Commercial synthetic acaricides have selected resistant populations of *Rhipicephalus microplus*, and generate residues in the environment or in milk/cattle products. In this study, aqueous extracts (AE) from *Melia azedarach* (Maz), *Allium sativum*, *Capsicum chinense*, *Nicotiana tabacum* (Nta) and *Dysphania ambrosioides* were evaluated for the bioactivity against the cattle tick. The treatment using Nta or Maz AE resulted in the lowest egg hatching rate (34.0 ± 11% and 25.0 ± 19%), and in the values of reproduction inhibition ranging from 89.0% to 85.3%. Phytochemical screening associated to RP-HPLC/DAD analysis suggested the presence of alkaloids for Nta and gallic acid derivatives and catechins, for Maz. Such results highlighted that the use of Nta and Maz AE can be a promising source of bioactive compounds for the control of infections caused by the cattle tick.

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1. Introduction

*Rhipicephalus microplus* is a hematophagous ectoparasite found on cattle, and is a vector for *Anaplasma marginale*, *Babesia bovis*, *Babesia bigemina*, and other microorganisms (Ghosh et al. 2015). The cattle tick *R. microplus* causes significant economic losses to cattle production (liveweight gain and milk production), and a high cost is associated with its control (Grisi et al. 2014).

Plant extracts have been pointed out as a fruitful source of new acaricides, by the action of different bioactive molecules, and may help to reduce the emergence of resistant strains and environmental contamination. Alternative farming techniques for tick control have also been applied to promote animal health, welfare, and sustainable food production (Chen et al. 2012). Some studies have reported that Meliaceae, Amaryllidaceae, Solanaceae and Amaranthaceae families have already shown to contain compounds capable of affecting *R. microplus* (Adenubi et al. 2018). However, phytochemical prospecting and acaricide effects of aqueous extracts obtained from plants of these species on cattle ticks adult females are still scarce (Oyagbemi et al. 2019). Thus, the aim of this study was the bioprospecting of aqueous extracts (AE) from *Melia azedarach* (Maz) present in barks (bMaz) or leaves (lMaz), *Allium sativum* (Asa) obtained in organic (esAsa) or conventional cultivation systems (mgAsa), *Dysphania ambrosioides* (Dam), *Capsicum chinense* (Cch), and *Nicotiana tabacum* (Nta) against *R. microplus*.

2. Results and discussion

2.1. Phytochemical analysis, total phenolics and total flavonoids

Alkaloids were observed in Nta and Cch aqueous extracts (AE) (Table S1). Alkaloids have cytotoxic, larvicidal and ovicidal effects, which can cause the reduction in the fecundity of various arthropods (Chowański et al. 2016). AE from all plants exhibited phenolics and terpenoids (Table S1). Some biochemical and pharmacological properties have been reported for phenolics and flavonoids present in AE of Meliaceae, Amaryllidaceae, Solanaceae and Amaranthaceae families (Adenubi et al. 2018). Total
phenolic (Tph) content varied from 17.83 to 11.90 mg GAE/g, while total flavonoids (Tfl) from 5.40 to 0.15 mg CTE/g (Table S2). The highest values for Tph and Tfl were observed in the bMaz and Dam AE, respectively, while the lowest values in the esAsa AE. In addition, values of Tph and Tfl showed some variation, comparing AE prepared with different parts of the plant (bMaz/lMaz) or when the same plant was grown in different crop conditions (esAsa/mgAsa). Differences in the chemical composition of AE can be related to plant genotype, edaphoclimatic conditions, type of vegetal tissue studied, and phytochemical methods (Mpofu et al. 2006; Sreelatha and Padma 2009; Cen-Pacheco et al. 2020; Parra-Palma et al. 2020).

2.2. PCA/HCA, HPLC-DAD and acaricide activity

The Hierarchical Clusters Analysis (HCA) indicated 2 plant species groups (A and B) identified by hatching (Hat) and reproduction inhibition (RI), taking into consideration dissimilarity ≥ 1.8. Group A, represented by bMaz and Nta, stood out as a separated group in Principal Components Analysis (PCA) and HCA (Figure S1). Tph and Tfl contents contributed more than the classes of secondary metabolites (anthraquinones, xanthones, catechins, flavonols, 2-deoxy sugars, or alkaloids—Table S1) to the separation of the groups. The PCA showed that the division of B Group (B1, B2 and B3) also was influenced by Tph and Tfl contents. Dam and Cch AE showed the highest values for Tfl, while esAsa AE the lowest value in the B Group (Figure S1-A). Although Tph and Tfl had contributed to the segregation of A and B Groups, it is also important to consider that the presence of other secondary metabolites classes from plants at A Group, than phenolics and flavonoids, may have contributed to reduce the Hat and RI of R. microplus.

Egg weights (Ewe) following exposure of Nta, esAsa, Dam, and lMaz AE were lower and differed significantly from the control treatment. When females of R. microplus were exposed to bMaz, Nta, esAsa, Dam and Cch AE, Hat values were reduced, and were also significantly different from the control treatment (Table S3). The lowest hatching (Hat < 35%) and the highest reproduction inhibition (RI > 85%) values were observed after exposure of R. microplus females to the bMaz and Nta AE (Table S3). HPLC/DAD analysis suggested the presence of alkaloids for Nta and gallic acid derivatives and catechins, for bMaz. These results were in accordance with phytochemical analysis (Table S1), and previous studies (Chen et al. 2012; Sen and Batra 2012). Acaricide and/or repellent effects of organic extracts containing phenolics, flavonoids, terpenoids, steroids, and alkaloids from plants belonging to the Lamiaceae, Papaveraceae or Fabaceae families against ticks have been reported in the literature (Adenubi et al. 2018). However, the majority of these experiments are related with the use organic compounds which were extracted under conditions other than presented here (Adenubi et al. 2018; Oyagbemi et al. 2019; Fernandez et al. 2020; Gonçalves et al. 2020; Pereira Junior et al. 2020). On the other hand, studies presenting data about the phytochemical composition and acaricide activity of aqueous extracts from plants of Meliaceae, Amaryllidaceae, Solanaceae and Amaranthaceae families on cattle ticks were not seen in the last years. Then, results presented here are showing a comparison of the chemical characterization and of the effects of a set of plants on R. microplus. These results could be used for the adoption of more sustainable practices by farmers, and for the improvement of agricultural products quality.
3. Experimental
See supplementary material.

4. Conclusions
*Melia azedarach* from barks (bMaz) and *Nicotiana tabacum* (Nta) aqueous extracts were effective in controlling hatching and for reproduction inhibition of *Rhipicephalus microplus* females. RP-HPLC/DAD analysis suggested the presence of alkaloids for Nta and gallic acid derivatives and catechins, for Maz, which could be associated with the most expressive effect on tick control. These results contribute to elucidate the chemical profile of bMaz (Meliaceae) and Nta (Solanaceae) aqueous extracts, and their potential use in veterinary medicine.

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