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Evaluation of the insecticidal potential of leaf extracts of *Anadenanthera colubrina* var. *cebil* (Griseb) Altschul against *Tetranychus urticae* Koch and *Sitophilus zeamais* Mots

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ABSTRACT

The deterioration and degradation of crops by insects causes economic losses of several billion dollars and affects food security. *Sitophilus zeamais* and *Tetranychus urticae* are two economically important species of crops around the world. The high toxicity of synthetic insecticides and the development of insect resistance to currently used chemicals stimulate the investigation of plant-derived insecticides as new alternatives for pest control. The insecticidal properties of secondary metabolites have been recognized, and have been confirmed scientifically in the last decades. The extract in cyclohexane (Ch) and ethyl acetate (Ea) of the leaves of *Anadenanthera colubrina* var. *cebil* collected in the dry and rainy seasons were evaluated for the survival and nutritional physiology of *S. zeamais* (maize weevil) and the acaricidal effect front *T. urticae* (two-spotted spider). All extracts induced mortality of *S. zeamais*; however, only cyclohexane dry season (Chd) and ethyl acetate in the rainy season (Ear) caused significant mortality ($p < 0.05$), more than 50%, promoting physiological damage to insects in all concentrations tested. In the mortality tests with the spider mite, extracts Chd and Ear showed an LC_{50} of 52.75 and 156.42 $mg.mL^{-1}$. Still, in the same test, it can be observed that oviposition was also susceptible to extracts. Therefore, the extracts of *A. colubrina* are a source of compounds capable of interfering with and impairing the metabolism of the two studied pests.

Keywords: Natural insecticides, toxicity, two-spotted spider, maize weevil

Introduction

The insect infestation in cultivation and storage regions cause considerable economic losses to agricultural production worldwide (Amante et al., 2017). Among the most damaging species are *Tetranychus urticae* (Two-spotted spider) and *Sitophilus zeamais* (Maize weevil) (Baker et al., 2017). These insects are cosmopolitan and attack various economically essential crops such as corn, wheat, and others. Two-spotted spider causes a decrease in production by directly attacking the plant, feeding on the sap, causing the appearance of chlorotic spots causing leaf death (Mercês et al., 2018). Maize weevil mainly attacks grains directly but also consumes processed products (Fazolin et al., 2010).

Traditional chemical insecticides are currently the primary form of control of these pests. However, its excessive and indiscriminate use has rendered pest insect populations resistant and, in addition to other problems associated with damage to non-target organisms, such as other non-harmful insects (e.g. pollinators) (Restello et al., 2009) of global crop production (Mazzoleni & Oliveira, 2010), besides being environmental contaminants, of food and affecting human health (Kodavanti & Loganathan, 2016). Recently several studies on molecules searches in plants that can act as an alternative method of combating insects have been developed (Pavela et al., 2016; Mercês et al., 2018).

Plants interact with the environment through the production of organic compounds, called secondary metabolites, which naturally deplete various biological functions and among them some molecules are responsible for protection against insect attack (Pavarini et al., 2012). Among alternative products for combating insect pests from agriculture, several plant extracts, organic and aqueous have been cited as effective in combating various pests (Benelli et al., 2017). They present a complex composition, with hundreds of biomolecules with diverse applications, and represent an ecologically correct alternative in the fight against pests in agriculture (Raliya et al., 2018).

Anadenanthera colubrina var. *cebil* (Griseb) Altschul is a tree widely distributed throughout Brazil, native to South America and Northeast Brazil. It is popularly known as angico, belongs to the Fabaceae family and can reach up to 7 m in height (Agra et al., 1993; Barretto & Ferreira, 2011). The presence of the flavonoid Anadanthoflavone can inhibit lipoxygenase (Gutierrez-Lugo et al., 2004). Routine and quercetin are also compounds found in the fruit. It presents a hallucinogenic and poisonous action in the hydroalcoholic extract demonstrating antioxidant and photoprotective activity of DNA (de Sousa Araújo et al., 2008). In the aqueous extract, it was observed the larvicidal activity (Farias et al., 2010). Phytochemical studies revealed that the leaves present tannins, flavonoids, and proanthocyanidins, proving the antimicrobial activity against strains of *Staphylococcus aureus* (Araújo et al., 2014).

Material and Methods

Plant material

Leaves of *Anadenanthera colubrina* var. *cebil* were collected in the Catimbau National Park, in the Northeast of Brazil, in January and June (dry and rainy season, respectively). The collected material was oven dried at 45ºC for 72h, then ground to obtain a thin powder, stored in an airtight container and kept at 4ºC until use. One specimen was identified and registered by the Herbarium of the Instituto Agronômico de Pernambuco (IPA), under voucher IPA - 80350.

Organic extracts

Organic extracts were obtained from 100 g of the powder of the sheets subjected to eluotropic series of organic solvents: cyclohexane (Ch), chloroform (Cf) and ethyl acetate (Ea) in Soxhlet at a temperature below the boiling temperature of the solvent, and each was kept under reflux for 24h. The extracts were then filtered, and the solvents were entirely removed on a rotary evaporator at 45°C under reduced pressure. The extracts were stored at room temperature in a desiccator. In this study, extracts obtained by extraction with cyclohexane and ethyl acetate were tested.

Insects

Sitophilus zeamais

Maize weevil colonies were obtained from the Protein Biochemistry Laboratory of the Department of Biochemistry of the Federal University of Pernambuco (Recife, Brazil) and kept in glass containers covered with voal at 28 ± 2 ºC, with 70% relative humidity and light 12:12h photoperiod: dark. The diet consisted of corn grains selected according to the integrity, sanitary conditions, size, and absence of contamination by insects. Adult insects (30 to 60 days old) were used per treatment.

Tetranychus urticae

The harvested mite was obtained from the rearing kept in bean (*Canavalia ensiformes* L.) plants of the Agricultural Acarology Laboratory of the Departamento de Agronomia of the Universidade Federal Rural de Pernambuco (Brazil). The insects were kept in glass containers, closed with delicate fabric (tulle type) to allow gas exchanges, at a temperature of 25 ± 1ºC, with 65 ± 3% relative humidity and a photoperiod of 12h: 12h light: dark. Ten adult females were released at baseline. Four replicates were used per treatment.

Evaluation of toxicity by ingestion of organic extracts

The tests were performed according to Napoleão et al. (2013). Each bioassay consisted of a Petri dish containing five discs of a mixture of wheat flour with the extracts of the dop angico leaves. Concentrations of 250 to 750 $mg.g^{-1}$ were evaluated. The disks were prepared using extracts mixed with 2 g of autoclaved flour, and solubilized in water to a final volume of 5 ml. After homogenization for 5 min, five aliquots of 200 µL were arranged in Petri dishes equidistantly and maintained in an oven at 56°C for 24 h, and then the mass of the discs was recorded.

Groups of 20 adult *S. zeamais* insects of known weight were transferred to each plate. The bioassays were maintained at 28 ± 2 ºC and carried out in triplicate. Dimethylsulfoxide 1% was used as a negative control. After seven days of the experiment, the mortality rate was determined, observing the motility of the insects to the naked eye and the weights of the diet discs and the insects were measured to determine the index of deterrence.

Bioassay

The food deterrence index (FDI) was calculated according to the formula: FDI = 100 x $[(MI_c - MI_t)] / MI_c$, where MI_c corresponds to the mass ingested in the control and MI_t to the mass ingested in the test. According to FDI values, the effect of the sample was classified as: non-deterrent (FDI < 20%), weak detergent (20% < FDI ≤ 50%), moderate detergent (50% < FDI ≤ 70%), or (FDI ≥ 70%) (Procópio et al., 2015).

The following nutrient indices were also calculated: Relative consumption rate (RCR) = (ingested mass) / (initial biomass of insects x days), Relative biomass gain rate (RBGR): (biomass acquired / (initial biomass of insects x days). For statistical analysis, significant differences ($p < 0.05$) were calculated between treatments and were calculated using the Tukey's test Data were expressed as mean ± standard deviation.

Residual contact test – Fumigation

The fumigation test was done, according to (Arena et al., 2017), with modifications to evaluate the residual contact toxicity of the organic extracts of *A. colubrina* var. cebil against the mite. The concentrations used ranged from 9 to 102.6 mg.mL^{-1} for extracts Ch and 9.5 to 500 mg.mL^{-1} for extracts Ea, the solutions being prepared by solubilizing extracts in methanol. Three leaf disks of bean (2.5 cm) were immersed in solutions previously prepared. After drying the discs were conditioned equidistantly in Petri dishes containing a filter paper disk saturated with water to prevent mite leakage and to maintain the turgidity of the leaves. Ten adult females were placed on each leaf disc, and mortality was determined after 24 hours of exposure. Mites were considered dead when unable to walk a distance greater than the length of their body after a slight touch with the end of a brush of fine bristles. The evaluation of the oviposition after exposure to extracts will be done after the number of eggs from the treatments and the control be quantified. The Azamax botanical acaricide was used as positive control and methanol with negative control. All experiments were performed in triplicate. The data were analyzed by the Probit model through the POLO-PC Software for the determination of the LC_{50} values, with 95% confidence intervals, validated through the SAS statistical program and generated in graphic form through SigmaPlot software.

Results

After the exposure of *S. zeamais*, the extracts for seven days observed that all presented a significant difference in insect mortality when compared to the control (Figure 1). It is important to note that the extracts EaR and ChD caused death above 50%. However, we observed that the former showed a higher efficiency with activity already from the lowest concentration tested (250 mg.mL^{-1}).

The relative consumption rates (RCR) of diets with *S. zeamais* extract were zero or lower than the control consumption, differing significantly from the control. These data suggest the rejection of the pre-ingestion diet (Figure 2), showing that there was a strong effect deterrent caused by the extracts tested, indicating that they were able to prevent the consumption of the diet when used to *A. colubrina*.

The rates of relative biomass gain in the treatments with all the extracts (Figure 3) were negative, indicating that the insects lost biomass, since the insects because they did not feed on the diet, consumed its body reserves. The calculation of the FDI values indicated a strong food deterrence effect in all the extracts and concentrations tested. Thus, leaf extracts of *A. colubrina* contain compounds capable of causing diet rejection.

The concentration-mortality responses of the split mite subjected to the ChD and EaR extracts as well as the Azamax, used as a positive control, were compared regarding the difference of the estimated LC_{50}. Table 1 shows that ChD and EaR extracts tested were toxic to *T. urticae* by residual contact after 24 hours of exposure, but Azamax showed superior efficiency due to a small concentration of LC_{50}. The susceptibility of the spider mite was higher for the ChD extract, which was about 1.39 times more toxic than the EaR extract. Azamax was about 170 times more toxic than the ChD extract and about 236 times more toxic than the EaR extract.

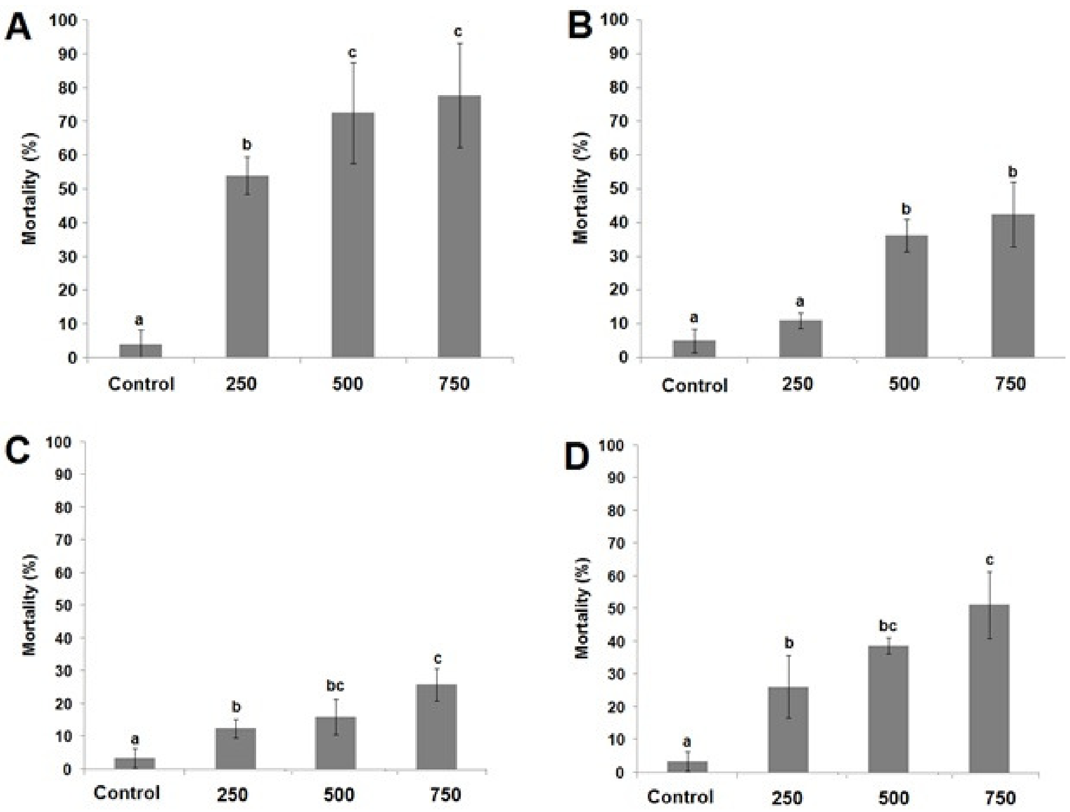

Figure 1. Mortality Rate (%) of *Sitophilus zeamais* under the effect of EaD (A), EaR (B), ChD (C), ChR (D) in different concentrations ($mg.g^{-1}$).

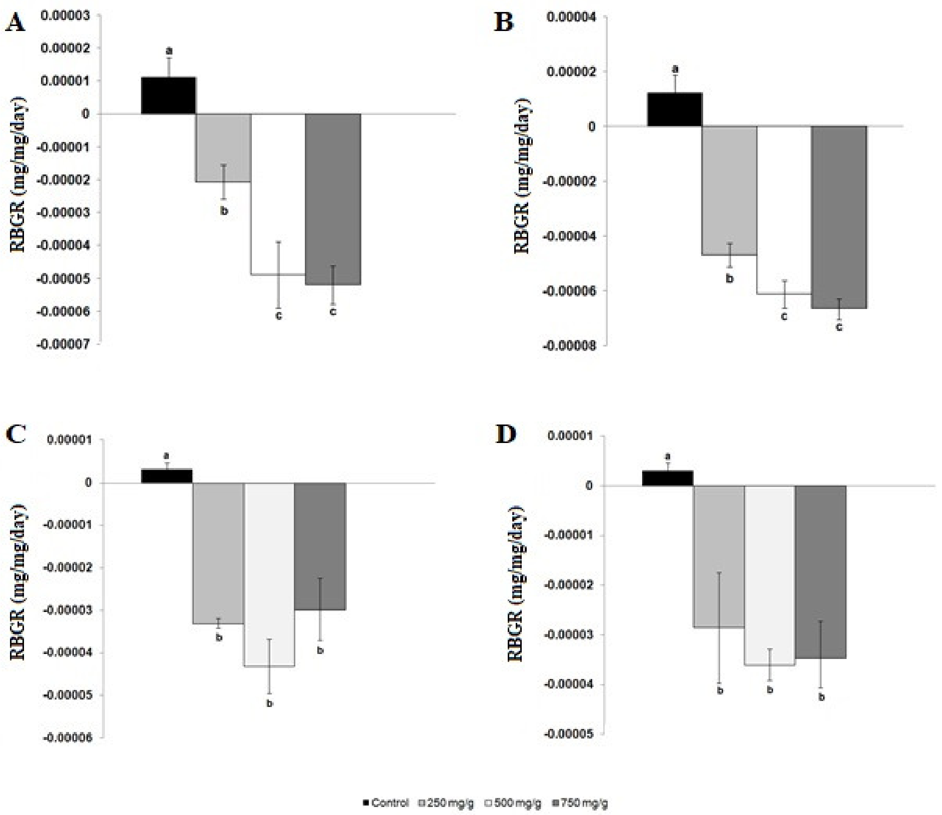

Figure 2. Relative consumption rate of artificial diets containing: EaD (A) EaR (B), CfD (C) CfR (D) from *Anadenanthera colubrina* by adult insects of *Sitophilus zeamais*. Different letters indicate significant differences among treatments.

However, it was observed that in sublethal concentrations the extracts of *A. colubrina* affected the fecundity in *T. urticae*. As the concentrations for both extracts were increased survival decreased, causing an effect, consequently reducing oviposition.

As can be seen in Figure 4, ChD extract at the concentration of 40 mg.mL^{-1} caused mortality of about half of the individuals with a 10% reduction in fecundity. At the highest concentration of 102.6 mg.mL^{-1}, mortality reached 100%. Regarding the EaR extract, the concentration capable of killing 100% of the individuals was 416 mg.mL^{-1}.

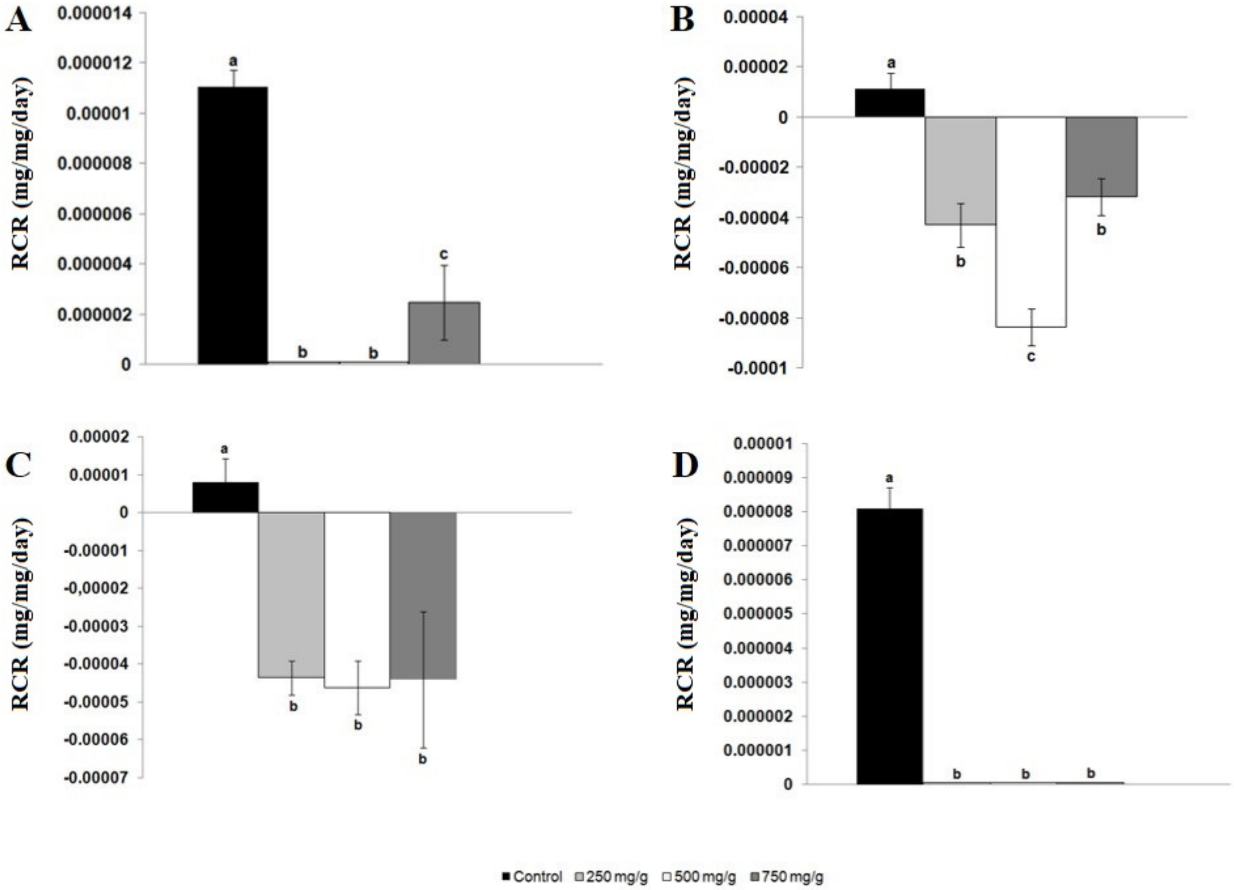

Figure 3. Relative gain rate of the biomass of *Sitophilus zeamais* adult insects kept in artificial diets containing: EaD (A) EaR (B), CfD (C) CfR (D) from *Anadenanthera colubrina*. Different letters indicate significant differences among treatments.

Table 1. Residual contact toxicity (LC_{50} in mg.mL^{-1}) of the extracts ChD and EaR from the leaves of *Anadenanthera colubrina* var. cebil and Azamax on *Tetranychus urticae*. N = individuals' Number; DF = Degree of Freedom; χ^2 = qui-square; TR = Toxicity Reason; RI = Reliability Index.

Extract	N	DF	Slope	LC_{50} (RI 95%)	χ^2	TR (RI 95%)
ChD	540	4	5.61 (5.12 – 6.10)	52.75 (46.28 – 59.86)	7.67	170.71 (150.50 – 193.64)
EaR	810	7	2.35 (2.06 – 2,64)	73.27 (65.41 – 81.39)	8.8	237.11 (203.00 – 276.98)
Azamax	630	5	2.46 (2.08 – 2,84)	0.31 (0.28 – 0.35)	8.3	-

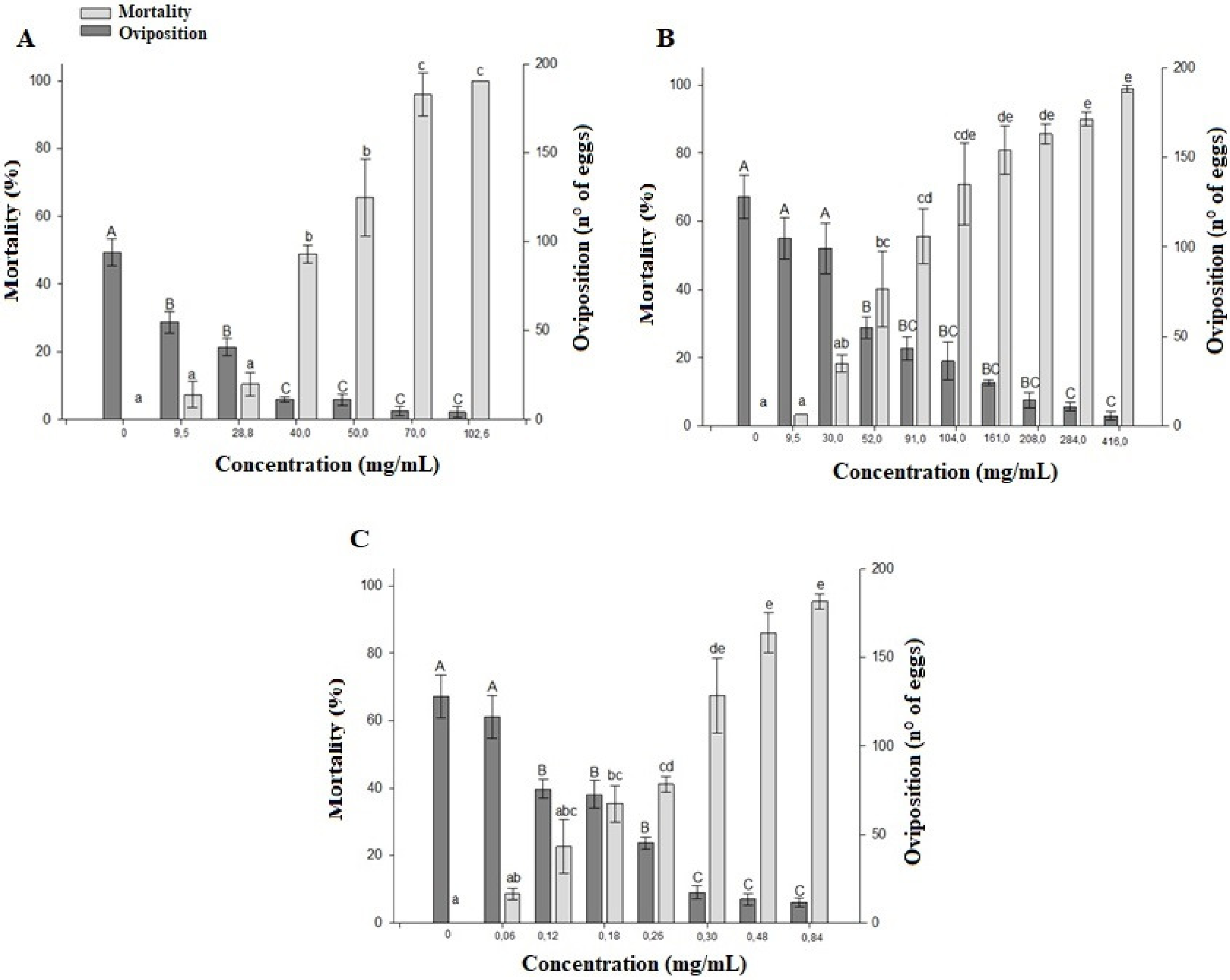

Figure 4. Mortality and oviposition of *Tetranychus. urticae* on leaf discs treated with different concentrations of ChD (A), EaR (B) from *Anadenanthera colubrina* and control with Azamax (C).

Discussion

Natural plant products can be used on a small scale by farmers to protect stored grains and vegetables against pests (Ngamo et al., 2006). In recent years, there has been considerable effort to evaluate the potential of secondary plant metabolites as sources of control agents and consequently new sources for the design of target-specific molecules (Ateyyat et al., 2009).

Secondary metabolites present in plants with insecticidal effects may act as inhibitors of insect feeding or hinder growth, development, and reproduction (Spochacz et al., 2018). The deterrent effect of an insecticidal product has great potential in preventing the harmful action of the pest as well as can cause death by starvation (Mello & Silva-Filho, 2002). Thus, the data obtained in this study can be explained due to the nature of the compounds present in the extracts that alter the processes of ingestion and absorption of nutrients, so that the insects begin to metabolize their body reserves to survive (Napoleão et al., 2013).

Changes in insect feeding behavior, as observed in *S. zeamais* results, may be the consequence of a process mediated by gustatory sensitivity (pre-ingestion effect). Due to the lack of ingestion of the disks containing the extracts, the observed nutritional disorders occur. In addition, the effect of deterrence may be related to intoxication (post-ingestion effect), where the insect avoids feeding after ingesting a small amount of food that activates the rejection response (Michiels et al., 2010; Napoleão et al., 2013; Sauvion et al., 2004; Sprawka & Goławska, 2010).

Several plants have insecticidal activities related to their composition of secondary metabolites, such as *Adathoda vasica*, *Cynodon dactylon*, *Eclipta alba*, *Morinda pubescens*, *Ocimum tenuiflorum*, *Phyllanthus amarus*, *Sesbania grandifolora*, *Solanum surattense*, *S. trilobatum* and *Vinca rosae* (Moshi & Matoju, 2017). Classes of compounds such as tannins and flavonoids have been related to those responsible for such activity, because they act in defense of plants against herbivores, causing a deterrent effect. *A. colubrina* presents these compounds are part of the phytochemical composition (Araújo et al., 2014). Tannins and other secondary metabolites may interfere with specific metabolic pathways or insect physiological processes (Lessard, 2004). Studies have shown that the number of metabolites with insecticidal activity has been increasing in recent years. Plants of the genus *Capsicum* contain substances such as alkaloids, flavonoids, coumarins, saponins and essential oils from secondary metabolism with high insecticidal potential and repellency (Ayil-Gutiérrez et al., 2018).

Previous studies have reported the ability of plants to reduce fecundity in *T. urticae* (Pontes et al., 2010; Ribeiro et al., 2016), but without indicating whether this reduction in fecundity was due to the phytochemical properties of the extracts used or to the death of mites. Reduction of egg numbers and inhibition of oviposition are essential effects of plant extracts on insect reproduction (Costa et al., 2004).

The use of plants or its extracts with insecticidal activity has an essential application in public health and agriculture (Matias et al., 2002). It is the first report of acaricidal activity of extracts of *A. colubrina*. Both extracts show promise for direct use of this plant matrix, cost and benefit studies in the preparation of formulations to be applied in open environments, in addition to the effects on predatory mites.

Conclusion

The leaf extract of *A. colubrina* is a natural source of insecticides, which acts against adults of *Sitoplhilus zeamais* and *Tetranychus urticae*. These extracts have the potential to be used to control both the maize weevil, dissuading the consumption and leading the insects to death by starvation, as in the brindle mite, killing the insects through the ingestion of compounds in the extracts.

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