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# Pyroligneous Acid Controls *Aphis craccivora* (Hemiptera: Aphididae) and Increases Cowpea [*Vigna unguiculata* (L., Walp)] Productivity

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**Abstract**

The aphid *Aphis craccivora* Koch (Hemiptera: Aphididae) is a major pest of cowpea crops [*Vigna unguiculata* (L., Walp.)]. Adults and nymphs feed on leaves, stems, flowers, and pods, leading to a reduction in plant productivity. Botanical insecticides can be an alternative for the control of *A. craccivora*, but studies under field conditions are necessary to evaluate these substances. We assessed the mortality of *A. craccivora* in cowpea crops for two seasons (dry and wet) using pyroligneous acid in three concentrations (2, 4 and 6 mL/L). Water was used as the control. Crop productivity was also evaluated. We performed the experiment using a completely randomized block design with four replications. Pyroligneous acid at 4 and 6 mL/L caused higher mortalities ($p < 0.0001$) and productivity was higher in plots sprayed with pyroligneous acid at these concentrations in both experimental seasons ($p < 0.0001$). In summary, pyroligneous acid controls *A. craccivora* and increases cowpea productivity.

***Keywords:*** alternative control; biostimulants; cowpea aphid; organic production.

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## 1. Introduction

A large number of studies are conducted to identify promising botanical insecticides to be used in crop management as an alternative to synthetic products, especially in organic farming, where these products can not be used [1]. However, problems such as plant production (obtaining enough plant material to extract), sophisticated extraction methods and bioactivity validation, and also a lack of field trials demonstrating their efficacy may limit their availability to farmers [2]. Thus, studies that assess promising substances using simple methods of plant extraction and also testing these materials under field conditions are necessary to increase the adoption of this alternative control. The cowpea aphid *Aphis craccivora* Koch (Hemiptera: Aphididae) is a major pest of cowpea crops [*Vigna unguiculata* (L.) Walp.] in tropical regions [3]. Adults and nymphs suck the plant sap from stems, terminal shoots and petioles during the plant vegetative phase, and pods and flowers when the plants are mature [4;5] This insect can cause yield losses of 50% or more in the absence of management practices [6]. Thus, the cowpea aphid must be controlled when its density reaches the economic threshold to mitigate these losses. Botanical extracts based on pyroligneous acid have been described as substances with insecticide activity against sap-sucking insects such as aphids [7]. Therefore, those substances can be an alternative for the control of *A. craccivora,* but there is a lack of information regarding their efficacy under field conditions. Thus, this study was conducted to assess: i) the insecticide activity of aqueous extracts of pyroligneous acid against *A. craccivora* under field conditions, and ii) the productivity of cowpea as a result of these treatments.

## 2. Materials and methods

### *2.1 Pyroligneous acid*

The pyroligneous acid was purchased (Extrato Pironlenhoso do Brasil®, São Paulo, Brazil) and distilled water was added until concentrations of 2, 4 and 6 mL/L were obtained.

### *2.2 Study area and experimental procedure*

We conducted the experiment in cowpea crops (*V. unguiculata* cv. BRS Tumucumaque) located at Picos, Piauí, Brazil (l07º04’37’’ S, 41º28’01’’ W, 206 m above sea level) cultivated during both dry (August-October, 2018) and wet (December-March, 2019) seasons. The climate is arid (BSk, Köppen classification) with irregular precipitation pattern and temperature ranging from 21 to 39 °C [8]. Before each crop establishment, the soil was plowed, barred, grooved and fertilized according to the recommendations for cowpea crops based on soil chemical analysis [9]. Sprinkler irrigation was applied during all the dry season. We performed the experiment using a completely randomized block design ($N$ = 4) with one plot of each treatment per block. We used aqueous extracts of pyroligneous acid at 2, 4, and 6 mL $L^{-1}$ and water as control ($N$ = 16). Each plot had five rows with an inter-row spacing of 0.5 m and intra-row spacing of 0.6 m (12 $m^2$ per plot). Blocks were spaced 1 m apart from each other. Control for other pests or diseases was not used, and weed control was performed every 15 days with a hoe. Treatments were sprayed, during the morning, three times at the end of the crop vegetative stage with a one-week interval between them. We applied one liter of solution per plot using a $CO_2$

pressurized backpack sprayer with a pressure of 275 kPa and a volume of 125 L/ha (model XR110.020, TeeJet®). The number of aphids was counted in each plot. Six evaluations were made in total, one before and another two days after each spraying. Assessments were conducted using only the three middle rows of each plot (we did not use border plants) using a shake cloth (1 m x 0.6 m) sampling technique and a magnifying glass (10x). The shake cloth was placed in each row and plants were beaten five times on it. Afterward, the number of aphids on the shake cloth was counted using the magnifying glass. Then aphid mortality was estimated for each plot as the proportional difference between aphid abundance before and after each spraying: $M$ (%) = $[(C_0 - C_f) / C_0] \times 100$, where $M$ (%) is aphid mortality estimated, $C_0$ is the number of aphids before spraying, and $C_f$ is the number of aphids after treatment application. Crop productivity was estimated in kg/ha using the number of pods and weight of grains from the three central rows where aphids were counted.

### *2.3 Statistical analysis*

We computed all analysis in R (version 3.5.0.) using the package 'agricolae' [10]. Data were tested for normality of errors and homogeneity of variances using, respectively, Shapiro-Wilk and Bartlett's tests. Mortality data were transformed (arcsine√) to fulfill these assumptions [11]. Then aphid mortality was submitted to an analysis of variance ($\alpha = 0.05$) with block, concentration, spraying and the interaction concentration*spraying as covariates, and means were compared using Tukey's test ($\alpha = 0.05$) for significant effects. Cowpea productivity was also submitted to an analysis of variance ($\alpha = 0.05$) with block and treatment as covariates, and means were compared using Tukey's test ($\alpha = 0.05$) for significant effects. Graphics were designed using Sigma Plot software version 11.

## 3. Results

The mortality of *A. craccivora* increased as the concentration and the number of sprayings of pyroligneous acid increased (Figure 1), indicating a significant interaction between these factors for both growing seasons (Table 1). In the dry season, aphid mortality was generally higher at the concentration of 6 mL/L. Highest mortalities were observed for this concentration after the second and third sprayings, as well as for 4 mL/L after the third (Figure 1a). We observed this same trend in the wet season, with highest mortalities caused by the concentration of 6 mL/L after the second and third sprayings (Figure 1b). Overall, in both seasons, pyroligneous acid at the concentration of 2 mL/L caused the lowest mortalities (Figure 1).

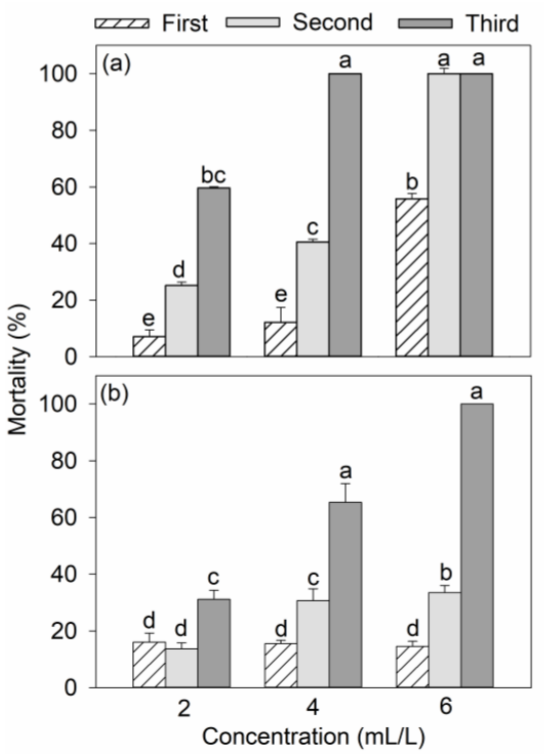


**Figure 1:** Estimated mortality (mean ± standard error) of *Aphis craccivora* for different concentrations of pyroligneous acid (2, 4, and 6 mL/L) and sprayings (first, second, and third) on *Vigna unguiculata* crops cultivated during both dry (a) and wet (b) seasons in Picos, Piauí, Brazil. Different letters indicate differences among concentrations and sprayings by the Tukey's test at $p < 0.05$.

**Table 1:** Summary of the analysis of variance (ANOVA) for the effects of pyroligneous acid at different concentrations, number of sprayings and their interaction in the mortality of *Aphis craccivora* on *Vigna unguiculata* cultivated for two seasons in Picos, Piauí, Brazil. Data were transformed (arcsine√) before ANOVA.

| Source | D.f. | Dry | | Wet | |
|---|---|---|---|---|---|
| | | F | *p* | F | *p* |
| Block | 3 | 0.91 | 0.44 | 2.20 | 0.11 |
| Concentration | 2 | 410.48 | <0.001 | 92.57 | <0.0001 |
| Spraying | 2 | 368.28 | <0.001 | 260.9 | <0.0001 |
| Concentration*Spraying | 4 | 123.58 | <0.0001 | 54.58 | <0.0001 |
| Residuals | 24 | | | | |

D.f. = degrees of freedom.

The control of *A. craccivora* increased the productivity of *V. unguiculata* (Table 2). All plots treated with pyroligneous acid had significant higher values compared to the control plots (Table 3). In both seasons, plots that received 6 mL/L of pyroligneous acid had the highest productivity, followed by the concentrations of 4 and

2 mL/L (Table 3).

**Table 2:** Summary of the analysis of variance (ANOVA) for the productivity of *Vigna unguiculata* according to the application of different concentrations of pyroligneous acid in two seasons of growth in Picos, Piauí, Brazil.

| Source | D.f. | Dry | | Wet | |
|---|---|---|---|---|---|
| | | F | *p* | F | *p* |
| Block | 3 | 2.33 | 0.14 | 2.75 | 0.12 |
| Treatment | 3 | 7298.54 | <0.0001 | 577.2 | <0.0001 |
| Residuals | 9 | | | | |

D.f. = degrees of freedom.

**Table 3:** Productivity (kg/ha) of *Vigna unguiculata* according to the applied concentration (2, 4, and 6 mL/L) of pyroligneous acid (PA) in two seasons of growth (dry and wet) in Picos, Piauí, Brazil. Different letters in the same column indicate differences by the Tukey's test at $p < 0.05$.

| Treatment | Mean (± standard error) | |
|---|---|---|
| | Dry | Wet |
| Control | 966.70 (±17.94) a | 1199.87 (±37.38) a |
| PA 2 $mL.L^{-1}$ | 1553.62 (±11.50) b | 1585.75 (±33.63) b |
| PA 4 $mL.L^{-1}$ | 2267.51 (±4.06) c | 2360.52 (±38.75) c |
| PA 6 $mL.L^{-1}$ | 2904.76 (±6.98) d | 3332.82 (±88.79) d |

## 4. Discussion

Simple methods for pest control are desirable and can help farmers to improve crop production, especially in regions at the tropical zone where the majority of the agriculture is of a small scale with few resources available [2]. Botanical insecticides are a good alternative for pest management under these circumstances and also have a great impact in more specific conditions like organic farming, where resources for pest control are even more limited. However, the validation of these alternative methods depends on field trials. In this context, the findings in this study are an improvement in cowpea production because they can enhance pest management programs of small-scale producers and organic farmers. Our study was done under field conditions and it shows that pyroligneous acid controls *A. craccivora* and increases cowpea productivity. This effect changed as a function of concentration with higher mortalities at the highest tested concentration (i.e., 6 mL/L). First, two aspects must be considered: i) the insecticide effect of pyroligneous substances, and ii) the induction of plant growth and development by this product. The bioactivity of pyroligneous substances has been reported for *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae), causing more than 90% mortality 48 hours after exposure [7]. Pyroligneous is a byproduct of wood carbonization process, and it has a high number of substances in its composition such as phenolic compounds, aldehydes and organic acids [9]. These compounds have an insecticide effect, but also a positive impact on plant growth and development (biostimulants) as demonstrated for tomato, soybean, rice and others crops [12]. Also, the concentration of a substance plays an important role on its biological effect and therefore we expected greater mortality rates at higher extract concentrations. This hypothesis was confirmed, especially for the highest concentration of the pyroligneous (6 mL/L). Altogether, these aspects may explain the results found in our study. Crop productivity depends on factors such as fertilizer, control of pest and diseases, cultivar, water availability and others. Among them, the relationship between yield loss and pest density is an important step to determine the economic injury level for pest control. For cowpea crops, an increase in *A. craccivora* density can raise yield losses by 50% or more [6]. Thus, the management of this insect is particularly relevant right before the beginning of the flowering period to reduce the dispersion of *A. craccivora* to these structures [5]. In this sense, our study shows that even low rates of *A. craccivora* control can increase cowpea productivity as highlighted by the contrast between treated plots at the minimum concentration and the lowest productivity observed in control plots. Our study indicates that pyroligneous extract can cause mortality to A. craccivora, increasing cowpea productivity. This study contributes to the use of a friendly insecticide on fields that does not impact the environment negatively. Further, we suggest that other pyroligneous extract concentrations can be tested to increase cowpea productivity.

## 5. Conclusion

Our study demonstrates that field control of *A. craccivora* using pyroligneous acid can be efficient and it increases cowpea productivity. This effect was due to its dual action (i.e., pesticide and increase in plant growth and development) and therefore its incorporation in management programs of cowpea aphid is recommended.

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