

## Artigos

### ***Leucoagaricus gongylophorus* provides protection for *Atta sexdens* against plant extracts**

*Leucoagaricus gongylophorus* confere proteção para *Atta sexdens* contra extratos vegetais

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## ABSTRACT

Plant extracts have been considered for the control the leaf-cutting ants. The objectives of this study were to evaluate the survival leaf-cutting worker ants after topical application of *Ageratum conyzoides* (mentrasto) and *Manihot esculenta* (cassava) extracts; and to evaluate the effect of *Manihot esculenta* hexane extract via ingestion on the survival of the workers of *Atta sexdens* Forel 1908 isolated or not from their colonies, in order to verify whether the symbiotic fungus *Leucoagaricus gongylophorus* confers protection to the workers against the extract. First, ten medium workers were removed from their colonies and received the application of 1  $\mu$ L of extract on the pronotum. The cassava extract (125  $\mu$ g.mL<sup>-1</sup>) was diluted in a solution of honey with water for the ingestion bioassay. Five repetitions (ten ants inside a transparent plastic container) were performed in each of the four colonies used in the experiment. The numbers of dead ants were recorded daily until the control also died. The solution of the hexane *Manihot esculenta* extract gave a significant result at the concentration of 100  $\mu$ g.mL<sup>-1</sup>, with 100% of mortality after 4 days. The ants maintained with the symbiont fungus that ingested the cassava hexanic extract showed a lower mortality rate (40% at the end of 25 days) than the workers who were kept isolated (90%). The symbiosis between the ants and the fungus means more than a food source for leaf-cutting ants because it reduces the toxic effect on the ants when the ants remain in contact with the fungus and is offered an unsafe vegetable.

**Keywords:** Leaf-cutting ants; *Ageratum conyzoides*; *Manihot esculenta*

## RESUMO

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Extratos vegetais foram utilizados para o controle das formigas cortadeiras. Os objetivos deste estudo foram avaliar a sobrevivência de operárias de formigas cortadeiras após aplicação tópica de extratos de *Ageratum conyzoides* (mentrasto) e *Manihot esculenta* (mandioca); e avaliar o efeito por ingestão do extrato de *Manihot esculenta* extraído com hexano na sobrevivência de operárias de *Atta sexdens* Forel 1908 isoladas ou não de suas colônias, a fim de verificar se o fungo simbiótico *Leucoagaricus gongylophorus* confere proteção às operárias contra o extrato. Primeiro, dez operárias médias foram removidas de suas colônias e receberam a aplicação de 1 µL de extrato no pronoto. O extrato de mandioca (125 µg.mL<sup>-1</sup>) foi diluído em uma solução de mel com água para o bioensaio de ingestão. Foram realizadas cinco repetições (dez formigas dentro de um recipiente de plástico transparente) em cada uma das quatro colônias utilizadas no experimento. O número de formigas mortas foi registrado diariamente até que o tratamento controle também morresse. A solução do extrato *Manihot esculenta* com hexano apresentou resultado significativo na concentração de 100 µg.mL<sup>-1</sup>, com 100% de mortalidade após 4 dias. Formigas mantidas com o fungo simbiote que ingeriu o extrato hexânico da mandioca apresentaram menor mortalidade (40% ao final de 25 dias) do que as operárias mantidas isoladas (90%). A simbiose entre as formigas e o fungo significa mais do que uma fonte de alimento para as formigas-cortadeiras, devido ao fato de reduzir o efeito tóxico de extratos vegetais nas formigas quando elas permanecem em contato constante com o mesmo.

**Palavras-chave:** Formigas-cortadeiras; *Ageratum conyzoides*; *Manihot esculenta*

## 1 INTRODUCTION

Leaf-cutting ants live in symbiosis with the fungus *Leucoagaricus gongylophorus* (Singer) grown inside their nests. To keep this association, the ants cut fresh vegetable materials and take them to the nest where they serve as substrate for the fungus (HOLLOBLER; WILSON, 1990). The fact, that the ants cut large quantities of fresh vegetables, makes them attain great economic importance (DELLA LUCIA; SOUZA, 2011). This group of ants is considered historically one of the great obstacles to the exploitation of forests, agricultural plantations and grazing land (MATRANGOLO *et al.*, 2010).

Controlling these ants usually require the use of insecticides, in the form of dry powder, of liquids applied as fogs or in the shape of baits, this last one the preferred type (MARINHO; DELLA LUCIA, 2016). However, the chemical control may cause detrimental effects on the environment and to men; as a result, sulfluramid, the principal compound now used, is about to be prohibited (STOCKHOLM CONVENTION, 2008). Hence, research has advanced in the quest for insecticides collected from toxic

plants supposed to be less aggressive to the milieu (BUENO; BUENO, 2011) but harmful to leaf-cutting ants (GANDRA *et al.*, 2011; SOUZA *et al.*, 2012; GOMES *et al.*, 2016). The application of their extracts is made directly on the fungus developed in culture media or on the ants themselves (BUENO *et al.*, 2004; FRANCO *et al.*, 2013; SANTOS *et al.*, 2013; MORAIS *et al.*, 2015). Santos *et al.* (2013), who used a methanol extract of cassava leaves (*Manihot esculenta* = Me) applied upon worker ants of *Atta sexdens rubropilosa* of different castes, found that, if the concentration of the extract was high enough, there occurred a reduction on survival of the insects. Morais *et al.* (2015) also observed an inhibition of 100% on the growth of the symbiotic fungus of *Atta sexdens rubropilosa* when a mentrasto (*Ageratum conyzoides* = Ac) was applied.

However, no insecticide containing plant extracts as active ingredient is so far available in the market. Leaf-cutter ants are social insects which have complex physiological and behavioral defense mechanisms that difficult the development of new products for their control (MARINHO; DELLA LUCIA; PICANÇO, 2006).

Among the defense mechanisms presented by leaf-cutting ants there is an enzyme called lacase, present at the tip of the specialized hyphae of the symbiont (gongylidia), a preferred structure by the ants for eating. The lacase thus ingested passes through the digestive system of the ants and is still active in the fecal particles liberated by the insect on the leaves that they add to the fungus garden (DE FINE LICHT *et al.*, 2013).

De Fine Licht *et al.* (2013), reported that fourteen species of leaf-cutting ants bear that enzyme. It is capable of detoxifying secondary compounds perchance present in the vegetable fragments; such fragments would no longer harm the colony. It is conceivable, then, that if lacasse is de-activated, the toxic substances would remain in the fragments and eventually kill or impair the ants. The enzyme has not yet been detected in *Atta sexdens rubropilosa*.

The present work had the objective of evaluating the effect of the symbiont fungus *Leucoagaricus gongylophorus* on the survival of workers of *Atta sexdens* when these are exposed to extracts of leaves of mentrasto (Ac) and of cassava (Me).

## 2 MATERIAL AND METHODS

The preparation of the vegetable extracts, the rearing of the ants and the bioassays were conducted at the Laboratories of Agricultural and Forest Entomology and of General Chemistry of the Federal University of São João del Rei, Campus of Sete Lagoas, state of Minas Gerais, Brazil.

The raising of the ants was conducted under controlled conditions of temperature ( $25 \pm 3$  °C), of humidity ( $60 \pm 10\%$ ) and photoperiod (escotofase of 24 hours) (ARAUJO *et al.*, 2011). The survival was evaluated daily, by counting the number of living ants and the volume of the fungus garden until the death of the control individuals.

### 2.1 Vegetable extractions

Leaves of mentrasto (Ac) and cassava (Me) were used in obtaining the extracts, the first being collected in Paraopeba - MG ( $19^{\circ} 16' 54''$  Sul,  $44^{\circ} 24' 32''$  Oeste) and the second in Sete Lagoas - MG state ( $19^{\circ} 28' 4''$  Sul,  $44^{\circ} 14' 52''$  Oeste), Brazil, on January of 2018. The fresh material was dried at the ambient temperature for 30 days and ground in an industrial blender until a very fine powder was obtained.

The extract of cassava was prepared with 10 g of the powder inside an Erlenmeyer. The powder was homogenized with 200 mL of methanol-hexane solvent. In the case of mentrasto, 8 g of powder were homogenized with 160 mL of the same solvent. The material was taken to a chapel where it remained for 15 days for the extraction of the molecules of the vegetables. The solution present in the Erlenmeyer were strained in filter paper. The filtered material was kept in the chapel for other 7 days, this time in beakers. After this, the solvent evaporated, leaving the chemical substances, the crude extracts. The beakers were then washed carefully with their respective solvents and the chemical so obtained were deposited in Petri dishes and kept in the chapel for 7 more days (SIMÕES; SPITZER, 2002). The crude extract was then diluted to obtain concentrations of  $50 \mu\text{g.mL}^{-1}$ ,  $100 \mu\text{g.mL}^{-1}$  and  $250 \mu\text{g.mL}^{-1}$ .

## 2.2 Topical application of the extracts on workers

Four colonies of *Atta sexdens* from the collection were selected to provide the ants. These colonies had all about 2 to 3 liters of fungus. Ten workers of medium size (1.75 to 2.4 mm of head capsule width) were taken to transparent plastic containers (500 mL capacity) with a perforated lid underneath wet cotton.

Every recipient was considered a sampling unit. Five repetitions were assayed for each concentration of each of the solutions. Three concentrations were tested (50, 100 and 250  $\mu\text{g}\cdot\text{mL}^{-1}$ ), in addition to the control, a pure quantity of the solvent of the respective extract solution, making a total of 80 plastic containers for each bio-assay. The methodology here utilized was adapted from Ribeiro *et al.* (2012). Together with a micro-pipet of 10  $\mu\text{L}$  capacity, both the extract solutions and the solvents were applied to the ant pronotum; each individual received 1.0  $\mu\text{L}$ . To feed the ants, two small tubes filled with cotton were placed on the sides of the plastic recipients. One of the tubes received water, the other honey diluted with distilled water 1:1.

## 2.3 Application by ingestion of vegetable extracts to ant workers set aside with or without the mass of fungus

Four colonies of *Atta sexdens* with fungus volume between 3 and 4 liters and similar foraging activities were chosen for this experiment. Ten medium sized workers (1.75–2.4 mm of average head capsule width) were isolated with two grams of fungus mass (but without larvae and pupae) inside plastic containers (500 mL) with perforated lids and a wet cotton swab. Each arrangement of ants and fungus was considered a replication. Five sampling units (repetitions) were prepared for each colony. The same quantity of ants and containers were again prepared but deprived of the fungus

A recipient was placed inside the container already described. This new recipient contained 1 mL of the hexane solution of cassava extract of the concentration of 125  $\mu\text{g}\cdot\text{mL}^{-1}$  diluted with a solution of 1:1 honey in water. This extract was applied in the 125  $\mu\text{g}\cdot\text{mL}^{-1}$  concentration, as the 100  $\mu\text{g}\cdot\text{mL}^{-1}$  concentration showed higher toxicity during the topical application bio-assay.

A plant fragment from the species used in rearing the ants was offered inside the recipient with the mass of fungus so that the ants could carry on their activities. The recipients without the fungus received no fragment but the same honey solution and the extract.

## 2.4 Application of the plant extract on the symbiont fungus

Two grams of fungus with larvae and pupae were separated in plastic recipients along with 10 adult workers of medium size (1.75–2.4 mm of head capsule width). Ten  $\mu\text{L}$  of the hexane solution of cassava at the concentration of  $100 \mu\text{g}\cdot\text{mL}^{-1}$  was spread over the fungus using a micro-pipet; this solution had indicated a higher toxic effect. To be better distributed, this application occurred in five portions.

Plant fragments were daily put inside the plastic container for the ants to carry on their activities. Water was also offered in small plastic vials kept humid by wet cotton swabs.

## 2.5 Statistical analysis

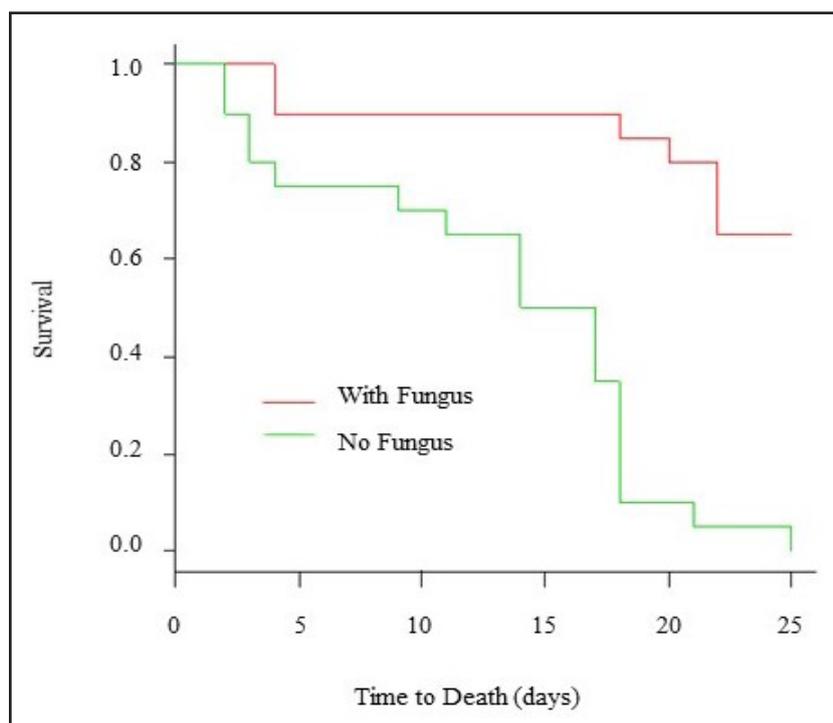
The data so collect were analyzed using the Kaplan-Meier distribution for survival analysis (CRAWLEY, 2013). The platform R, the package Survival, the function survreg (R DEVELOPMENT CORE TEAM, 2014) and the exponential distribution were used to adjust the models at the 5% significance level.

## 3 RESULTS AND DISCUSSION

The workers isolated from the mass of fungus exhibited a smaller survival rate within 25 days (less than 10% survival) when compared to the ants kept in contact with the fungus (more than 60% of survival) ( $X^2= 14.85$ ,  $df= 1$ ,  $p=0.00012$ ) (Figure 1). According to De Fine Licht *et al.* (2013), the fungus produces lacase, which detoxifies plants cut by the species *Acromyrmex echinator*, *Acromyrmex octospinosus*, *Atta colombica*, *Atta cephalotes*, *Sericomyrmex amabilis*, *Trachymyrmex cornetzi*, *Trachymyrmex* sp. 3, *Cyphomyrmex longiscapus*, *Cyphomyrmex muelleri*, *Cyphomyrmex costatus*, *Mycocepurus smithii*, *Myrmicocrypta ednaella*, *Apterostigma collare* and by *Apterostigma dentigerume*. As already said, this was not confirmed for *Atta sexdens*. This capacity for cancelling

toxic effects is only true if the workers swallow the fungus and keep the enzyme in their intestines. The ants are not able to produce this enzyme and need the fungus continually (DE FINE LICHT *et al.*, 2013). This result deserves mention because it confirms that the existence of fungus together with ants gives a protective shield to the insects that experienced contact with the toxic plants. It also emphasizes the obligation of the association between the fungus and the ant. In other words, the ants are not safe without the fungus because their chances of survival can be reduced.

Figure 1 – Survival of worker ants of *Atta sexdens* held in isolation with or without the mass of fungus after the application by ingestion of a hexanic extract solution of leaves of cassava (*Manihot esculenta*) in the concentration of  $125 \mu\text{g}\cdot\text{mL}^{-1}$  ( $\chi^2 = 14.85$ ,  $\text{df} = 1$ ,  $p = 0.00012$ )



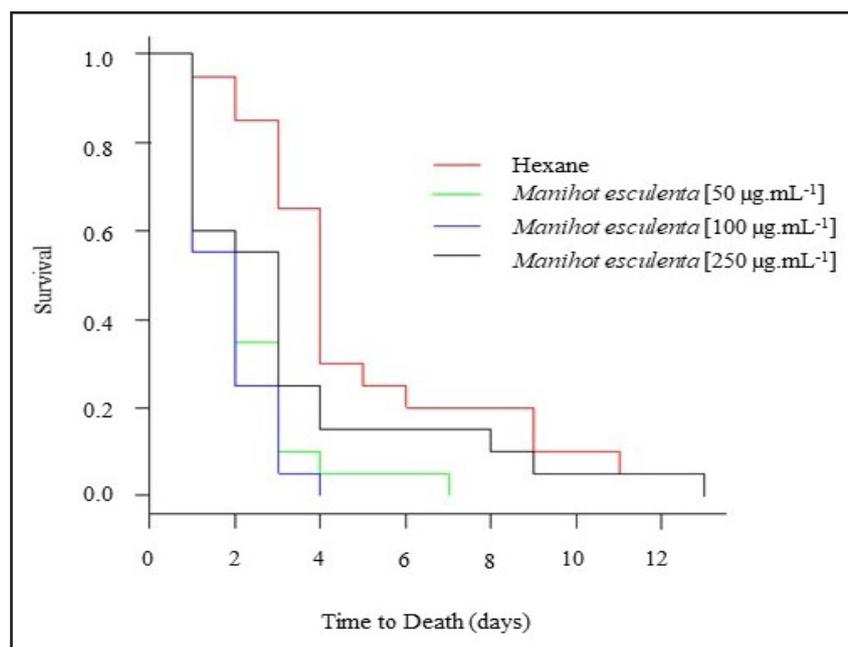
Source: Authors (2020)

This result is made more evident if the mortality rate of the ants isolated from their colonies and submitted to topical application of a  $100 \text{ mg}\cdot\text{mL}^{-1}$  hexanic extract of leaves of cassava (Figure 2) is compared to the mortality during the ingestion bio-essay and using the  $125 \text{ mg}\cdot\text{mL}^{-1}$  of the same extract (Figure 1), with the ants kept in contact

with the fungus. In topical application, 100% of the ants died within 4 days of the application of the extract (Figure 2). In the ingestion bio-essay, even after 25 days from the beginning of the essay, only 48% had died (Figure 1). That is because the fungus not only protects the ants but also feeds them. This fact shows that research with the objective of testing plants for a possible toxic effect should modify its test protocol and add the fungus to the bio-essays; this should give a more authentic portrayal of the behavior of the colonies in the field. At the end, if fungus and ants were joined and an extract was added, it would be possible to draw accurate inferences about toxicity, the moment the ants die or not.

The topical application of a hexane solution of cassava leaves did affect the survival of *Atta sexdens* workers ( $\chi^2 = 12.58$ ,  $df = 3$ ,  $p = 0.006$ ), the concentration of  $100 \mu\text{g.mL}^{-1}$  the one that showed a faster negative effect upon the survival, killing all individual within 4 days. It took the  $50 \mu\text{g.mL}^{-1}$  concentration 7 days to also kill 100% of the ants (Figure 2). The two other concentrations employed (control, pure hexane and the  $250 \mu\text{g.mL}^{-1}$  of cassava extract) took 11 and 13 days, respectively, to kill all the ants. This suggests that the highest concentration is not necessary to control the ants.

Figure 2 – Survival of worker ants of *Atta sexdens* after the topical application of a hexanic extract solution of cassava leaves (*Manihot esculenta*) ( $\chi^2 = 12.58$ ,  $df = 3$ ,  $p < 0.01$ )

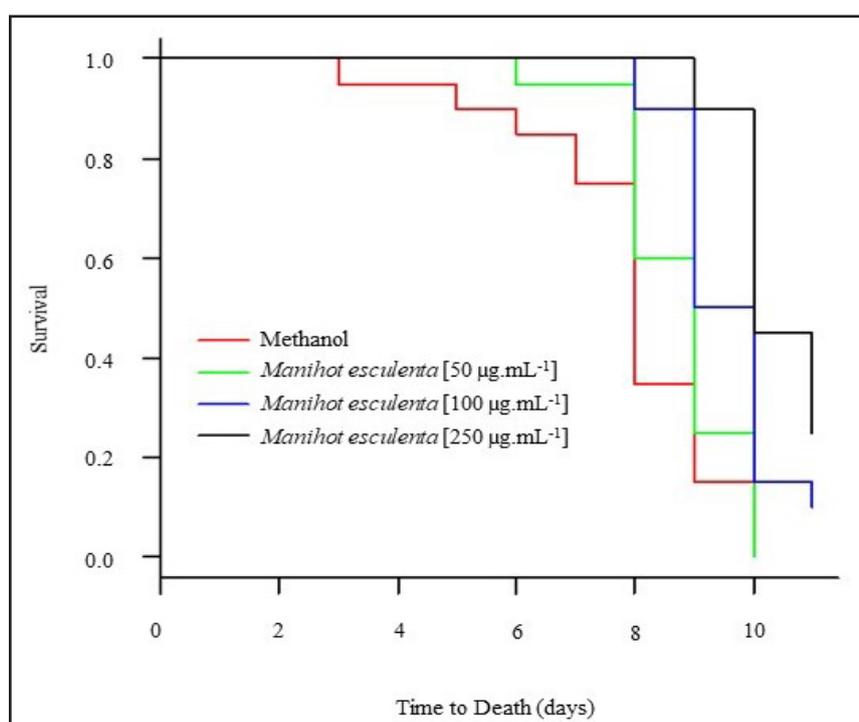


Source: Authors (2020)

The results showed that there was no difference between the topical application of the methanol extract of cassava in a  $50 \mu\text{g.mL}^{-1}$  concentration and the control ( $\chi^2=3.17$ ,  $df = 3$ ,  $p= 0.37$ ) (Figure 3). Both the extract and the control killed all the ants within 10 days. The concentrations of  $100 \mu\text{g.mL}^{-1}$  and of  $250 \mu\text{g.mL}^{-1}$  killed all the ants but at the end of 11 days. This result differs from that found by Santos *et al.* (2013), in which case the methanol extract when applied topically on ants of different castes of *Atta sexdens rubropilosa* reduced survival time in a rate proportional to the increase in concentration (they used  $250 \text{mg.kg}^{-1}$ ,  $500 \text{mg.kg}^{-1}$ ,  $1000 \text{mg.kg}^{-1}$  and  $1500 \text{mg.kg}^{-1}$ ). In other words, death came more rapidly as concentration increased due to toxicity increasing.

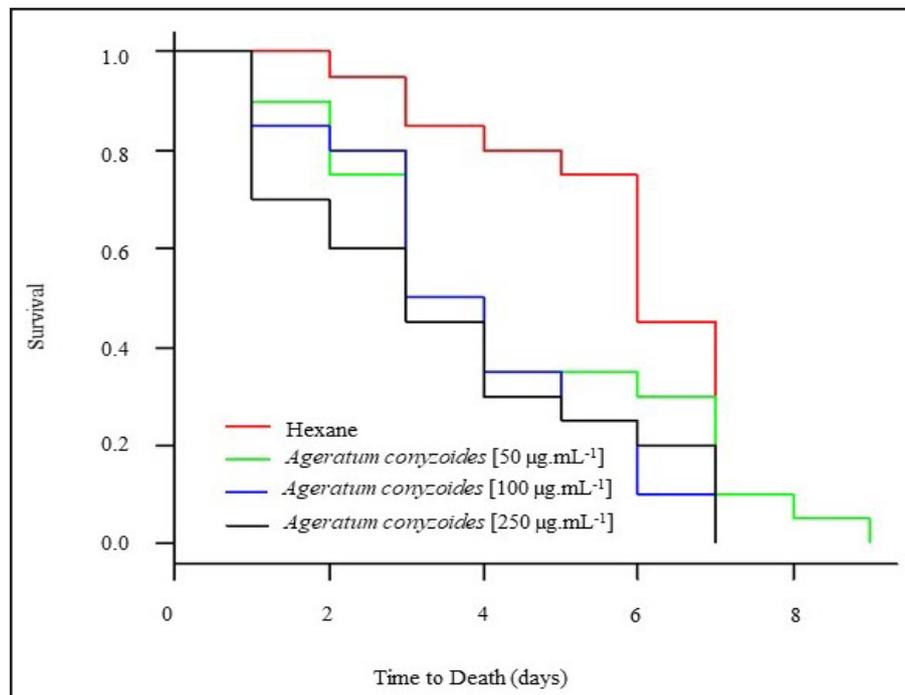
It required 9 days for the  $50 \mu\text{g.mL}^{-1}$  hexanic extract of mentrasto to kill 100% of workers. The other two concentrations and the control did not differ among themselves: it took them 7 days to exterminate all the ants (Figure 4).

Figure 3 – Survival of worker ants of *Atta sexdens* after the application of a methanol extract of cassava leaves (*Manihot esculenta*) ( $\chi^2 = 3.17$ ,  $df = 3$ ,  $p= 0.37$ )



Source: Authors (2020)

Figure 4 – Survival of worker ants of *Atta sexdens* after the topical application of a hexanic solution of mentrasto leaves (*Ageratum conyzoides*) ( $\chi^2 = 3.34$ ,  $df = 3$ ,  $p = 0.34$ )



Source: Authors (2020)

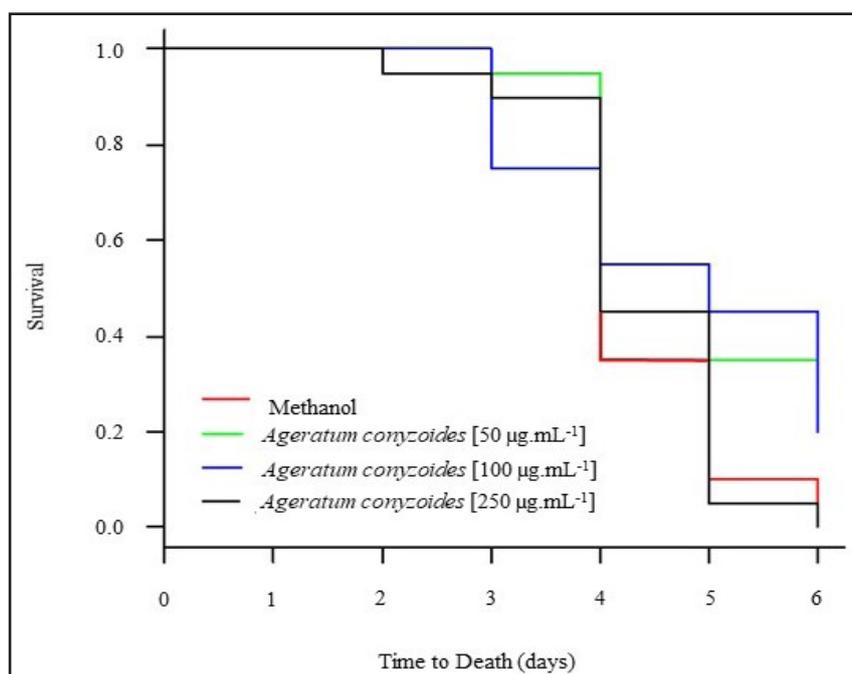
Araújo *et al.* (2008), also tested topically the extract of mentrasto but in a higher concentration ( $1,0 \text{ mg.mL}^{-1}$ ) than the ones used in this work. The authors found the extract was effective, increasing the mortality of the cutting ants of the species *Atta laevigata* and *Acromyrmex subterraneus subterraneus*. Morais *et al.* (2015), reported that mentrasto extract in the concentrations of 25, 50 and  $100 \text{ mg.mL}^{-1}$  was capable of interfering negatively with the growth of symbiont fungus. It can be assumed that hexanic extract of this plant is harmful for colonies of *Atta sexdens rubropilosa*.

Nogueira *et al.* (2010), have shown that mentrasto extract in hexane has a composition of precoceno II (46.35%), precoceno I (42.78%), cumarin (5.01%) e trans-carofilen (3.02%). Singh and Kumar (2011) applied to the notum of the larvae of *Chrysomya megacephala* (Diptera: Calliphoridae) dosages of precoceno II and found that this chemical is capable of negatively affecting the growth and causing death of these larvae. This insecticidal influence was also related for the substance cumarin but

concerning other insect orders (MOREIRA *et al.*, 2007). Thus, it is safe to say that the hexanic extract of menstrato has an unquestionable potential for the control of leaf-cutting ants. It is necessary, however, that it is used in larger concentration than the ones utilized in this work (50, 100 and 250  $\mu\text{g}\cdot\text{mL}^{-1}$ ), to determine the concentration used in the field.

In what concerns the methanol extract of menstrato leaves applied topically, the results have shown a 100% worker mortality in all concentrations of the extract within 6 days. Likewise, the dilutions did not differ from control ( $\chi^2 = 1.86$ ,  $df = 3$ ,  $p = 0.60$ ) (Figure 5).

Figure 5 – Survival of ant workers of *Atta sexdens* after the topical application of a methanol extract of menstrato leaves (*Ageratum conyzoides*) ( $\chi^2 = 1.86$ ,  $df = 3$ ,  $p = 0.60$ )



Source: Authors (2020)

Several plant species have already been tested using leaf-cutting workers, either topically or via ingestion, and showed positive results for mortality, thus revealing a toxic effect (BUENO *et al.*, 2004; GOUVÊA *et al.*, 2010). Bigi *et al.* (2004) prepared leaf extracts of *Ricinus communis* and applied them, in a liquid artificial diet or topically,

to workers of *Atta sexdens rubropilosa* and found the ricinin, present in the extracts, caused the death of the ants. Another study with this same ant species showed that the survival rate decrease during a topical application of a crude oil of cashew nuts (*Anacardium occidentale*) and of virola, *Carapa guianensis* (SANTOS-OLIVEIRA *et al.*, 2006). In this last work, the authors also related a reduction in the survival of *Atta sexdens rubropilosa* when oils of *Anacardium occidentale*, *Anacardium indica*, *Carapa guianensis*, *Elaeis guineensis*, *Ricinus communis* and of *Sesamum indicum* were added to an artificial diet, i.e., ingested. It should be mentioned that, in all these researches, the ants had been kept completely isolated, that is, in the absence of the fungus.

In our work, we did not test the effect of the hexanic solution of cassava leaves applied directly over the fungus mass in the concentration of 100  $\mu\text{g.mL}^{-1}$ . The mass suffered fragmentation after such application. It was visually observed, however, that the ants kept in isolation with the fungus rapidly recomposed the mass (they restructured the fungus) in less than 48 hours). The application of vegetable extracts via a pipet does not seem to be the best method of treatment: the product does not contact the whole fungus nor touches the ant workers. As the solution did not perfectly treat the whole mass, even when applied in fractions, the ants did not immediately suffer and lived longer (27 days), compared to those treated topically (13 days) or via ingestion (24 days).

The results have shown, then, that methanol extracts of cassava and of mentrasto leaves are not efficient to promote death of *Atta sexdens* ants, nor harm the fungus. However, the hexanic solution of cassava in the concentration of 100  $\mu\text{g.mL}^{-1}$  and used topically killed the insects in 4 days. This same solution used in a liquid diet in a concentration of 125  $\mu\text{g.mL}^{-1}$ , and applied when the fungus was kept with or without the ants, did in fact influence the mortality of the insects. The participation of the symbiont fungus during the realization of bio-essays increases the capacity of the *Atta sexdens* ants in surviving the contact with extracts of toxic plants.

## 4 CONCLUSION

*Leucoagaricus gongylophorus* provides protection for *Atta sexdens* against plant extracts *Ageratum conyzoides* and *Manihot esculenta* in leaf-cutting ant workers.

## 5 FINAL SUGGESTIONS

This suggests a need for reformulating the protocols for testing the extracts on ants using the symbiont fungus together. Research using vegetable extracts for a possible control of leaf-cutting ants should continue. We suggest, though, that higher concentrations than the ones here defined must be used.

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## REFERENCES

ARAÚJO, M. S. *et al.* Fundação e estabelecimento de formigueiros. *In*: DELLA LUCIA, T. M. C. (ed.). **Formigas Cortadeiras**: da bioecologia ao manejo. Viçosa, MG: Universidade Federal de Viçosa, 2011. p. 173-188.

ARAÚJO, M. S. *et al.* Toxicidade de Extratos Hexânicos de Plantas às Operárias de *Atta laevigata* e *Acromyrmex subterraneus subterraneus* (Formicidae: Attini). **Revista Brasileira Agrociência**, Pelotas, v. 14, n. 3, p. 106-114, jul./set. 2008.

BIGI, M. F. M. A. *et al.* Activity of *Ricinus communis* (Euphorbiaceae) and ricinine against the leafcutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) and the symbiotic fungus *Leucoagaricus gongylophorus*. **Pest Management Science**, New York, v. 60, n. 9, p. 933-938, set. 2004.

BUENO, O. C.; BUENO, F. C. Plantas Inseticidas: perspectivas de uso no controle de formigas-cortadeiras. In: DELLA LUCIA, T. M. C. (ed.). **Formigas Cortadeiras**: da bioecologia ao manejo. Viçosa, MG: Universidade Federal de Viçosa, 2011. p. 359-372.

BUENO, O. C. *et al.* Toxicity of Sesame Extracts to leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). **Sociobiology**, Chico, v. 44, n. 3, p. 599-606, 2004.

CRAWLEY, M. J. **The R book**. 2nd ed. United Kingdom: Wiley, 2013. 975 p.

DE FINE LICHT, H. H. *et al.* Laccase detoxification mediates the nutritional alliance between leaf-cutting ants and fungus-garden symbionts. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 110, n. 2, p. 583-587, jan. 2013.

DELLA LUCIA, T. M. C.; SOUZA, D. J. Importância e história de vida das formigas-cortadeiras. In: DELLA LUCIA, T. M. C. (ed.). **Formigas-Cortadeiras**: da bioecologia ao manejo. Viçosa, MG: Universidade Federal de Viçosa, 2011. p. 13-26.

FRANCO, A. A. *et al.* Ação de extratos vegetais sobre o desenvolvimento de fungos simbiotes das formigas cortadeiras. **Revista Engenharia Ambiental: Pesquisa e Tecnologia**, Espírito Santo do Pinhal, v. 10, n. 1, p. 103-113, jan./fev. 2013.

GANDRA, L. C. *et al.* Mortality and respiratory rate of *Atta bisphaerica* ant workers (Hymenoptera: Formicidae) after application of a plant (*Ageratum conyzoides*) extract. **Sociobiology**, Chico, v. 57, n. 3, p. 487-493, 2011.

GOMES, M. C. A. R. *et al.* Toxicity of Plant Extracts from Bahia, Brazil, to *Atta sexdens* (Hymenoptera: Formicidae) Workers. **Sociobiology**, Chico, v. 63, n. 2, p. 770-776, jun. 2016.

GOUVÊA, S. M. *et al.* Lethal and behavioral effects of amazonian plant extracts on leaf-cutting ant (Hymenoptera: Formicidae) workers. **Sociobiology**, Chico, v. 56, n. 3, p. 1-13, 2010.

HÖLLDOBLER, B.; WILSON, E. O. **The ants**. Cambridge: Harvard University Press, 1990. 732 p.

MARINHO, C. G. C.; DELLA LUCIA, T. C. M.; PICANÇO, M. C. Fatores que dificultam o controle das formigas cortadeiras. **Bahia Agricola**, Salvador, v. 7, n. 2, p. 18-21, abr. 2006.

MARINHO, C. G. S.; DELLA LUCIA, T. M. C. Formigas-cortadeiras. In: SILVA, N. M. *et al.* Crescimento de eucalipto sob efeito de desfolhamento artificial. **Pesquisa Agropecuária Brasileira**, Brasília, v. 45, n. 9, p. 952-957, set. 2010.

MORAIS, W. C. C. *et al.* Extracts of *Ageratum conyzoides*, *Coriandrum sativum* and *Mentha piperita* inhibit the growth of the symbiotic fungus of leaf-cutting ants. **Industrial Crops and Products**, [s. l.], v. 65, n. 1, p. 463-466, mar. 2015.

MOREIRA, M. D. *et al.* Compounds from *Ageratum conyzoides*: isolation, structural elucidation and insecticidal activity. **Pest Management Science**, New York, v. 63, n. 6, p. 615-621, jun. 2007.

NOGUEIRA, J. H. C. *et al.* *Ageratum conyzoides* essential oil as aflatoxin suppressor of *Aspergillus flavus*. **International Journal of Food Microbiology**, [s. l.], v. 137, n. 1, p. 55-60, nov. 2010.

R DEVELOPMENT CORE TEAM. **R**: a language and environment for statistical computing. Vienna: R Foundation for Statistic Computing, 2014. Available in: <http://www.r-project.org>. Accessed: 1 aug. 2018.

RIBEIRO, M. M. R. *et al.* Diversity of fungi associated with *Atta bisphaerica* (Hymenoptera: Formicidae): the activity of *Aspergillus ochraceus* and *Beauveria bassiana*. **Psyche**: A Journal of Entomology, London, ID 389806, 2012.

SANTOS, M. A. I. *et al.* Extrato metanólico de folhas de mandioca como alternativa ao controle de lagarta-do-cartucho e de formigas cortadeiras. **Semina**: Ciências Agrárias, Londrina, v. 34, n. 6, p. 3501-3512, maio/jun. 2013.

SANTOS-OLIVEIRA, M. F. S. *et al.* Toxicity of *Azadirachta indica* to leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). **Sociobiology**, Chico, v. 47, n. 2, p. 423-431, 2006.

SIMÕES, C. M. O.; SPITZER, V. Óleos voláteis. In: SIMÕES, C. M. O. *et al.* **Farmacognosia da planta ao medicamento**. Porto Alegre: Universidade Federal do Rio Grande do Sul; Florianópolis: Universidade Federal de Santa Catarina, 2002. p. 397-426.

SINGH, S., KUMAR, K. Sensitivity of last larval stadium of *Chrysomya megacephala* (Fabricius) to anti-allatin ageratochromene Precocene II. **Indian Journal of Experimental Biology**, New Delhi, v. 49, n. 8, p. 600-608, aug. 2011.

SOUZA, M. D. *et al.* Desenvolvimento in vitro do fungo simbiote de *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae) em meio de cultura com diferentes extratos vegetais. **Ciência Rural**, Santa Maria, v. 42, n. 9, p. 1603-1609, sep. 2012.

STOCKHOLM CONVENTION. **The 12 initial POPs under the Stockholm Convention**. Genève, 2008. Available in: <http://chm.pops.int/TheConvention/ThePOPs/The12InitialPOPs/tabid/296/Default.aspx>. Accessed: 21 aug. 2019.

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