International Journal of Agriculture Innovations and Research
Volume 9, Issue 5, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 23/02/2021 | Accepted on: 10/03/2021 | Published: 26/03/2021

Influence of Toxic Bait Diameter During Chemical Control of *Atta sexdens* Leaf-Cutting Ants

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***Abstract* – This study investigates to know the interference of particle diameter in the control of leaf-cutting ants, *Atta sexdens*, and from the different diameters used in the manufacture of the granulated toxic baits. The hypothesis addressed questions whether the particle size can influence the efficiency of the control. In this case, verifying whether the increase or decrease in particle size of the toxic baits could perform better than the commercial size currently adopted by the industry. Three diameters used to make baits: 1.5 mm; 2.0 mm and 3.0 mm. The baits were supplied to the colonies under laboratory conditions. Seven treatments were used: control (2.0 mm); sulfluramid [0.3%] (1.5 mm, 2.0 mm and 3.0 mm) and indoxacarb [0.15%] (1.5 mm, 2.0 mm and 3.0 mm). The treatments divided into three groups: i) control; ii) sulfluramid [0.3%], (delayed action) and iii) indoxacarbe [0.15%] (fast acting). It concludes that the diameter toxic bait not influenced the mortality the colony, however, the action mode of the insecticide influences the time mortality colony.**

***Keywords* – Active Ingredient, Diameter, Leaf-Cutting Ants, Toxic Baits.**

I. Introduction

Leaf-cutting ants of the genus *Atta* Fabricius, 1805 are eusocial insects found exclusively in the Neotropical region [1]. The relevance of the genus *Atta*, is mainly due to its great distribution throughout the Brazilian territory and intense foraging activity throughout the annual period [2], its importance is mainly from an economic point of view, since this genus is one of the main pests in forestry crops, such as *Eucalyptus* ssp. [3].

Because it is considered a potential pest in silviculture systems and represents this group of defoliating insects well, *Atta* spp. has often been used as a model of study in the development of new substances with formicide characteristics [4], another characteristic that facilitating research with the genus *Atta* is the possibility of maintaining and growing the colonies under laboratory conditions, allowing a large number of tests to be carried out under controlled conditions. The practice of some specific laboratory studies thus aims to reduce the economic losses caused by the foraging activity of these insect pests, in view of the fact that recurrent defoliation caused by leaf-cutting ants ends up significantly compromising the production and profitability of the enterprise, which may generate the economic unfeasibility of areas with high nest densities [5].

The use of toxic granulated baits is currently the main method of controlling these pest insects. In addition, toxic baits represent the only method that presents technical, economic and operational viability in the control of leaf-cutting ants. The active ingredient sulfluramid is the most used in bait formulations, mainly due to the efficiency of this substance in controlling all species of leaf-cutting ants [6]. However, it´s necessary that new active ingredients are developed and produced on a commercial scale to replace sulfluramid, or even the proposal for the optimization of product application processes in a more efficient and assertive way by research centers and companies that must seek new ways to guarantee less dependence in only one active ingredient. In addition, toxic baits must also have physical and chemical characteristics capable of attracting ants, to facilitate the process of loading this type of substrate into the nest. Greater speed in carrying bait particles probably implies a reduction in the time of exposure of the baits to adverse environmental conditions such as climate change, natural enemies or other insects that may compete for the same resources in the area [7].

The optimization of the loading of baits by leaf-cutting ants also ends up decreasing the probability of exposure and risks of intoxication resulting from the active principle present in the formulation of the bait, to Brazilian fauna animals such as birds and rodents and other non-target organisms in the different ecosystems. Other factors relevant to the acceptance of toxic baits refers to two aspects: mass and diameter, in this case, mass determines the concentration of the asset and the dimensions of the bait are adequate to optimize the loading and processing inside the nest. In this study, the use of two insecticides was addressed to test sulfluramid and indoxacarb, as well as, the interference of the size of the bait particle in mortality colony.

II. Material and Methods

A. *Studied Colonies*

Forty colonies of *A. sexdens* were collected in the city of Botucatu, Sao Paulo, Brazil, and maintained under controlled conditions (temperature at 24 ± 2 °C, relative humidity at 70% ± 10 °C, and photoperiod of 12 hours of light) in the Laboratory of Social Insects-Pests of the Sao Paulo State University. Each colony was stored in a 1000 mL plastic container with a 1.0 cm plaster layer at the bottom to keep the fungus garden moist and two containers with capacity for 250 ml were connected, one for plants provision and one for residue deposition (waste). Daily, leaves of the *Acalypha* spp. plant were provided to maintain the growth of the symbiotic fungus.

B. *Bait (Pellets) Preparation*

Oranges were peeled and cut into small pieces, which were then dried in oven at 50 ◦C for 72 h; after drying, the pulp was crushed and stored in freezer until pellets preparation [8]. A pelletizer machine was necessary, it must be of uniform particle size [9]. The pelletizing process involves extrusion through a die. The molten extrudate passes through the rings and is cut when it emerges from the extrusion die through knives; the pellets are subsequently dried in an oven. The pelletizing device used converted the product into pellets of different dimensions: with an average length of approximately 2.0 mm (similar to that of the standard commercial bait) and ring diameter sizes (1.5 mm; 2.0 mm and 3.0 mm). The treatments employed were: first control (no active ingredient), standard control 0.3% sulfluramid and finally, 0.15% indoxacarb [6], [10]. Pellets preparation used citric pulp (75%), carboxymethylcellulose (CMC) (15%), corn oil solution (5%), tracer dye (5%), and insecticide's concentration was discounted from the citrus pulp (0.30% or 0.15%). Furthermore, was used distilled water to form a pasty mixture. The mixtures were put inside pelletizer machine. Pellets were arranged on aluminum trays and oven dried at 50 ◦C for 21 h; after drying, they were stored in freezer until use in the study [11]. We used the following treatments:

Treatments	Formulation
Treatment 1 (T1)	Negative control: Bait 0% a.i - (bait without toxic) - Citrus pulp
Treatment 2 (T2)	Bait 0.30 % a.i sulfluramid + Diameter 1.5 mm + Citrus Pulp
Treatment 3 (T3)	Bait 0.30 % a.i sulfluramid + Diameter 2.0 mm + Citrus Pulp

Treatments	Formulation
Treatment 4 (T4)	Bait 0.30 % a.i sulfluramid + Diameter 2.0 mm + Citrus Pulp
Treatment 5 (T5)	Bait 0.15 % a.i indoxacarb + Diameter 1.5 mm + Citrus Pulp
Treatment 6 (T6)	Bait 0.15 % a.i indoxacarb + Diameter 2.0 mm + Citrus Pulp
Treatment 7 (T7)	Bait 0.15 % a.i indoxacarb + Diameter 3.0 mm + Citrus Pulp

The colonies of *A. sexdens* had containing approximately 1000.0 cm3 of fungus garden, and there was a provision of 0.5 g of bait for each treatment on the day of application, the baits were offered in the container referring to the foraging arena, after 24 h was offered leaves of the Acalypha spp. keep the growth of the symbiotic fungus. After bait supply, the fungus garden chamber was closed with a transparent glass lid to allow the observation of the behaviors performed in the colony.

We observed 6 behavioral acts: Loading the pellet (B1), Holding the pellet (B2), Licking the pellet (B3), Chopping/Chewing the pellet (B4), Incorporating pellet fragment in the fungus garden (B5), deposition of hypha into the pellet fragment deposited in the fungus garden (B6), These behaviors were quantified by the frequency of the workers involved in each of them. In addition, the mortality assessment was carried out on the 1st, 2nd, 3rd, 5th, 7th, 9th, 11th, 14th, 17th and 21st days according to the methodology of Nagamoto et al [4].

C. *Statistical Analysis*

The data were submitted to Analysis of Variance (ANOVA), using the software R [12], and, when significant, to the Scott-Knott group of averages test (P≤ 0.05).

III. Results

The behavior of licking and holding the pellet was the first and second, respectively, the most performed behavior by workers of *A. sexdens* in all treatments, it occurred with a higher percentage in treatment 7 (3 mm) (Table 1). The behavior of cutting, incorporating and depositing hyphae were statistically similar among treatments (Table 1). On the other hand, the transporting behavior differed among treatments, being most frequent in treatments 2 and 5 (Table 1).

Table 1. Percentage of worker behavior of *Atta sexdens* (Hymenoptera: Formicidae) during the preparation of the toxic baits for cultivation of the fungus garden.

Behavior	T1 (%)	T2 (%)	T3 (%)	T4 (%)	T5 (%)	T6 (%)	T7 (%)
Licking the pellet	36,4 a	35,6 a	34,9 a	35,8 a	34,4 a	35,9 a	38,0 b
Holding the pellet	29,7 a	28,4 a	28,4 a	28,2 a	28,6 a	28,9 a	31,5 b
Chopping/Chewing the pellet	19,4 a	19,4 a	21,4 a	20,9 a	20,1 a	21,3 a	19,0 a
Incorporating pellet fragment	6,4 a	6,1 a	7.0 a	6,5 a	5,9 a	6.5 a	5,5 a
Loading the pellet	6,1 a	8,4 b	5.9 a	6,34 a	8,7 b	5,5 a	4,5 a
deposition of hypha	2,0 a	2,1 a	2,4 a	2,2 a	2,3 a	1,9 a	1,5 a
Total	**100**						

[*] Means followed by the same letter, per line, do not differ by the Scott-Knott test (p ≤ 0.05).

The control treatment (T1) did not occur colony mortality during 21 days of experiment (Fig. 1). For treatments with active ingredient – sulfluramide-, mortality of colonies occurred between the 14th and 17th day for treatments T2, T3 and T4. For the active ingredient – indoxacarb- , mortality occurred on the 7th day for treatments T5, T6 and T7 (Fig. 1).

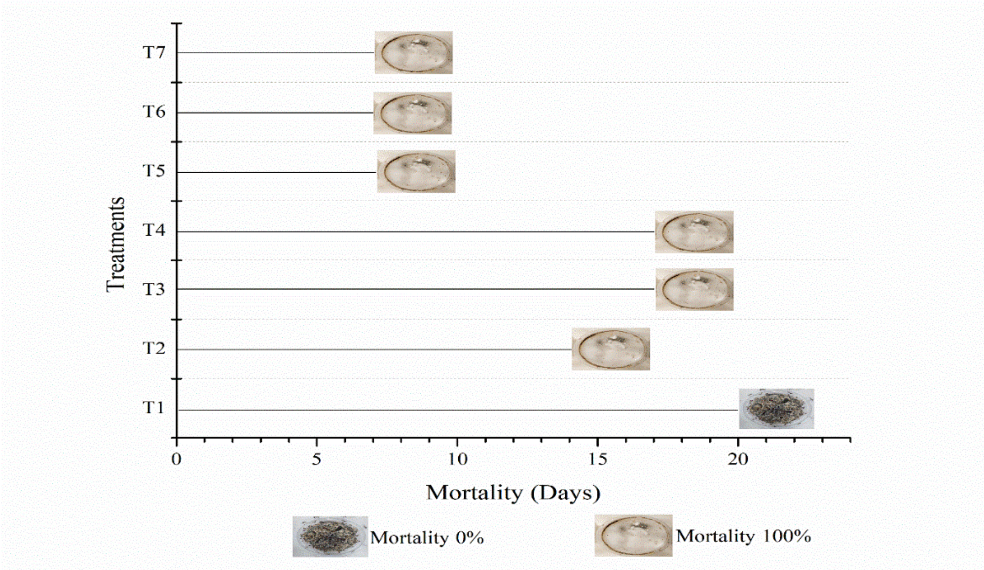

Fig. 1. Mortality in colonies of *Atta sexdens* (Hymenoptera: Formicidae) after toxic bait supply.

IV. Discussion

The behavior of licking pellets occurred more frequently in all treatments (Table 1). The results corroborate those obtained by Forti et al. [13], who studied the behavioral acts performed by *Acromyrmex subterraneus*, during the cultivation of fungus garden with toxic baits. According to Silva et al. [14] this behavior in leaf-cutting ants becomes frequent during the processing of pellets for cultivation of fungus garden, as it is responsible for moisturizing and softening the pellets thus facilitating the other behavioral acts for later incorporation into fungus garden. For treatment 7, there were higher values of the behavior of licking and cutting the pellet in relation to the other. This was due to the fact that the pellets have larger dimensions (3 mm), requiring a greater number of workers to lick and hydrate the surface of toxic bait. For treatment 2 and 5, a high frequency of transporting behavior was observed in relation to the other. The transporting behavior is the one that demands the most energy expenditure by leaf-cutting ants workers [15] and is the first direct contact of the workers with the active ingredient contained inside the toxic baits. In addition, it can become the behavior that promotes the longest contact time between worker and insecticide.

Regardless of the particle size, all insecticides caused the mortality in colonies (Fig. 1). However, for sulfluramid the death was delayed, because its action mode. This molecule inside the insect's organism is broken down, becoming a main component called DESFA (perfluorooctane sulfonamide), which acts in the oxidative phosphorylation process (aerobic respiration), interrupting the production of adenosine triphosphate (ATP) in the mitochondria [16]. The faster mortality with indoxacarb insecticide, because its action mode is a neurotoxic fast-acting substance that, when inside the insect body, after bioactivation, results in the formation of the metabolite IN-JT333 (N-decarburomethoxylate, DCJW). This metabolite blocks the sodium channels, that is, it compromises nerve function, interrupts food, paralyzes and leads to death [17]. This action id so fast that once absorbed or ingested by the insect, it stops feeding, which can occur in 2–8 hours [17], causing mortality in 48 h [18].

V. Conclusion

The particle size of toxic bait does not interfere with colony mortality. However, baits with indoxacarb caused faster mortality in the colonies in relation to toxic baits with sulfluramid.

Acknowledgment

Tarcisio Marcos Macedo Mota Filho, Ramon De Marchi Garcia and Luis Eduardo Pontes Stefanelli thank the support of the Coordination for the Improvement of Higher Education Personnel [Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (Higher Level Personnel Improvement Coordination)] – Brazil (CAPES) - Finance Code 001. Luiz Carlos Forti gratefully acknowledges the support of the National Council for Science, Research and Technology [Conselho Nacional de Ciencia de Pesquisa e Tecnologia (National Council for Research Science and Technology)] (CNPq-PQ) (donation No. 301938/2017-2).

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