ACTIVITY OF LEAF-CUTTING ANT *Atta sexdens piriventris* SUBMITED TO HIGH DILUTION HOMEOPATHIC PREPARATIONS

[ACTIVIDAD DE LA HORMIGA CORTADORA *Atta sexdens piriventris* SOMETIDA A PREPARADOS HOMEOPATICOS EN ALTAS DILUCIONES]

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SUMMARY

The effect of high dilution preparations on the movement and foraging activities of *Atta sexdens piriventris* was evaluated. Five colonies of ants were located on each of the five experimental areas using a randomized complete block design. Three main forage trails from each colony were selected from where evaluations were made. Ten mL of high dilution preparation of *Atta sexdens piriventris* nosodes and *Belladonna* homeopathy solution were sprayed over 0.5 m of each selected trail, 1.0 m far from the nest. The controls were pure water and non treated trails. Applications were made daily during 10 days. The total number of ants moving on each trail one meter away from the nest, carrying or not plant fragments, were assessed before the daily application. Dilution preparations at 30CH (thirtieth centesimal Hahnemannian dilution) of *A. sexdens piriventris* nosodes and *Belladonna* reduced the activities of ants from the fifth day after the first application. The treatment effect lasted more than 20 days after the last application. The use of preparation at 30CH dilution order to reduce the foraging activity of leaf-cutting ants is a potential non residual method to manage leaf-cutting ants.

Keywords: *Atta*; foraging; non residual preparation.

RESUMEN

Se evaluó el efecto de preparados en altas diluciones en el movimiento de *Atta sexdens piriventris* y en su actividad forrajera. Cinco hormigueros en cinco áreas experimentales fueron utilizados como bloques al azar. Se seleccionaron tres transectos forrajeros principales de cada hormiguero donde las evaluaciones fueron hechas. Se asperjaron 10 mL de nosodios de *Atta sexdens piriventris* y la solución homeopática de *Belladonna* en 0.5 m de cada transepto seleccionado, a una distancia de 1.0 m del hormiguero. Los testigos consistieron en aplicación de agua pura y hormigueros no tratados. Las aplicaciones fueran hechas diariamente durante10 días. Las evaluaciones fueron hechas antes de la aspersión diaria y consistieron del conteo de hormigas que transportaban o que no transportaban fragmentos vegetales por un minuto a un metro distante del hormiguero. Las altas diluciones de *A. sexdens piriventris* y la solución homeopática de *Belladonna* de 30CH (trigésima dilución centesimal Hahnemanniana) pudieron reducir consistentemente las actividades de la hormiga a partir de la quinta aplicación diaria. El efecto de reducir el movimiento y la actividad de forraje se extendió por más de 20 días después de la última aplicación. El uso de preparados en diluciones de 30CH de nosodios de hormiga y *Belladonna* tienen el potencial para el manejo de la hormiga cortadora.

Palabras clave: *Atta*; forrajeo; preparación no residual**.**

INTRODUCTION

Leaf-cutting ants of the genus *Atta* are important generalist herbivores largely occurring throughout South America, especially in tropical regions (Fowler *et al.*, 1989; Fernández and Ospina, 2003). Species of *Atta*, together with those of *Acromyrmex* genus represent the true leaf-cutting ants overspread in the Neotropics (Fernández, 2003). However, *Atta* ants are highly polyphagous and they defoliate not only native forest species but also many agricultural and horticultural crops (Correa *et al*., 2005). *Atta sexdens piriventris* Santschi [1919] is one of the predominant species in Southern Brazil agricultural areas (Link and Link, 2007; Grürzmacher *et al.,* 2002). Leaf-cutting ants cut young plant parts such as sprouts, buds, leaves, and flowers and they use it as substrate for their symbiotic fungus *Leucoagaricus gongilophorus* inside their nest (Roces, 1990). The fungus is the main food source for the colony, mostly required by larvae, young ants, and the queen (Della Lucia and Moreira, 1993; Jolivet, 1998). The population of leaf cutting ants have been increased due to deforestation, large area cattle pasture and crop monoculture. Also, degraded areas maintained by intensive agricultural systems and the use of pesticides have reduced natural enemies and facilitated some insect species to assume the status of pest as do the leaf-cutting ants (Cherrett *et al*., 1986). Foraging activities of ants can seriously affect agriculture by reducing yields in annual and perennial crops (Grürzmacher *et al*., 2002). Most modern conventional control methods of leaf-cutting ants are based on the application of large amounts of synthetic insecticides, which are costly, non selective, and hazardous to the environment (Della Lucia and Vilela, 1993; Jaccoud *et al*., 1999). On the other hand, leaf-cutting ants in natural ecosystems assume important position in the food chain by recycling organic matter, breaking down the dormancy of some seeds, and restoring the soil structure (Jolivet, 1998; Kaspari, 2003; Delabie *et al.,* 2003).

Ecologically based technologies must be developed in such a way that management strategies of pests and diseases should aim to keep down its population levels and improve resilience to the crop, instead of total elimination of the causal agent. Moreover, as stated by García-Hernández *et al*. (2009) the increase of organic food demand has required several methods of pest management mainly at large plantations, which restrictions or opportunities are regulated by certificated agencies (Brasil, 2008). One possibility that has recently emerged is the use of high dilution preparation to treat plants (Betti *et al*., 2003). High dilution preparations consist of successive one hundredth dilution started from mother tincture followed by 100 sucussions that are vertical 90^{o} angular movements (Brasil, 1997). Intervention with high dilution preparations can restore health to the organism and its entire habitat without substantial changes in the ecological niche (Boff, 2008). This is a suitable technology aimed to design sustainable agriculture systems, which may consider all the complex interaction present in the agroecosystem. Despite the fact that very little is known about the mechanism of action of high dilutions preparations, its effects can be measured throughout experimental studies (Brizzi *et al.,* 2005). According to Bonato (2004), high dilution preparations can be used for restoring the dynamic balance of agroecosystems by targeting a particular point of disturbance; such as pests and diseases, or to improve the resilience throughout inducing resistance/tolerance to the crop. Boff (2008) pointed out that high dilution preparations obtained from local resources such as nosodes, can provide the farmers a simple, versatile, and cheap tool to quickly overcome local problems.

The use of nosodes in agroecosystems could be appropriate to small family farms which represents more than 80% of the farmers in Southern Brazil. Nosodes are bio-therapeutics preparations that are made from insect/pests present in the plant, or parts of the actual diseased plant. Another source of high dilution preparations is the normal homeopathic remedies found in the manipulation pharmacies that are steadily increasing in Brazil (Boff, 2008). In both cases, the high dilution preparations are prepared by combined successive dilution and sucussion (sequence of rhythmically vertical 90^{o} angular movements) until the desirable potency is reached (Andrade, 2001). The laboratory protocols for obtaining homeopathic preparations are well documented in the Farmacopéia Homeopática Brasileira (Brasil, 1997), that follows similar procedures done in Mexico and USA (Tichavský, 2009). In Brazil, homeopathy became a recognized specialization by the Medicine, Veterinary, Pharmacist, and Agronomist Federal Council that are supported by law.

The efficacy of high dilution preparations in agriculture has been recently demonstrated by several works. However, it is still a new area for research in the field of Agroecology. Almeida (2003) observed a significant reduction in the incidence of corn caterpillar *Spodoptera frugiperda* by using high dilution preparations made from larvae of *S. frugiperda,* also called nosodes. Indication of a suitable high dilution preparation can also be found throughout the Homeopathic Matéria Médica, a compendium describing the therapeutic properties of homeopathy remedies for human treatments that were collected from studies made with formal protocols (Brasil, 1997). According to Andrade (2001) we can make analogies with the Matéria Médica and carry out further studies to evaluate its effect on agriculture problems. This is possible because the principle of similitude in homeopathy is concerning to all living

systems. However, the cure properties pointed out in Homeopathic Matéria Médica must be tested in agricultural systems, since living organisms might respond differently among kingdoms (Bonato, 2004).

The present study was carried out in 2007 during four field-work months, was restricted to *Atta sexdens piriventris* colonies in Planalto Serrano Catarinense region. Its objective was to study the effect of high dilution preparations of *Atta sexdens piriventris* nosodes and *Belladonna* homeopathy solution on the movement and foraging activities of the leaf cutter ant.

MATERIAL AND METHODS

Study area

The selected nests for study were located in highland fields comprising about 2,500 m^2 of the Planalto Serrano Catarinense region, Santa Catarina State, Brazil. This region is part of the Atlantic Forest, at an altitude of 600 to 800 m above the sea level and situated between 27º 52' and 27º 43' W; 50º 28' and 50º 31' S. The field areas consisted of highland fields originally covered by native grass associated with *Araucaria angustifolia* forest ecosystem. Nowadays, this region has been modified by agriculture and re-forestation with exotic pine species. The research was carried out during the summer season (December-March).

Experimental set up

Data were collected from 25 colonies of *A. sexdens piriventris* located in the field and randomly submitted to high dilution treatments, with five repetitions. The ants of *Atta* genus were selected by the presence of three spine pairs on the dorsal thorax of worker ants and by the presence of crescent-shaped mounds of soil on the soil surface. The selected colonies were generally more than 50 m away from the closest nest and not more than 100 m away from one another. The distance of 100 m was also kept far from pinus re-forestation and the surrounding area of nests was predominantly covered by tough grasses. A sample of worker ants was collected and their species was confirmed at the laboratory following the key of Della Lucia and Moreira (1993). Treatments were randomized in five blocks each one in different areas. Each ant colony was one experimental plot receiving a single treatment. In each experimental plot, the three main foraging trails were labeled at one meter from the nest, from where applications of high dilution preparations and evaluations of ant activity were made. The ant nests area was calculating by measuring the minimum and maximal diameter and estimated according to Zanetti *et al.* (2002).

High dilution preparation treatments

The treatments were: a) ant nosodes prepared from maceration adults *A. sexdens piriventris*, b) ant nosodes prepared from trituration of adults *A. sexdens piriventris*, c) *Belladonna* homeopathy solution (*Atropa belladonna*) obtained from a homeopathic pharmacy, d) pure water, and e) non-treated ant colony. Both, the last two as control plots. All the following homeopathic dilutions were prepared in the Homeopathy and Plant Health Laboratory of Lages Experimental Station in EPAGRI (Agriculture Research and Extension Service Agency of Santa Catarina State, Brazil), according to Farmacopéia Homeopática Brasileira (Brasil, 1997), which describes standard procedures about homeopathic pharmacotechniques in Brazil and elsewhere (Brizzi *et al*., 2005).

The macerated and triturated ant nosodes were obtained from a sample of 50 worker ants collected randomly from foraging trails of well-established colonies in the research area. For the trituration method, one gram of pre-ground ants was mixed with 99 g of lactose into a porcelain grail. The mixture was ground during 1(one) hour in the sequential period of time, being 10 min in circular movements and 5 min recollecting the material from the grail wall to the centre in vertical movements. This procedure produced the 1CH trit. (first centesimal Hahnemannian dilution of trituration) ant nosode. Again, by taking one part of 1CH trit. and adding 99 parts of lactose into a porcelain grail and ground it during one hour as described above, it was reached the 2CH trit. This procedure was repeated until the 3CH trit. was obtained. After that, each potency level until the 28CH (twentieth centesimal Hahnemannian dilution - stock) was prepared taking one part from the previous potency into 99 parts of alcohol 70%, and followed by 100 sucussion (sequence of rhythmically vertical 90º angular movements) using a mechanical dynamizer (Autic®, Mod. Denise 10-20).

The macerated preparation was originated from the mother tincture of nosode, which was obtained by the mixture of 1 gram of active worker ants mixed with 45 mL of water+alcohol+glycerine (1:1:1) solution and kept all into an amber glass bottle at room temperature for 48 h (Brasil, 1997). The homeopathic dinamization protocol was then followed: 0.2 mL of mother tincture and 19.8 mL of alcohol were placed in a 30 mL amber bottle. The bottle was placed in a mechanic dinamizer (Autic®, Mod. Denise 10-20) followed by 100 sucussions in order to obtain the 1CH (first centesimal Hahnemannian dilution). The same procedure was repeated to obtain the 2CH and the successive potencies until 28CH (the stock homeopathic matrix). The *Belladonna* homeopathy at the level of third centesimal Hahnemannian dilution (3CH) was

obtained from a drug store and prepared until 28CH potency, using alcohol at 70%.

The high dilution preparations were turned to 30CH potency only on the day of application, just before going to the field, using water instead of alcohol. The water treatment was obtained throughout the same procedure, starting with 100 sucussions of pure water until 30CH was reached.

Treatment applications

Application of treatments was performed as double-blind, where neither the applicator nor the evaluator knew the identity of the treatment. The treatments were individualized and identified by codes. Each high dilution preparation of ant nosodes, *Belladonna* and pure water were applied with an individual garden sprayer (Brudden®). Applications consisted of spraying 10 mL of homeopathic preparation over 0.5 m on each of the three main foraging trails of each of the five colonies per treatment. The application sites were located 1.0 m away from the ant nest. Daily applications were made in the morning, from 7:00 to 9:00 during the first 5 days and in the afternoon, from 17:00 to 19:00 during the last 5 days. This schedule was followed due to practical reasons about the travel and facilities.

Evaluation and data analysis

The foraging activity and total movement were recorded just before the applications of high dilution preparations. Foraging activity was considered when ants moved carrying green plant fragments. Total movement was considered when all ants were moving with and without green plant fragments. Both activities were estimated separately by counting the ants passing throughout a given point of the forage trail located 1.0 m away from the ant nest in one min. Evaluations were made just before each daily application during the 10 day treatment period. The last evaluation was made 20 days after the last application.

Statistical analysis was made using the statistical software SAEG®. Data analysis was performed considering the sum of the three foraging trails of each ant colony nest. Analysis of variance was performed to analyse the interaction between application days and homeopathic preparations, and the significant reduction of activities due to treatments. Total movement and foraging activity reduction were calculated by comparing the ninth or 29^{th} day to the first day just before starting high dilution applications. These reductions were compared by Tukey test ($p = 0.05$).

RESULTS

The species of ants in all studied ant nests was confirmed to be *Atta sexdens piriventris* Santschi, 1919. The ant nests in our study had an area ranging from 111 to 234 m^2. No significant difference (F test; df=4, p=0.98) was detected among treatments after randomizing them.

No significant interaction between "treatment" and "application time" factors was obtained for total movement (F test; df=40, p=0.33) and foraging activity (F test; df=40, p= 0.98). However, the total movement was highly correlated with foraging activity for all treatments, thus *Belladonna* (r=0.99, p<0.001), triturate of *Atta* (r=0.99, p<0.001), macerate of *Atta* (r=0.98, p<0.001), pure water (r=0.97, p<0.001) and no application (r=0.95, p<0.001). High dilution preparation of *Belladonna*, ant macerated, and ant triturated reduce steadily the total movement and foraging activities in comparison to control with pure water or no application from the fifth application onwards, and maintained this effect for more than 20 days (Figure 1 and 2).

The total movement and foraging activity of ants were significantly reduced at the ninth and at the 29^{th} day after application in comparison to the first application day when ants were submitted to the macerated or triturated ant nosodes and homeopathy solution of *Belladonna* (Table 1). *Belladonna* at 30CH showed to be the most effective treatment, by consistently reducing total ant foraging activities. Pure water did not reduce total movement or the foraging activity when compared with the non intervention treatment.

The daily rate of movement and forage activity indicated that after 5 days of applications, the reduction was maintained in the ant nests treated by nosodes and *Belladonna* homeopathic treatment (Figures 3 and 4). Control plots (pure water and no application) tended to keep the reduction rate close to zero, while high dilution treatments of triturated and macerated *Atta* reduced in more than 0.1 the daily rate of ant foraging activity after seven days of applications (Figure 4).

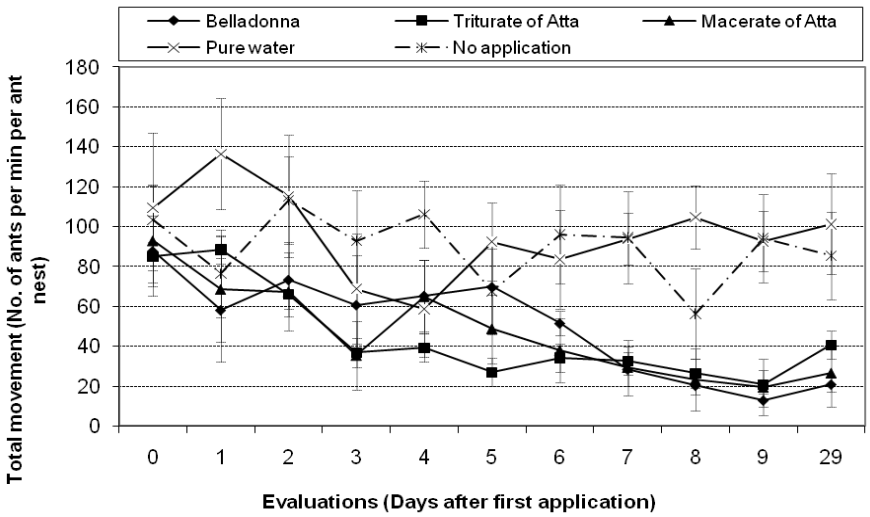

Figure 1. Ant movement of *Atta sexdens piriventris* submitted to high dilution preparations at 30CH (thirtieth centesimal Hahnemannian dilution). Santa Catarina State, Brazil, 2007. Bars are standard error of mean from five replicates.

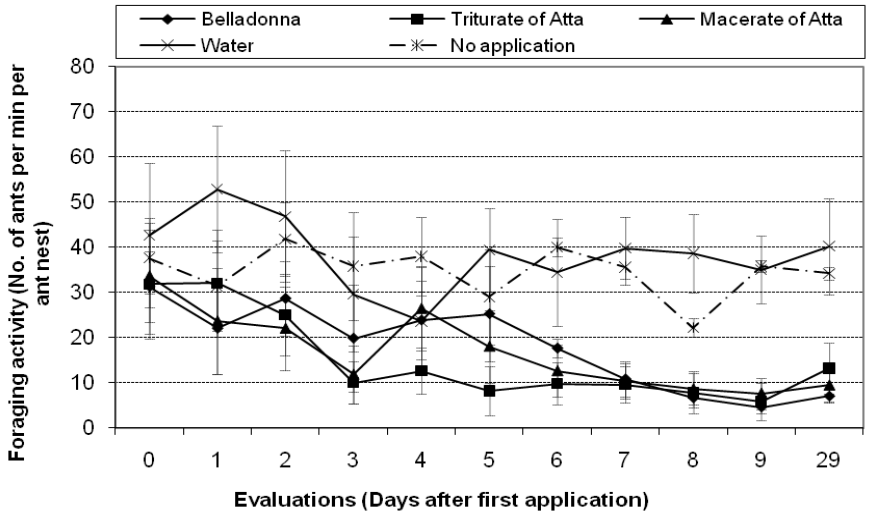

Figure 2. Foraging activity of *Atta sexdens piriventris* submitted to high dilution preparations at 30CH (thirtieth centesimal Hahnemannian dilution). Santa Catarina State, Brazil, 2007. Bars are standard error of mean from five repetitions.

DISCUSSION

High dilution preparations made from *Atta sexdens piriventris*, as macerated or triturated nosodes and *Belladonna* homeopathy could significantly reduce the total ant movement and foraging activities of the specie in the main ant trails. Due to possible yield losses caused by ant foraging activities, a variety of control methods, including use of synthetic insecticides, thermal fogging, poison baits, and explosives to blow up the colonies are usually recommended (Link and Link, 2007). Such conventional practices aim to eliminate the entire colony. Despite the damage that leaf-cutting ants may cause, the conventional control methods do not consider the important role in nutrient recycling and soil aeration of tropical ecosystems that ants may play (Zeh *et al.* 1999; Farji-Brener and Medina, 2000). Reducing the activities of leaf cutter ants without causing the colony extinction is an important strategy to have a low side effect and allow maintaining the ant's colonies playing their role in the food-chain. A ten-day application period of high dilution preparations could reduce up to 85% of foraging activities and the method reported here carries a minimum risk to biological diversity, frequently associated with the synthetic pesticides. The reduction of foraging activities without leading the colonies to extinction allows farmers to consider ants as part of the agroecosystem and accept their presence without unnecessary alarm (Fernández, 2003). Farmers in Panama have protected plants from the attack of *Atta*

cephalote by using a refused plant material of this leaf cutting species colony dumped as a deterrent while ant colonies still are in the field (Zeh *et al*., 1999). It is also important to maintain the biodiversity which improves the resilience property in the agroecosystem as a whole. According to Boff (2008) resilience is a function of complexity of a given ecosystems and the complexity is built up with diversity.

Farmers could use the high dilution method to reduce or suppress ant foraging activity during the most critical period for crop losses. Further studies are needed to see the consistence to manage ant foraging by high dilution homeopathic preparations under farmer conditions. Adopting this method, farmers could be able to establish an acquaintanceship with the leaf cutter ants in their agricultural systems. From our results we can state that 30 days is a particularly good period to protect plants from foraging attacks by leaf cutter ants in annual or perennial crops since sprouting and flowering occur in a short period of time. It is just necessary that farmers are able to identify critical periods in crop cycles when high dilution applications are needed. In case of changing ant paths or nests we should also consider a new situation and design another strategy. Also, it would be expected to protect the flowering period of most crops and, if necessary, indicate a new set of ten day applications. Nevertheless, this must be tested for different production system conditions (Zanetti *et al.*, 2002).

The frequency of applications is also an important factor to obtain an increasing effect of high dilution preparations. Significant reductions of ants' activities were observed after five days applications. It means that a single dose of a high dilution preparation, as sometimes recommended to animal and human treatments, probably will not work on the management of leaf cutting ants. In the case of *A. sexdens piriventris*, more than one application was necessary most probably for reaching the minimum number of worker ants that could cause changes in behaviour of the colony. Considering the ant nest as a society, we may also argue that a high dilution preparation treatment could take a while to have an effect, but when it does so the ant colony changes as a whole. In our study, it was observed a high correlation between reduction of foraging activities and reduction on total activities. This data means that the high dilution preparation acts on the behaviour of the entire ant colony with direct impact to ant movement and foraging activities. However, Della Lucia and Moreira (1993) pointed out that frequency must be also adjusted according to the size of ant nest. The nests we have studied had an area ranging from 111 to 234 m^2. This can give an idea to the farmer in how to manage ant leaf-cutting in colonies of similar size and also to take into account the residual effect. Moreover, we did not find new ant colonies surrounding those we have treated by high dilution preparations until 30 days after starting the applications. This means that high dilution preparations did not perturb the ant colonies at a level to stimulate them to move into new areas. Bonato (2004) reported that when high dilution preparations in agriculture have a prolonged effect, it could be an indication that such intervention approached to the *simillimum* of the agricultural system. This is particularly important for perennial crops, such as orchards because it gives a longer effect in the same treated area. Organisms sensitive to high dilution treatments became more easygoing and less exciting which may also reduce the feeding activities.

Table 1. Reduction of foraging and total movement of *Atta sexdens piriventris* observed at the 9th and 29th days compared to the day zero (0) after starting applications of high dilution preparations. Santa Catarina State, Brazil, 2007.

Treatments	9 days applications*		After 29 days start application*	
	Movement reduction (%)	Foraging reduction (%)	Movement reduction (%)	Foraging reduction (%)
Belladonna 30CH	85.19 a	85.25 a	76.08 a	77.56 a
Triturated *Atta* 30CH	75.52 a	81.76 a	52.23 a	58.49 a
Macerated *Atta* 30CH	79.14 a	77.23 a	71.39 a	72.02 a
Water 30CH	15.35 b	17.84 b	7.49 b	5.63 b
No application	8.89 b	4.78 b	17.40 b	9.04 b
C.V.(%)	49	50	60	59

*Columns followed by the same letter for the same activity (total movement or foraging) did not differ significantly (Tukey, p = 0.05). Mean of five replications.

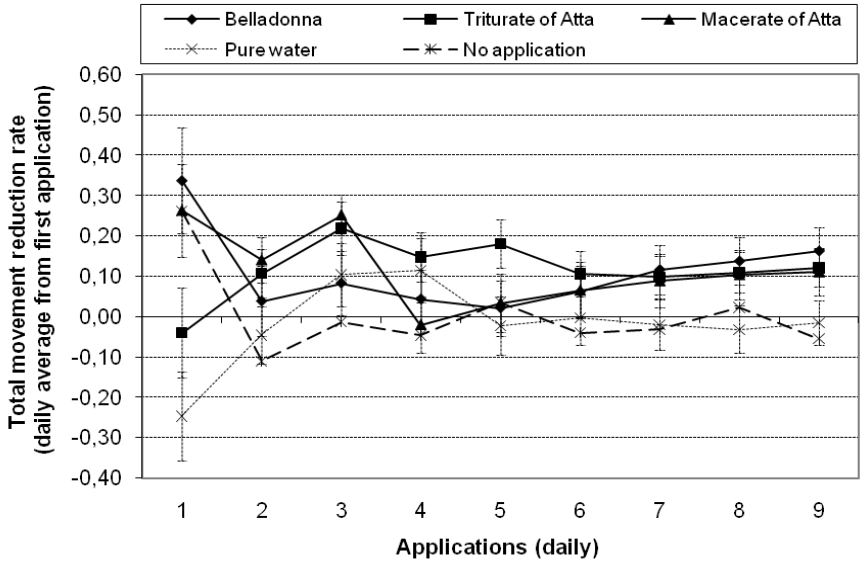

Figure 3. Reduction in daily total movement rate of ant *Atta sexdens piriventris*, considering the time from the first application. Lages, Santa Catarina State, Brazil, 2007. Bars are standard error of mean from five repetitions.

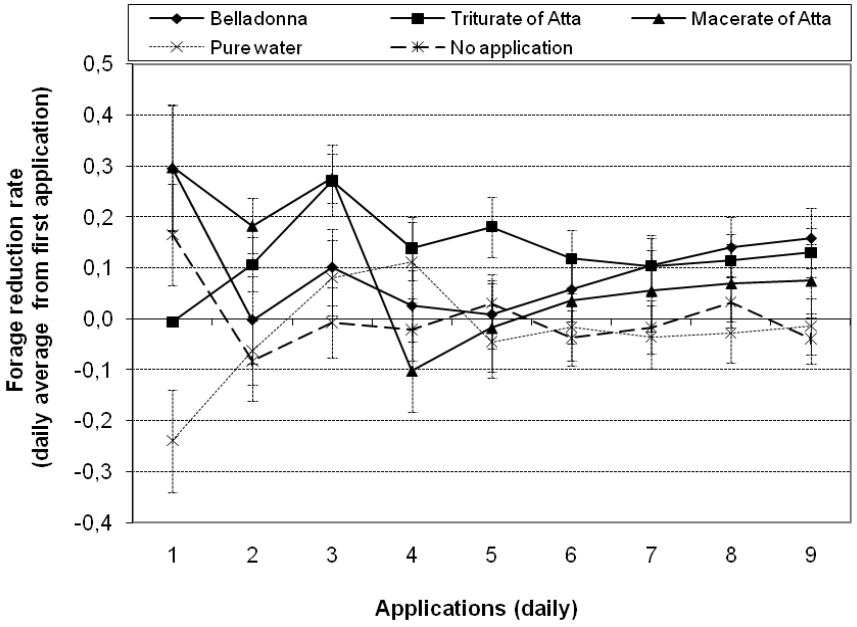

Figure 4. Reduction of daily foraging activity rate of ant *Atta sexdens piriventris*, considering the time from the first application. Lages, Santa Catarina State, Brazil, 2007. Bars are standard error of mean from five repetitions.

Daily applications for such period of time showed to be a practical way to apply homeopathic preparation to manage leaf cutting ants in family farms and organic systems. This is feasible because daily inspections around the orchards and garden are common in small farms and it may be included other routine checking ant foraging activities to do the spraying.

Concerning the method to obtain the mother tincture and get ant nosodes, both procedures, maceration and trituration, were similar on their effect on ant activities. *Belladonna* at 30CH was also as good as ant nosodes at 30CH for reducing foraging activities. Since *Belladonna* homeopathy can be found in pharmacies that manipulate other remedies, it would be a good option for farmers that are not trained for managing high dilutions, or in cases which local conditions are not suitable to prepare the high dilution preparation according to standard protocols.

The homeopathic therapy in human treatments is used everywhere in the world despite criticisms and the pretext of placebo effect (Brizzi *et al.*, 2005). The non effect of pure water at 30CH and similar results in the non intervention plot refused the placebo effect in our studies. In Brazil, there are several successful examples where animal production systems and companion animals are treated with high dilution preparations only (Boff, 2008; Sampaio, 1995). Nevertheless, this is not normal for plant health or plant protection strategies. The lack of research in crop systems with high dilution preparations can be the main cause of the scarce use of agro-homeopathy. However, homeopathy as an applied science implies a change in our perception of life, where co-existence and mutual aid would be the most important principle to develop sustainable living systems. In this sense, it is believed that high dilution preparations applied to agriculture is more feasible to implement under organic crop systems. In addition to that, high dilution preparations are an unprotected right medicine that means they are under public domain. Agro-ecology scientists must take the challenge to improve agro-ecosystems with technologies such high dilution preparations with no side effect and easy to implement.

CONCLUSIONS

High dilution preparations obtained from worker ants (nosodes) and *Belladonna* at 30CH dinamization, reduce the foraging activity of *A. sexdens piriventris* and their effects last more than 20 days. This is a potential strategy to manage leaf-cutting ants on several agricultural and horticultural crops considering further research showing consistent results on its efficacy. High dilution preparations are potential measures to manage leaf-cutting ants without colonies extermination, as do conventional strategies. Additional studies are necessary to check whether the *Atta sexdens periventris* nosodes and *Belladonna* homeopathy could be useful for controlling this insect species in large community areas.

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