Received: August 12, 2002
Accepted: November 19, 2002
Published online: February 14, 2004

J. Venom. Anim. Toxins incl. Trop. Dis.
V.10, n.1, p.77-85, 2004.
Original paper - ISSN 1678-9199.

USE OF REPELLENTS FOR HONEYBEES (*Apis mellifera* L.) *IN VITRO* IN THE YELLOW PASSION-FRUIT (*Passiflora edulis* Deg) CROP AND IN CONFINED BEEF CATTLE FEEDERS

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ABSTRACT. The presence of *Apis mellifera* in places such as candy and soft drink factories, restaurants, and ice-cream shops has been a concern to many people. In the yellow passion-fruit crop, *Apis mellifera* is able to collect all anther pollen but has no active role in pollination. Honeybees also visit animal feeders with chopped sugar cane, preventing the cattle from eating. This work studied the effect of natural and synthetic substances as *Apis mellifera* repellents *in vitro* in the yellow passion-fruit crop and in confined beef cattle feeders. There was a repellent effect *in vitro* with the following substances in decreasing order: tobacco, rue, garlic, parsley, and century plant extracts; average effect was twenty-five minutes. For the yellow passion-fruit, garlic extracts and 2-heptanone were equally efficient with a two and a half hour repellent action. Garlic and citronella extracts were efficient in repelling *Apis mellifera* from confined beef cattle feeder for six hours. Garlic repellent action was higher than citronella.

KEY WORDS: honeybees, repellents, yellow passion fruit crop, beef cattle feeder, pheromones.

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INTRODUCTION

In Brazil, deforestation of huge areas of native vegetation has dislocated many swarms to urban areas. They are attracted to products of candy and soft drinks factories, restaurants, and ice-cream shops and in search of places to build nests. Another place that is visited by honeybees is animal feeders where chopped sugar cane is used. The cattle stops eating, consequently the cattle raiser has losses. *Apis mellifera* also visits the yellow passion fruit flowers. It cannot be considered a pollinating agent to this crop due to the peculiarities of yellow passion-fruit flower anatomy and honeybee small size. During its visit, the honeybee collects all anther pollen, even before the stigma is receptive. Thus, the use of repellents or other methods to keep the honeybees away from these places would be of great interest. These repellents could also be used in the future associated with pesticides so that beehive losses due to poisoning and honey contamination would be prevented. Since the beginning of the last century, the use of repellents has been studied to decrease the damage caused by insecticide (8). In general, repellents are volatile, which makes their effective period short. Therefore, the repellent power of certain substances and vehicles that enable longer effective period, with gradual liberation should be studied. Nicotine sulphate repellent action was efficient in England's but not in Israel's climate, even though concentration was increased by 80 times (7). A synthetic repellent (2-heptanone), similar to a honeybee alarm pheromone was applied at a lucerne *(Medicago sativa* L.) bed, repelling the bees for 45 minutes. Laboratory data showed that its usage would not be practical in agriculture (10). Iso-penthil-acetate and 2-heptanone were pulverized in sunflowers and a great decrease in the number of foragers was seen during twelve minutes (4). In rape (*Brassica napus*) and beans beds, with high concentrations of the same components, foraging was reduced in 80 and 40%, respectively. The repellent effective period lasted 30 minutes. The objective of this work was to study the effects of natural and synthetic substances used as repellents to *Apis mellifera in vitro* in the yellow passion-fruit crop and confined beef cattle feeders.

MATERIALS AND METHODS

This experiment was divided into three parts with tests *in vitro*, tests in the yellow passion-fruit crop, and confined beef cattle feeders.

Analysis of variance used ESTAT to compare averages. Data were considered significant at 5% probability level, and the Tukey test was used to compare averages.

First part – In vitro *tests*

Extracts of garlic, rue, century plant, tobacco and parsley were obtained by maceration with water in a blender at the proportion of 3:1 of extract and water, respectively. Six round pots with syrup (water and sugar at proportion of 1:1) to attract the honeybees were placed on 170 cm posts, two meters apart. This phase was performed in Ribeirão Preto, São Paulo State, between July and August 2000. Twenty observations were obtained at different times. Plastic recipients with the extracts were placed in the pots. Honeybee counting per pot was done until the treatments with extracts received the same number of visits as the control. No extract was placed in the control treatment.

Second part – Tests in passion-fruit crop

A passion fruit plantation in Catanduva, São Paulo State, with an area of 1.5 ha was submitted to four treatments with an area of 50 square meters and five plants each, with three-day repetitions in December 2000. The first treatment consisted of the application of dissolved garlic in solid glycerine (1:3) in tablets coated with tulle, hung near the buds from noon, the time of the flower opening. In the second treatment, 112 ml of 2-heptanone was added to 3 kg of glycerine and 100 ml of water. In the third treatment, 30 plates with pollen, available for honeybee collection were hung on the plants. In the fourth treatment (control), neither repellent nor pollen were used. The number of honeybees per treatment was obtained every thirty minutes, from 10:00 to 17:30 hours.

Third part – Tests in confined beef cattle feeder

The repellent garlic and citronella extracts were obtained the same way as in the First Part in July in Jaboticabal, São Paulo State. They were analyzed at confined animal feeders containing chopped sugar cane and a concentrated mixture of soy and corn middlings. These

extracts were prepared in two different ways; dissolved in solid glycerine forming tablets, and in liquid glycerine soaking hygienic internal-pads (OB) in proportion 3:1, respectively to glycerine and extract which were put inside of square wood recipients that had lateral sides covered with screen. These recipients were placed together with the bovine food. Honeybees frequency from 08:00 to 14:00 hours in feeders where the extracts were used was compared to the control.

RESULTS AND DISCUSSION

First part – In vitro *tests*

Table 1 and Figure 1 show that the extracts have different grades of repellency. The most significant effect was obtained from tobacco extract, followed by rue, garlic, parsley, century plant, and the control. The relative repellent effect of each extract was constant over time. All extracts presented a reduction in the repellent effect over time. After 30 minutes, they were similar to the control, with an average repellent time of 25 minutes. In Piracicaba, São Paulo State, turpentine, cattail leaf extract, garlic oil, clove oil, and *Lantana camarae* leaf extract were tested. No repellent effect was found in evaluations performed every 30 minutes in sunflower crops (9). In Jaboticabal, São Paulo State, citronella oil and 2-heptanone were efficient to keep *Apis mellifera* away from the pots also *in vitro* tests (12).

Table 1. Average number of *Apis mellifera* foragers and repellent action percentage against the control (in parentheses) for the 6 treatments up to 30 minutes after application of the extracts in twenty tests.

Treatment	5´	10´	15’	20´	25´	30´
Garlic	10±11a *(50)	16±14ab (47)	26±16abc (42)	38±19bc (36)	48±19bc (32)	64±18a
Rue	10±10a (50)	15±14ab (50)	24±16abc (47)	34±17bc (42)	44±18c (38)	66±21a
Century plant	16±14a (20)	25±21ab (17)	40±20ab (11)	50±20ab (15)	64±18ab (10)	70±22a
Tobacco	8±7a (60)	12±11b (60)	20±13c (56)	27±14c (54)	39±17c (45)	63±19a
Parsley	13±12a (35)	20±18ab (33)	30±20abc (33)	40±17abc(32)	55±17abc (20)	69±20a
Control	20±20a	30±21a	45±23a	59±24a	71±20a	72±22a

Similar small letters in the same column do not differ statistically from the 5% probability level.

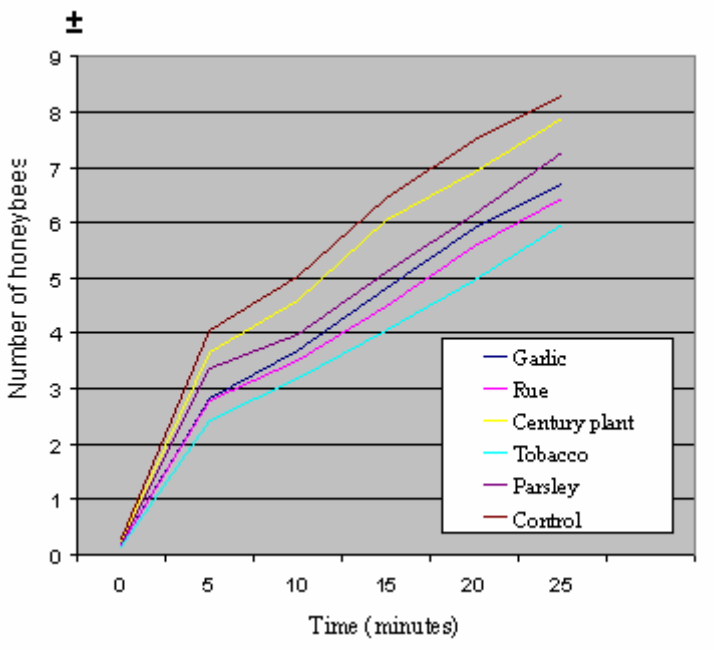

Figure 1. Average number of honeybees in the *in vitro* tests after extract application.

Second part – Tests in the passion-fruit crop

The honeybees collected all passion-fruit flower pollen two hours after their opening. There were fewer honeybees per flower in the garlic treatment, followed by the 2-heptanone. There was no statistical difference. The repellent effect of these extracts lasted two and a half hours. The number of honeybees in the control treatment was 3.5 greater in the areas where the extracts were applied (Table 2 and Figure 2). Many authors proved the efficiency of 2-heptanone as *Apis mellifera* repellent (3,5,6,11). The presence of pollen also kept the honeybees away from the flowers for about two hours. After this period, there was an increase in the number of honeybees in the area with pollen. This number exceeded the number of honeybees observed in the control treatment. The results show repellent substances may be used to keep honeybees away from passion-fruit flowers for a period long enough to allow producers to remove pollen for manual pollination. However, the use of pollen should be better studied. Although the honeybees are kept away from the flowers in the beginning, they can be attracted in the future**.**

Table 2. Average number of honeybees in passion-fruit flowers in the 4 treatments from 10:00 to 17:30 hours.

Time (hours)	Garlic	2-heptanone	Pollen	Control
13:00	0	0	0	23
13:30	5	5	5	33
14:00	13	14	16	41
14:30	24	23	17	61
15:00	4	6	24	41
15:30	14	18	27	44
16:00	0	0	4	0
16:30	0	0	0	0
17:00	0	0	0	0
17:30	0	0	0	0

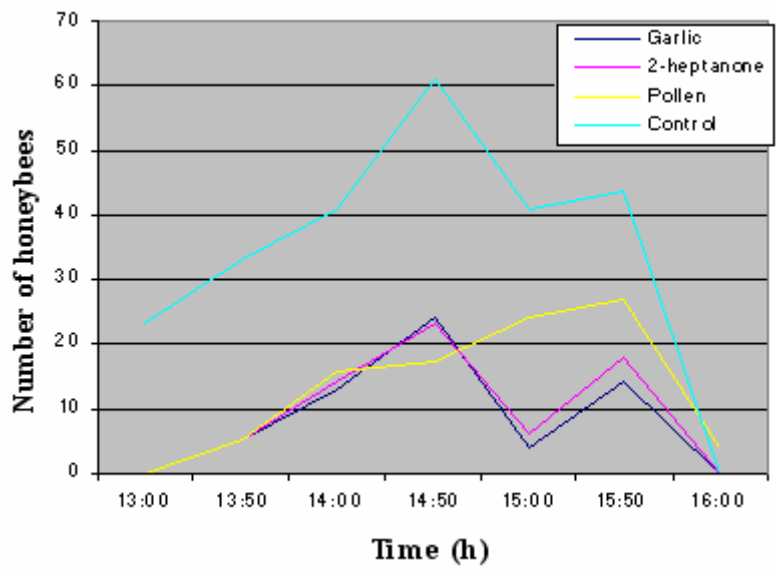

Figure 2. Average number of honeybees in passion fruit-flowers in the 4 treatments from 10:00 to 17:30 hours.

Third part – Tests in confined beef cattle feeders

When compared to control, there was a reduction of 82, 68, 60, and 60% in the number of honeybees for of garlic extract in glycerine, garlic extract on internal-pads, citronella extract in glycerine, and citronella extract on internal-pads, respectively (Figure 3). On average, garlic repelled 75% of honeybees and citronella 60%. The superiority of garlic in repelling honeybees is reinforced when comparing application method. Treatments that had glycerine as vehicle reduced 71% of honeybees and those that had internal-pads as vehicle reduced 64%, showing that glycerine is better than internal-pads in repelling honeybees. Some authors used pyrethroids and deltamethrin to repel honeybees (1,2). These substances are harmful to honeybees.

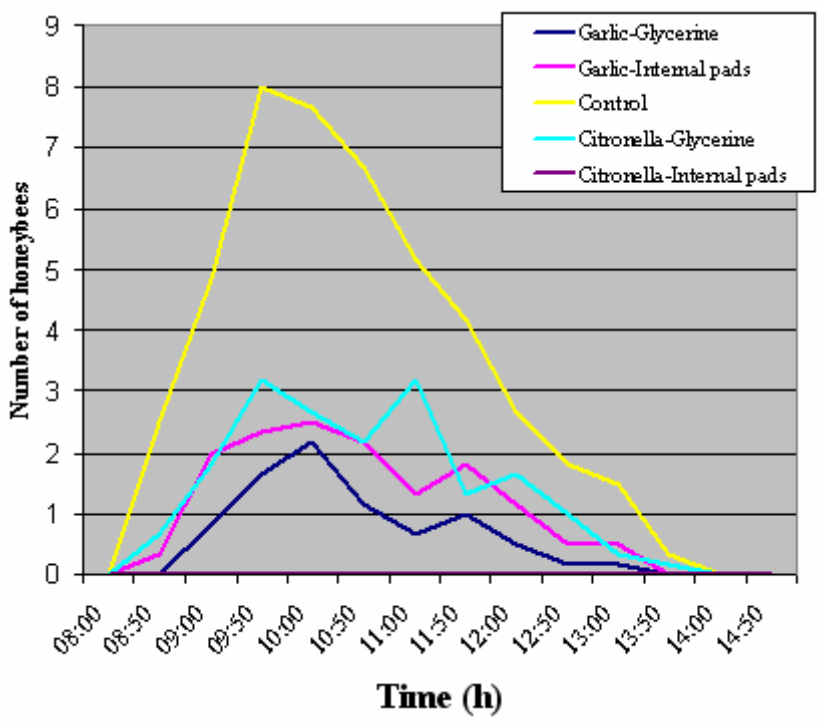

Figure 3. Average number of foragers in confined beef cattle feeders in Jaboticabal.

CONCLUSIONS

1. There was a repellent effect *in vitro* with the tested substances, in the following decreasing order: tobacco, rue, garlic, parsley, and century plant extracts with average effect duration of 25 minutes.

2. In yellow passion-fruit, garlic and 2-heptanone extracts were equally efficient in repelling honeybees. The effect lasted two and a half hours. Pollen collection allows the manual pollination of passion-fruit flowers and stimulates a larger number of honeybee visits in the area over time.

3. Garlic and citronella extracts were efficient in keeping *Apis mellifera* away from confined beef cattle feeders for 6 hours. The repellent action of garlic was higher than the citronella.

4. Repellents may be used to keep honeybees away from passion-fruit flower and confined beef cattle feeders for 2 hours and a half and 6 hours, using garlic, 2-heptanone, and garlic and citronella extracts, respectively.

ACKNOWLEDGEMENTS

Supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP (process n^{o} 01/05460-2).

REFERENCES

1 BOCQUET JC., L'HOTELLIER M., FEVRE F., BAUMEISTER R. A five-year study on the effect of deltamethrin on bees under natural conditions. In: ***SYMPOSIUM ON HARMONIZATION OF METHODS FOR TESTING THE TOXICITY OF PESTICIDES TO BEES,* 2**, Honenhein, 1982**. Proceedings...** Honenhein: International Bee Research Association, **1982**:21-3.

2 DAVID BV., SOMASUNDARAN L. Synthetic pyrethroids – an evaluation of their potential effects non-target organisms**.** ***Pesticides*, 1985**, **19**, 9-12.

3 FERGUSON AW., FREE JB. Production of a forage marking pheromone by the honeybee. ***J. Apic. Res*., 1979, 18,** 128-35.

4 FREE JB**.** ***Pheromones of social bees***. London: Chapman and Hall, **1987**. 218p.

5 FREE JB., FERGUSON AW., SIMPKINS JR. Honeybee responses to chemical components from the worker sting apparatus mandibular glands in field tests**.** ***J. Apic. Res*., 1989, 28**, 7-21.

6 FREE JB., FERGUSON AW., SIMPKINS JR., AL-SA'AD BN. Effect of honeybee Nasonov and alarm pheromone components on behaviour at nest entrance. ***J. Apic. Res*., 1983, 22**, 214-23.

7 HARPAZ I., LENSKY Y. Experiments on bee repellents. ***Bee World*, 1959, 40,** 146-53.

8 JOHANSEN CA. Pesticides and pollinators. ***Annu. Rev. Entomol*., 1977, 22,** 177-92.

9 MORETTI ACCC. ***Estudo sobre a polinização entomófila do girassol* (*Helianthus annuus* L.) *utilizando diferentes métodos de isolamento da flora***. Piracicaba: Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiróz", **1989**. 126p. (Tese - Doutorado).

10 RIETH JP., WILSON WT., LEVIN MD. Repelling honeybees from insecticide treated flowers with 2-heptanone. ***J. Apic. Res*., 1986, 25**, 78-84.

11 SIMPSON J. Repellency of the mandibular gland scent of worker honey bees. ***Nature*, 1966, 209**, 531-2.

12 SOUZA DTM. ***Efeitos de atrativos e repelentes sobre o comportamento forrageiro da abelha Apis mellifera***. Jaboticabal: Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, **1996**. 119p. (Tese - Doutorado).