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Black Queen Cell Disease in Honeybees. Effect of a Citric Extract Adsorbed to a Titanium Nanoparticle

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ABSTRACT

Titanium nanoparticles with citrus extracts adsorbed to its structure dissolved in water was used to control this viral disease of bees. Initially, two nuclei of two- and three-frame bees, four well-populated three-frame nuclei with a progenitor queen selected and inseminated to obtain larvae for translarvae, two starter hives consisting of two jumbo hive bodies separated by a queen excluder, two finisher hives with a queen excluder and a queen excluder were used, two finishing hives with a capacity of one and a half jumbo hives (fifteen frames), with a queen excluder placed vertically at the height of the tenth frame acting as a separator between the queen part and the queenless section. 1ST STAGE. A dilution of 1 litre of product in 10,000 litres of water, equivalent to 0.1 ml of product per litre of water. 2nd STAGE A dilution of 1 litre in 200 litres of water, equivalent to 5 ml of product per litre of water. The dilution was sprayed directly to each of the honeycombs with the bees on the surface. Three applications, one every week, the product has a life of 72 hours. After the last application there have been at least 5 translarvae without signs of the disease in the real cells of 10 to 11 days of age and transferred to their nuclei of fertilization, there were deaths of pupae or queens almost to be born but these are fully formed situation or symptom that does not correspond to the disease that concerns us. It is concluded that NANOCYT is effective in controlling black queen cell disease in honey bees.

KEYWORDS: Black queen cell disease, Nano therapy

INTRODUCTION

Beekeeping is an important activity worldwide providing honey, pollen, royal jelly, etc. And medicinal products such as propolis, apitoxins and toiletries components of some beauty products.

The importance of this activity is the environmental service of pollination that they perform and through which many plant species reproduce, including some plants do not reproduce if bees or other pollinating insects do not intervene. It is estimated that they pollinate up to 30% of the vegetable crops on which human food depends and also intervene in the pollination of approximately 90% of wild plants, some commercial crops increase their production by up to 40% as well as improving the quality of the fruit.

The production of queen bees is of fundamental importance as they are the basis of every hive as they are the only fertile female in the colony, they are responsible for the generation of worker bees, their number and their characteristics define the productivity of the hive.

For these reasons the production of queen bees of selected breeds and lines, free of diseases and with the best practices, is key for a productive beekeeping.

All animal species used or exploited for the benefit of mankind are affected by various diseases and parasitosis, caused by fungi, bacteria, viruses or by external or internal parasites, which limit, decrease productivity, render useless or cause the death of productive animal species.

Black queen cell disease of honey bees, which causes the death of queen bees before they emerge from their cocoon, resulting in losses to commercial queen bee farms that can be substantial.

Black queen cell disease (BQCV) is caused by an RNA-like virus of the genus Crypavirus, which exclusively affects queen bee larvae and may be present in adult bees without symptoms in any other stage or individual in the hive, Nurse honey bees transfer BQCV horizontally from infected cells to healthy larvae in brood food, and queens can also transmit it vertically to eggs (Spurny et al. 2017,Al Naggar & Paxton 2020).

BQCV can be present year-round in adult bees and it is estimated that the Varroa mite and the microsporidium Nosema spp. may be vectors.

The virus is inoculated into the larvae via the food provided to the actual larvae by the nurse bees.

To date, hygienic measures are recommended for materials or tools used in daily activity or the removal of genetic material (parent bees) showing susceptibility to this disease, adequate feeding, young queen bees with stocked hives, rotation of combs every 3-4 years and placing the hives in a warm and sunny position during the autumn, winter and spring periods. The use of antibiotics such as fumagilin or oxytetracycline hydrochloride may help to reduce or eliminate the presence of BQCV in the hive. We do not recommend this and suggest that this method only be used if other control/elimination strategies are ineffective.

In the autumn of 2017, a major outbreak of this disease occurred in Apiarios Sto Domingo, a company dedicated to the production and sale of queen bees and nucs, established in the state of San Luis Potosi, Mexico, in the municipality of Cerritos, which affected more than 50% of the production of queen bees, resulting in a drop in the production of queen bees for sale, which was around 70 queen bees per week.

Titanium nanoparticles (NPs) with antibacterial, antifungal and especially antiviral properties have been used as an antiviral alternative ((Sagavedan et al. 2022, Maduray & Parboosing, 2020). The antiviral properties of these NPs are enhanced when citrus extracts are adsorbed on their structure (Fall et al. 2021, Balta et al. 2020, Al Naggar et al. 2020). Taking into account the properties of these nanoparticles, they were considered for use as an alternative therapy against BQCV.

MATERIALS AND METHODS

A titanium nanoparticle with citrus extracts adsorbed on its structure (NANOCYT) dissolved in water base was used as an alternative for the control of BQCV, a viral disease of bees. With chlorine-free purified water for dilution and as an application vehicle, a two-litre hand-held sprayer and an insulin application syringe for dosing.

As test subjects, two nuclei of two- and three-frame bees were used initially, four well-populated three-frame nuclei with a selected and inseminated queen progenitor in each to obtain the larvae for the translarvae, two starter hives consisting of two jumbo hive bodies separated by a queen excluder, 17 heavily populated frames and a fully developed queen less than one year old in the lower body of this hive including an internal frame feeder were also used.

Two finishing hives with a capacity of one and a half jumbo hives (fifteen frames) were also used, with a queen excluder positioned vertically at the height of the tenth frame acting as a separator between the queen and queenless sections, with two external lids, which could be opened independently for the ten-frame section and the queenless section (capacity five frames).

The dose to be used in the test was 1 : 10,000 of the product dissolved in water without chlorine, considering two stages, the first using the dose recommended for drinking water for animal use, and if no effect was seen, using the dose recommended on the product package.

1ST STAGE .... A dilution of 1 litre of product in 10,000 litres of water, equivalent to 0.1 ml of product per litre of water.

2ND STEP.... A dilution of 1 litre in 200 litres of water, equivalent to 5 ml of product per litre of water.

Once the dilution had been made directly in the sprayer, shaking it vigorously, pressure was applied by means of the built-in hand pump and the dilution was sprayed directly onto each of the honeycombs with the bees that were on the surface of the honeycombs.

It was considered that the cleaning instinct of the bees against foreign liquids on their bodies or combs, and for which they use their mouthparts to ingest these liquids, would be sufficient for the rapid incorporation of the active ingredients into their bodies without the need to use any food to induce the consumption and incorporation of the active ingredients into their bodies.

The product with systemic effects on any surface could act on the causative agent which could be found in the stored food or on the surface of the comb.

MATERIALS AND METHODS

The method of production of queen cells is the Doolittle method by grafting larvae of the appropriate age in artificial co-cells, the Cloake board method is used for the initiation and completion of the queen cells, and a part of the initiated cells as two frames are initiated in the initiator hive and one is passed to a finisher hive for the correct feeding and sealing of the queen cells, After seven days, when another production cycle of queen cells is started, they are taken to an incubator for their finalisation and use at ten or eleven days of age. This method and rhythm is the one used in the company during the whole production season in such a way that a batch of fertilised queens ready for sale is obtained every week.

The situation in queen cell production was serious as the mortality of larvae in the queen cells became very high, as an example of the queen cells achieved in one of the grafting racks was 10 dead larvae out of 25 finished cells checked on 25, said November 2017 which represents 40%, even though several sanitisation activities were carried out, such as disinfecting the plastic co-cells with chlorine, clean water for food preparation and disinfection of the grafting instruments without obtaining any results.

Two nuclei of bees of two frames were chosen to carry out preliminary tests and to check if there was no negative effect on the bees, these nuclei were sprayed with the product and the dilution of the first stage, comb by comb together with the bees and brood that they had on November 17th, checking on

November 18th and 20th not presenting any negative effect neither in the adult bees nor in the larvae.

It was decided to make three applications, one every week, because the product has a life of 72 hours and most of the bee diseases have a cycle of three weeks.

The applications were made on 20 and 27 November and 4 December 2017, applying to four brood hives, two starter hives and two finisher hives, trying to cover all possible sources of the disease and covering comb by comb according to the above mentioned, these applications were made in the course of the morning, until one o'clock in the afternoon.

The rest of the activities were carried out normally, the most important being the grafting of larvae, which was carried out in the afternoon of the same days.

RESULTS

The larvae affected in the last grafting before application, which was carried out on 14 November and checked on the 25th of the same month when they were ready to be placed in their fertilisation nucleus, were 10 out of 25 cells, giving a 40% affectation rate.

The cells of the grafted larvae, after the first application on 20 November were checked on 1 December without any apparent death of larvae, so they were placed in the fertilisation nucleus with the intention of checking their hatching in three days and verifying if they were not affected by BQCV, but there were some meteorological phenomena such as strong cold, which did not allow us to verify the rate of affectation.

Subsequent graftings from the dates of the other two applications showed no symptoms of disease, as none of them showed the characteristic blackish colouring in the bud of the queen cell, and two to three suspect cells of the grafted larvae from each of the dates were opened and found to be healthy.

After the last application, at least 5 graftings have been carried out and there have been no signs of BQCV in the queen cells, which are 10 or 11 days old and have been placed in their fertilisation nuclei. We should mention that there have been deaths of pupae or queens almost ready to hatch, but these are fully formed, a situation or symptom that does not correspond to the disease in question.

The company has increased the production of queens to the expected levels, even though the season is not the right one.

In all the colonies treated, an increase in vigour and general health of the hive was noted and they are successfully overwintering.

CONCLUSIONS

The use of titanium NPs with citrus extracts adsorbed to their structure can control or cure the Black Royal Cell disease, since after at least 6 grafting events, no symptoms of the disease have appeared.

It was not necessary to increase the concentration of the product and repeat treatments, the dose used was sufficient to control and possibly eliminate the virus causing the disease.

These studies with all the experimental rigour to verify and corroborate the results obtained in this work, both at the level of adequate dose of NPs and to detect the vector and the way in which the bee larvae are infected.

It would be interesting to study the effect of the product used on other pathogens that affect bees such as those that cause the diseases nosemiasis, tracheal acariosis and lime brood as these diseases are very difficult to combat and some products used in their control are highly polluting and therefore currently banned in Mexico.

There is no conflict of interest

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Assistants: Victor Pérez y Rubén Medina

Company: Apiarios Sto Domingo, property of Mr. Felipe Arias G.

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