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Potential of the chemical dinotefuran in the control of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) semi-engorged female ticks

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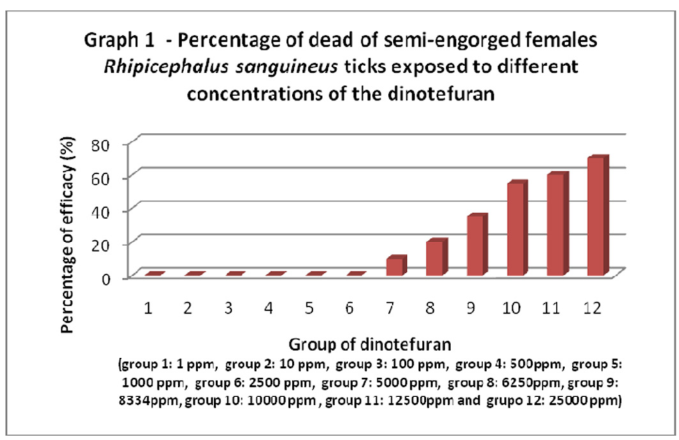
HIGHLIGHTS

- The results showed the daily number of dead *Rhipicephalus sanguineus* semi-engorged females.
- The mortality data were subjected to Probit analysis.
- An LC_{50} of 10,182.253 ppm (8725.987–13,440.084) was estimated.
- The acaricide effect is dose-dependent.
- The action of dinotefuran was slow and gradual.

GRAPHICAL ABSTRACT



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ABSTRACT

Ticks are vectors of several pathogens to vertebrates, including the human being. They produce lesions on the hosts during the blood feeding and great economic losses. Several chemical acaricides have been used in an attempt to control tick infestations; however these substances are harmful to both the human being and non-target organisms, and to the environment. Therefore, there is a need to fight these ecto-parasites through less harmful methods, less aggressive to the environment, non-target organisms and

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to the human health. The present study examined the efficacy of dinotefuran on the susceptibility of *Rhipicephalus sanguineus* semi-engorged females exposed to different concentrations of the product. Its lethal concentration of 50% (LC_{50}) at 95% confidence interval was determined. The ticks were immersed in Petri dishes containing different concentrations of dinotefuran or distilled water for 5 minutes and then dried and maintained in an incubator for 7 days. The results showed the daily number of dead *R. sanguineus* semi-engorged females after being treated with different concentrations of dinotefuran. The mortality data in bioassay 2 were subjected to Probit analysis, where a LC_{50} of 10,182.253 ppm (8725.987–13,440.084) and 95% confidence interval were estimated. The susceptibility of *R. sanguineus* semi-engorged females to dinotefuran in higher concentrations of the acaricide was demonstrated, indicating that its effect is probably dose-dependent. In addition, the action of dinotefuran was slow and gradual, interfering in the development and growth of the individuals throughout the observation period (7 days).

1. Introduction

The tick *Rhipicephalus sanguineus* is one of the main ectoparasites of medical and veterinary importance worldwide (González et al., 2004; Labruna and Pereira, 2001; Linardi and Nagem, 1973; Soares et al., 2006; Szabó et al., 2001).

This arthropod is the main vector and reservoir of *Ehrlichia canis*, being also responsible for the transmission of other pathogens such as *Babesia canis*, *Babesia caballi*, *Babesia equi* (Sexton et al., 1976), *Hepatozoon canis* (Craig, 1990), *Anaplasma platys* (French and Harvey, 1983) and *Haemobartonella canis* (Woldehiwet and Ristic, 1993). Some studies have reported its participation in the transmission of canine visceral leishmaniasis (Coutinho et al., 2005). Others stated that *R. sanguineus* would be the vector of *Coxiella burnetii* – causative agent of the human Q fever (Stephen and Achyutharao, 1980), *Rickettsia rickettsii* – causative agent of spotted fever, *Rickettsia conori* – causative agent of botonous fever (Merle et al., 1998), of *simile* Lyme borreliosis (Yoshinari et al., 1997) and of the bacteria *Francisella tularensis*, the causative agent of tularemia (Walker et al., 2000).

In addition, the *R. sanguineus* causes direct and indirect damage to their hosts, such as blood spoilage and skin lesions (Sonenshine, 1991).

Several studies have focused on the search for an efficient strategy to control ectoparasites. The chemical control (via synthetic acaricides) is still the main method to control ticks, despite having many disadvantages, such as the high cost of the products, facilities and qualified manpower for the application of the substances, damage to the environment, contamination of the soils, rivers and animals with chemical residues – also affecting human health (Nolan, 1985; Oliveira et al., 2008, 2009, 2011; Pruett, 1999).

Another concern is the selection of tick lineages resistant to the different active ingredients mainly due to the incorrect use of chemicals (Crampton et al., 1999). Therefore, the search for new acaricides with lower toxicity and less contaminants has been intensified.

A new category of compounds with the potential to control ticks is the neonicotinoids, presenting high selectiveness for the insect nicotinic acetylcholine receptors, and low toxicity for mammals. Since the commercialization of the pyrethroids, this is the fastest growing category in the marketplace (Kiriyama and Nishimura, 2002; Nauen and Bretschneider, 2006).

Among the neonicotinoids is dinotefuran, the most recently synthetized, belonging to the third generation (Wakita, 2011; Wakita et al., 2003, 2005). Dinotefuran is unique in its structure, as it is based on the acetylcholine molecule, not on nicotine as the other neonicotinoids (Wakita, 2011). Compared with the other neonicotinoids, it is highly toxic for insects, mainly Hemiptera, Coleoptera, Diptera, Dictyopera and Thysanoptera (Tomizawa and Casida, 2005; Wallingford et al., 2012). Toxicological and ecotoxicological studies have demonstrated that dinotefuran presents low toxicity for birds, aquatic animals (Kagabu, 1997; Uneme et al., 1999; Wakita et al., 2005), and the environment (Wakita et al., 2005; Wakita, 2011). Still regarding its toxicology, it was demonstrated that dinotefuran does not have genotoxic, teratogenic or carcinogenic effects on mice, rats, rabbits or guinea pigs (Wakita et al., 2003). Its excellent chemical-physical, biological and toxicological properties make dinotefuran a promising option to control plagues and vectors of public importance (Wakita et al., 2005; Zaim and Guillet, 2002), confirming its important role in the present context and motivating the development of studies focusing on its effects and mechanisms of action.

Dinotefuran was shown to be effective against *Ctenocephalidade felis felis* fleas (Dryden et al., 2011) and also mosquitoes (Corbel et al., 2004). In the case of *R. sanguineus*, few studies on the use of this chemical in tick control are currently available in the literature.

Recently, Bissinger et al. (2011), Erdmanis et al. (2012) and Lees et al. (2014) studied the nicotinic acetylcholine receptors (nAChR) in ticks. According to Erdmanis et al. (2012) and Lees et al. (2014), the Acari, including both ticks and mites, are largely insensitive to imidacloprid (a neonicotinoid) and that the basis of this insensitivity may reside at the nAChR itself.

The efficacy of a chemical is usually assessed through *in vitro* bioassays, which are relatively simple to be performed, inexpensive and require little specialized equipment (Scott, 1995).

Considering the information above, the present study was aimed to evaluated the efficacy of dinotefuran and the susceptibility of adult semi-engorged female *R. sanguineus* to different concentrations of this chemical through *in vitro* bioassay (AIT), daily monitoring, estimating LC_{50} (lethal concentration 50%) and 95% confidence interval.

2. Materials and methods

2.1. Chemical substance

2.1.1. Synthetic: dinotefuran (CAS 165252-70-0)

Dinotefuran is a compound of the neonicotinoid chemical class, molecular formula $C_7H_{14}N_4O_3$. The chemical was obtained from the commercial acaricide Protetor Pet®, produced by "Ouro Fino Saúde Animal", Cravinhos, SP, Brazil, in tubes of 0.48 mL, concentration 25%, for animals up to 5.0 kg.

2.2. *Rhipicephalus sanguineus* ticks (Latreille, 1806)

R. sanguineus semi-engorged females, weighing 27 mg on average (about 5 days of feeding), were used throughout the experiment. They were supplied by the tick colony maintained under controlled conditions (28 °C, 85% humidity, and 12-h photoperiod) in a BOD (Biological Oxygen Demand) incubator, in a room of the Animal Facility of the Department of Biology – UNESP, Rio Claro campus/São Paulo, Brazil. Semi-engorged females were obtained after unfed *R. sanguineus* couples (25 couples/infestation) were allowed to feed on naive New Zealand white rabbits following Bechara et al.