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Tagetes minuta Linnaeus (Asteraceae) as a Potential New Alternative for the Mitigation of Tick Infestation

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Abstract

Ticks are hematophagous parasites of most vertebrate animals and can transmit various pathogens. After mosquitoes, ticks are considered the most prevalent group of ectoparasitic arthropods to transmit pathogens to humans and rank first in the transmission of agents that cause disease in animals. The primary tool used to control these ectoparasites is the use of chemical products; however, resistance to several of these chemical compounds has already been reported in various locations worldwide. Considering this reality, several studies of plant extracts have been developed aiming to identify new compounds that are able to control ticks. In this context, the essential oil of *Tagetes minuta* may be a promising alternative in the control of some species of ticks. *T. minuta* is an annual herbaceous plant belonging to the family Asteraceae and is popularly known in Brazil as "cravo-de-defunto" or wild marigold. In this review, we highlight four species of ticks that are considered important for both animal and public health in Brazil. Here, we address the methods of tick control to provide a foundation for new studies and highlight the use of phytotherapeutic *T. minuta* as a promising alternative in the control of these ectoparasites.

Keywords: Control tick; Phytotherapeutics; Ectoparasites

Introduction

Ticks are hematophagous parasites [1] of most vertebrate animals and can transmit various pathogens [2]. After mosquitoes, ticks are considered the most prevalent group of ectoparasitic arthropods to transmit pathogens to humans and rank first in the transmission of agents that cause disease in animals [3,4]. Ticks belong to the Phylum Arthropoda, Class Arachnida, Order Acari and Suborder Ixodida and have a wide geographical distribution.

Currently, there are over 896 cataloged species of ticks that are divided into three families: Argasidae, Ixodidae and Nuttalliellidae (which has only one species) [5]. The Brazilian Ixodidae fauna is currently composed of 66 species [6] belonging to nine genera: *Ornithodoros*, *Antricola*, *Argas*, *Carios*, *Amblyomma*, *Ixodes*, *Haemaphysalis*, *Rhipicephalus* and *Dermacentor* [7].

The species of ticks that parasitize domestic animals are usually the ones that are most studied, with their biology, vector capacity and forms of control being the subject of many studies in the country [8]. However, the following ticks have the highest incidence in Brazil: *Rhipicephalus microplus*, *R. sanguineus*, *Amblyomma cajennense* and *Dermacentor nitens.*

The control of these ectoparasites is still performed through the use of chemicals. According to the Brazilian Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e do Abastecimento - MAPA), for new products to be registered as acaricides, they must present an efficacy of at least 95% [9]. The lack of a program to control these parasites allows the majority of producers to define the criteria for control. The emergence and selection of tick strains that are resistant to these compounds remains a major motivation to develop new antiparasitic products [10].

Considering this reality, several studies with plant extracts have been developed aiming to identify new compounds that are able to control ticks. The use of phytotherapeutics obtained from the essential oil of *Tagetes minuta* is a promising alternative [11,12], but there have been very few studies on it to date. *T. minuta* is an annual herbaceous plant belonging to the family Asteraceae. Its best-known common name in Brazil is "cravo-de-defunto" [13]. This plant is used in folk medicine and grows in temperate regions of South America [14].

The tick species discussed in this review are largely important for domestic and production animals and are immensely important to public health in Brazil. Difficulties related to the methods of control are discussed to encourage further research. The use of phytotherapeutic *T. minuta* is highlighted in this review as a promising alternative for controlling these ectoparasites.

Species of Ticks used in Tests with *T. minuta* in Brazil

Rhipicephalus microplus

R. microplus is known as "cattle tick" (carrapato-do-boi) in Brazil, and cattle are its main host, with preference for *Bos taurus* compared with *B. indicus*. Although this tick can parasitize other animals, domestic or otherwise, it is a monoxenous (one-host) tick.

This species was most likely introduced in Brazil during the early 18th century and is currently found in all regions of the country, with the intensity of infestation varying according to climatic conditions and cattle breeds [15]. This tick causes major losses in livestock worldwide, in addition to transmitting several pathogens, most importantly the pathogens that comprise two well-known diseases collectively known in Brazil as "*tristeza parasitária bovina* (TPB)" [16] babesiosis, which is caused by the protozoa *Babesia bigemina* and *B. bovis*, and anaplasmosis, which is caused by *Anaplasma marginale* [17].

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R. sanguineus

R. sanguineus is a trioxenous (three-host) tick that feeds primarily on dogs and accidentally on other hosts, including humans [18]. Dogs are the only known primary hosts for the parasitic stages of this tick [19]. This tick is an important transmitter of pathogens and is considered the main vector of *Ehrlichia canis* in Brazil, which has been established as an important zoonotic disease since 1992 [20].

This ectoparasite can also transmit pathogens such as *Babesia canis* to dogs and *Rickettsia conorii* to humans [21]. In the American continent, this species of tick transmits other diseases such as Brazilian spotted fever (BSF), which is caused by *Rickettsia rickettsii*, and in Brazil, it is the main transmitter of *Hepatozoon canis* [22].

The tick *R. sanguineus*, which is also known as "brown dog tick" or "carrapato vermelho do cão" in Brazil, is a cosmopolitan species and most likely has a widespread geographical distribution [18,23]. This tick is originally from the African continent, where there are approximately 79 species of the genus *Rhipicephalus* [24].

Amblyomma cajennense

Commonly known as "cayenne tick" ("carrapato-estrela" or "carrapato-do-cavalo" in Brazil), *A. cajennense* has a three-host life cycle and low host specificity and, thus, is able to parasitize several species of domestic and wild animals [25].

It is believed that tapirs (*Tapirus terrestris* L.) and capybaras (*Hydrochoerus hydrochaeris* Erxleb.) are the main primary hosts for *A. cajennense* in South America [26]. After the introduction of horses to Latin America during the European colonization, *A. cajennense* became a serious pest for these animals, which are also primary hosts for all stages of this ectoparasite [27].

This species is the main species that parasitizes humans in Central America and Brazil [26,28] and is one of the main vectors of *Rickettsia rickettsii*, which is the causative agent of BSF in humans [29,30].

Argas miniatus

Argas miniatus Kock (1844) is the only species of the genus *Argas* occurring in Brazil, and domestic birds are their host. In nature, this species is found in small flocks of *Gallus gallus* and causes productivity losses, anemia, spoliation and the transmission of pathogens. *Borrelia anserina* is an important pathogen transmitted by *A. miniatus* [31].

A. miniatus is a heteroxenous tick that feeds at night. During the feeding process, the larva remains, parasitizing the host for multiple days, while nymphs and adults feed on the blood for only a few minutes. During the free-living stage, these ticks are found in the shelters and nests of their hosts, which are the locations where molting and copulation occur [32].

Types of controls

To avoid losses due to the spoliation effect caused by ticks, there are some methods that seek to minimize this problem, such as the use of chemicals (acaricides), vaccines, phytotherapeutics, genetic selection and the preservation and/or use of natural enemies (biological control). These methods are even more effective when used in the form of "Integrated Management" and/or "Strategic Management".

The constant exposure of ticks to acaricides, which is associated with a lack of proper management, accelerates the selection pressure for resistant individuals in the population, inevitably worsening the resistance problem, as already reported by several authors in various global locations [33-35].

The lack of new molecules adds an additional layer of complication to satisfactory tick control. These difficulties are directly related to the high costs of research and the lengthy process involved in the development of new chemical formulations.

Natural control is the spontaneous regulation by living organisms (antagonists) of populations of other species of animals with no human intervention [36]. The identification of natural control agents, so-called natural enemies, allows man to manipulate these organisms, producing them under controlled conditions for subsequent release in areas of interest.

This form of manipulated natural control, deemed biological control, includes artificial, classical and applied controls [37]. Although the use of acaricides remains the primary tool for control, other methods such as biological control methods have previously been studied and include options involving the use of microbial agents, such as fungi [38], and the action of natural predators, such as the cattle egret *Egretta ibis*, which prefers insects but also feeds on ticks [39] and on ants [40,41]. Although biological control is a much more attractive cost/benefit approach compared to other methods, it still does not have satisfactory applicability in the field.

Over the last few decades, studies involving the development of vaccines to control ticks have intensified because of the need to replace chemical controls. As previously mentioned, the residues of chemical controls cause damage to public health and to the environment, among other undesirable effects. Currently, only vaccines for *R. microplus* are available for import into Brazil. These vaccines were developed from a protein called Bm86, which confers partial protection to cattle against future infestations by reducing the number of ticks, egg production and fertility [42].

The Bm86 protein is a "hidden antigen" obtained from the intestine of ticks [43]. This protein is the basis of two commercial vaccines available on the market: the TickGARD vaccine, which was developed in Australia [44], and the Gavac vaccine, which was developed in Cuba [45]. Although they are an important control alternative, the protection levels provided by vaccines are not yet sufficient to replace the use of acaricides [46,47]. This reinforces the need for further research in the search for candidate antigens that may confer greater control efficiency.

Brazil has a large plant biodiversity with approximately 55,000 cataloged species; however, only 1% of these plants have been submitted to chemical and/or pharmacological studies. Medicinal plants are consumed by all social classes and make up a national market worth US$ 400 million. Moreover, their use is recommended by the United Nations (UN), which recognizes that two-thirds of the world's population uses medicinal plants. Although the use of medicinal plants is often rejected by physicians, there are at least 300 medicinal plants that are part of the Brazilian popular therapeutic arsenal [48].

Several studies with plants extracts have been developed with the objective of using plant extracts as an alternative method to reduce or even replace the use of synthetic products. Currently, *R. microplus* and other tick species have been the subject of these studies due to the emergence and selection of strains that are increasingly resistant to various chemical groups that are used in different parts of the world [49]. Phytotherapeutics have some advantages over synthetic compounds, such as a slower development of resistance due to the presence of different compounds with different mechanisms of action [50-52].

Approximately 55 plant species belonging to 26 families have been tested against *R. microplus*; however, only a few compounds have been identified and proven to have acaricidal action [53]. The main challenge in the development of alternative acaricides is the difficulty of transposing the efficacy obtained *in vitro* to the field, which is partly due to the difficulty in stabilizing the various chemical compounds present in the extract [54] and also to the high volatility of natural products, which have low persistence in the environment [55].

Tagetes minuta

Tagetes is a genus of herbaceous plants and shrubs that includes some species of the composite family of plants. This genus is native to Central and South America and was naturalized in other tropical and subtropical regions. *Tagetes spp.* are commonly known as marigolds ("cravos"), and some species, such as *T. erecta*, *T. tenuifolia* and *T. patula*, are grown as ornamental plants. However, *T. minuta* Linnaeus can grow under natural conditions, and in some countries, such as Australia and South Africa, this plant has been classified as a noxious plant [56].

T. minuta was introduced in Brazil several years ago and is perfectly acclimatized, even becoming a sub-spontaneous plant [57]. *T. minuta* is classified as follows [58]:

- Family: Compositae or Asteraceae
- Subfamily: Asteroideae
- Tribe: Helenieae
- Genus: *Tagetes*
- Specie : *Tagetes minuta* Linnaeus

This plant is commonly known as the southern cone marigold, Mexican marigold, black mint, wild marigold or stinking Roger, while in Brazil, its common names include vara-de-rojão, rabo-de-foguete, cravo-de-defunto, cravo-de-urubu, chinchilho, coari, coari-bravo and estrondo. Its essential oil is used as an anthelmintic in folk medicine. *T. minuta* is a plant that reproduces by seeds that germinate in spring and summer; in southern Brazil, its cycle lasts 120 to 150 days until the formation of seeds. *T. minuta* received its name due to the size of its flowers and not the size of the plant, which can grow as high as 2 meters. The plant is found in dry terrains and develops better in cultivated areas, areas with good fertility and in burned areas [58].

Several species of this genus have been investigated as possible sources of different biological activities that can be used in industry and medicine. This possibility is due to the presence of secondary metabolisms that produce compounds that are not distributed in all parts of the plants and are not strictly necessary but that play an important role in the interaction between the plants and the environment. Terpenes (derived from mevalonic acid or pyruvate and 3-phosphoglycerate), phenolic compounds (derived from shikimic acid or mevalonic acid) and alkaloids (derived from aromatic amino acids) are the three major groups of secondary metabolites [59].

Several compounds are formed in the leaves, flowers or fruits and then accumulate in specific organs of *Tagetes spp.* in the form of essential oils that possess insecticidal and antimicrobial properties [60,61]. For example, flavonoids have antioxidant properties [62], and carotenoids, especially lutein esters that are found only in the flower's petals, are used in pharmaceutical preparations [63,64] and as food additives and colorants [65]; they are also known for their anticancer effects [66].

An analysis of the essential oil of *Tagetes minuta* L. flowers from the northwest Himalayas identified and characterized the following components: (Z)-β-ocimene (39.44%), dihydrotagetone (15.43%), (Z)-tagetone (8.78%), (E)-ocimenone (14.83%) and (Z)-ocimenone (9.15%), in addition to demonstrating that ocimenone has a larvicidal activity against mosquitoes [67].

Later, Moghaddam [68] and Garcia [11] corroborated these results when they demonstrated that the major components of *T. minuta* oil are α-terpineol, (Z)-β-ocimene, dihydrotagetone, (E)-ocimenone, (Z)-tagetone and (Z)-ocimenone. The composition of the essential oil of *T. minuta* varies according to the different parts of the plant and its stage of growth/maturation; however, the composition does not differ in relation to the geographic origin [69].

T. minuta is a very common plant throughout Brazil [70]. This species is the subject of studies that have shown promising results, with the species being effective against microbial agents, such as fungi [71], viruses [72] and bacteria [73]. Recently, an *in vitro* study conducted by Garcia [11] tested the essential oil of *T. minuta* in the control of four species of ticks: *R. microplus*, *R. sanguineus*, *A. cajennense* and *A. miniatus*. In that study, at a concentration of 20%, the authors observed efficacies higher than 95% for all of the species analyzed and concluded that *T. minuta* has acaricidal potential for controlling both the larvae and adults of these species. Nchu [74,75] had previously suggested an acaricide action for *T. minuta* when they tested its essential oil against *Hyalomma rufipes* and observed satisfactory results.

Another study using *T. minuta* from the same group conducted by Andreotti [12] observed the *in vivo* acaricidal potential of this oil in the control of *R. microplus* and concluded that at a concentration of 20%, its efficacy was greater than 95%, consistent with previous results reported by Garcia [11]. Both results suggest that *T. minuta* is a promising acaricide.

Final considerations

Given the information described above, limitations in the performance of these different tools have been observed that restrict their satisfactory control of ticks. The need for new compounds combined with directed public policies, ranging from oversight of the trade of these tick control products to the correct application of these products, are the main factors that decrease tick control efficiency and, consequently, influence the failure of control.

This information reaffirms the importance of studies involving the use of phytotherapeutics in the control of ticks and highlights the acaricidal potential of new species. In this context, the use of *Tagetes minuta* essential oils is promising for both the control of the species mentioned in the text and its potential action against other tick species from other geographical regions. Thus, further studies are needed to identify which species are sensitive to this physiotherapeutic agent.

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