Industrial Crops and Products 67 (2015) 55–61

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Action of ethanolic extract from aerial parts of *Tagetes patula* L. (Asteraceae) on hatchability and embryogenesis of *Rhipicephalus sanguineus* eggs (Acari: Ixodidae)

Flávio Augusto Sanches Politi[a], Glyn Mara Figueira[b], Maria Izabel Camargo-Mathias[c], Jean-Michel Berenger[d], Philippe Parola[d], Rosemeire Cristina Linhari Rodrigues Pietro[a,*]

[a] *Drugs and Medicines Department, School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara-Jaú Highway, Km 01, CEP 14801-902 Araraquara (SP), Brazil*
[b] *Biological and Agricultural Multidisciplinary Research Center, State University of Campinas (UNICAMP), CP 6171, CEP 13081-970 Campinas (SP), Brazil*
[c] *Institute of Biosciences of Rio Claro, Department of Biology, São Paulo State University (UNESP), Avenue 24-A, 1515, Bela Vista, CEP 13506-900 Rio Claro (SP), Brazil*
[d] *Unité de Recherche Sur Les Maladies Infectieuses et Tropicales Emergentes (URMITE), UMR 6236, Faculté de Médecine, Université de la Mediterranée, Boulevard Jean Moulin 27, 13358, Marseille, Cedex 5, France*

ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form
16 December 2014
Accepted 9 January 2015
Available online 21 January 2015

Keywords:
Rhipicephalus sanguineus
Tagetes patula
Hatchability
Embryogenesis
Ethanolic extract

ABSTRACT

Rhipicephalus sanguineus (Latreille, 1806) presents medical importance, being the principal vector of *Rickettsia conorii*, the causative agent of Botonous fever, occurring in various regions of Europe and Africa. In the Americas, has been reported as a vector of *Rickettsia rickettsii*, the causative agent of Mountain Spotted Fever. It presents veterinary importance because is responsible by the transmission of zoonosis like babesiosis, ehrlichiosis, hepatozoonosis and others. The resistance that these ticks show to some of the active principles used in acaricides has risen wildly. In other hand, the use of herbal medicines has been outstanding recently. Among the advantages of phytotherapics that justify their use are synergistic effects of its components and easy degradation in the environment. In this scenario, *Tagetes patula* L. (Asteraceae) appears as a species with great biocide potential. The phytochemical investigation of *T. patula* has resulted in the isolation of several chemical constituents such as benzofurans, carotenoids, flavonoids and thiophenes. This study aimed to test the effect of the ethanolic extract obtained from aerial parts of *T. patula* against eggs of *R. sanguineus* by Egg hatchability test and to verify, for the first time, the action of this sample on ovary cells of engorged females submitted to the Adult Immersion Test. The extract was very effective, inhibiting egg hatching in 96.98% (±0.025) with LD_{50} = 6.312 mg/mL (4.064–8.497 mg/mL). Microscopic analysis of the structure of the ovaries showed significant morphological changes in the structure of oocytes II–V and pedicel cells, interfering directly in its normal embryogenesis, impairing or impeding the formation of healthy larvae, breaking the life cycle of this ticks in the beginning. Despite the difficulty to control adult populations of ticks, these findings are important and provide an alternative to disrupt development on the environment.

1. Introduction

The intense agricultural activity, misuse of pesticides the interaction of man with domestic animals and the current context of climate change in the world favor the spread of infectious agents transmitted by ticks, leading to the emergence and resurgence of different etiologic agents (Massard and Fonseca, 2004).

The ticks belong to the order Ixodida (class Arachnida, family Ixodidae) and are a very heterogeneous group, with a huge variety of habits and habitats (Guimarães et al., 2001). Currently, the tick *Rhipicephalus sanguineus* (Latreille, 1806) is the species with the largest worldwide spread, favored by the wide distribution of its natural host, the dog, and by the capacity to parasitize other animals, like humans, for example (Dantas-Torres, 2008; Dantas-Torres et al., 2006). These ticks presents medical importance, being the principal vector of *Rickettsia conorii*, the causative agent of Botonous fever, which occurs in various regions of Europe and Africa (Merle et al., 1998). In the Americas,

* Corresponding author. Tel.: +55 16 33016965/60.
E-mail address: pietrorc@fcfar.unesp.br (R.C.L.R. Pietro).

http://dx.doi.org/10.1016/j.indcrop.2015.01.014
0926-6690/

has been reported as a vector of *Rickettsia rickettsii*, the causative agent of Mountain Spotted Fever (Demma et al., 2005). In Brazil it is indicated as a potential vector of Lyme simile, caused by *Borrelia* sp. (Yoshinari et al., 1997). It presents veterinary importance because may cause anemia as well as the transmission of zoonosis like babesiosis, caused by *Babesia canis* (Woldehiwet and Ristic, 1993) or *Babesia gibsoni* (Trapp et al., 2006), tropical canine pancytopenia or ehrlichiosis, caused by *Erlichia canis*, hepatozoonosis, caused by *Hepatozoon canis*, canine cyclic thrombocytopenia, caused by *Anaplasma platys*, haemobartonellosis, caused by *Mycoplasma haemocanis* (Woldehiwet and Ristic, 1993) and it is suspected that is involved in the transmission of *Leishmania chagasi*, responsible for canine visceral leishmaniasis (Coutinho et al., 2005).

The tick *R. sanguineus* has three hosts in its life cycle, with the molt occurring in the environment. The eggs are very small, spherical and dark brown. From the hydrolysis of the proteins of corium, Jaskoski and Butler (1971) determined its biochemical composition, identifying the amino acids lysine, arginine, aspartic acid, serine, glycine, glutamic acid, alanine, threonine, valine, tyrosine, leucine and isoleucine. The engorged females of *R. sanguineus* usually deposit around 4.000 eggs (Koch and Tuck, 1986). The incubation period ranges from 6 to 23 days (Pegram et al., 1987). After this, small larvae hatch and immediately begin to search for a host. Under favorable conditions, the life cycle can be completed between 63–91 days (Bechara et al., 1995).

The tick resistance to some of the active principles used in commercial formulations of acaricides such as fipronil, amitraz, carbaryl and pyrethroids like deltamethrin, permethrin and cypermethrin, has been described (Jernigan et al., 2000; Miller et al., 2001; Otranto et al., 2005; WHO, 2006; Vatsya and Yadav, 2011). The exclusive use of acaricide chemicals is becoming unfeasible in practical and economic terms, requiring the adoption of alternative methodologies that aim to mitigate environmental problems, since the indiscriminate use cause accumulation of toxic waste in nature (Paião et al., 2001).

The use of herbal medicines in animals increased in recent years, directly related to the emerging market of pets. Among the advantages of phytotherapics that currently justify their use are synergistic effects of its components, easy degradation in the environment, the share of compounds acting on different molecular targets, the lowest risk of side effects and the relatively lower costs in research (Yunes et al., 2001). Some plant extracts have been reported in the literature for the control of certain species of ticks as *Hyalomma anatolicum* (Abdel-Shafy and Zayed, 2002; Singh et al., 2014), *Rhipicephalus microplus* (Barbosa et al., 2013; Kongkiatpaiboon et al., 2014; Pazinato et al., 2014), *Rhipicephalus annulatus* (Kaaya et al., 1995; Ravindran et al., 2012) and even *R. sanguineus* (Fernandes, 2007; Arnosti et al., 2011; Denardi et al., 2012; Sampieri et al., 2012; Politi et al., 2012, 2013).

Tagetes patula L. (Asteraceae), popularly known as dwarf marigold or French marigold, is an annual plant, 20–30 cm tall, native to North America and widely disseminated throughout the world. It is very easily for the cultivation and propagation, producing flowers and seeds throughout the year. A large number of studies have reported the biocide properties of extractives obtained from different species of the genus *Tagetes*, some of which are shown in Table 1. The phytochemical investigation of different parts of *T. patula* has resulted in the isolation of several chemical constituents in many different classes of secondary metabolites, such as benzofurans, carotenoids, flavonoids and thiophenes, which have good insecticide potential (Massera et al., 1998). From different parts of *T. patula*, Bano et al. (2002) isolated thiophenes, steroids and terpenoids. Politi et al. (2012) identified twelve *O*-glycosylated flavonoids in the same ethanolic extract of the aerial parts of *T. patula* used in this work: kaempferol, patuletin, quercetin-3-*O*-pentoside, quercetin-3-*O*-glucoside (isoquercitrin) and quercetin-3-*O*-galactoside (hyperoside), patuletin-7-*O*-glucoside (patulitrin) or 6-*O*-methyl-quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnosyl-*O*-xyloside, quercetin-3-*O*-di-rhamnoside, quercetin-3-*O*-glycosyl-7-*O*-rhamnosyl, quercetin-3-*O*-rhamnosyl-7-*O*-glycosyl, kaempferol-3-*O*-di-hexoside and quercetin-3-*O*-galloyl-hexoside.

The objective of this study was to investigate, for the first time, the action of 70% ethanolic extract of aerial parts of *T. patula* on *R. sanguineus* eggs by Egg hatchability test (EHT). Besides, in order to verify the effect of these plant extract on the embryogenesis, histological analyzes were performed in ticks submitted to the Adult immersion test (AIT) for 5 min, looking for changes in ovary cells.

2. Material and methods

2.1. Plant material

Aerial parts of *T. patula* (stems, leaves and flowers) were provided by Collection of Medicinal and Aromatic Plants (CPMA) of the Multidisciplinary Center for Chemical, Biological and Agricultural Research (CPQBA), State University of Campinas (UNICAMP). The planting was done from seeds of Top Seed Garden line (Agristar®). A voucher specimen was deposited in the CPQBA herbarium with number of process 1421.

2.2. Sample preparation

The dried aerial parts of the plant were triturated into cutting mill. The powdered drug was used for preparing the extract by percolation using ethanol 70% (v/v) as the extractor liquid, with average flow rate of 40 drops/min. After complete evaporation of the solvent, this extract was lyophilized and stored in a desiccator to avoid incorporation of humidity and/or contamination.

2.3. Collection of ticks

Engorged adult females of *R. sanguineus* were obtained from the colony maintained at the Unité de Recherche en Maladies Tropicales et Infectieuses Emerging (URMITE), Faculté de Médecine, Marseille (France), under controlled conditions (25 °C, 80% RH, 12/12 h photoperiod) in BOD incubator. These ticks were fed in fabric chambers fixed in the ears of New Zealand rabbits (*Oryctolagus cuniculis*), acquired from Charles River Laboratories. The experimental protocol was approved by the Ethic Committee of Aix-Marseille Université, C2EA-14 (France) and authorized by the "Ministère de l'Enseignement Supérieur et de la Recherche (France)", under the number 1078.01. Rabbits were handled according to the rules of "Décret n° 2013-118, février 7, 2013".

2.4. Egg hatchability test (EHT)

Approximately 200 eggs of *R. sanguineus* were placed in test tubes and immersed for 5 min in 1.0 mL of the following dilutions of 70% ethanolic extract of aerial parts of *T. patula* ($AP_{EtOH70\%}$): 12.5, 25.0, 50.0 and 100.0 mg/mL. Distilled water was used as negative control. Subsequently, the egg masses were dried on filter paper at room temperature for 15 min. The tubes were dried with the aid of strips of filter paper and the eggs were then relocated therein. After, they were transferred to environmental chamber and kept at 28 °C and 80% RH (12/12 h photoperiod) for 3 weeks (Fig. 1). The test was run in triplicate. The percentage of hatchability was calculated according to Eq. (1):

$$H_{eggs}(\%) = \frac{L_h}{N_{eggs}} \times 100 \quad (1)$$