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IN VITRO ACTION OF *Tagetes patula* L. (ASTERACEA) ESSENTIAL OIL ON EGGS OF *Haemonchus contortus*, THE GASTROINTESTINAL NEMATODE OF SMALL RUMINANTS

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Abstract

The sheep breeding has suffered huge economic losses due to parasitism by gastrointestinal nematodes. Among these, *Haemonchus contortus* has shown greater importance due to its wide distribution and high prevalence in herds in tropical countries. These hematophagous parasites cause in the infected animals since acute hemorrhagic anemia, until the submandibular edema formation, damage to the gastric functions and hypoproteinemia. The main way to control this parasitic disease is through the use of synthetic anthelmintics, however, its indiscriminate use has resulted in the selection of highly resistant parasites. This scenario has influenced the course of scientific research in healthcare and the search for alternatives that aim to minimize this problem have been proposed, such as the use of botanical insecticides based on plant extracts. *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 has high economic and commercial importance due to the production of secondary metabolites, mainly terpenes and thiophenes, which have a variety of biocidal effects, such as nematicides, bactericides, fungicides and insecticides. This study aimed to test the efficacy of the essential oil of the aerial parts of *T. patula* in inhibiting hatching of *H. contortus* eggs by Egg Hatch Test (EHT). The eggs were obtained from feces, directly collected from the rectum of host lambs (*Ovis aries*) experimentally infected with the Embrapa 2010 isolate, which has shown anthelmintic resistance to benzimidazoles, macrocyclic lactones and imidazotiazols (Chagas et al. 2013). The eggs were taken from feces according to the methodology described by Bizimenyera et al. (2006), an adaptation of the original method proposed by Coles et al. (1992). In the EHT the efficacy of each treatment was determined based on hatching percentage according to the following equation: (%) Inhibition = 100 x (P*Test* / P*Total*), where, P*Test* refers to the number of eggs and P*Total* corresponds to the number of eggs plus L1 larvae (first instar larvae). The highest and the lowest concentrations evaluated were 12.0 mg/mL and 0.006 mg/mL.The negative controls consisted of distilled water as well Tween 80 solution used to solubilize the essential oil. In all cases (treatments and controls) there were six replicates. From 12.0 mg/mL to 0.75 mg/mL the oil showed 100% inhibition on hatching. At the lowest concentration tested, the oil was responsible for only 10.9% (± 1.514%) of inhibition. The 50%, 90% and 99% lethal concentration (LC50, LC90 and LC99) were determined by Probit analysis (SAS Institute, 2003). The values were, respectively, 0.049 mg/mL (0.045-0.052 mg/mL), 0.261 mg/mL (0.235-0.294 mg/mL) and 1.628 mg/mL (1.327-2.05 mg/mL). Politi et al. (2013) described the composition of the oil, identified as major compounds 4-vinyl guaiacol (8.55%), gamma-terpinene (8.40%), limonene (6.32%), 3,9-epoxy-para-mentha-1,8(10)-diene (6.21%), (E)-tagetone (5.32%), rotundifolone (4.64%), 1,3,8-para-menthatriene (3.93%), alpha-ocimene (3.44%), cariofilene oxide (3.66%), nerolidol (3.23%), cis-epoxy-ocimene (2.71%), dihydrotagetone (2.31%) and trans-beta-ocimene (2.27%). It can be conclude that the essential oil of *T. patula* have effective activity in low concentrations when tested *in vitro*. Thus, the larval development test (LDT) and *in vivo* tests should be performed to prove the potential of this matrix in combating the parasite. **Finantial Suport**: FAPESP (Process Number 2013/03493-8) and

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