

**Potential of Integrating *Calpurnia aurea* with Entomopathogenic  
Fungus *Metarhizium anisopliae* for the Control of *Rhipicephalus  
appendiculatus* and *Rhipicephalus pulchellus***

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## ABSTRACT

Ticks of the genus *Rhipicephalus* are important parasites of livestock in the world. *Rhipicephalus* ticks cause huge economic losses to cattle and have a great capacity to develop resistance to chemical acaricides. There is the need therefore to develop alternative strategies that could complement the existing control methods of this ectoparasite. Entomopathogenic fungi are being considered as a promising option for the control of ticks on-host and off-host. The aim of this study was to assess the potential of using extracts from *Calpurnia aurea* as attractant in association with *Metarhizium anisopliae* for the control of *Rhipicephalus* spp. as an affordable and environmentally friendly technology. Experiments were first carried out to assess the response of two tick species, *Rhipicephalus pulchellus* and *R. appendiculatus* to different concentrations of extracts (acetone, aqueous and oil) of dry leaves of *C. aurea* in both an inverted glass tube and a dual choice T-olfactometer. The oil extract at the concentrations of 50 and 100 mg/ml attracted 46.7 and 65.9% of *R. appendiculatus*, respectively, in the inverted glass tube assay, which was comparable to the attraction-aggregation-attachment pheromone (AAAP) used as a check (47.8%). The attraction of both tick species to plant extract was also tested in semi-field plot experiments using a trap baited with different concentrations of emulsifiable extract of *C. aurea*. A dose of 100 mg/ml attracted 52.2% of *R. pulchellus* and 44.4% of *R. appendiculatus* from a distance of 1 m, respectively, while 14.4% of *R. pulchellus* and 12.2% of *R. appendiculatus* were attracted from 5 m distance. Addition of CO<sub>2</sub> to the plant extract-baited trap at the dose of 100 mg/ml increased the range of attraction of adult *R. pulchellus* to 44.4% and to 33.3% of adult *R. appendiculatus* tick from a distance of 5 m. The compatibility of the fungus *M.*

*anisopliae* with emulsifiable extract of *C. aurea*, and AAAP was evaluated in the laboratory in terms of fungus vegetative growth, conidia production and viability. Compared to AAAP which inhibited fungus vegetative growth and conidial viability, emulsifiable formulation of the plant extract was compatible with the fungus at all the concentrations tested. The prospects of attracting and infecting *R. appendiculatus* ticks were evaluated in semi-field experiments. Ticks were released at various distances and then attracted to the trap baited with a mixture of emulsifiable extract and fungal conidia. Half of these ticks exposed to *M. anisopliae* were brought to the laboratory and incubated at  $26 \pm 2$  °C and  $85 \pm 5\%$  RH. The other half was allowed to feed on rabbit in order to evaluate the reproduction potential. Eighty three (83%) per cent of the ticks brought to the lab died of fungal infection. Male *R. appendiculatus* ticks were attracted and exposed to conidia in a plant extract-baited trap. These males were collected and placed together for five weeks with uncontaminated females in proportions of 1:1. This study showed that autodissemination of fungal inoculum between *R. appendiculatus* ticks did occur under semi-field conditions. The results of the current study revealed that *C. aurea* extracts with *M. anisopliae* in a trap system can potentially be used as an environmentally friendly and low cost strategy to control *Rhipicephalus* ticks in the field.