Compatibility study of *Duddingtonia flagrans* conidia and its crude proteolytic extract on gastrointestinal nematodes

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Chemical control is the conventional approach to mitigate the prevalence of gastrointestinal nematodes (GIN), which represents a serious challenge for agriculture. However, this method often faces challenges, such as its excessive use after the proliferation of more resistant organisms, resulting in secondary environmental contamination, which poses potential risks to human health. In this context, the nematophagous action of the *Duddingtonia flagrans* fungus is considered beneficial for biocontrol, as it is a natural antagonist of these parasites. In addition to fungi, enzymes can be used to control nematodes, as they catalyze the degradation of the cuticle, leading to its death. This study aimed to evaluate the concomitant use of the nematophagous fungus *D. flagrans* (AC001) and its crude fungal extract (rich in proteases) in the in vitro control of *Haemonchus* spp. and *Trichostrongylus* spp. The fungus was isolated using the commercial product Bioverm® in Petri dishes containing Potato Dextrose Agar (BDA) in an oven at 25±1°C for seven days. Next, approximately 500 µL of a solution containing 10^6 conidia/mL in 50.0 mL of liquid culture medium for enzyme production was added to the flasks. After six days, the medium was collected, filtered through Whatman No. 1 filter paper, and centrifuged at 10,000 *g* for 10 min. The obtained supernatants were called cell-free crude extracts. The proteolytic activity was evaluated using the caseinolytic method, obtaining 5.69 U/mg. Five experimental groups were set: (1) one control group (G1) and (4) four treated groups –G2 – active crude extract; G3 – denatured crude extract; G4 – fungus, and G5 – fungus + active extract. Plates were incubated at 28 °C for 24 h, and then the larvae were recovered using the Baermann technique. The treatments carried out in the groups (G2, G4, and G5) showed statistical significance ($p<0.01$) according to the t-test, compared to the control group (G1), with the average percentages of reduction 59%, 86%, and 76%, respectively. The group treated with fungus supplemented with active extract (G5) showed a significant difference ($p<0.01$) from the group with active extract (G2). This fact corroborates the hypothesis that supplementation of the fungus with an active extract did not cause an antagonistic effect but rather a "friendly" action between the fungus and the extract when used concomitantly, increasing the nematicidal potential of each one separately. The present study demonstrated the in vitro compatibility of the crude fungal extract (rich in proteases) and *D. flagrans* for the first time.

Keywords: nematophagous fungi, *Duddingtonia flagrans*, crude proteolytic extract, nematicidal activity

Estudo de compatibilidade de conídios de *Duddingtonia flagrans* e extrato bruto proteolítico sobre nematoides gastrointestinais

Avaliou-se o uso concomitante do fungo nematófago *D. flagrans* (AC001) e seu extrato fúngico bruto (proteases) no controle in vitro de *Haemonchus* spp. e *Trichostrongylus* spp. Foram montados 5 grupos experimentais: (G1) Controle, (G2) extrato ativo; (G3) extrato desnaturado; (G4) fungo e (G5) completo, incubados por 24h a 28°C. Os grupos (G2, G4 e G5) apresentaram diferença estatística ($p<0,01$) conforme teste t para o controle (G1), e percentuais médios de redução 59%, 86% e 76% respectivamente. Demonstrou-se pela primeira vez a compatibilidade do extrato fúngico bruto e *D. flagrans* sobre nematoides parasitas gastrointestinais.

Palavras-chave: fungos nematófagos, *Duddingtonia flagrans*, extrato bruto proteolítico, atividade nematicida

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