Rev Argent Microbiol. 2017;xxx(xx):xxx–xxx

asociación argentina de microbiología

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

ORIGINAL ARTICLE

In vitro effect of *Chrysosporium indicum* and *Chrysosporium keratinophylum* on *Toxocara canis* eggs

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Received 9 March 2017; accepted 22 August 2017

KEYWORDS
Biological control;
Nematophagous fungi;
Ovicidal activity;
Geohelminths

Abstract The degree of antagonism exercised by fungi on geohelminth development varies according to the morphological alterations caused by different fungal species. Saprophytic fungi may exert ovicidal or ovistatic effects. The aim of this study was to apply scanning electron microscopy (SEM) to observe the action of two soil saprophytic species of *Chrysosporium* (*C. indicum* and *C. keratinophylum*) on *Toxocara canis* eggs. The fungal strains to be tested were incubated for 28 days at 28 °C in 2% water agar with a suspension of unembryonated *T. canis* eggs. A suspension of *T. canis* eggs in 2% water agar was used as control group. The assay was done in triplicate for each fungus and the control group. SEM observations were performed on the 4th, 7th, 14th, 21st, and 28th day after inoculation. The effect of the fungi on eggs was evaluated in accordance with the alterations observed on the surface and the changes in the normal characteristics of the eggs. Hyphae around the eggs, *appresoria* penetrating the shell and changes in the typical egg membrane were observed in this assay. Type 3 effect (alterations that occur both in the embryo and the shell, and hyphal penetration of the eggs) was the prevalent effect. SEM allowed us to observe clearly the morphological alterations in *T. canis* eggs due to the effect of *C. indicum* and *C. keratinophylum*. Both saprophytic species of *Chrysosporium* alter the egg structure and alterations increase as exposure increases.

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https://doi.org/10.1016/j.ram.2017.08.001

Please cite this article in press as: Bojanich MV, et al. *In vitro* effect of *Chrysosporium indicum* and *Chrysosporium keratinophylum* on *Toxocara canis* eggs. Rev Argent Microbiol. 2017. https://doi.org/10.1016/j.ram.2017.08.001

PALABRAS CLAVE
Control biológico;
Hongos nematófagos;
Actividad ovicida;
Geohelmintos

Efecto *in vitro* de *Chrysosporium indicum* y *Chrysosporium keratinophylum* sobre huevos de *Toxocara canis*

Resumen El grado de antagonismo ejercido por los hongos sobre el desarrollo de los geohelmintos depende de la especie fúngica y las alteraciones morfológicas que causan. Los hongos saprófitos pueden tener efecto ovicida u ovistático sobre los huevos. El objetivo fue aplicar la microscopía electrónica de barrido (MEB) para observar la acción de 2 especies de *Chrysosporium* (*C. indicum* y *C. keratinophylum*) saprófitas de suelos, sobre huevos de *Toxocara canis*. Las especies a ensayar se sembraron en agar agua al 2% con una suspensión de huevos no embrionados de *T. canis* y se incubaron 28 días a 28 °C. Como grupo control se utilizó una suspensión de huevos de *T. canis* en agar agua al 2%. El ensayo se realizó por triplicado para cada hongo y el grupo control. Las observaciones con MEB se realizaron a los 4, 7, 14, 21 y 28 días de incubación. La acción de los hongos se evaluó según las alteraciones en la superficie y los cambios en las características normales de los huevos. En este ensayo se observaron: hifas rodeando los huevos, *appresorios* penetrando la cubierta y cambios en la membrana característica del huevo, prevaleciendo el efecto tipo 3 (alteraciones que se producen tanto en el embrión como en la cubierta y penetración de hifas al interior de los huevos). La aplicación de la MEB permitió observar claramente que las 2 especies de *Chrysosporium* saprófitas de suelos, afectan el normal desarrollo de los huevos de *T. canis*, alteran su estructura y las alteraciones aumentan con el tiempo de exposición.

Introduction

Toxocariasis is the clinical presentation of human infection by *Toxocara* spp., a roundworm that lives in the small intestines of domestic dogs and cats. The infection occurs by the accidental ingestion of embryonated *Toxocara* eggs present in contaminated soil or on dirty hands[6,14]. Toxocariasis is present worldwide and is a consequence of the human habit of keeping dogs and cats for company, which favors the persistence of the parasite in the environment and its transmission[13,22]. Despite its extensive geographical distribution, infection is more frequent in tropical and subtropical regions, especially in populations with poor sanitary conditions[20]. Human *T. canis* infection is a public health concern in the Americas, Europe and in all developing countries. However, a full appreciation of the global burden of this disease may be greatly underestimated[18].

Toxocara spp. eggs, as the eggs of other geohelminths, have a high degree of resistance to adverse environmental conditions and diverse chemicals, since they are protected by a thick, complex shell. This shell is made up of three membranes or layers – the outer vitelline layer, a middle chitinous layer, and the inner lipidic layer – that makes eggs resistant to chemicals and temperature changes, allowing them to survive outside the host for long periods[22]. Consequently, soil contamination with infective eggs is a worldwide health issue, and that is why the interest in finding biological control agents to reduce this contamination has increased in the past decades[4].

Fungal parasitism on nematode eggs is a natural biological phenomenon that can be used for biological control of geohelminth eggs in the environment, since they are the most resistant stage in the life cycle of nematodes[4,5]. The penetration process of the hypha through the egg shell has not been completely elucidated yet. In 1995, Bonants et al.[8] were the first to mention that the fungi colonization mechanism may be mechanical and/or enzymatic. Special penetration organs (''*appresoria*'') formed from the hypha help the fungus apply pressure on the egg shell (mechanical effect). Other investigations suggest the involvement of exoenzymes such as proteases and chitinases breaking up egg shells (enzymatic mechanism)[4].

The degree of antagonism exercised by fungi on geohelminth development varies according to the morphological alterations caused by the different fungal species. Thus, a saprophytic fungus may exert ovicidal or ovistatic effects, where the ovistatic ability is shown by the delay in embryo development or inhibition with no morphological damage to the egg shell[12]. Therefore, several researchers have assayed the *in vitro* effect of different fungi on geohelminth eggs. Knowledge about the effect of the genus *Chrysosporium* is scarce, since there is no register of studies on the subject except for the study carried out by Ciarmela et al.[10], who characterized *Chrysosporium merdarium* species as having very high ovicidal activity on *Toxocara canis* eggs, along with other soil saprophytic fungi.

The purpose of this study was to apply scanning electron microscopy (SEM) to observe the action of two soil saprophytic species of *Chrysosporium*, *Chrysosporium indicum* and *Chrysosporium keratinophylum*, on *T. canis* eggs.

Materials and methods

Fungal strains

Strains IMR-MF-816 *C. indicum* and IMR-MF-40 *C. keratinophylum* deposited in the culture collection at the Mycology Department, Instituto de Medicina Regional, Universidad Nacional del Nordeste, Argentina, were assayed. Both

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