Colonization and destruction of ants of the genus *Camponotus* sp. (Hymenoptera: Formicidae) in vitro by the fungus *Pochonia chlamydosporia* in the southeast region of Brazil

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Abstract

The objective of this study was to evaluate, in vitro, the colonization and destruction of ants of the genus *Camponotus* sp. by the ovicidal fungus *Pochonia chlamydosporia* (VC4 isolate), in the southeast region of Brazil. The insects used in the experiment were worker ants of the genus *Camponotus* sp., collected periodically in the environment and immediately transported to the laboratory in test tubes. Then, VC4 growth was promoted in 2% chitin agar medium (2% WQ) to obtain a fungal solution containing conidia and/or chlamydospores. Two experimental groups were formed. Treated group consisted of Petri dishes containing 2% agar–water culture medium (2% WA) with nine live insects and 20 µL of fungal solution at the concentration of 15,000 conidia/chlamydospores. Control group consisted of Petri dishes containing 2% WA culture medium and nine live insects. The dishes in the treated and control groups were incubated in BOD at 25 ± 1 °C and $80 \pm 10\%$ relative humidity for 4 days. After 4 days, it was observed that the VC4 had grown, colonized, and caused the destruction of the ants. The fungus *P. chlamydosporia* was efficient at colonizing and destroying the urban ants collected on an experimental basis. Thus, it could open up new ways to reduce the use of chemical compounds in the future, decreasing health and environmental problems.

Keywords Urban pests · Public health · Biological control · Nematophagous fungus

Arthropods are considered mechanical vectors once which can become contaminated when they come in contact with infectious agents, transporting them around the environment. Ants are highly diversified social animals and there

are more than 2000 known species. They perform important ecological functions, such as promoting the decomposition of organic matter (Beadson 1972; Baccaro et al. 2015).

The ant genus *Camponotus* is a vector of pathogens that are extremely harmful to human and animal health in hospital environments, because their populations have high rate of reproduction and dispersion. Controlling these insects is difficult and may compromise hospital dynamics and put immunocompromised patients at risk (Fowler et al. 1993; Rodovalho et al. 2007; Pesquero et al. 2008; Santos et al. 2009; Castro et al. 2015). In addition, other problems caused by ants in hospitals include various types of skin irritations and lesions (Eichler 1990).

Among the microorganisms transported by ants are bacteria, which have an affinity for these insects and may be resistant to antimicrobials. Some are considered multiresistant, such as the genera *Acinetobacter* spp., *Gemella* spp., *Klebsiella* spp., and *Streptococcus* spp. (Moreira et al. 2005; Costa et al. 2006). Some genera of fungi of medical importance, such as *Aspergillus* spp., *Fusarium*

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spp., *Purpureocillium* spp., and *Staphylococcus aureus* also bind to the exoskeleton of the ants, and are thus transported (Pantoja et al. 2009; Gopal et al. 2012). These authors further mention that, because of their dominant behavior, ants can cause serious problems for humans. Beadson (1972) reported, for the first time, the presence of ant infestation in nine UK hospitals which were strains of *Salmonella* spp., *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Streptococcus* spp., and *Clostridium* spp.

Therefore, based on the literature, it can be affirmed that these arthropods present a high level of contamination, and may be vectors of agents that are harmful to human and animal health (Fowler et al. 1993; Rodovalho et al. 2007; Lima et al. 2013).

The means used to control these insects may be mechanical, biological, or chemical, the latter being the most used by the human population. Chemical products can be applied directly to the nests, in liquid, powder, or granulated bait forms that are generally applied near the colonies (Boaretto and Forti 1997). On the other hand, chemical methods are becoming increasingly unviable, because they cause serious damage to the environment, increase the risk of human contamination, and generate high economic costs for the population. As a result, in a short period of time, the propagation of these insects in urban areas tends to increase (Oliveira and Campos-Farinha 2005).

Research has shown that combatting insect pests using biological control with entomopathogens may be a very efficient alternative. These include the use of fungi, viruses, bacteria, nematodes, and protozoans (Valicente 2009). However, the use of so-called “nematophagous” fungi has attracted the interest of researchers due to their high potential for biocontrol of plant and animal parasites, being natural enemies of nematodes, as well as being easily cultivated in the laboratory and providing an excellent model for interaction studies (Braga and Araújo 2014; Sufiate et al. 2018).

Among the various species of nematophagous fungi, *Pochonia chlamydosporia*, an ovicidal fungus, has the potential for use as a biological control agent, and has been widely tested in the control of helminth eggs due to its efficiency in preying on eggs (Braga and Araújo 2014; Larriba et al. 2015). It produces proteases responsible for the destruction of phytonematode eggs, producing enzymatic actions on the eggs of geo-helminths (Carvalho et al. 2010). It is worth noting that this fungus is natural, non-toxic when handling, and does not cause any type of environmental pollution, as it is a spontaneous process that occurs in nature (Braga et al. 2011).

However, recently, Braga et al. (2013) reported a new use for this fungus. These authors showed that nematophagous fungi can be used in the control of arthropods. Based on this premise, the objective of this study was to evaluate the

colonization and destruction of ants of the genus *Camponotus* sp. in vitro by the fungus *P. chlamydosporia*.

The experimental trial was conducted in the Experimental Parasitology and Biological Control Laboratory and the Clinical Veterinary Parasitology Laboratory of the Universidade Vila Velha (UVV). The insects used in the experiment, namely, worker ants of the genus *Camponotus* sp. (Baccaro et al. 2015), were collected periodically in the environment and immediately transported to the laboratory in test tubes.

The fungus *P. chlamydosporia* (VC4) was used. This fungus came from the collection of the Parasitology Laboratory of the Department of Veterinary Medicine of the Federal University of Viçosa (UFV), in Viçosa, Minas Gerais. It was isolated and maintained in a BOD incubator at 25 ± 1 °C and $80 \pm 10\%$ relative humidity. Next, in the Experimental Parasitology and Biological Control Laboratory, the VC4 isolate was transferred by means of inoculation to the 2% chitin agar culture medium, according to the method published by Braga et al. (2013). After being left to grow for approximately 10 days in a BOD-type chamber at 26 °C, the fungal solutions were prepared.

Ten milliliters of sterile distilled water plus Tween 90 (0.1%) adhesive spreading agent were added to the dishes in which the VC4 isolate was grown. The resulting suspensions were filtered through sterile gauze and a concentration of 20 µL was measured by quantification in a Neubauer chamber, using an optical microscope according to the methodology of Azevedo et al. (2015).

The treated group consisted of two Petri dishes containing the 2% agar–water culture medium with nine live insects. A fungal solution of 20 µL of the fungus *P. chlamydosporia* containing a concentration of 15,000 chlamydospores was then applied to the insects in the dishes. The control group was comprised of two Petri dishes containing the 2% WA culture medium and nine live insects placed on the surface of each. The dishes in the treated group were incubated in a BOD at 25 ± 1 °C and $80 \pm 10\%$ relative humidity for 4 days.

At the end of 4 days, the insects of both groups were analyzed under a stereo light microscope. In addition, to verify possible fungal growth, isolation in a Petri dish with agar–water (2%) WA culture medium was performed for 7 days to confirm the presence or absence of the VC4 isolate by means of its characteristics (Braga et al. 2013), Figs. 1a–d and 2b–e; Fig. 2a (control group, without fungus).

This was an experimental study conducted under laboratory conditions. At the end of the assay, it was proven that there was interaction, growth, colonization, and destruction of the specimens of *Camponotus* sp. ants by the VC4.

The literature mentions that some fungi of the entomopathogenic group have been used in laboratory and field experiments for the control of arthropods (Machado et al. 2009). Because ants are social insects and live in colonies, these environments are favorable for the development

Fig. 1 Interaction, growth, colonization, and destruction of specimens of ants of the genus *Camponotus* sp. by VC4. **a** Start of interaction of the fungus *Pochonia chlamydosporia* on *Camponotus* sp.; **b**, **c** evolu-

tion and fungal interaction of *P. chlamydosporia* on *Camponotus* sp.; **d** fungal structure of the chlamydospores type of fungus *P. chlamydosporia*

of fungi because of their low light intensity and humidity. However, this is the first report of interaction of a nematophagous fungus in the possible control of the genus *Camponotus*.

To understand the different activities of entomopathogenic and nematophagous fungi, it should be made clear that the predatory action of nematophagous fungi was limited to helminth eggs and larvae (Araújo et al. 2009), but new premises of predatory activity have been discussed. Braga et al. (2013) reported the interaction of the predatory nematophagous fungus *Duddingtonia flagrans* on ticks of the species *Amblyomma cajenense* and achieved isolation of a chitinase enzyme during the interaction, and thus, the authors recorded the production and characterization of this enzyme under laboratory conditions and also suggested that this production was related to the culture medium of the fungal growth.

In this study, we used 2% chitin agar culture medium as a source of chitin for the growth of the VC4 isolate and, subsequently, its possible enzymatic action. Our results agree with other studies that suggest the production of certain enzymes by nematophagous fungi, depending on the type of substrate in which they are cultivated (Soares et al. 2013).

According to Braga and Araújo (2014), because nematode eggs and arthropod exoskeletons are rich in chitin in general, nematophagous fungi are able to infect them due to their production of extracellular enzymes, e.g., chitinases. Thus, since arthropods have an exoskeleton composed of this carbohydrate, there was fungal action on the specimens

of *Camponotus* spp. In our study, the possible chitinase produced by the nematophagous fungus *P. chlamydosporia* was essential for fungal colonization in Formicidae, emphasizing a rapid and possible entomopathogenic action (Fig. 2b–e).

Although chemical methods are the most frequently used in the control of Formicidae in urban environments (Boaretto and Forti 1997), they are increasingly less feasible, because they cause serious damage to the environment, increasing the risk of animal and human intoxication and generating high economic cost for the population (Diel et al. 2003). Therefore, the interaction of the nematophagous fungus *P. chlamydosporia* (VC4) with household ants may become a new control alternative in the future, due to the absence of toxicity to the environment, animals, and human beings.

Carvalho et al. (2010) and Braga and Araújo (2014) in important communications mentioned that the fungus *P. chlamydosporia* is one of the most studied in the control of helminths harmful to human, animal health, and agriculture. This fungus is harmless to human and animal health, with its action directed to the target organism.

In this study, the fungus *P. chlamydosporia* was efficient at “colonizing” and “destroying” the urban ants collected on an experimental basis, but could open up new ways to reduce the use of chemical compounds in the future, decreasing health and environmental problems. However, it is important to stress the need for new research in the Southeast region of Brazil, since this area is still little explored, and the biological control of household ants of the genus *Camponotus* is a pioneering study.

Fig. 2 a Control group and b–e colonization of *Pochonia chlamydosporia* (VC4) on ants during 4 days of the experiment

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Compliance with ethical standards

Conflict of interest There were no conflicting interests that could have influenced the conduct and reporting of this study.

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