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Effects of different temperatures and mineral salt on pellets of *Monacrosporium thaumasium* - - a nematode-trapping fungus

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ARAÚJO, J. V., W. M. SAMPAIO, R. S. VASCONCELLOS, A. K. CAMPOS: Effects of different temperatures and mineral salt on pellets of *Monacrosporium thaumasium* - a nematode-trapping fungus. Vet. arhiv 70, 181-190, 2000.

ABSTRACT

Experiments were performed to determine whether the nematode-trapping fungus *Monacrosporium thaumasium* is able to survive encapsulation in sodium alginate and the effects of different temperatures and mineral salt. Pellets of sodium alginate were treated with paraffin, mineral salt or without these elements. They were placed in 250 ml Erlenmeyer flasks at 4 °C, room temperature, 25 °C, 30 °C and 35 °C. Over a 16-week period, once a week one pellet was placed in the center of an 8.5 cm Petri dish containing 20 ml of 2% Potato Dextrose agar, and radial growth was monitored for nine days. The best treatment ($P<0.01$) involved fungal pellets without paraffin, at 4 °C, and which remained viable for up to 16 weeks of storage. Pellets without paraffin induced higher growth than pellets with paraffin at room temperature, as well as pellets without mineral salt in all temperatures ($P<0.01$). This formulation of the fungus proved to be a powerful tool for use as biological control of nematodes and will be limited by storage conditions.

Key words: biological control, nematophagous fungi, nematode-trapping fungi, *Monacrosporium thaumasium*, nematode, sodium alginate

Introduction

Non-chemotherapeutic approaches to nematode parasite control of livestock have passed from being largely of academic interest. Alternatives, or at least adjuncts, to anthelmintic control of nematode parasites are absolutely necessary. Towards this objective, significant advances have recently been made in the development of ruminant

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vaccines against parasites (MEEUSEN, 1996), in the breeding of animals for parasite resistance (WOOLASTON and BAKER, 1996) and in the biological control of parasites, particularly by exploiting nematophagous fungi (ARAÚJO et al., 1998).

The fungi antagonistic to nematodes consist of a wide variety of organisms including nematode-trapping or predacious fungi, endoparasitic fungi, parasites of nematode eggs and cysts, and those producing nematotoxic metabolites. It is remarkable that fungi belonging to highly divergent orders and families occur in each of the above groups (MANKAU, 1980).

The predacious group, which includes the genus Monacrosporium, produce an extensive system of hyphae in the environment. Along the hyphae there are organs that are able to capture living nematodes (BARRON, 1977). The Monacrosporium genus was classified by COOKE and DICKINSON (1965) and LIU and ZHANG (1994) as belonging to the subdivision Deuteromycotina. Species of Monacrosporium can control phytonematodes, free-living nematodes and parasitic nematodes of cattle (ARAÚJO et al., 1992; GOMES et al., 1999).

The final challenge is to develop an efficient, low cost means of scaling up production of fungal material and to develop a formulation to satisfy industrial needs for commercial exploitation of this technology. In addition, formulation may facilitate shipping and storage of the bio-control agent.

The present study was designed to determine whether the nematode-trapping fungus *Monacrosporium thaumasium* is able to survive encapsulation in alginate, the effects of temperature and mineral salt.

Materials and methods

One isolate of nematode-trapping fungus *Monacrosporium thaumasium* was obtained from Brazilian soil and kept in small flasks containing 2% Potato-Dextrose-Agar (2% PDA) at 4 °C. Mycelium grown of the fungi was performed in liquid medium of KADO and HESKET (1970) after nine days of incubation at 25 °C in the dark. Sodium Alginate pellets were made as described by WALKER and CONNICK (1983) and modified by LACKEY et al. (1993). A part of pellets was treated with paraffin in their surface and 100g of mineral salt (50.0% bone meal, 49.64% sodium chloride, 0.18% zinc sulfate, 0.15% copper sulfate, 0.01% cobalt sulfate, 0.01% potassium iodate and 0.01% sodium selenite) per 7.5g of pellets was added. The pellets were weighed, divided into 7.5 g portions and placed in 250 ml Erlenmeyer flasks at 4 °C, room

temperature, 25 °C, 30 °C and 35 °C. Over a 16-week period, once a week one pellet was placed in the centre of an 8.5 cm Petri dish containing 20 ml of 2% PDA and radial growth was monitored for 9 days.

These assays were repeated three times and analysed by the minimal significant difference test at a level of ($P<0.01$).

Results and discussion

Figures 1, 2, 3, 4 and 5 show the radial growth of the mycelium from the pellets with and without paraffin at 4 °C, room temperature, 25 °C, 30 °C and 35 °C, respectively. Treatment of pellets without paraffin at 4 °C produced the best results ($P<0.01$) and remained viable for up to 16 weeks storage at this temperature. Pellets without paraffin induced higher growth than those with paraffin at room temperature, as well as pellets without mineral salt in all temperatures ($P<0.01$).

It is very important to screen nematophagous fungi in each location as they can be effective, or even be found, in certain ecological niches. Future experiments will demonstrate whether this fungus isolate can be used as a biological agent in the control of gastrointestinal nematodes of grazing calves under natural conditions. According to LARSEN et al. (1991), the major problem in the use of nematophagous fungi as biocontrol agents is the deposition of the fungal material in dung pats where the entrapment of the parasite larvae should take place. The most obvious possible course would be to add nematophagous fungi to the alimentary tract without loss of viability. After this material passes through the gastrointestinal tract of animals and is eliminated together with the faeces to the environment, faecal material is colonized by these fungi and a close contact between the recently hatched larvae and the fungi takes place, promoting the production of traps and further capture and death of nematodes. However, most nematode-trapping fungi are very susceptible to destruction by the severe conditions in the gastrointestinal tract. In previous experiment, ARAÚJO et al. (1999) achieved the passage of this isolate through the gastrointestinal tract of calves without loss of viability to prey-infective *Haemonchus placei* larvae.

Several techniques have been employed for the delivery of bio-control agents. For example, bio-control organisms have been applied in liquids (KERR, 1980; MAGALHÃES et al., 1997, 1997a), in organic matter (WELLS et al., 1972), as seed or seed-piece treatments (FRAVEL et al.., 1985; HARMAN et al., 1980; MAGALHÃES and FRAZÃO, 1996; WINDELS, 1981) and in vermiculite or in clays such as Pyrax® (FRAVEL et al., 1983). The final challenge is to develop an efficient, low cost means of scaling up

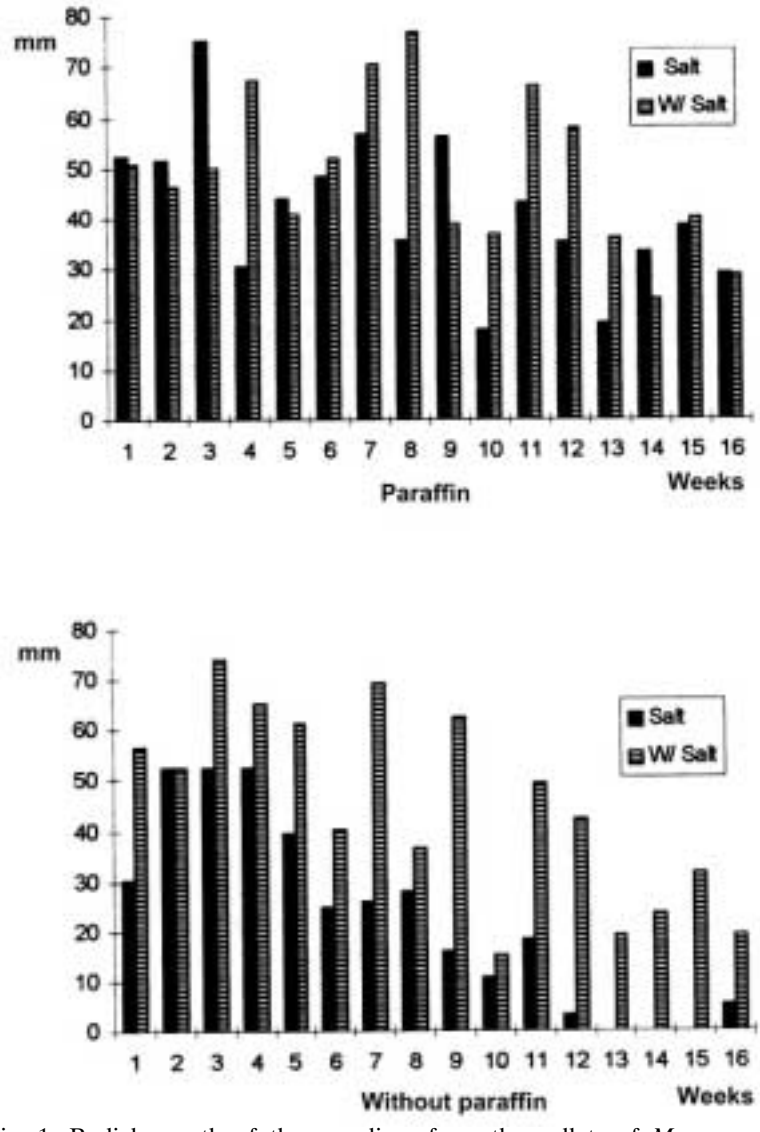

Fig. 1. Radial growth of the mycelium from the pellets of *Monacrosporium thaumasium* with paraffin and without paraffin at 4 °C, with mineral salt (salt) and without mineral salt (w/salt) during 16 weeks

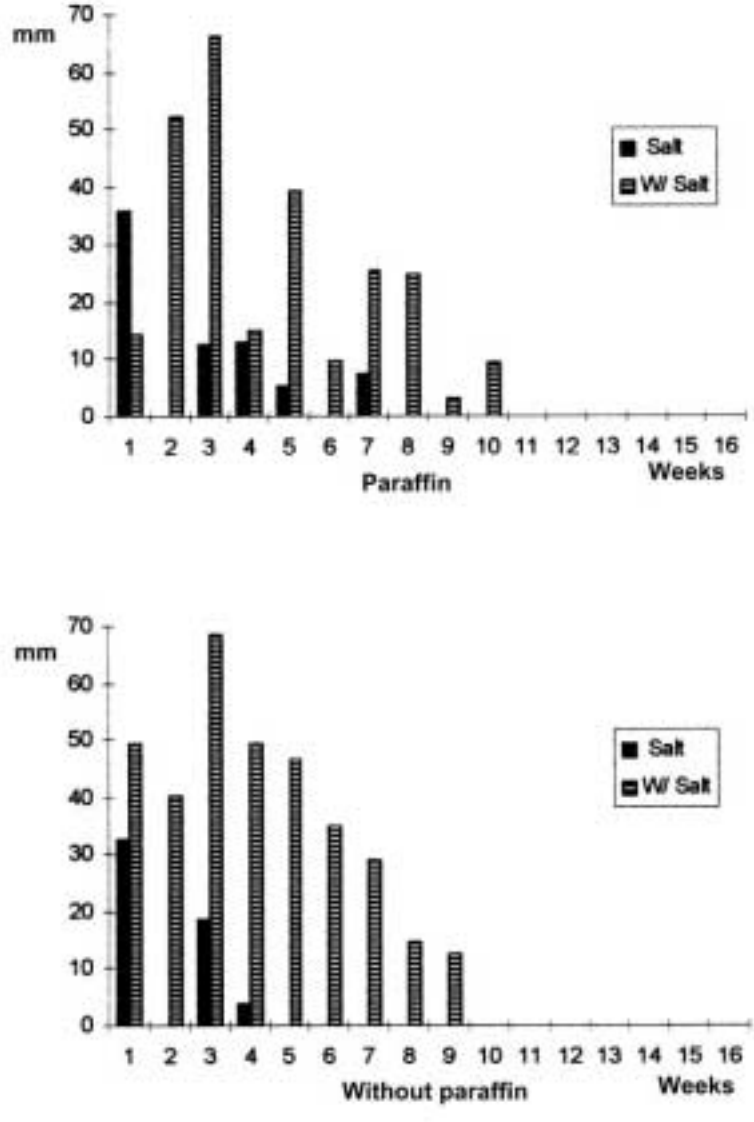

Fig. 2. Radial growth of the mycelium from the pellets of *Monacrosporium thaumasium* with paraffin and without paraffin at room temperature, with mineral salt (salt) and without mineral salt (w/salt) during 16 weeks

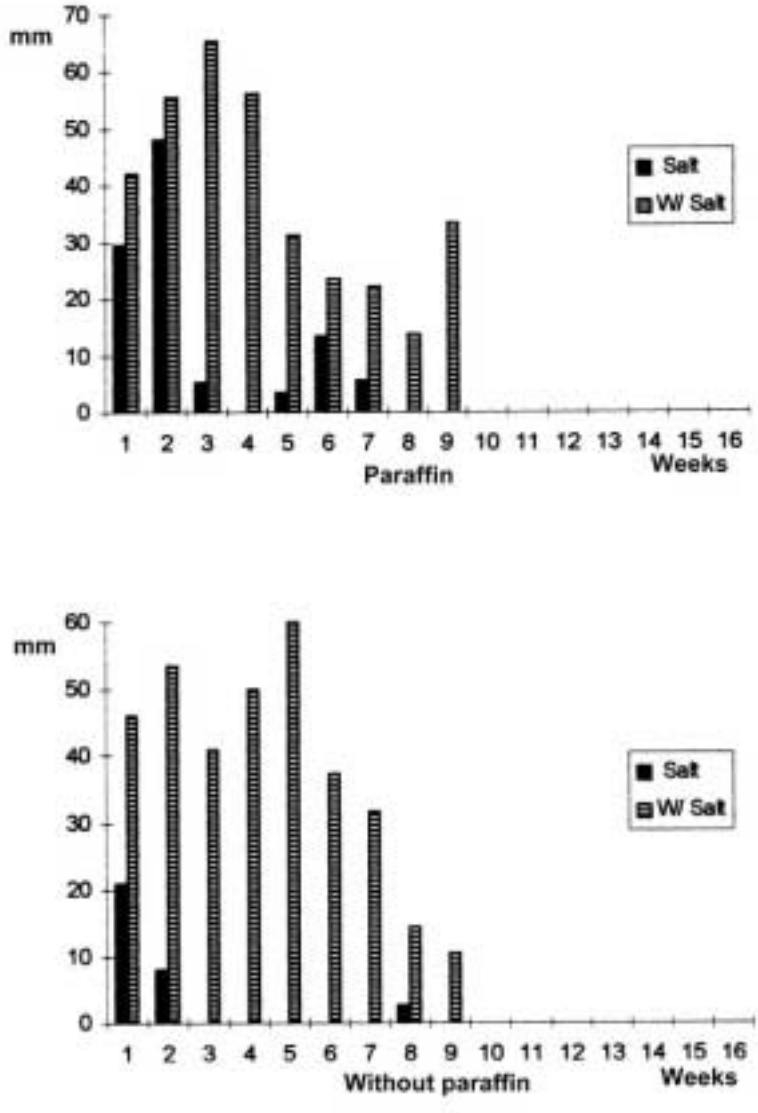

Fig. 3. Radial growth of the mycelium from the pellets of *Monacrosporium thaumasium* with paraffin and without paraffin at 25°C, with mineral salt (salt) and without mineral salt (w/salt) during 16 weeks

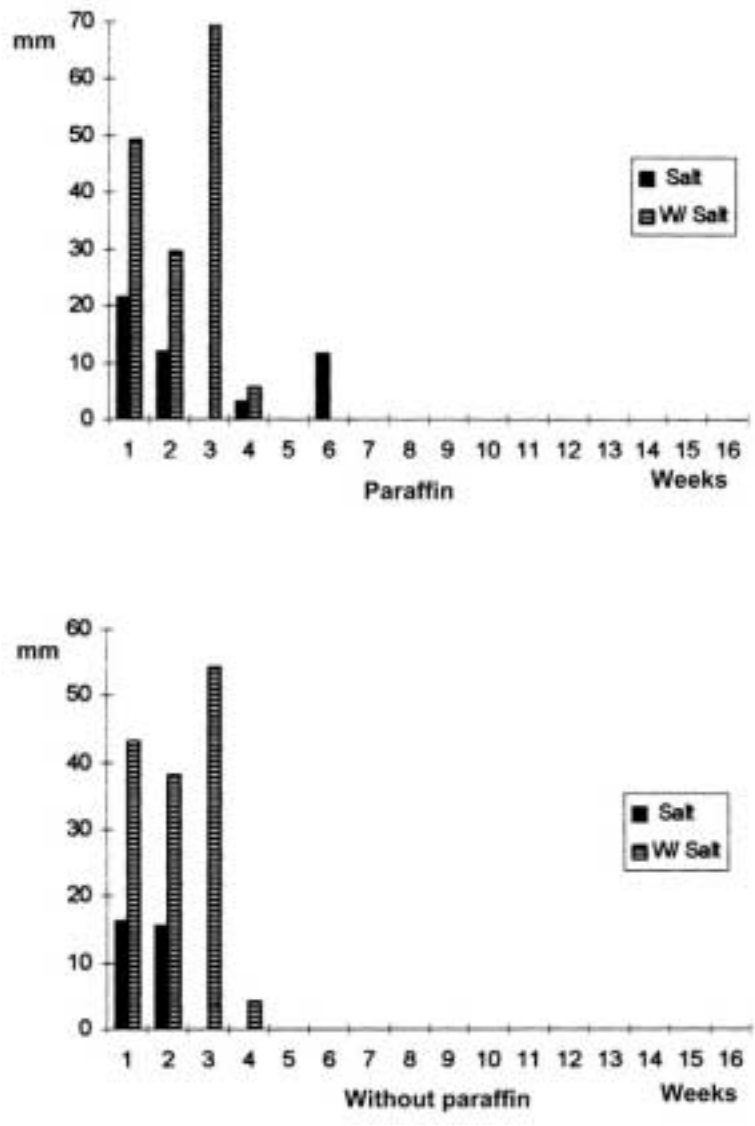

Fig. 4. Radial growth of the mycelium from the pellets of *Monacrosporium thaumasium* with paraffin and without paraffin at 30 °C, with mineral salt (salt) and without mineral salt (w/salt) during 16 weeks

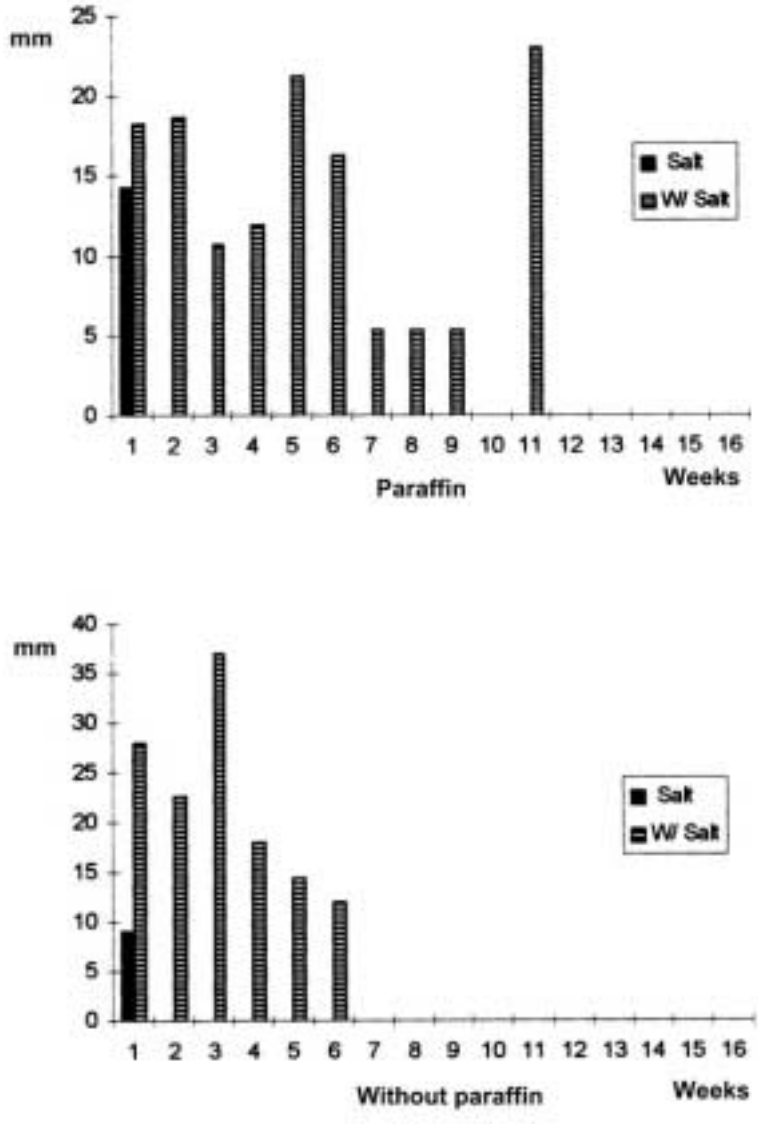

Fig. 5. Radial growth of the mycelium from the pellets of *Monacrosporium thaumasium* with paraffin and without paraffin at 35 °C, with mineral salt (salt) and without mineral salt (w/salt) during 16 weeks

production of fungal material to satisfy industrial needs for commercial exploitation of this technology. Sodium alginate and $CaCl_2$ are commonly used as food additives and are considered to be non-toxic to non-target organisms (FRAVEL et al., 1985).

This formulation of the fungus proved to be a powerful tool to be used for biological control of nematodes and will be limited by storage conditions.

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ARAÚJO, J. V., W. M. SAMPAIO, R. S. VASCONCELLOS, A. K. CAMPOS: Učinci različitih temperatura i mineralnih soli na pelete *Monacrosporium thaumasium* - gljivice koja napada obliće. Vet. arhiv 70, 181-190, 2000.

SAŽETAK

Pokusi su provedeni da se utvrdi da li *Monacrosporium thaumasium*, gljivica koja napada obliće može preživjeti svoju inkapsulaciju u natrijevom alginatu pri različitim temperaturama i uz mineralne soli. Pelete natrijevog alginata bile su podvrgnute različitim temperaturama, i to bez prethodne obrade, ili nakon obrade parafinom ili mineralnom soli. U Erlenmajerovim posudicama od 250 ml držane su na 4 °C, na sobnoj tempereaturi, na 25 °C, 30 °C i 35 °C. Tijekom 16 tjedana, jednom tjedno je po jedna peleta stavljana u sredinu Petrijeve posudice s 20 ml 2% agara od krumpirove dekstroze, te je promatran njen radijalni rast tijekom 9 dana. Najbolji rast ($P<0.01$) pokazale su gljivične pelete bez obrade parafinom i držane na 4 °C, koje su sačuvale sposobnost rasta do 16 tjedana pohranjivanja. Pelete bez parafina su i na sobnoj temperaturi pokazivale bolji rast od onih obrađenih parafinom, kao što su bolje rasle i pelete bez mineralne soli na svim temperaturama ($P<0.01$). Ovakva primjena gljivica se pokazala kao moćno sredstvo biološke kontrole nametničkih oblića, ali je njegova uporaba ograničena uvjetima pohranjivanja.

Ključne riječi: biološka kontrola, gljivica koja napada nametničke oblića, *Monacrosporium thaumasium*, nematodi, oblići, natrijev alginat

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