Encapsulation of fungi in novel formulations for soil pest control

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INTRODUCTION AND OBJECTIVE

Today in formulation science, there are only few reports on systematic investigations on encapsulation of agrobiologicals with regard to materials, methods and technology for mass production. Only a few systematic investigations on beads based on other biopolymers besides alginate exist for encapsulation of BCAs. Also hollow beads and coated beads (Vemmer & Patel 2013) have not been tested successfully so far. Furthermore, the formulation trend of mixing polymers with different physicochemical and biochemical properties into one formulation with improved characteristics such as improved persistence at high mechanical strength was not investigated. Additionally, the persistence of those formulations in different soil types was rarely investigated.

EPF such as *Metarhizium anisopliae* or *Beauveria bassiana* have been formulated by encapsulation in conventional alginate beads supplemented with nutrients (e.g. Burges, 1998; Gerding-Gonzalez *et al.*, 2007) but establishment in soil is still slow and biomass content too high, making the formulations uneconomic.

That is why in the scope of the EU funded project INBIOSOIL we will aim at developing novel mechanically stable beads containing EPFs as well as novel synergistic co-formulations of EPF with entomopathogenic nematodes or semiochemicals.
Here, we report investigations on influence of bead material and soil type on persistence in soil as well as influence of bead materials on growth of *M. anisopliae* out of beads.

MATERIALS AND METHODS

Bead formation for persistence investigations

Beads were formed by dripping polymer solutions with a concentration of 2 % into a crosslinking solution containing 2 % Ca^{2+} ions using ionic gelation. Different polymers and polymer combinations were used: alginate (Protanal LF20/40, FMC Biopolymer, USA) as well in combination with lignin (Lignex, Chemische Werke Zell-Wildhausen, Germany), pectinate (Amid CU-L 66/10, Herbstreith & Fox KG, Germany). Chitosan (Low molecular weight 448869, Sigma-Aldrich, Germany) beads were formed by dripping chitosan solutions (dissolved in 1 % acetic acid) with a concentration of 2 % into a crosslinking solution of 6 % sodium-tripolyphosphate (Sigma-Aldrich, Germany). Besides alginate-gelatin beads were formed by dripping a warm biopolymer solution consisting of 5 % gelatin (Gelatin Bloom 280 Gelita GmbH, Germany) and 2 % alginate into a cold $CaCl_2$ solution (AppliChem GmbH, Germany) using thermal gelation. A bead combination with alginate, gelatin, lignin and pectinate was formed. Biopolymer concentrations were the same as used above, respectively. These bead systems were used for investigations in three different soils: Peat soil and two different field soils which were provided by Mario Schumann, University of Göttingen. 2.5 g beads were buried 5 cm below the soil surface and the bead diameter was measured regularly with a digital measuring slide. To ensure that the bead diameter is not only decreasing by loss of water the moisture in the soil was adjusted to 30 % (w/w) constantly.

Encapsulation of fungus

M. anisopliae (Bipesco 5) was encapsulated in different polymers as well as in combinations of polymers. Beads were produced via ionic or thermal gelation as described above. Therefore 10 mL of polymer mixture was dripped into 100 mL crosslinking solution under gentle stirring for 20 min to form beads. The solidified beads were removed from the crosslinking solution and washed three times with distilled water. Mycelium growth was investigated by placing a single bead on a water agar plate (20 g agar with 1 L H_2O) and measuring the mycelium growth out of the beads.

RESULTS AND DISCUSSIONS

Persistence investigations

Beads for persistence investigations were formed by ionic and thermal gelation. Three polymer combinations and three polymers showed stable bead formation (data not shown). Initially the influence of polymer on persistence in peat soil at room temperature was investigated (figure 1). Alginate and alginate combinations e.g. alginate-gelatin still showed a bead diameter of 50 % and chitosan of 30% after 18 days. Whereas in the case of pectinate it was observed that the beads were completely degraded by soil microorganisms after 15 days.

In an ongoing experiment the influence of three different soil types on persistence of 6 bead types at room temperature is investigated. Figure 2 exemplarily depicts two bead types in three soil types, respectively. The figure shows that the soil type has no influence on bead persistence.

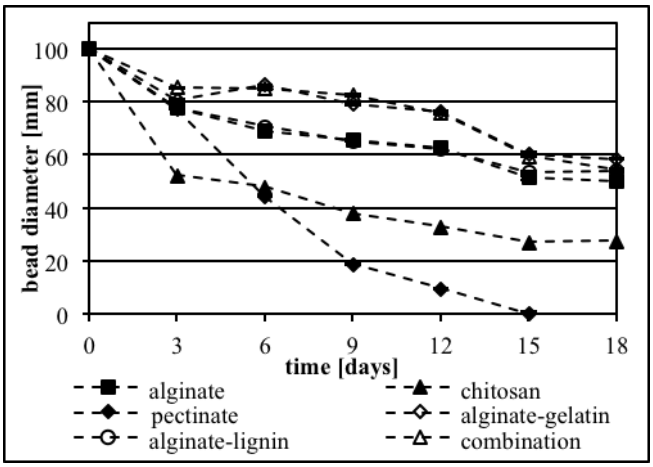

Figure 1 : Influence of polymer on persistence in peat soil

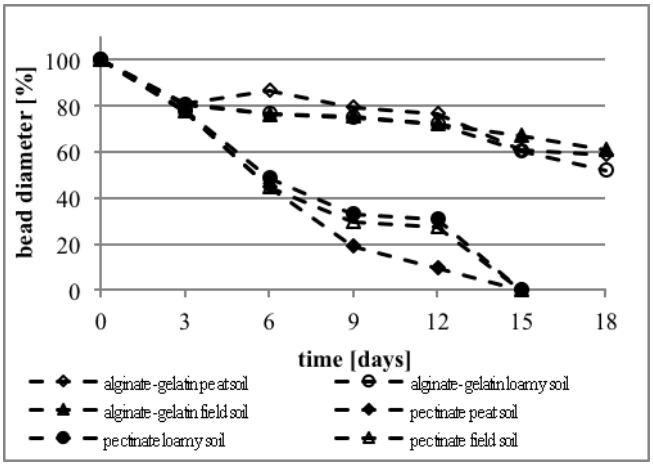

Figure 2 : Influence of soil type on alginate-gelatin and pectinate beads shown exemplary for several bead types

Mycelium growth

Encapsulated fungi investigations were aiming on the influence of polymers on the mycelium growth of *M. anisopliae* out of beads.

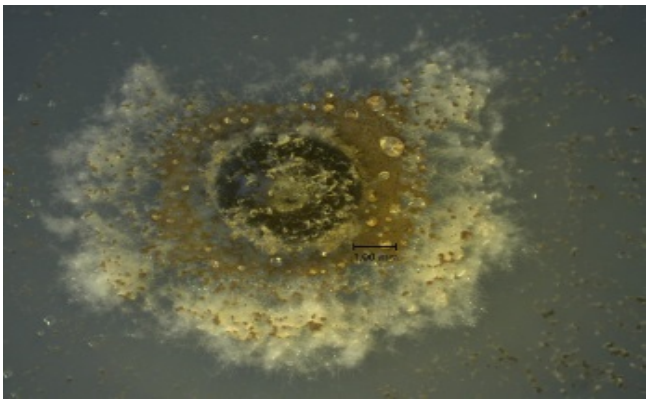

Figure 3 : Radial growth of *M. anisopliae* out of an alginate-gelatin bead on water agar (20 x maginifation)

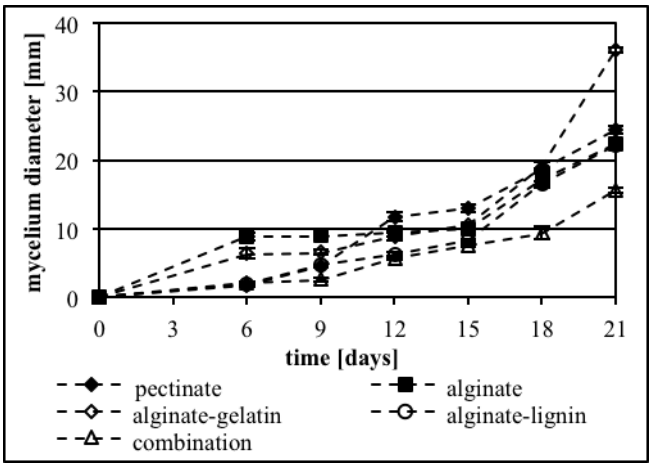

Figure 4 : Influence of the bead material on mycelium growth of *M. anisopliae* on water agar

It was observed that at day 18 the mycelium growth increased in the case of the gelatin amended alginate beads indicating that the fungus can utilize the bead structure as nutrient. However, by the combination of those four polymers the increase of growth was not as high indicating an inhibiting effect which is unknown yet (figure 4).

CONCLUSIONS

It may be concluded that it is worthwhile investigating different bead materials for the encapsulation of fungi, because they apparently have an effect on the mycelium growth of the fungus. In further experiments, persistence and growth in different soils with more polymers, crosslinkers and polymer combinations resulting in beads, hollow beads and coated beads as well as nutrients and also two encapsulated *M. anisopliae* strains will be investigated. Besides, synergistic co-formulations with entomopathogenic nematodes will be developed.

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