

Short Communication

Effect of formulation on the viability of biocontrol agent, *Trichoderma harzianum* conidia

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A zeolite-water heterogeneous mixture (1:2 w/v) was found suitable medium for growing Turkish isolate of *Trichoderma harzianum* T1. Pelletized formulations of zeolite containing conidia of Turkish isolate of *Trichoderma harzianum* T1 in alginate were prepared. Formulations were more viable at 4°C than at 30°C.

Key words: *Trichoderma harzianum* T1, formulation, sodium alginate, zeolite, survival.

INTRODUCTION

Isolates of *Trichoderma harzianum* have received considerable attention in recent years because of their potential use as a biological control agent (Biçici et al., 1994; Okigbo and Ikedugwu, 2000; Küçük and Kivanç, 2001). *Trichoderma* spp., for example, is able to produce biocontrol agents against soilborne plant pathogens. This fungus can be used for the biocontrol of *Fusarium culmorum*, *F. moniliforme*, *Sclerotium rolfsii* and *Gaeumannomyces graminis* var. *tritici* (Sivan et al., 1984; Okigbo and Ikedugwu, 2000; Küçük and Kivanç, 2001).

Despite the experimental application of *T. harzianum* for disease control (Küçük and Kivanç, 2001; Biçici et al., 1994; Okigbo and Ikedugwu, 2000; Sivan et al., 1984), very little information is known about the establishment, proliferation and survival of indigenous or introduced isolates of these fungi in naturally substrated cultures (Sivan et al., 1984; Lewis and Papavizas, 1985).

The development of formulation and delivery systems for biocontrol (antagonistic) microorganisms to reduce the

incidence of diseases caused by soilborne pathogens is of great importance in the field of biocontrol. One of the recent technologies for the formulation of biocontrol organisms is the immobilization of wet or dry biomass within cross linked polymers such as alginate and carrageenan (Cho and Lee, 1999). For example incorporation of fungal mycelia in alginate pellets has been found to be successful for the delivery of biocontrol fungi (Papavizas et al., 1987).

The biocontrol microorganisms were immobilized wet or dry as a formulated pellets (Walker and Connick, 1983). Alginate type pellets were used in formulations of chemical and microbial herbicides (Walker and Connick, 1983). In the biotechnology industry, cell entrapment is often used to enhance production rates of bioproducts to reduce mortality of cells, and to facilitate their recovery (Gousen, 1987). Recently, alginate pellets containing spores of various biocontrol fungi (Lewis and Papavizas, 1983, 1985) and yeast cells have been formulated (Serp et al., 2000). Such preparations offer many advantages compared with conidial suspensions. For example, pellets can be stored dry. Zeolite is a mineral whose structure is constituted by SiO₄ and/or AlO₄. It is most significant features are the huge amount of gaps and

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eavities it contains. Today, zeolite are used in fields such as agriculture and stockbreeding, pollution control, energy applications and mining and metallurgy.

T. harzianum T1 was isolated from Turkey soils (Küçük and Kıvanç, 2001). *T. harzianum* T1 is an effective antagonist of several soilborne plant pathogens, including *F. culmorum*, *F. moniliforme* and *G. graminis* var. *tritici*. In this study, we tested different food based substrates for the viability of *T. harzianum* T1, and of propagules in soil and storage.

MATERIALS AND METHODS

Fungal culture and soils

T. harzianum T1 isolate obtained from Turkey soils was used (Küçük and Kıvanç, 2001). Soils were a silty loam (pH 7.97; 1.45% OM) and loamy (pH 7.94; 2.38% OM). Clinoptilolite type natural zeolite was used in the experiments. Zeolite were kindly provided by Department of Physics, The Anadolu University.

Growth of *T. harzianum* T1 on natural substrates

The growth potential of *T. harzianum* in zeolite, molasses- brewer's yeast and wheat bran containing media was studied. Substrate water content was adjusted to 50% (w/v) with sterilized distilled tap water and autoclaved for 1 h. Each medium was inoculated with 0.1 ml of a conidial suspension containing 1×10^7 conidia of *T. harzianum* per milliliter and incubated for 7 week at 30°C. Viability testes were carried out using the dilution method in *Trichoderma* selective medium (TSM) as described in the literature (Elad et al., 1981).

Preparation of pellets

Conidia of *T. harzianum* T1 was collected from the surface of colonies on Potato Dextrose Agar (PDA, MERCK) plates by washing. The concentration of conidia was determined with a haemocytometer prior to pellet preparation (Lewis and Papavizas, 1985).

The pellets containing the fungus, zeolite and alginate preparation was made by the methods of Lewis and Papavizas (1985). Sodium alginate (20 g) was dissolved in distilled water (750 ml) at 40°C using a magnetic hotplate stirrer. The zeolite (50 g) was mixed in a blender with 250 ml distilled water autoclaved for 30 min and cooled. Then *T. harzianum* T1 isolate and sodium alginate solution were added to and this mixture was comminuted for 30 min at high speed. The concentration of conidia added way 1×10^7 propagules per litre.

This final containing *T. harzianum* T1, alginate and zeolite was added dropwise into 500 ml of a gellant solution (0.25 M CaCl_2 , pH 5.4). After 20 min, beads formed in the gellant solution were separated by gentle filtration, washed and dried for 24 h at 28°C. All experiments were done twice.

Biological activity of pellets

One grams pellets was comminuted with 100 ml of water in a mixer until pellets disintegrated. Serial dilutions of the homogenate were prepared and 1 ml aliquants from the dilutions were spread on the TSM medium. Colonies were counted on the agar plates after 10

days of incubation at 30°C and populations were reported as colony-forming-units (cfu) per g of pellet.

Effects of storage temperature and soil

Portions of a sterile silty loam and loamy were amended with air-dried pellets (0.5%) at 4°C and 30°C in jars. After 0, 1, 3, 6, 9, 12, 15, 18 and 24 weeks of inoculation, 100 g of soil was with drawn from each jar and passed through a 1 mm mesh sieve to separate pellets from soil (Lewis and Papavizas, 1985). Soil dilutions were prepared and populations of the isolate (cfu/g soil) were assayed on TSM medium. To determine the effects of storage temperature on the viability of *T. harzianum* T1 in the pellets and on its subsequent behavior after its removal from soil were also studied.

RESULTS AND DISCUSSION

Comparing to the bran and molasses media zeolite was found to be more suitable medium for growth and sporulation of *T. harzianum* T1 at 30°C (Figure 1). Longer survival time was achieved for the alginate-zeolite beads and after 8 weeks, about half of the colonies survived. Lewis and Papavizas (1983) showed that several isolates of *Trichoderma* spp. can develop large amounts of biomass containing conidia and chlamydozoospores in both liquid and solid media containing different substrates. Moreover, growth on this mixture, the viable *T. harzianum* T1 population persisted more than any other tested substrate (Figure 1).

Although the alginate-bran pellets prepared from *T. harzianum* resulted in higher numbers of cfu/g of pellets than those prepared with zeolite and molasses after 1 week, there were 10 , 10^3 and 10^5 cfu/g viable colonies for the bran, molasses and alginate-zeolite pellets after 5 weeks, respectively. In a previous study, the ability of young mycelium of *Trichoderma* spp., and other fungi grown on bran to colonize the soil and proliferate as indicated by the great numbers of cfu were demonstrated (Lewis and Papavizas, 1984).

Sodium alginate is commonly used in many food products, moreover, any residues in plants or soil should not be toxic to human (Gousen, 1987). After storing at 4°C and 30°C the alginate-zeolite formulation containing the fungus produced roughly the same number of colonies (data not shown).

Similar results were obtained for formulations of *Trichoderma* sp. with and without kaolin that were stored at ambient conditions for 6 to 8 month (Walker and Connick, 1983). In Table 1, the effects of soils and storage temperatures on the viability of *T. harzianum* T1 formulated in alginate-zeolite beads are presented. More than 70% viability of the colonies was observed at 4°C and 30°C storage temperatures for 6 weeks. No viability was observed in all soils at 30°C after 9 weeks and beyond whereas there was viability in all soils 4°C even after 24 weeks. *T. harzianum* in pellets showed less than 1% viability when stored for 24 weeks at 30°C, but these pellets in soil allowed the populations to increase 10^8

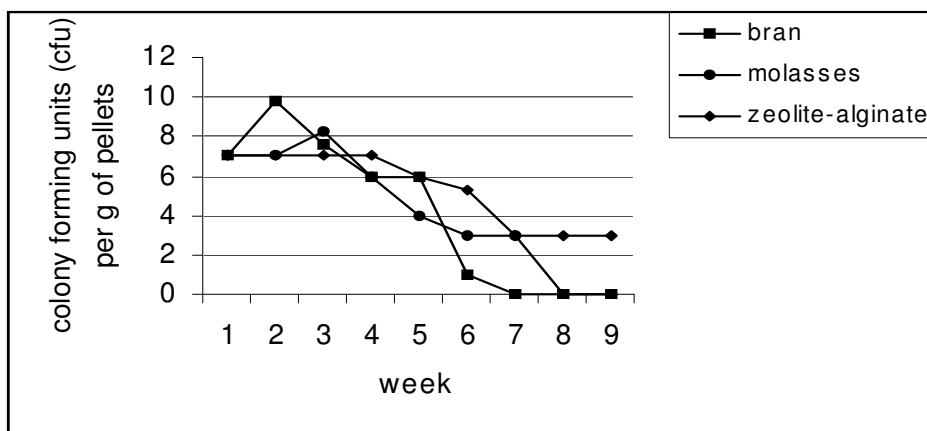


Figure 1. Comparative colony-formation rate of *T. harzianum* T1 grown in a zeolite/water, molasses and wheat-bran containing cultures (colony forming units Per g of substrates).

Table 1. Effects of soils and storage temperatures on viability of *T. harzianum* T1 formulated in zeolite alginate pellets.

Soil sample	1 weeks		3 weeks		6 weeks		9 weeks		12-15 weeks		18-24week	
	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C	4°C	30°C
Sterilized silty loam	77.5	74.6	76	72	70.4	69.8	77.4	-	72.2	-	61	-
Sterilized loamy	91.25	76.2	85	76	72.5	71	66.3	-	62.5	-	52	-
Silty loam	72.5	63.8	72.5	72	72.5	83	72.5	-	63.1	-	43	-
Loamy	70	72.2	70	78.2	76.3	82	80	-	76.4	-	46	-

Viability is expressed as percentage of the colony-forming units per g of pellets

cfu/g of soil. This number was similar to the obtained when stored at 4°C.

Although there is considerable loss of viability with some fungi after 24 weeks at 30°C, the pellets appear to retain enough viable propagules to increase cfu/g of soil as effectively as freshly prepared pellets. Storage at 4°C rather than 30°C would be preferable. The viability of the fungus in the pellets was higher in the sterilized soils than in unsterilized soils. In addition loamy soil was more suitable for survival of the fungus than silty loam soil (Table 1).

The number of immobilized fungi in alginate pellets approached the maximum values at 3-4 weeks and then decreased sharply, even when wheat bran was coentrapped in alginate as a nutritional addition for the proliferation of fungi (Lewis and Papavizas, 1985). Ground wheat brand was used rather than an inert clay (Fravel et al., 1985) as the bulking agent in pellet formation bran is a good food base for the antagonist. With some of the effective fungi high maintained at least 9 weeks after soil amendment with pellets. A previous report indicated similar results in soil.

Amended with mycelial preparations of antagonists (Lewis and Papavizas, 1984). A wheat bran preparation was as a food base for the growth and application of *T. harzianum* to soil as a biocontrol agent by Sivan et al. (1984).

The results of present paper proposes the use of alginate with zeolite that is relatively cheap and harmless to the environment, as the formulation and delivery of a biocontrol fungus, *T. harzianum* T1. Alginate formulations of mycoherbicides could be applied directly to soil as pellets or applied as foliar sprays. Alginate and zeolite formulations may be satisfactory for microbial biocontrol agent.

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