EVOLUTION OF BIOCHEMICAL PARAMETERS IN THE SUPPRESSION OF THE DAMPING-OFF CAUSED BY *RHIZOCTONIA SOLANI* AFTER THE ADDITION OF SEWAGE SLUDGE COMPOST

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ABSTRACT

A calcareous clay loam soil, with low organic matter content (1.85%) was infected with *Rhizoctonia solani*, a pathogenic fungus responsible for the sugar beet damping-off. The suppression of the pathogenic activity was analyzed over a fifteen week period of incubation at 25ºC and 60% of the highest water retention capacity (HWRC), in the original soil, and in the infected soil without and with increasing amounts of mature sewage sludge compost: 0.1, 0.5 and 1% (w/w). The evolution of dehydrogenase activity (DhA), esterase (FDAH), arginine ammonification (ArgA), alkaline phosphatase (AlcPA) and β-D-glucosidase activities (βGA), were analysed at 0, 2, 5, 7, 14, 21, 35, 70 and 105 days. The activity of *R. solani* was increased at the end of the incubation period; however, increasing quantities of compost, had a positive effect in the number of viable plants in the infected soil. Endocellular enzymatic activities, DhA and FDAH, decreased during the incubation period showing quick responses to the initial addition of organic matter or water replacement. More stable activities, such as AlcPA or βGA, showed an increase after 105 days of incubation, mainly in the highest doses of compost, which could provide an important source of nutrients for the microbial antagonistic groups of the pathogen.
KeyWords*: enzymatic activities, Rhizoctonia solani, compost, damping-off suppression.*

INTRODUCTION

Different mechanisms are involved in the biological control of pathogenic fungi based on competition, antibiosis, hyperparasitism, and the induction of systemic acquired resistance in the host plant (Hoitink et al., 2001). Mature composts from different organic residues are media in which it is possible to find important amounts of fungal biocontrol agents, such as fungi like *Trichoderma* or *Gliocadium* (Kuter et al., 1983), or bacteria, such as *Bacillus* spp. or *P. fluorenscens*, or to induce their growth in the soil (Pascual et al., 2000). The organic matter surplus added as a soil amendment, has as a consequence a higher microbial activity and an increased soil biomass which could prevent the germination of spores of the pathogens and the infection of the host (Boehm et al., 1993). The rate of hydrolysis of fluorescein diacetate (FDA) is a way of measuring the microbial activity and can be used to predict the soil or compost suppressiveness against some fungal diseases (You and Sivasithamparam, 1994). However, the addition of fresh residues or immature composts may enhance the saprophytic growth and the disease activity of damping-off pathogens; on the other hand, excessively stabilised organic matter may not support the activity of biocontrol agents (Chen et al, 1988). In conclusion, the quality of the residues and the stage of decomposition may transform the soil to a suppressive or to a conductive medium against fungal pathogens (Hoitink and Boehm, 1999). The aim of this work is to test the capacity of suppression of sugar beet (*Beta vulgaris*) damping-off caused by *R. solani* through the addition of a mature compost of biosolids, with more than six months of curing step, at the ratios normally used in field applications. During the incubation experiment, the activity of INT-dehydrogenase, FDA-hydrolase, alkaline phosphatase and β-D-glucosidase were periodically determined, as well as the germination index of *Lepidium sativum* at the beginning and

at the end of the incubation period.

MATERIAL AND METHODS

The incubation experiment was conducted with a calcareous clay loam soil with an alkaline pH (8.78), low organic matter content (1.85%), total N (1.25‰) and total P (0.518‰). The compost was obtained from a mixture of sewage sludge and tree bark in a process with four weeks of thermophylic fermentation and a curing phase of six months at ambient temperature. Its characteristics were: pH 7.45, organic matter 37.9%, total N 2.59%, total P (as P_2O_5) 4.60%, total K (as K_2O) 0.42%, Heavy Metals (in μg g^{-1} dry weight): Cd 1.5, Cu 332.8, Ni 53.0, Pb 231.1, Zn 1214.7, Hg 2.2, Cr 341.

R. solani was grown on PDA (Potato Dextrose Agar) for ten to fourteen days at room temperature. The contents of one to four Petri dishes, microorganisms and agar plus sclerotia and mycelia, were blended in 160 ml of sterile, deionised water in a warning blender at high speed for 45-60 s. The suspension was then filtered through one layer of cheese cloth and mixed with the soil in a ratio of 50 ml kg^{-1}. This sample (S_p), the original soil (S_o) and mixtures of S_p with increasing amounts of compost: 0.1, 0.5 and 1% (w/w), were adjusted to 60% HWRC with deionised water and incubated at 30ºC in 500 ml plastic boxes with perforated caps to avoid anaerobiosis. Periodically, deionised water was sprayed on the soil samples to replace the initial water content. After an incubation period of 0, 2, 5, 7, 14, 21, 35, 70 and 105 days, all of the samples were stored frozen at -20ºC until the biochemical analyses. Enzymatic activities were determined according to Alef and Nannipieri (1995) in thawed samples after three days at 4ºC. The suppression of the fungal pathogenic effect was assayed with sugar beet seeds in 250 ml pots in a mixture of 100 g of soil and 100 g of sterilised vermiculite and grown during 45 days of incubation in a climatic chamber under controlled conditions of temperature (23±1ºC), photoperiod (12 hours) and humidity. The germination experiment was carried out in quadruplicate Petri dishes with a water extract and twelve *Lepidium sativum* seeds per dish, for 48 hours in the dark (Zucconi et al., 1981).

RESULTS AND DISCUSSION

Enzymatic activities showed a heterogeneous pattern during the soil incubation (**Figure 1**). Endocellular enzymes, such as FDA-esterase, were strongly dependent on the metabolic activity of soil microorganisms, and were sensitive to organic matter addition or water replacement. Deshydrogenases in S_o, after an initial increase in activity, decreased in the course of incubation as a consequence of microbial consumption of readily available soil nutrients. The arginine ammonification was positively affected by the pathogen addition (S_p) due to the increase in degradative potential introduced into the soil. Compost addition exerted a negative effect during the five first days of incubation, depending on the dose of compost; the highest doses of compost showed negative values of activity probably due to a net N-immobilisation in a very mature compost. Other enzymes, in which an important amount of enzyme could be in a stable extracellular location, linked to soil colloids, such as alkaline phosphatases or β-D-glucosidases, showed a less sensitive response to the environmental variables and could reflect better the effect of compost addition in the nutritional status of soil microorganisms. Both of them showed an increase in activity at the end of incubation which depended on the compost/soil ratio.

The soil inoculation with *R. solani* had a clearly negative effect on the percentage of survi-

val of seeds, which decreased with the incubation time as is shown in **Table 1** (S_o and S_p columns). The initial effect of compost addition depended upon the dose; the lowest dose (0.1%) had no effect, but the intermediate dose (0.5%) had a strongly negative effect and the highest dose (1%) had a positive effect in terms of dumping-off suppression. At the end of incubation, the pathogen increased it effectiveness in the soil without compost or with the lowest dose of it. Highest doses of compost restore the seed survival percentage to the levels of the soil without fungal addition. The Zucconi's test with *Lepidium sativum* showed that the presence of *R. solani* affected negatively to the Germination Index and this effect was corrected by the compost addition; although, after the incubation period, this effect was lost.

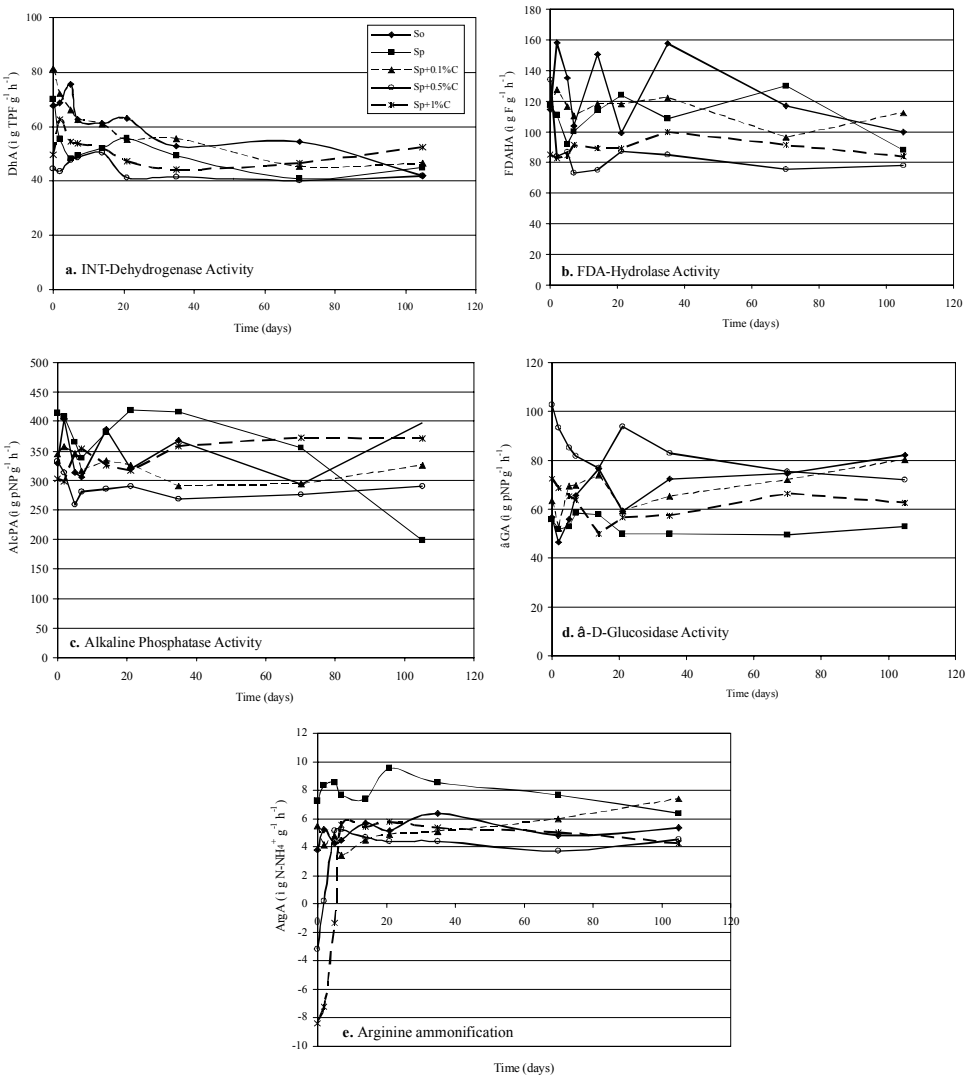

Figure 1. *Evolution of the enzymatic activities during 15 weeks of incubation of soil, with (S_p) and without (S_o) R. solani inoculation, and increasing amounts of compost (0.1, 0.5, 1%).*

Table 1. *Percentage of plant survival in the original and in the infected soil with or without different amounts of compost at the beginning and at the end of 15 weeks of incubation.*

Sample	S_o[a]	S_p[a]	S_p + 0.1% C[a]	S_p + 0.5% C[a]	S_p + 1% C[a]
% Survival Initial Sample	65.85	55.26	54.35	25.53	86.05
% Survival Incubated Sample	66.67	39.47	21.95	61.82	67.44
% G.I. Initial Sample[b]	1.26	0.73	0.90	1.10	1.14
% G.I. Incubated Sample[b]	0.83	1.04	0.90	0.82	0.88

[a]*So: original soil, Sp: soil infected with R. solani, Sp + C: soil infected with different amounts of compost.*
[b]*G.I.: Germination Index with Lepidium sativum test.*

CONCLUSIONS

The addition of mature compost exerted a positive effect in the control of sugar beet dumping-off caused by *R. solani*; although this response was not proportional to the compost dose. In this work the incubation was made with the range of compost amount normally applied to soil in extensive agriculture; higher doses of compost could have a greater effect in pathogen control.

Alkaline phosphatase and β-D-glucosidases are enzymes which better reflected the effect of the compost in the increase of soil supressiveness. These enzymes, in which important amounts of activity could be stabilised through immobilisation onto soil colloids, are better indicators than other strictly endocellular enzymes.

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