J. Mycopathol. Res. 31(1) : 35-42 (1993) Printed in India

# Inhibition of *Rhizoctonia solani* by *Streptomyces arenae* and *S. chibaensis* in soil

P. K. KUNDU* AND B. NANDI
*Department of Botany, University of Burdwan*
*Burdwan 713 104*

Damping-off of cauliflower by *Rhizoctonia solani* was significantly reduced in presence of antagonists viz., *Streptomyces arenae* and *S. chibaensis* in soil. Maximum reduction of the disease incidence occurred when the antagonists (as spore suspensions) were added to soil in a mixture (1 : 1) in advance of the pathogen. Reduction in disease incidence was found to be due to increased population of the antagonists in soil with concomitant lowering in number of the pathogen.

Key words : Biological control, Cauliflower Damping-off, *Rhizoctonia solani*, *Streptomyces arenae*, *Streptomyces chibaensis*

## INTRODUCTION

Chemical control of plant diseases, which is one of the most effective methods, is now being considered hazardous because of the residual detrimental effects of these chemicals on man and the environments. Besides, many pathogenic microorganisms often develop partial to complete resistance to these chemicals and thus survive. As a consequence, biological control of diseases is receiving increasing importance in recent years, although only a few have so far been successful in field trials ( Papavizas *et al*, 1975 ; Papavizas and Lumsden, 1980 ; Harman *et al*. 1981 ; Chattopadhyay and Nandi, 1982 ; Kundu and Nandi, 1984, 1985 ; Ichielevich-Auster *et al*., 1985 ).

Damping off of cauliflower seedlings caused by *Rhizoctonia solani* was found to be effectively controlled when either of the antagonists, *Streptomyces arenae* and *S. chibaensis*, was used as seed augmentation ( Kundu and Nandi, 1984 ). In the present investigation efficacy of the two antagonists were tested when applied to soil.

*Department of Life Science, Dianhat School, Burdwan.

## MATERIALS AND METHODS

Experiments were undertaken in sandy-clay soil free from *R. solani* in earthen pots of 20 cm diameter ( Kundu and Nandi, 1985 ). The pathogen, grown on sand-corn meal medium for two weeks at 28°C, was added to the pot soil at the rate 2% ( w/w ) of air dry soil ( 1 kg soil/pot ) following Lewis (1979) and iucubated for 10 days.

In the first set, 10 ml of spore suspension ( $1.5 \times 10^7$, $1.5 \times 10^6$ and $1.5 \times 10^5$ spores/ml of distilled water containing 0.1% w/v Tween 80 ) of each antagonist was added to the pathogen infested soil, either seperately or in a mixture ( 1:1 ), mixed thoroughly and incubated for 10, 20 and 30 days.

In a parallel set, both the antagonist (s) and the pathogen were added to the soil simultaneously and incubated for 10, 20 and 30 days.

In the third set, the antagonists either separately or in a mixture ( 1 : 1 ) were added to the soil 10 days in advance of the pathogen and were further incubated for a period 10, 20, and 30 days before seeding.

Soil infested with either *R. solani* or *Streptomyces* spp. and *R. solani* and *Streptomyces* spp. ( autoclaved ) together kept as control.

All sets were then seeded ( 16 seeds/pot ) with apparently healthy seeds of two popular cauliflower cultivars viz., 'Early Market' and 'Sutton Pusi' after surface sterilizing them using 2% NaOCl solution followed by washing in sterile distilled water. Pots were watered daily with and identical volume to keep the soil moisture at its original level of 15.5%. When the seedlings were 28 days old, number of infected seedlings were counted. Population of *R. solani* in soil was recorded following soil clamp plate method of Ko and Hora(1971)and that of *Streptomyces* spp. were studied following dillution plate count method of Kuster and Williams (1964) after 10, 20, 30 and 40 days of incubation in soil in all cases.

## RESULTS

When added to soil, each of the antagonists at the tested spore concentrations considerably reduced the disease incidence. Maximum reduction of the disease was recorded when highest concentration of the antagonists (s) was applied. Disease incidence showed further reduction when the two antagonists, at the highest concentration, were incorporated simultaneously into the soil in advance of the pathogen. With this treatment, disease incidence was reduced to 6.07% and 4.27% in 'Early Market' and 'Sutton Pusi' cultivar respectively ( Table 3 ) while control showed 81.86% and 76.16% of plants infected with *R. solani*.

**Table 1.** Disease incidence in antagonist(s) amended soil : antagonist(s) added to pathogen infested soil and incubated for 10, 20 and 30 days before seeding

| Organism / Spores/ml | Disease incidence (%)* (± S. E.) | | | | | |
|---|---|---|---|---|---|---|
| | Early Market | | | Sutton Pusi | | |
| | Incubation in days | | | Incubation in days | | |
| | 10 | 20 | 30 | 10 | 20 | 30 |
| *S. arenae* | | | | | | |
| $1.5x10^7$ | 34.01 (0.90) | 28.73 (0.84) | 18.83 (0.81) | 31.72 (0.89) | 26.22 (0.94) | 14.20 (0.80) |
| $1.5x10^6$ | 36 87 (0.25) | 31 20 (0.65) | 20 84 (0.66) | 33.60 (0.68) | 29 93 (0.48) | 16.90 (0.63) |
| $1.5x10^5$ | 41.62 (0.44) | 36.56 (0.52) | 24 81 (0.31) | 38 43 (0 49) | 33.19 (0.27) | 22.76 (1.18) |
| *S. chibaensis* | | | | | | |
| $1.5x10^7$ | 40 35 (0.83) | 32.89 (0.62) | 20.79 (0 64) | 37.30 (0.62) | 30.08 (0 63) | 18 06 (0.95) |
| $1.5x10^6$ | 42.64 (0.93) | 34.25 (0.64) | 22.57 (0.24) | 41.34 (0.67) | 33.18 (0.66) | 21.66 (0.60) |
| $1.5x10^5$ | 46.39 (0.87) | 41.05 (1.39) | 26.78 (0.38) | 43.68 (0.33) | 37.98 (0.52) | 25.24 (0.56) |
| *S. arenae* + *S. chibaensis* | | | | | | |
| $1.5x10^7$ | 30.32 (0 29) | 26 08 (0 58) | 16.90 (0.91) | 29.30 (1.10) | 24.58 (0.79) | 13.92 (0.78) |
| $1.5x10^6$ | 32.83 (0.39) | 29.13 (0.70) | 18.17 (0.23) | 31.90 (0 57) | 27.01 (0.51) | 15 02 (0.42) |
| $1.5x10^5$ | 38.59 (1.24) | 31.69 (0.27) | 22.41 (0.26) | 36.19 (0.27) | 30.21 (0.56) | 19.85 (0.54) |
| Controls only *R. solani* | 81.76 (1.04) | NC | NC | 76.16 (1.02) | NC | NC |
| *R. solani* + *Streptomyces* spp. (Autoclaved) | 81.76 (0.62) | NC | NC | 76.16 (0.85) | NC | NC |
| *Streptomyces* spp. (Living) | ND | ND | ND | ND | ND | ND |

* Mean of five replicates ;

NC = No change

ND = No disease

**Table 2.** Disease incidence in antagonist(s) amended soil : both antagonist(s) and pathogen were added to soil simultaneously and incubated for 10, 20 and 30 days before seeding

| Organism Spores/ml | Disease Incidence (%)* (± S. E.) Early Market Incubation in days 10 | 20 | 30 | Sutton Pusi Incubation in days 10 | 20 | 30 |
|---|---|---|---|---|---|---|
| *S. arenae* | | | | | | |
| $1.5x10^7$ | 26.63 (0.98) | 21.38 (0.61) | 15.05 (0.49) | 22.07 (1.04) | 16.97 (1.29) | 11.20 (0.37) |
| $1.5x10^6$ | 31.32 (1.54) | 25.25 (0.54) | 17.84 (0.69) | 26.46 (0.54) | 20.14 (0.60) | 14.30 (0.84) |
| $1.5x10^5$ | 34.14 (0.92) | 28.84 (0.49) | 21.01 (0.52) | 31.48 (0.58) | 22.92 (0.41) | 16.95 (1.23) |
| *S. chibaensis* | | | | | | |
| $1.5x10^7$ | 30.05 (0.99) | 27.03 (1.89) | 18.79 (1.24) | 26.32 (0.60) | 20.35 (0.83) | 15.06 (1.12) |
| $1.5x10^6$ | 34.27 (1.46) | 31.09 (1.01) | 20.57 (0.63) | 30.63 (0.56) | 24.53 (1.21) | 18.66 (0.62) |
| $1.5x10^5$ | 37.34 (1.01) | 32.24 (1.07) | 23.78 (0.50) | 32.30 (0.42) | 27.31 (0.46) | 20.24 (1.03) |
| *S. arenae* + *S. chibaensis* | | | | | | |
| $1.5x10^7$ | 23.78 (1.23) | 19.21 (0.74) | 14.02 (0.41) | 20.11 (0.97) | 14.07 (0.93) | 9.35 (0.93) |
| $1.5x10^6$ | 29.91 (0.79) | 24.98 (1.07) | 16.37 (0.57) | 14.16 (0.76) | 18.07 (0.76) | 12.66 (0.40) |
| $1.5x10^5$ | 33.98 (0.79) | 28.01 (0.24) | 18.03 (1.01) | 28.14 (0.53) | 22.25 (1.22) | 15.45 (0.73) |
| Controls only *R. solani* | 81.76 (1.14) | NC | NC | 76.16 (1.02) | NC | NC |
| *R. solani* + *Streptomyces* spp. (Autoclaved) | 81.76 (0.62) | NC | NC | 76.16 (0.85) | NC | NC |
| *Streptomyces* spp. (Living) | ND | ND | ND | ND | ND | ND |

* Mean of five replicates

NC = No change

ND = No disease

**Table 3.** Disease incidence in antagonist(s) amended soil : antagonist(s) added to soil 10 days in advance of the pathogen and incubated for a period of 10, 20 and 30 days before seeding

| Organism / Spores ml | Disease Incidence (%)* (± S. E.) | | | | | |
|---|---|---|---|---|---|---|
| | Early Market | | | Sutton Pusi | | |
| | Incubation in days | | | Incubation in days | | |
| | 10 | 20 | 30 | 10 | 20 | 30 |
| *S. arenae* | | | | | | |
| $1.5x10^7$ | 20.36 (0.48) | 11.70 (0.30) | 10.83 (0.42) | 16.14 (0.30) | 9.02 (1.10) | 6.94 (1.08) |
| $1.5x10^6$ | 24.35 (0.90) | 16.25 (0.58) | 14 82 (1.03) | 19.23 (0.73) | 10.17 (0.59) | 8.05 (0.71) |
| $1.5x10^5$ | 28.10 (0.65) | 21.14 (0.43) | 20 16 (0.27) | 23.65 (0.89) | 16.15 (1.64) | 15.90 (1.20) |
| *S. chibaensis* | | | | | | |
| $1.5x10^7$ | 29.83 (0.75) | 19.23 (0.43) | 18.65 (1.07) | 24.12 (0.91) | 15.23 (0.54) | 13.19 (0.58) |
| $1.5x10^6$ | 33.70 (0.18) | 21.60 (0.63) | 22.15 (0.13) | 28.60 (0.63) | 20.50 (0 62) | 17.44 (0 51) |
| $1.5x10^5$ | 36 91 (0.83) | 26 85 (1.14) | 25.06 (0.18) | 31.17 (1.03) | 24.16 (1.22) | 21.66 (1.62) |
| *S. arenae* + *S. chibaensis* | | | | | | |
| $1.5x10^7$ | 18.91 (0.54) | 12.85 (0.62) | 6.70 (0.66) | 16.05 (0.45) | 6.38 (0.64) | 4.26 (1.38) |
| $1.5x10^6$ | 23.72 (0.46) | 16.27 (0.55) | 10 91 (0.73) | 20.62 (1.18) | 9.23 (0.75) | 7.95 (0.71) |
| $1.5x10^5$ | 27.12 (0.79) | 18.95 (0.29) | 17 12 (0.72) | 24.81 (0.63) | 14.31 (1.10) | 13.50 (1.67) |
| Controls only *R. solani* | 81.76 (1.4) | NC | NC | 76.16 (1.02) | NC | NC |
| *R. solani* + *Streptomyces* spp. (Autoclaved) | 81.76 (0.61) | NC | NC | 76.16 (0 85) | NC | NC |
| *Streptomyces spp.* (living) | ND | ND | ND | ND | ND | ND |

* Mean of five replicates

NC = No change

ND = No Disease

Control sets treated with *R. solani* and autoclaved *Streptomyces* spp., together showed same degree of infection but showed no improvement in seedling growth. Again control set treated with living *Streptomyces* spp. showed no diseased plant and some improvement in seedling growth.

## DISCUSSION

A gradual increase in antagonist(s) population ( as was evident from dilution plate counts ) and the associated antibiosis ( possibly through disintegration of

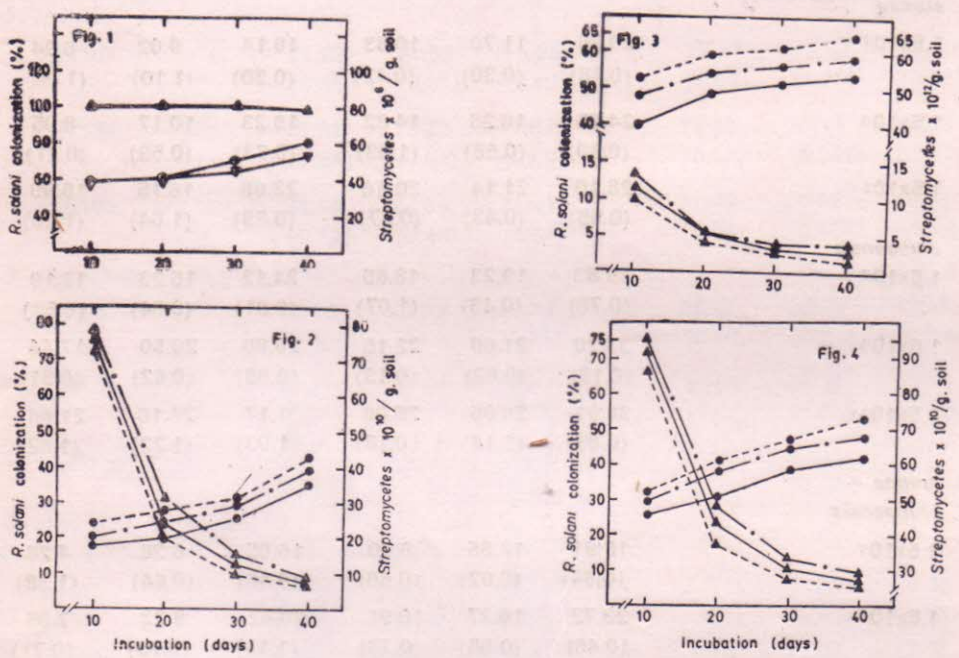


Fig. 1. Population of *R. solani* (△——△). *S. arenae* (●——●) and *S. chibaensis* (△——△) in control soil after 10, 20, 30 and 40 days of incubation.

Figs. 2-4. Population of *R. solani* and *Streptomyces* spp. ( antagonists )

*R. solani* (△——△) Vs. *S. chibaensis* (●——●)
*R. solani* (△——△) Vs. *S. arenae* (●——●)
*R. solani* (△——△) Vs. *S. arenae* + *S. chibaensis* (●——●)

a) Antagonists were added either in pathogen infested soil ( Fig. 2 ) or added simultaneously with the pathogen ( Fig. 3 ) and incubated for 10, 20 and 40 days.

b) Antagonists were added 10 days in advance of the pathogen and the pathogen-antagonists, combinations were further incubated for 10. 20, 30 and 40 days ( Fig-3 ).

fungal protoplasm, followed by death of the hyphae as was found in *in vitro* studies ) with concomitant decrease of the pathogen population in soil ( Figs. 2, 3 and 4 ) might be the reason for significant reduction of the disease ( Cf. Elad *et al.* 1980 ; Lewis and Papavizas, 1985 ; Merriman *et al.*, 1974 ; Mew Rosalas, 1986 and Odovody *et al* 1980). Maximum reduction of the disease, when the antagonists were used simultaneously, might be due to synergistic effect in reducing the pathogen population to a higher extent. Appearance of the disease in some plants might be due to localised adsorption on soil particles of the compound(s) produced by the antagonists which lead to decreasing their antifungal activities and thus allowing the pathogen to infect the hosts. *Streptomy es* spp. biomass ( autoclaved ) did not affect seedling growth but their metabolic products resulted in significant change in the seedling growth. The present findings clearly indicated that soil treatment rather than seed treatment with the antagonist(s) was more effective in reducing the pathogen population and hence, the disease incidence, than seed treatment with the antagonist(s) ( Kundu and Nandi, 1984 ). This method of disease control proved to be an effective alternative of costly method of chemical control and at the sametime will reduce the pollution problem through indiscriminate uses of fungicides.

**ACKNOWLEDGEMENT**

The authors thank the University of Burdwan, Burdwan for financial assistance and necessary laboratory facilities to carry out the investigation. Thanks are also due to Prof. K. D. Domsch, Institute for Bodenbiology, Braunschweigh, W. Germany, for his valuable help.

**REFERENCES**

Chattopadhyay, S. K. and Nandi, B. (1982). Inhibition of *Helminthosporium oryzae* and *Alternaria solani* by *Streptomyces longisporus* ( Krasil' nikov ) *Pl. Soil*, **69**, 171-175.

Elad, Y., Chet, I. and Katan, J. (1980). *Trichoderma harzianum* & biocontrol agent effective against *Sclerotium rolfs i* and *Rhizoctonia solani*. *Phytopathology* **70**, 119-121.

Harman, G. E., Chet, I. and Baker, R. (1981). Factors effecting *Trichoderma hamatum* applied to seeds as a biocontrol agent. *Phytopathology* **71**, 569-572.

Ichielevich-Auster, M., Sneh, B. and Koltin, Y. (1985). Supression of damping off *Rhizoctonia solani* by a non pathogenic isolate of *R. solani Phytopathology* **75**, 1080-1084.

Ko W and Hora F K (1971). A selective medium for quantitative determination of *Rhizoctonia solani* in soil. *Phytopathology* **61**, 707-710

Kundu, P. K. and Nandi, B. (1984). Control of cauliflower damping-off by using antagonist coated seeds. *Pedobiologia* **27**, 43-48.

Kundu, P. K. and Nandi B. (1985). Control of *Rhizoctonia* disease of cauliflower by competitive inhibition of the pathogen using organic amendments in soil. *Pl. Soil* **83**, 357-362.

Kuster, E. and Williams, S. T. (1964). Selection of media for isolation of *Streptomycetes*. *Nature* (London) **202**, 928-929.

Lewis, J. A. (1979). Influence of soil texture on growth and survival of *Rhizoctonia solani* in soil. *Can J. Microbiol.* **25**, 1310-1314.

Lewis, J. A. and Papavizas, G. C. (1985). Effect of mycelial preparation of *Trichoderma end Gliocladium* on population of *Rhizoctonia solani* and incidence of damping-off. *Phytopathology* **75**, 812-817.

Merriman, P. R., Price, R. D., Kollomorgan, J. F., Piggot, T. and Ridge, E. H. (1974). Effect of seed inoculation with *Bacillus subtilis* and *Streptomyces griseus* on the growth of cereals and carrot *Auster. J. Agric. Res.* **24**, 219-226.

Mew, T. W. and Rosales, A. M. (1986). Bacterization of rice plants for control of sheath blight caused by *Rhizoctonia solani*. *Phytopathology* **76**, 1260-1264.

Odovody, G. N., Boosalis, M. G. and Kerr, E. D. (1980). Biological control of *Rhizoctonia solani* with a soil inhabiting basidiomycetes. *Phytopathology* **70**, 655-658.

Papavizas, G. C., Adams, P. B., Lumsden, R. D. Lewis. J. A., Dow, R. L, Ayers, W. A. and Kantzes, J. G. (1975). Ecology and epidemology of *Rhizoctonia solani* in field soil. *Phytopathology* **65**, 871-877.

Papavizas, G. C. and Lumsden, R. D. (1980). Biological control of soil-borne fungal propagules. *Ann. Rev. Phytopathol* **18**, 389-413.

*(Accepted for publication 17th November, 1992)*