Neth. J. Pl. Path. 90 (1984) 127-132

Suppressive effect of Azotobacter chroococcum on Rhizoctonia solani infestation of potatoes

SUDHIR U. MESHRAM[1]

Institute for Soil Fertility, P.O. Box 30003, 9750 RA Haren (Gr.), the Netherlands

Accepted 15 May 1984

Abstract

Isolates of *Azotobacter chroococcum* were found to be promising for the control of infestation of potato plants with *Rhizoctonia solani*. Inoculation with an isolate of *Verticillium biguttatum* in combination with isolates of *A. chroococcum* effectively protected sprouts, stems and stolons against infestation with *R. solani*. The effect of inoculation varied with soil temperature.

No sclerotia were formed on potatoes harvested from soil in pots inoculated with isolates of *A. chroococcum* plus *V. biguttatum* under glasshouse conditions; the yield increased significantly over the control.

Additional keywords: antagonism, interaction, *Verticillium biguttatum,* biological control.

Introduction

Pesticides can effectively control plant diseases in agriculture, but they may cause environmental pollution. Besides, chemical control is costly and therefore often beyond the means of farmers in developing countries. Microbiologists are therefore looking for 'biological control' methods integrated into plant disease control programmes.

Novak and Dvorzhakova (1955) reported that *Azotobacter chroococcum* inhibited the growth of phytopathogenic fungi such as species of *Alternaria, Venturia, Sclerotinia, Rhizoctonia* and *Pythium.* Lakshimi Kumari et al. (1972) found that *Azotobacter* spp. produced a thermolabile, ether-soluble fungistatic substance which inhibited the growth of *Fusarium moniliforme* in vitro.

Meshram and Jager (1983) reported the screening and selection of isolates of *Azotobacter chroococcum* on the basis of their antagonism towards *Rhizoctonia solani* on agar plates and in soil. The present paper presents the results of further tests with isolates of *A. chroococcum* alone and in combination with a hyperparasite of *R. solani, Verticillium biguttatum,* in a 'micropot' experiment with soil, conducted at four temperatures. In addition, a trial with pots of normal size (29 cm diam.) was performed under glasshouse conditions.

Materials and methods

'Micropot' (11 cm diam.) experiment. The soil was a clay loam with pH-KCl 8.1. The

[1] Guest worker. Present address: Division of Microbiology, IARI, New Delhi – 110012, India.

same five isolates of *A. chroococcum* (J2, J4, J5, J6 and SM6) as used in a previous study (Meshram and Jager, 1983) were used here. An isolate of *V. biguttatum* (M 73) and a culture of *R. solani* were obtained from stock cultures maintained by the Department of Soil Biology. Preparation of the cultures, inoculation of the soil, planting of potatoes, and incubation at 10, 15, 20 and 25 °C were done as described before (Meshram and Jager, 1983). For inoculation with *V. biguttatum,* 15-day-old cultures were mixed with the soil. Where the soil was inoculated with combinations of isolates of *A. chroococcum* and *V. biguttatum,* 10 ml of each broth culture was used.

The incidence of disease on the potato sprouts was recorded after 18 days of incubation.

Pot trial under glasshose conditions. A marine sandy loam (pH-KCl 7.6) with shell fragments was inoculated with *A. chroococcum* isolates in various combinations with *V. biguttatum* and *R. solani,* as shown in Table 2. The amounts per pot and the densities of the inocula were

1) 200 ml *A. chroococcum,* 16 days old, 10-25 $\times$ 10^9 cells per ml,
2) 200 ml *V. biguttatum,* 3 weeks old, 10^8-10^9 propagules per ml (fragmented),
3) 150 ml *R. solani,* 3 weeks old, about 10^9 propagules per ml (fragmented).

Soil treated with 200 ml sterilized Ashby's broth medium served as a control. In the treatment with *R. solani* alone, the soil was inoculated with a 150 ml broth culture of the fungus and 200 ml sterilized Ashby's broth medium.

Four pregerminated potatoes (cv. Irene) infested with sclerotia were planted in 29-cm diameter pots on 2 June 1982. Each pot contained 12.5 kg soil, which had been fertilized with 1.5 g N as NH_4NO_3 and 1.5 g K_2O as K_2HPO_4. The 16 treatments were replicated three times in a randomized-block design. The crop was harvested on 23 August 1982.

Maximum daily air temperature varied from 14 to 26 °C and relative humidity from 65 to 95%.

The occurrence of disease on stems and stolons, and of sclerotia on the tubers was recorded separately. The degree of infection of sprouts, stems, stolons and tubers (Fig. 1) was given a numerical rating from 0 to 5 based on the size of the diseased area as a percentage of the total area (Meshram and Jager, 1983). The disease index was calculated as mentioned in an earlier paper (Meshram and Jager, 1983).

The presence of *A. Chroococcum* on the plants grown in inoculated soil was tested by placing pieces of stems, stolons and tubers on Jensen's agar medium. Treatment effect on tuber yield was evaluated statistically by analysis of variance and a test of significance at the 5% level (Fisher, 1958).

Results and discussion

'Micropot' experiment. The results are summarized in Table 1. The disease index was low at all temperatures when the soil had been treated with *A. chroococcum* J4 or J6. These two isolates effectively controlled *R. solani.* Isolates J2 and J5 were moderately effective, but SM6 performed poorly. *V. biguttatum* was also effective, but seemingly not at 25 °C in sterilized soil. The effect of temperature was clearly present when effective isolates were used.

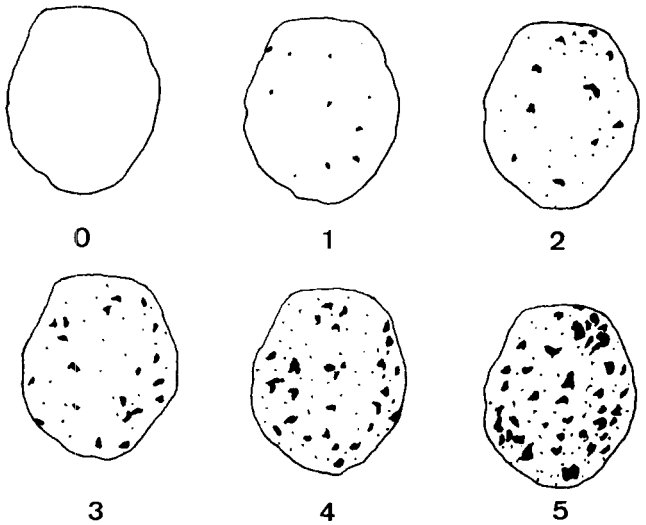

Fig. 1. Disease assessment key of potatoes showing the numerical rating (0-5) for the tuber surface area covered by sclerotia of *Rhizoctonia solani.*

Pot experiment in the glasshouse. The results are summarized in Table 2. As expected, inoculation with *R. solani* alone resulted in the highest sclerotium formation on the tubers (36%) and also gave infection of stems and stolons (54%). Among the antagonists, J4 was superior to other *A. chroococcum* isolates, and about as effective as *V. biguttatum.* No sclerotium formation was found on tubers when the soil was inoculated with *A. chroococcum* J4 + *V. biguttatum* and the symptoms of infection on stems and stolons were negligible.

A. chroococcum was abundant on tubers and stolons, which is a prerequisite to their protective action. Inoculation with *A. chroococcum,* especially isolate J4, increased the yield of potato tubers significantly as compared with the control, *R. solani* and other isolates when inoculated alone. Further increases were obtained when *A. chroococcum* was applied to the soil together with *V. biguttatum.* The yield of plants on soil inoculated with *R. solani* did not differ significantly from that of the control plants.

Some isolates of *A. chroococcum* kept infection at a low level in both pot experiments. Under laboratory conditions, the infection by *R. solani* in sterilized control soil was higher than in the unsterilized control soil (Meshram and Jager, 1983). This is probably due to the presence of an antagonistic microflora, competing with *R. solani.* In some cases the suppression of *R. solani* by *A. chroococcum* isolates and *V. biguttatum* varied with temperature.

The increase of growth and yield of several agricultural crops following bacterization with *A. chroococcum* is possibly due to a multiple action of this organism, i.e. (1) nitrogen fixation, (2) suppression of plant-pathogenic microorganisms, (3) production of growth-promoting substances, (4) favourable effect on other beneficial microorganisms in the soil, and (5) mobilization of soil phosphate (Jackson et al., 1964; Brown, 1974; Shende et al., 1975; Meshram, 1981).

Table 1. Disease indices (0-100) of potato grown at four temperatures in sterilized and unsterilized soil inoculated with different organisms (average of two replications).

Treatment	Sterilized soil				Unsterilized soil			
	temperature of incubation (°C)				temperature of incubation (°C)			
	10	15	20	25	10	15	20	25
1. *R. solani* (control)	47	53	67	51	33	52	43	49
2. *V. biguttatum* + *R. solani*	8	0	9	28	12	8	3	3
3. *A. chroococcum* SM6 + *R. solani*	17	54	69	63	23	73	63	47
4. *A. chroococcum* J2 + *R. solani*	16	10	6	7	20	10	8	3
5. *A. chroococcum* J4 + *R. solani*	8	0	3	5	0	6	0	0
6. *A. chroococcum* J5 + *R. solani*	34	6	9	9	4	13	13	0
7. *A. chroococcum* J6 + *R. solani*	7	0	0	2	0	0	3	0
8. *A. chroococcum* SM6, J2, J4, J5, J6 + *R. solani*	13	3	2	0	20	43	10	35
9. *A. chroococcum* SM6, J2, J4, J5, J6 + *V. biguttatum* + *R. solani*	0	0	0	3	6	10	0	0

Table 2. Disease indices of stems and stolons, sclerotium indices of tubers and the yield of tubers as influenced by the various treatments (antagonists added to the soil).

Treatment	Disease index of stems and stolons (0-100)	Sclerotium index of tubers (0-100)	Yield (g/pot)
1. None	48 ± 6 a[1]	28 ± 9 a c[1]	285.7[2]
2. *R. solani*	14 ± 13 a	36 ± 12 c	351.7
3. *V. biguttatum* M73	54 ± 11 b	8 ± 4 b	395.1
4. *A. chroococcum* SM6	30 ± 8 b	16 ± 8 a	358.0
5. *A. chroococcum* J4	15 ± 9 b	4 ± 3 b	560.7
6. *A. chroococcum* J6	19 ± 4 b	10 ± 8 b	375.6
7. *V. biguttatum* + *R. solani*	15 ± 14 b	8 ± 8 b d	523.5
8. *A. chroococcum* SM6 + *R. solani*	60 ± 16 a	12 ± 14 a	472.1
9. *A. chroococcum* J4 + *R. solani*	20 ± 10 b	5 ± 4 b d	512.1
10. *A. chroococcum* J6 + *R. solani*	33 ± 25 a	12 ± 11 b d	444.2
11. *A. chroococcum* SM6 + *V. biguttatum*	21 ± 18 b	4 ± 2 b d	502.2
12. *A. chroococcum* J4 + *V. biguttatum*	0 ± 1 b	0 ± 0 b d	597.2
13. *A. chroococcum* J6 + *V. biguttatum*	22 ± 25 a	1 ± 1 b d	470.5
14. *A. chroococcum* SM6 + *V. biguttatum* + *R. solani*	24 ± 19 a	2 ± 1 b d	560.5
15. *A. chroococcum* J4 + *V. biguttatum* + *R. solani*	9 ± 4 b	3 ± 4 b d	539.0
16. *A. chroococcum* J6 + *V. biguttatum* + *R. solani*	23 ± 13 b	4 ± 3 b	455.2

[1] Values followed by different letter differ significantly (P = 0.05).
[2] L.S.D. (5%) = 144.5.

The results indicate that some *A. chroococcum* isolates can protect the subterranean parts of potato plants against infection by *R. solani.* This protection is more effective when these isolates are applied in combination with *V. biguttatum,* which can also increase the yield of potatoes. The success of the inoculation varies with temperature, and also depends on the selection of appropriate isolates.

Acknowledgement

The author is indebted to the Institute for Soil Fertility, Haren, the Netherlands, for providing the necessary facilities and to Dr H. van Dijk and Drs G. Jager for helpful suggestions. The awarding of a Dutch Government fellowship 1981-82 and the sponsorship of the Ministry of Education, Govt. of India, are gratefully acknowledged.

Samenvatting

Onderdrukkend effect van Azotobacter chroococcum op de aantasting van aardappelen door Rhizoctonia solani

Enkele isolaten van *Azotobacter chroococcum* bleken sterk antagonistisch ten opzichte van *Rhizoctonia solani* en leken goed bruikbaar voor een biologische bestrijding van deze ziekteverwekker van de aardappel.

Beënting van de grond met *Verticillium biguttatum* en *A. chroococcum* gaf – in potproeven – een effectieve bescherming van spruiten, stengels en stolonen tegen aantasting door *R. solani.*

De vorming van sclerotiën bleef achterwege op knollen die gevormd waren in grond die was beënt met *A. chroococcum* plus *V. biguttatum.* De opbrengst uit behandelde grond was hoger dan die uit onbehandelde.

De effectiviteit van de behandeling is afhankelijk van de temperatuur.

References

Brown, M.E., 1974. Seed bacterization. A. Rev. Phytopath. 12: 181-197.

Fisher, R.A., 1958. Statistical methods for research workers. Harnew Pub. Co. Inc., New York.

Jackson, R.M., Brown, M.E. & Burlingham, S.K., 1964. Similar effects on tomato plants of *Azotobacter* inoculation and application of gibberellins. Nature (Lond.) 192: 575.

Lakshimi Kumari, M., Vijay Lakshmi, M. & Subba Rao, N.S., 1972. Interaction between *Azotobacter* species and fungi. I. In vitro studies with *Fusarium moniliforme* Sheld. Phytopath. Z. 75: 27-30.

Meshram, S.U., 1981. Effect of *Azotobacter chroococcum* on maize crop. Ph.D. thesis, New Delhi.

Meshram, S.U. & Jager, G., 1983. Antagonism of *Azotobacter chroococcum* isolates to *Rhizoctonia solani.* Neth. J. Pl. Path. 89: 191-197.

Novak, A. & Dvorzhakova, H., 1955. The destruction of some phytopathogenic fungi by *Azotobacter.* Sb. čsl Akad. Zeměd. Věd. Rostlinná výroba 28: 304.

Shende, S.T., Apte, R.G. & Singh, T., 1975. Multiple action of *Azotobacter.* Indian J. Genet. Pl. Breed. 35: 314-315.