

Effect of Antibiotics on Growth of Rhizobia and on Nodulation in *Trifolium alexandrinum*

S N SHUKLA* and R S DWIVEDI

University Botany Laboratory, Banaras Hindu University,
Varanasi 221005

(Received 2 August 1978)

The efficacy of four antibiotics viz. nystatin, griseofulvia, tetracycline (chloro) and chloramphenicol against 5 different strains of *Rhizobium* was studied *in vitro* at 50, 100, 200 and 400 ppm. Tetracycline (chloro) and chloramphenicol inhibited the growth of all rhizobia at all the concentrations. Nystatin and griseofulvin were ineffective.

The effect of foliar spray of three antibiotics viz. nystatin, griseofulvin and tetracycline (chloro) on nodulation in *Trifolium alexandrinum* was studied. All the antibiotics sprayed reduced the number of nodules significantly at 150 ppm. Nystatin reduced the weight and volume of nodules significantly at all the concentrations while tetracycline (chloro) did so only at 150 ppm.

Keywords: Antibiotics, rhizobia, nodulation, *Trifolium alexandrinum*

Introduction

Different antibiotics have been tested for their efficacy to control different diseases of nodulating legumes (Sakurai & Shimada 1971, Bowler & Atherton 1972, Taylor 1972). However, the after-effect of treatment of antibiotics on nodulation in leguminous plant is not known. There is also paucity of information regarding the effect of antibiotics on growth of different strains of Rhizobia. In the present study, experiments were conducted to find out the effect of four antibiotics, e. g. nystatin, griseofulvin, tetracycline (chloro) and chloramphenicol on growth of 5 different strains of Rhizobia. The effect of foliar spray of three antibiotics e. g. nystatin, griseofulvin and tetracy-

cline (chloro) on nodule number, weight and volume in *Trifolium alexandrinum* was also studied.

Materials and Methods

Experiment 1: Five different strains of *Rhizobium* viz. *R. trifolii*, *R. leguminosarum*, *R. phaseoli* strain 1, *R. phaseoli* strain 2, *Rhizobium* sp. (cow pea group) were isolated from root nodules of *Trifolium alexandrinum*, *Pisum sativum*, *Phaseolus mungo*, *P. radiatus* and *Vigna catjang* respectively and were maintained on yeast mannitol agar medium. Four concentrations e. g. 10, 50, 200 and 400 ppm of each antibiotic were prepared separately and added in sterile cooled (40°C) yeast mannitol agar medium so as to

*Present address: Scientist S-1, Central Rice Research Institute, Cuttack 753006, Orissa, India

maintain the respective concentrations. The mixture was shaken well and poured into sterile Petri dishes. The medium was allowed to solidify. Streaks were made on solidified agar discs from individual strain of *Rhizobium* separately. Three replicates were prepared for each concentration. Streaks made on yeast mannitol agar discs without antibiotics served as control. Petri dishes were incubated at 25°C for 48 hrs and thereafter the results were recorded.

Experiment 2: The seeds dressed with effective strain of *Rhizobium* were sown in earthenware pots containing equal amount of uniformly mixed unsterile soil. After a week, only five healthy seedlings were retained in each pot. 50, 100 and 150 ppm solutions of each of the antibiotic were prepared separately in distilled water and sprayed on the plants. First spraying with each concentration was done when the plants were 15 days old followed by another spraying after 5 days of first spraying.

Plants were watered regularly to maintain the moisture content. After 20 days of second spraying, plants were dug out gently and the root system was washed in running water. The nodules were detached from the roots, counted, dried in folds of blotting paper and weighed. The volume of nodules was determined by dipping the known number of nodules in a known quantity of water in graduated measuring cylinder. The weight and volume/100 nodules were recorded for comparison.

Results and Discussion

Amongst the antibiotics assayed, tetracycline (chloro) and chloramphenicol inhibited the growth of all the strains of *Rhizobium* tested at all the concentrations while nystatin and griseofulvin were ineffective (table 1). Actinomycin, tyrrithricin, tyrocidine hydrochloride and gramicidin had antirhizobial effect (Trussel & Sarles 1943). Keczke's and Manninger (1962) observed

Table 1 *In vitro* studies on effect of antibiotics on growth of rhizobia

Test antibiotic	Concentration in ppm	<i>Rhizobium trifolii</i>	<i>R. leguminosarum</i>	<i>R. phaseoli</i> strain 1	<i>R. phaseoli</i> strain 2	<i>Rhizobium</i> sp. (cow pea group)
Nystatin	10	+	+	+	+	+
	50	+	+	+	+	+
	200	+	+	+	+	+
	400	+	+	+	+	+
Griseofulvin	10	+	+	+	+	+
	50	+	+	+	+	+
	200	+	+	+	+	+
	400	+	+	+	+	+
Tetracycline (chloro)	10	—	—	—	—	—
	50	—	—	—	—	—
	200	—	—	—	—	—
	400	—	—	—	—	—
Chloramphenicol	10	—	—	—	—	—
	50	—	—	—	—	—
	200	—	—	—	—	—
	400	—	—	—	—	—
Control	—	+	+	+	+	+

+ Indicates growth of rhizobia; — Indicates inhibitory effects

the antagonistic effect of penicillin, streptomycin (dihydro), vionactan, chloramphenicol and terramycin towards growth of different strains of rhizobia. Streptomycin, aureomycin, terramycin and ledermycin are known to exhibit antirhizobial effect (Graham 1963).

Amongst the antibiotics sprayed, nystatin was relatively more toxic to the plants which reduced the number of nodule significantly at 150 ppm and decreased the weight and volume of nodules markedly at all the concentrations (table 2). Tetracycline (chloro) reduced the number, weight and volume of nodules at 150 ppm. Griseofulvin reduced the number of nodules

significantly at 150 ppm but had no effect on weight and volume at any concentrations. Nystatin and griseofulvin were ineffective against the growth of *Rhizobium in vitro* but when applied as foliar spray on the plants, they affected the number, weight and volume of nodules in a manner mentioned above. The reason for fall in number, weight and volume of nodules in the treated plants may be due to changes in metabolic activities of plants after treatments.

Acknowledgement

The authors are thankful to Late Dr R N Singh, Head of Botany Department for providing facilities.

Table 2 Effect of foliar spray of antibiotics on nodulation in the test plant

Antibiotic sprayed	Concentration in ppm	No. of nodule/plant (mean of 5 values)	't' value	Wt. in g/100 nodules (mean of 5 values)	't' value	Volume in ml/100 nodules (mean of 5 values)	't' value
Nystatin	50	137.00	0.250	0.153	5.633*	0.233	3.975*
	100	120.00	1.566	0.146	5.466*	0.216	4.750*
	150	92.66	4.606*	0.081	11.409*	0.186	5.063*
Griseofulvin	50	141.33	0.448	0.360	0.102	0.35	0.970
	100	109.33	1.976*	0.307	1.187	0.33	2.161
	150	101.33	4.517*	0.316	1.136	0.34	1.890
Tetracycline (chloro)	50	145.00	1.151	0.303	1.547	0.33	2.083
	100	136.2	0.666	0.296	2.090	0.30	1.083
	150	90.30	4.598*	0.171	4.829*	0.20	4.166*
Control	—	138.0	—	0.364	—	0.386	—

*Significant at 1% level

References

- Bowler J W and Atherton J G 1972 Effect of tetracycline antibiotics on plants affected by legume little leaf disease; *Aust. J. Biol. Sci.* **25** 43-51
- Graham P H 1963 Antibiotic sensitivities of root nodule bacteria; *Aust. J. Biol. Sci.* **16** 557-559
- Keczke's H and Manninger E 1962 Effect of antibiotics on growth of rhizobia; *Can. J. Microbiol.* **8** 157-159
- Mukewar P M and Bhide V P 1969 Effect of seed treatments with fungicide and antibiotics on efficacy of nodulation by *Rhizobium* strains of groundnut; *Hindu Antibiot. Bull.* **11** 172-176
- Sakurai H and Shimada T 1971 Studies on the antimicrobial effects of actinomycin-c group antibiotics on phytopathogenic micro-organisms; *Bull. of Agri. Chemicals Inspection Station* No. **11** 1122-1126
- Taylor J D 1972 Field studies on Halo blight of beans (*Pseudomonas phaseolicola*) and its control by foliar sprays; *Ann. Appl. Biol.* **70** 191-197
- Trussel P C and Sarles W B 1943 Effect of antibiotic substances upon rhizobia; *J. Bacteriol.* **45** 29

