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# PHYTOLOGICAL STUDIES OF POLYENE ANTIBIOTIC A-435

BY

S. C. SANTRA AND A. L. CHANDRA

*Department of Microbiology, Bose Institute, Calcutta-700 009*

Germination of mung and rice seeds was normal in the presence of <100 μg/ml A-435. At and above 100 μg/ml stunted growth of the seedlings was noted. Foliar sprays of <1000 μg/ml A-435 produced no toxic symptoms in rice and maize plants. But growth of the plants was arrested in liquid cultures containing 100 μg/ml of the antibiotic. A-435 was translocated upwards and retained its antibiotic activity for 48 hr. Rice plants infected with *Helminthosporium oryzae* when sprayed with 500 μg/ml A-435 showed a reduction in infected spots.

## INTRODUCTION

The polyenes are highly toxic to fungi but due to their unstable nature and toxic side effects in human beings only a few have been found suitable for clinical use. Still fewer have been studied for their effects on plants and plant diseases. Filipin exerted no effect on pea and tomato germination at 100 μg/ml when applied by soaking. It had no toxic effect on young tomato plants sprayed with 415 μg/ml (in 25% methanol) of filipin (Ammann *et al.* 1955). Chakraborty (1971) reported on the transportability of filipin in rice and pigeon pea. Aureofungin, dermostatin and nystatin were found to be systemic in activity in plants (Kadkol and Gopalkrishnan, 1971). Aureofungin has also been found to be active against many plant fungal diseases and widely used in many parts of our country (Naik, 1981).

A-435 is an extensively studied polyene antibiotic (Pal and Nandi, 1964, 1970; Chandra and Jarvis, 1972, 1975) isolated in our laboratory. In this investigation A-435 is studied for its toxic effects on plants and for controlling infection of *Helminthosporium oryzae* in rice plants.

## MATERIALS AND METHODS

10 mg A-435 was dissolved in 1 ml methanol. Further dilutions were made in sterile distilled water. Potato dextrose agar (PDA) was used for the growth of *Helminthosporium oryzae*. Rice (*Oryza sativa* L. cv Jaya) mung (*Phaseolus mungo* L. vc B-1) and maize (*Zea mays* L.) were obtained from the State Agricultural Farm, Kalimpong, W. Bengal. Incubations/growth were made at 30°C in dark chambers to minimize the light effect on A-435. Seeds/plants in sterile distilled water were used as controls.

*Phytotoxicity (germination)*

One hundred apparently healthy seeds of rice and mung were soaked in antibiotic solutions at concentrations of 0.1, 1, 10, 100 and 1000 μg/ml for 24 h on sterile moist papers in petridishes. Twenty five seeds were placed in each petridish and incubated. After 8 days shoot and root lengths were measured.

*Foliar spray*

Seedlings of rice and maize were raised in soil. After 15 days, 3 sets each with 50 seedlings were sprayed with 100, 500 and 1000 μg/ml A-435 for 5 consecutive days.

*Liquid cultures*

Fifteen days old rice and maize seedlings raised in soil, were uprooted, washed with water to remove adhering soil particles and placed in Knop's nutrient solution in 500 ml conical flasks and grown for 7 days A-435 was added at final concentrations of 50, 100 and 1000 μg/ml. The nutrient solution was changed on every alternate day and fresh antibiotic solutions were added to maintain constant concentrations. The experiments were conducted for 15 days, examining the seedlings for toxic effects at 5 day intervals.

*Translocation and persistence*

Seedlings of rice and maize were maintained in Knop's solution containing 50, 100 and 1000 μg/ml A-435 for 24 and 48 h. 1 cm pieces of the stem cut at different places were crushed and extracted with 5 ml n-butanol. The butanol extracts were dried, dissolved in 1 ml methanol-water (1 : 1 v/v) and assayed against *Saccharomyces cerevisiae* by the agar cup method of assay.

Persistence of the antibiotic translocated in the stem 12-72 h after the addition of A-435 was noted by the same method.

*Effect on rice plants infected with Helminthosporium oryzae*

The fungus was grown on PDA slants for 4 days and the spore suspension was fitered through sterile cotton pad to remove mycelial fragments. Spores were counted with haemocytometer and the concentration adjusted to $10^5$ spores/ml. 20 ml spore suspension was sprayed over 100 15-day-old rice seedlings and incubated. When pinhead sized infection spots were visible, the seedlings were sprayed with 20 ml of 500 μg/ml solution of A-435, 2 sprays per day for 5 consecutive days. Infected plants with no A-435 sprays were used as controls.

## RESULTS AND DISCUSSION

*Phytotoxicity*

In the untreated controls 87% of rice and 95% of mung seeds germinated. There was no significant difference in the percentage of germination of seeds treated with 0.1—100 μg/ml A-435. At 100 μg/ml, 88 and 94 per cent of rice and mung seeds germinated into normal seedlings. At 1000 μg/ml the percentage germination of the rice seeds was 46 and that of mung seeds only 32. Roots of germinated plants were shorter at 100 μg/ml (Table 1). Shoot growth was unaffected in mung but in rice plumule length was shortened by 10 mm when compared to the control. The sprouts of 1000 μg/ml treated seeds were too short and were not measured.

TABLE 1. *Effect of A-435 on growth of rice and mung seedlings**

| Seedling | | Length in mm at A-435 μg/ml concentrations | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 0.1 | 1.0 | 10 | 100 | 1000 |
| Rice | Shoot | 35 | 30 | 32 | 32 | 25 | — |
| | Root | 65 | 65 | 55 | 50 | 48 | — |
| Mung | Shoot | 75 | 78 | 65 | 75 | 75 | — |
| | Root | 35 | 40 | 50 | 40 | 25 | — |

* Average of 5 seedlings, measured after 8 days
\- not measured due to feeble growth.

In foliar sprays of 1000 μg/ml of A-435 yellowing and wilting of rice and maize plants were noticed in 9 days. Concentrations less than 1000 μg/ml produced no toxic symptoms.

In liquid cultures 100 μg/ml of the antibiotic arrested the growth of rice and maize seedlings in 15 days. 50 μg/ml had no effect. At 1000 μg/ml wilting and yellowing of the stunted plants were noticed in 15 days. Control plants in foliar sprays and liquid cultures showed no abnormalities.

*Translocation and persistence*

A-435 was translocated upwards, the translocation being faster in maize than in rice. Table 2 indicates that in 48 hr A-432 was translocated to 1 4 and 1 cm in maize and rice plants respectively. Assay of the stem after A-435 treatment showed that A-435 persisted in the plants for 48 hr without loss of activity.

*Effect on infected plants*

Rice plants infected with *Helminthosporium oryzae* when sprayed with 500 μg/ml A-435 showed 58.3% reduction in the number of infection points.

TABLE 2. *Translocation of A-435 in rice and maize plants*

| A-435 μg/ml | Translocation above ground level in cm in hours* | | | |
|---|---|---|---|---|
| | Rice | | Maize | |
| | 24 | 48 | 24 | 48 |
| 0 | — | — | — | — |
| 100 | 0.5 | 1.0 | 0.9 | 1.4 |
| 500 | 0.6 | 1.0 | 0.9 | 1.4 |
| 1000 | 0.6 | 1.1 | 0.8 | 1.5 |

*A-435 detected by agar cup assay with *Saccharomyces cerevisiae*

Since A-435 was found to be nontoxic to seeds and plants below 100 μg/ml and could be translocated into plants with retention of inhibitory activity, it could be used in nurseries to prevent fungal infections in young plants. Rice seedlings infected with *H. oryzae* could also be cured with 500 μg/ml A-435.

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