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GROWTH AND NITROGENASE ACTIVITY OF *AZOSPIRILLUM BRASILENSE* AS INFLUENCED BY FUNGICIDES

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THE advantages of using pesticides in agriculture prompted a rapid increase in their utilization in recent years. This caused concern among scientists about the possibile direct or indirect effects of using pesticides on human beings and animals, as well as other non-target organisms including soil micro-organisms of particular interest to the soil fertility. Although the role of *Azospirillum*, an associative symbiotic nitrogen-fixing bacterium in crop production[1,2] is well established, no information is available on the direct effect of pesticides on their growth and nitrogen fixing ability. Most of the studies have been conducted on symbiotic nitrogen-fixing bacterium, *Rhizobium*[3,4] as well as on heterotrophic nitrogen fixation[5,6]. The present study aims at evaluating the effect of certain commonly used fungicides on the growth and nitrogen-fixing ability of the strains of *Azospirillum brasilense*.

The fungicides with their active ingredients used in this study were Bavistin (carbendazim), Topsin (methyl thiophanate), Difolatan (copper oxychloride), and Hexacap (captan'). The strains S14 and S54 of *A. brasilense* isolated from the roots of *Cyanodon dactylon* and *Pennisetum typhoides* respectively were used.

To study the effect of fungicides on growth, nitrogen-free liquid malate medium supplemented with 250 ppm ammonium sulphate was used as the basal medium. Fungicides were incorporated before sterilization to get 100, 200 and 300 ppm concentrations. The medium was later distributed in 20 ml tubes of 6 ml each. Inoculation was done with 0.2 ml cell suspension (OD : 0.6) of the bacteria grown in

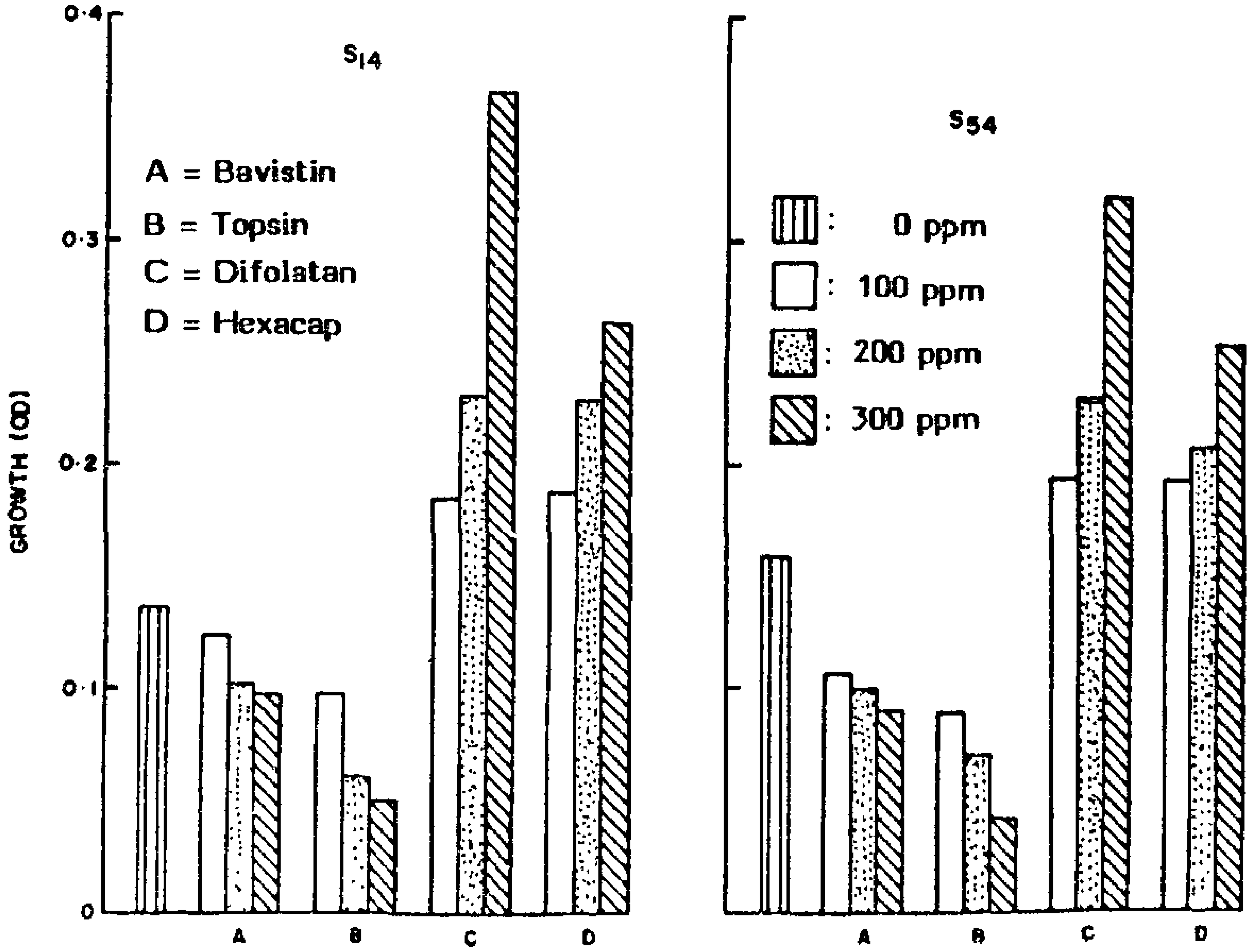

Figure 1. Growth of *A. brasilense* strains as influenced by fungicides.

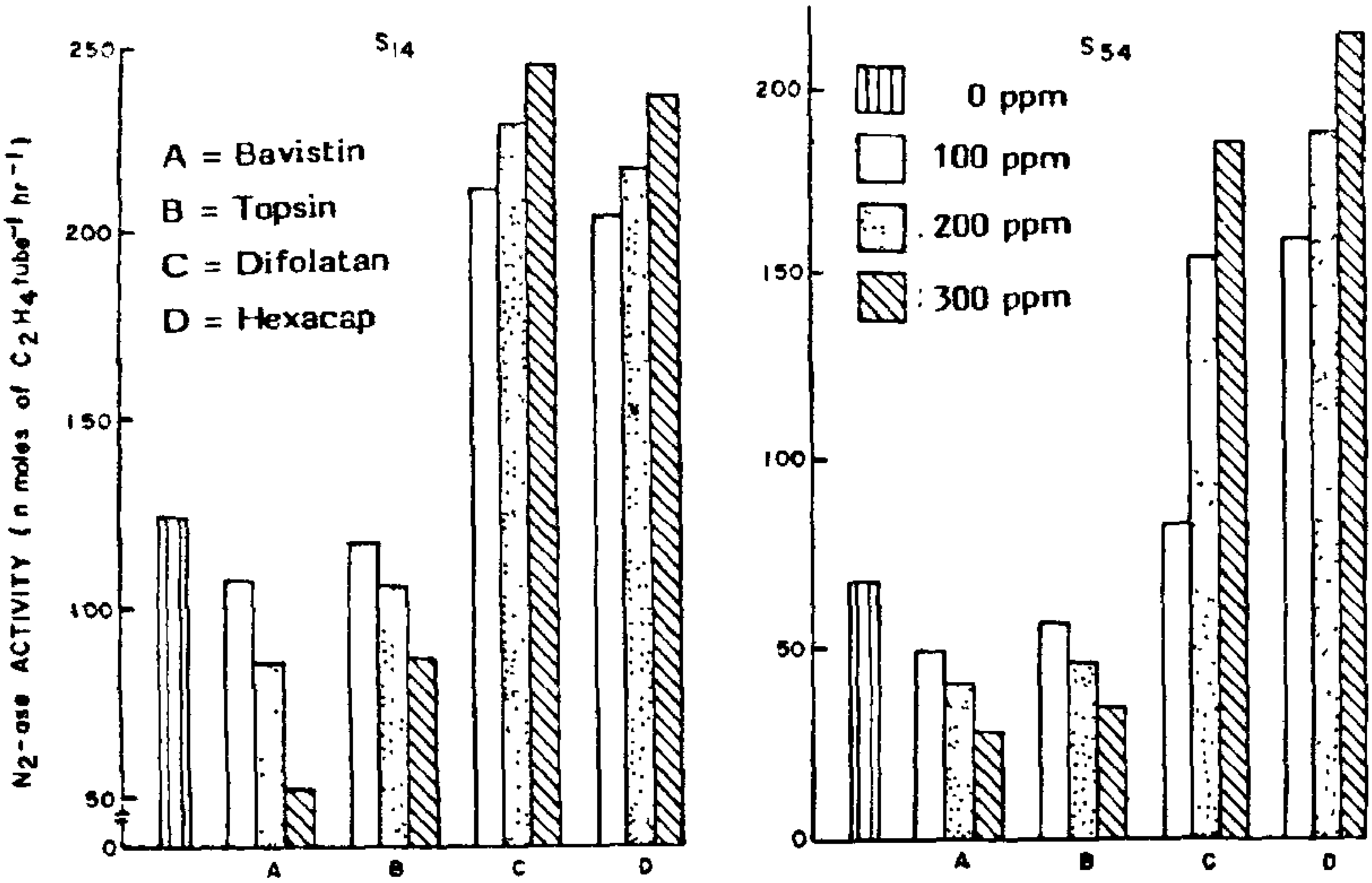

Figure 2. Effect of fungicides on nitrogenase activity of strains S14 and S54.

nutrient broth for 4 days. The tubes were incubated at 30 ± 1°C for 72 hr and the growth was measured as O.D at 520 nm in a Systronics spectrophotometer. The effect of fungicides on the N_2-ase activity was studied by incorporating different concentrations of the fungicides in 3 ml of N-free semi-solid malate medium. After 48 hr of incubation at 30 ± 1°C, the N_2-ase activity was estimated[2] and expressed as nmol of C_2H_4 produced per hour per tube.

The different strains of *A. brasilense* showed similar response to fungicides with regard to their growth in complete liquid medium (figure 1). Bavistin and Topsin inhibited the growth of both the strains. The growth inhibition increased with increase in the concentration of fungicides. Among the strains, the inhibition was greater in the S54 strain although it is a fast grower as compared to S14. Difolatan and hexacap were found to be stimulatory for the growth of both the strains. But the stimulation was greater with S14 as compared to S54. Similarly, consistent stimulatory or inhibitory effects of certain fungicides on the growth of *Rhizobium* spp. had been reported under controlled laboratory conditions[4]. Nayak and Rao[6] also reported the differential behaviour of *Azospirillum* and *Azotobacter* to different insecticides when treated in soil.

The strain S14 showed higher activity as compared to the S54 strain. With regard to N_2-ase activity, the response of these strains to different fungicides was similar to that of growth (figure 2). Bavistin and Topsin inhibited the activity while Difolatan and Hexacap stimulated N_2-ase activity. Similarly heterotrophic nitrogen fixation was stimulated by certain pesticides[5,6]. It was further reported that the growth of *Rhizobium* spp. was stimulated by a herbicide amitrole while dalapon was inhibitory[7]. The effects of pesticides on microorganisms or their activities varied with the pesticide and the ecological conditions[6,8]. The stimulation of growth and N_2-ase activity might be due to the availability of some micro/essential nutrients present in certain fungicides. Improvement in growth and productivity of certain crops upon fungicidal sprays besides controlling the diseases has been reported earlier[9,10]. Our studies lead us to conclude that fungicides should be used judiciously such that the important soil microbial process (N_2-fixation) is not adversely affected.

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INHERITANCE OF A SPIKELETLESS CHARACTER IN PEARL MILLET

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DURING the rainy season of 1983, an S_2 progeny of pearl millet (*Pennisetum americanum* (L.) Leeke), derived from IP 9503, an accession collected in Ghana[1], was found to be segregating for plants with normal and spikeletless spikes. The spikeletless mutant is characterized by the complete absence of spikelets from its entire spike (figure 1), although involucral bristles occur normally in mutant plants. The mutant plants produce relatively more tillers than the normal plants. Koduru and Krishna Rao[2] have recently summarized the literature on the inheritance of various qualitative characters in pearl millet. They did not report the occurrence or inheritance of the spikeletless trait amongst 21 spike and reproductive traits. The present note reports the inheritance of this character.

Remnant seed of the S_2 progeny segregating for the spikeletless mutant was replanted in the post-rainy season of 1983. The segregation pattern based on 52 plants showed a good fit to a ratio 3 normal : 1 spikeletless (table 1), indicating that the spikeletless trait may be inherited as a monogenic recessive. This hypothesis was tested by studying the segregation ratios in (i) S_3 progenies derived from selfing normal plants of the S_2 progeny, and (ii) F_2 progenies derived from crosses between two normal inbred lines and the plants heterozygous for the spikeletless allele.

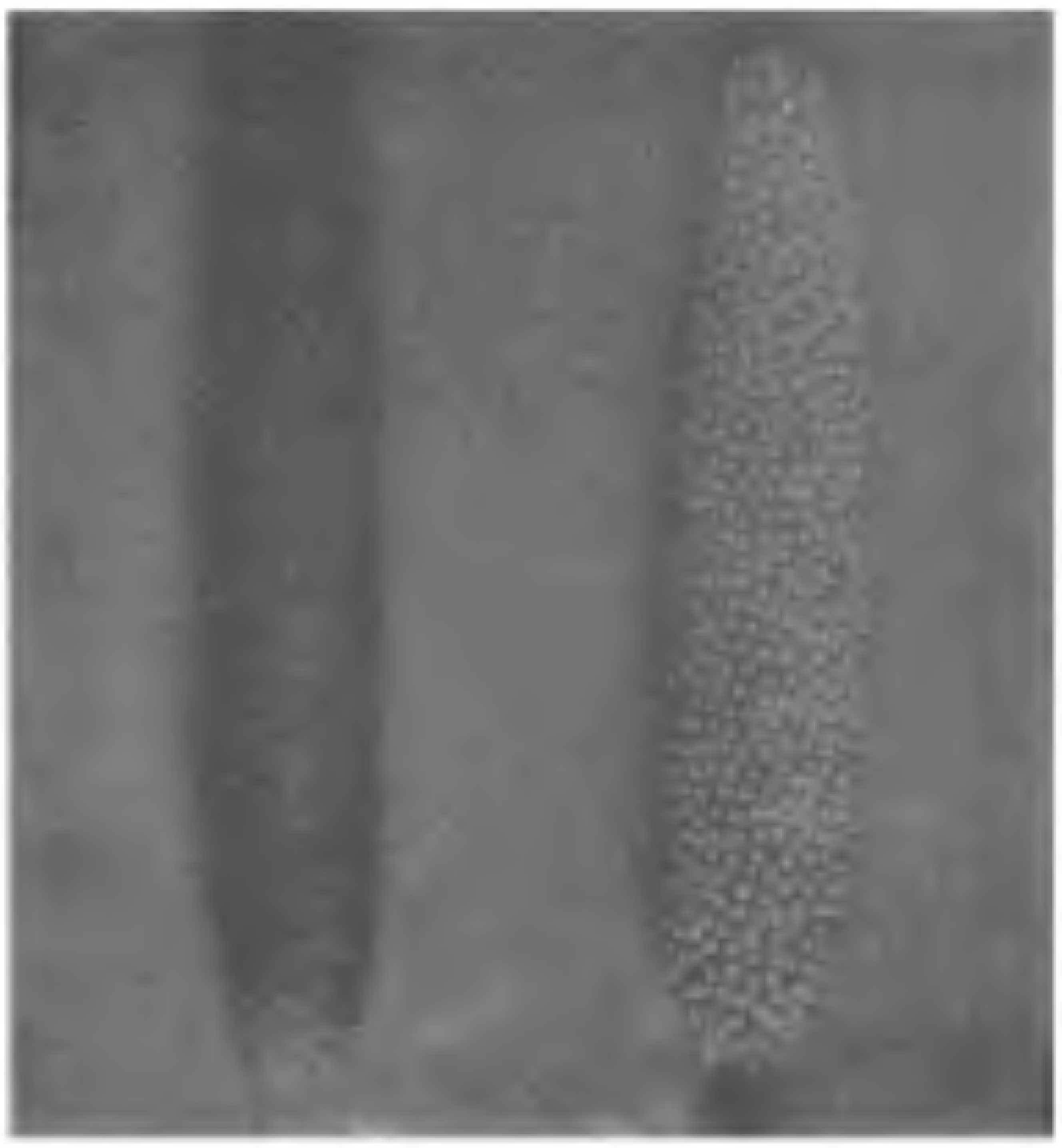

Figure 1. Pearl millet. Spikeletless spike (left) and normal spike (right).

According to the one-gene hypothesis, one-third of the normal plants in the S_2 progeny would be dominant homozygous and would breed true, whereas two-thirds of the normal plants, would be heterozygous and would segregate in the ratio of 3 normal : 1 spikeletless. Out of 13 S_3 progenies, 3 bred true for normal plants, and 10 segregated for normal and spikeletless plants, showing a good fit to 2:1 ratio ($\chi^2 = 0.615$; $P = 0.5$–0.3). The segregating S_3 progenies, consisting of 447 plants, showed a good fit to 3 normal : 1 spikeletless plants and the heterogeneity test[3] showed the segregation ratio across progenies to be homogeneous (table 1).

Normal plants of the segregating S_3 progenies were selfed as well as crossed onto two inbred lines: 81 B and 843 B. The S_4 progenies were grown to identify those segregating for normal and spikeletless plants. This enabled us to finally select the F_1 hybrids involving those plants from S_3 progenies that were heterozygous for the mutant trait. Four such F_1 hybrids, two each involving 81 B and 843 B, were selected and selfed to produce F_2 progenies. According to the one-gene hypothesis, half of the F_2 progenies would breed true for normal plants, and the other half would segregate for spikeletless plants. Five F_2 progenies were grown from each of the four crosses. Out of 10 F_2 progenies involving