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NITROGEN FIXATION BY *AZOSPIRILLUM* SP. ISOLATED FROM BENOMYL-AMENDED RICE SOIL

THE participation of nitrogen-fixing *Azospirillum* in several grass-bacteria associations and its high nitrogen-fixing potential in various ecosystems have been recognised recently[1, 3]. The occurrence of nitrogen-fixing *Azospirillum* in rice roots and soils has been reported recently[2,4,6]. Although the intensive use of pesticides in agriculture can lead to ecological disturbances in the environment, the effect of pesticides on *Azospirillum* population and nitrogen fixation has not been investigated. The present report deals with the effect of benomyl, a systemic wide-spectrum fungicide, on the population of *Azospirillum* and nitrogen-fixing efficiency of cultures isolated from benomyl-amended soils.

Technical grade benomyl [methyl-1-(butyl-carbamoyl)-2-benzimidazole carbamate] was applied at *0, 10, 20 and 100 ppm concentrations to a flooded paddy soil. The population of Azospirillum sp. in* unamended and benomyl-amended soils was estimated as follows: Tenfold dilution of the soil samples were *transferred to 10 ml of nitrogen-free semi-solid malate* medium[3]. The inoculated culture tubes, replicated five times, were then incubated for 48 hr. at 30° C. Positive identification of *Azospirillum* sp. was recorded when there was the formation of very characteristic white, dense undulating fine pellicle a few mm below the surface of the semi-solid medium and microscopic observations for the confirmation of the presence of the typical *Azospirillum*. Population density of *Azospirillum* in soils was determined by most-probable numbers on 10, 20 and 30-day incubation under flooded conditions. The nitrogen-fixing efficiency of *Azospirillum* cultures obtained from benomyl-amended and unamended soils incubated for 10, 20 and 30 days was also determined.

Striking stimulation of *Azospirillum* population occurred at all concentrations (10, 20 and 100 ppm) of benomyl used, but this stimulation was less marked at the highest concentration of 100 ppm (Table I). *Azospirillum* cultures isolated from benomyl-amended soil, in general, exhibited greater nitrogen-fixing efficiency than the cultures from unamended soils irrespective of the duration of the soil incubation (Table II). Although *Azospirillum* cultures from 100 ppm benomyl-amended soils exhibited greater nitrogen fixation than the cultures from 10 and 20 ppm levels, nitrogen fixation was low in cultures isolated from 30-day incubated samples with 100 ppm benomyl.

TABLE I

Effect of benomyl on the population of Azospirillum *sp. in a flooded rice soil*

Benomyl concentration (ppm)	MPN of *Azospirillum* × 10^5/g dry soil		
	Incubation (days)		
	10	20	30
0	20	14	5
10	20	200	44
20	20	200	200
100	3	115	44

TABLE II

Nitrogen-fixing efficiency of Azospirillum *cultures isolated from benomyl-amended flooded soil*

Benomyl concentration (ppm)	mg N fixed/g. malate by *Azospirillum* sp.		
	Soil incubation (days)		
	10	20	30
0	2·97	5·07	6·10
10	5·20	5·87	10·23
20	7·17	11·80	10·10
100	9·33	12·00	7·13

Stimulation of nitrogen fixation in soil following pesticidal application has been reported[7]. It has

been recently demonstrated that application of certain herbicides also stimulate *Azospirillum* population in soils[5]. These studies also suggest that benomyl besides fungicidal in its effect also stimulates certain beneficial microorganisms involved in nitrog n fixation in soils under flooded conditions.

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A NEW RECORD OF PROTOMYCES MACROSPORUS UNG. ON *FOENICULUM VULGARE* L.

DURING a survey of diseases of Bareilly region, the authors observed for the first time the galls on the stems of *Foeniculum vulgare* in March 1978. The pathogen was identified as *Protomyces macrosporus* Ung. Characteristic tumor-like swellings initially observed on stem and leaf sheaths, subsequently spread on the peduncle and inflorescence. Peduncles were hypertrophied and curved. Some of them did not bear flowers due to cessation of growth while others had fewer flowers as compared to normal ones. In later stages seeds also got infected and hypertrophied (Fig. 1–A, B and C). Stem galls were about 3 mm broad and upto 20–25 mm long and were confined to upper region of the stem.

It was intersting to note that the disease was confined only to the plants growing among heavily infected coriander fields. The plants away from the coriander fields showed rare infection. The disease flared up after a brief spell of rain.

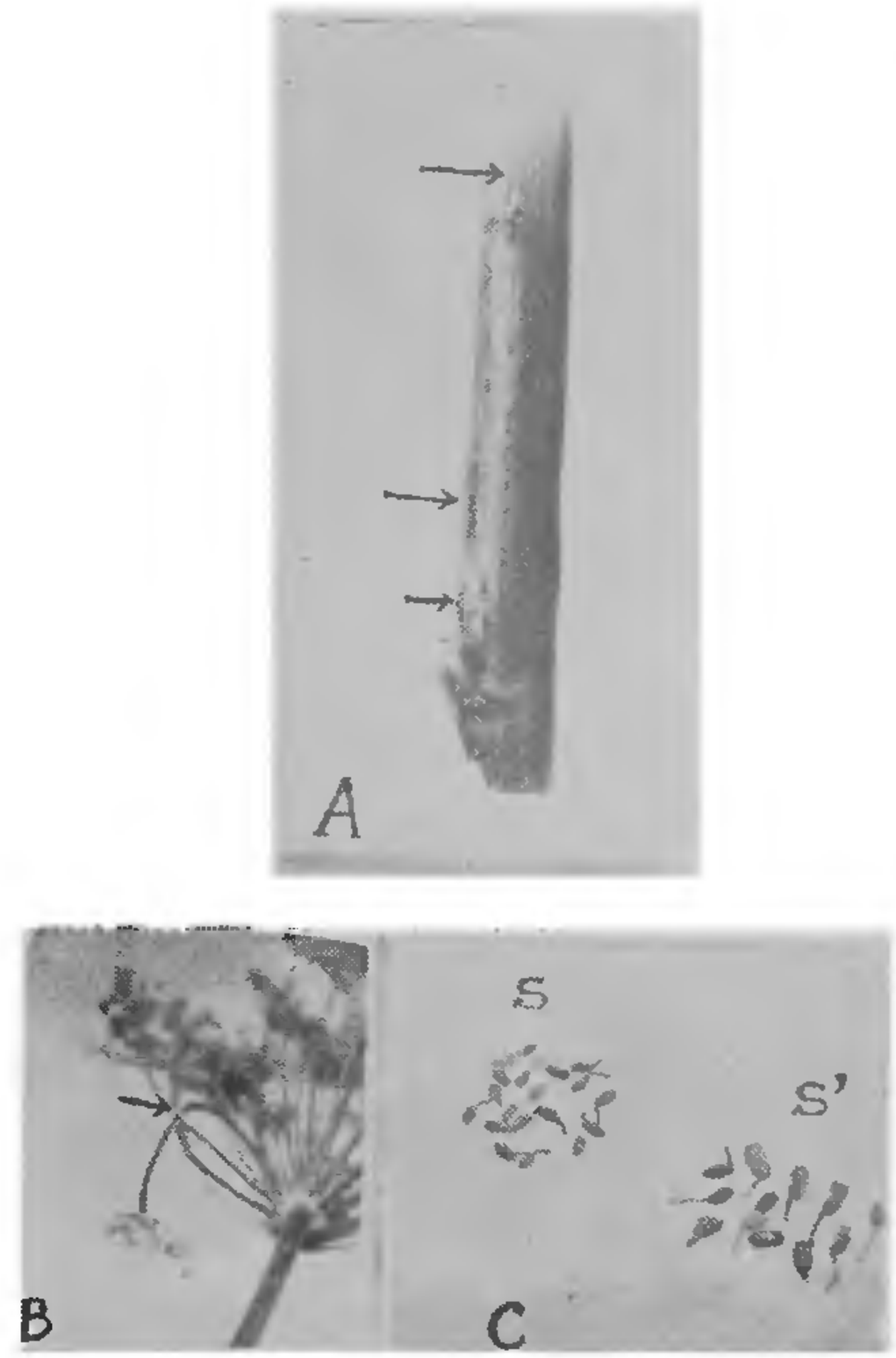

FIG. 1. Symptoms of *Protomyces macrosporus* Ung. on *Foeniculum vulgare* L. A—Tumor-like swellings on the stem (marked). B—Hypertrophied and curved peduncle (marked). C—Normal (S) and hypertrophied infected (S') seeds.

Microscopic investigation revealed the occurrence of chlamydospores and mycelium in the cortex, phloem, xylem parenchyma and pith of the host. The fungus was restricted to the tumors. Mycelium was intercellular, irregularly branched, closely septate, 10 to 12·5 μ broad; mature chlamydospores golden yellow, thick walled, three layered, attaining a diameter of 40 to 65 μ.

Foeniculum vulgare L. (vern. saunf, fam. Umbelliferae) is widely cultivated for its uses as medicine, spices and masticatories. The infection in floral parts and seeds severely damages the quality and yield. A perusal of available literature revealed that it is a first record of *Protomyces macrosporus* on *Foeniculum vulgare* from India and abroad. The report of Dr. Sivanesan of CMI, Kew, England, has confirmed that "*Protomyces macrosporus* Ung. has not been reported on *Foeniculum* before". The only host record of this fungus, so far available in India,