



FIG. 1. Negative staining of *Azospirillum* isolated from *O. vulgaris*.

ab. Nitrogenase activity of these isolates was estimated after 7 days of incubation of the cultures containing 3 ml of N₂-free semi-solid malate medium inoculated with 0.1 ml of 4-day old inoculum.

The results (Table I) indicate that the species of *Opuntia* are able to fix atmospheric nitrogen through the association of *Azospirillum* as was reported in other plants^{1,2,3}. The nitrogenase activity of these species varied from 32.2 to 75.7 nmoles/g of dry roots/24 h. Microscopic observations of cross-sections of the roots revealed that the cortical cells are

TABLE I

Nitrogenase activity of roots of Opuntia spp. and strains of Azospirillum isolated from the roots

Plant species	Nitrogenase activity (n moles of C ₂ H ₄)	
	Roots g/24 h	Pure culture/h
<i>Opuntia coccinellifera</i>	75.7	72.9
<i>O. microdays</i>	54.5	96.2
<i>O. vulgaris</i>	32.2	273.6

filled with small rod-like bacteria. *Azospirillum* sp. was isolated from within the roots of all the species. These cultures were highly motile showing characteristic spiral movements and formed white subsurface pellicles in N₂-free semi-solid malate medium indicating the microaerophilic nature of these bacteria. The pure cultures exhibited nitrogenase activity varying from 72.9 to 273.6 nmoles/h, with the maximum activity with the isolate made from *O. vulgaris*. The activities of these cultures compare with those of the strains isolated from graminaceous

plants⁴. These cultures generally preferred organic acids as carbon source as compared to sugars. The nitrogenase activity of the cultures was reduced by the addition of 100 ppm of ammonium sulphate indicating the repression of the enzyme. The association of *Azospirillum* with the roots of *Opuntia* spp. might be due to the accumulation, and root exudation of organic acids especially malate in these plants having crassulacean acid metabolic pathway of carbon dioxide assimilation⁶.

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OBSERVATIONS ON SEEDLING ALLELOPATHY IN WEEDS

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THE term allelopathy in current literature refers to the detrimental effects of one plant on germination, growth or development of another, through the production of toxic chemical compounds that are released into the environment^{1,2,3}. Investigations of allelopathic interactions thus far have been limited to mature plant parts. Since seedlings are actively growing juvenile plants with more vigorous metabolic activities, they could also produce allelopathic substances, which would be added to the environment. This fact could be ecologically very significant. Keeping this in view ten species of weed seedlings were examined for their allelopathic potential and the results are presented in this communication.

Air-dried seedlings (5 g) of 10 species (Table I) were crushed and soaked in 50 ml of distilled water for 24 h. The aqueous extracts prepared thus were filtered

TABLE I

Effect of seedling extracts of some weeds on early seedling growth of til (after 48 h) at $27^{\circ} \pm 2^{\circ} \text{C}$.

Plant species	Seedling extracts in percentage					
	1		5		10	
	R	H	R	H	R	H
<i>Alysicarpus vaginalis</i> (L.) DC.	3.5 ± 1.0	4.0 ± 1.0	2.0 ± 0.5	3.5 ± 0.5	nil	nil
<i>Borreria articularis</i> (L.) F. N. Will	8.0 ± 1.0	4.5 ± 0.5	4.5 ± 0.5	4.0 ± 0.2	nil	nil
<i>Crotalaria medicaginea</i> Lamk.	2.0 ± 0.5	3.0 ± 0.5	1.5 ± 0.5	1.7 ± 0.7	nil	nil
<i>Cucumis callosus</i> (Rottl.) Cogn.	8.0 ± 2.0	4.5 ± 0.5	4.5 ± 0.5	4.5 ± 0.5	nil	nil
<i>Indigofera cordifolia</i> Heyne ex Roth.	10.5 ± 0.2	4.5 ± 0.5	5.5 ± 0.2	4.0 ± 1.0	vlp	nil
<i>Ipomoea pes-tigridis</i> Linn.	7.0 ± 2.0	4.2 ± 0.5	4.0 ± 0.5	4.0 ± 1.0	nil	nil
<i>I. sindica</i> Stapf.	9.0 ± 1.0	4.5 ± 0.5	5.0 ± 0.2	4.0 ± 1.0	vlp	nil
<i>Merremia aegyptia</i> (L.) Urban	5.5 ± 0.2	4.0 ± 1.0	3.2 ± 0.2	3.5 ± 0.5	nil	nil
<i>Tephrosia purpurea</i> (L.) Pers.	4.2 ± 0.7	4.0 ± 1.0	3.0 ± 0.2	4.5 ± 0.5	nil	nil
<i>Trichodesma sedgwickianum</i> Banerj.	11.5 ± 1.5	4.5 ± 0.5	5.5 ± 0.2	4.0 ± 1.0	vlp	nil

Control: R = 17.0 ± 1.0 , H = 5.5 ± 0.5 ; R = radicle, H = hypocotyl, vlp = very little protrusion.

through Whatman filter paper No. 1. A portion of the filtrate (10%) was diluted with distilled water to prepare 5 and 1% extracts. Seeds of til (*Sesamum indicum* L.) were kept for germination in sterilized petridishes lined with a single layer of filter paper moistened with 2 ml of aqueous extracts. For the control, distilled water was used as germination medium. 20 seeds were used for the bioassay and the experiment was conducted in triplicate sets at $27^{\circ} \pm 2^{\circ} \text{C}$ for 48 h. At the end of the experiment the radicle and hypocotyl lengths were measured to the nearest mm.

It was observed that the aqueous extracts suppressed the early growth of til (Table I) at 1 and 5% concentrations; whereas at 10% concentration even the seed germination was inhibited. There was very little protrusion of radicle but no further growth of til in 10% extracts of *Indigofera cordifolia*, *Ipomoea sindica* and *Trichodesma sedgwickianum*. Germination of til was completely inhibited by this concentration of aqueous extracts from all other species of weed seedlings used in the present study. Extracts of the three species mentioned above were most effective even at the lower concentrations and the effect was more marked on the radicle than on the hypocotyl.

Of all the techniques and solvents employed for the extraction of inhibitors and bioassaying the extracts, the petridish culture and the use of cold water have been regarded as the most efficient¹. Aqueous extracts are ecologically more significant than organic solvents⁴. The effects of aqueous extracts of seedlings were more marked on radicle since young roots are known to be more sensitive than young shoots⁵. The inhibitory properties of aqueous extracts of weed seedlings is attributed to the presence of germination

inhibitors in the extracts since germination inhibitors are known to occur in a wide variety of mature seed plants and in their different parts⁵. It is therefore speculated that all these 10 species of weed seedlings could affect the crop growth by producing allelopathic substances even in the field conditions. Since til is the only commercial oil crop in Indian arid zone, these weed species are agro-ecologically very significant from the very seedling stage of their appearance in the field.

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MITOMYCIN C INDUCED MORPHOLOGICAL ABNORMALITIES IN A GREEN ALGA, *RHIZOCLONIUM HIEROGLYPHICUM* (AG.) KUETZ. (CHLOROPHYCEAE)

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THE effects of Mitomycin C (MMC), a known mutagen are studied well in prokaryotes as well