

pure culture synthesis of ectomycorrhizae between *S. citrinum* and *P. patula* and serves as a basis for future experimentation.

Based on its abundant occurrence even during dry seasons (author's observation, unpublished) *S. citrinum* appears to have potential value in reforestation programmes in India similar to *Pisolithus tinctorius* in USA<sup>2</sup>.

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## INDUCTION OF PROLINE ACCUMULATION BY METHYLPARATHION IN SORGHUM (*SORGHUM BICOLOR* L.)

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PLANTS have been known to accumulate high levels of proline when subjected to water stress<sup>1</sup>, salinity<sup>2,3</sup>, high and low temperatures<sup>4-7</sup>, nutrient deficiencies<sup>8,9</sup>, water-logging<sup>10</sup>, fungal infection<sup>11</sup> and air pollutants<sup>12-14</sup>. The biochemical mechanism leading to the induction of proline accumulation and its physiological significance have not been eluci-

dated although it has been suggested that proline may be involved in osmoregulation<sup>15,16</sup>. Since many of the stress factors induce proline accumulation, and no information is available on the effects of pesticides with regard to proline accumulation, it was of interest to study the effect of pesticide stress in plants with reference to this physiological response. Methylparathion, an organophosphorus pesticide, is widely used as an insecticide as a spray and the residual effect of this pesticide has been known to remain in the environment for a long time<sup>17,18</sup>. Therefore, it was decided to study the action of this pesticide using both as a spray and for seed treatment since the seeds when sown are exposed to the pesticide residue in the soil.

Certified seed of sorghum (*Sorghum bicolor* L.), var. CSH-1, was purchased from the Karnataka Seeds Corporation. Technical grade methylparathion was a gift from Bayer (India) Ltd. The seeds were soaked for 1 hr in different concentrations of methylparathion (as given in the legend to figure 1), surface-washed and then sown on moist filter papers in petri dishes. Fresh and dry weights were measured after 72 hr of growth of the seedlings and the results are plotted according to Prat *et al*<sup>19</sup>. Proline contents were estimated<sup>20</sup> in the seedlings grown in plastic pots containing soil, at different time intervals after 5 days of initial growth and the results are plotted in figure 2. Proline estimations were terminated on the third or fourth day after the initial 5 days

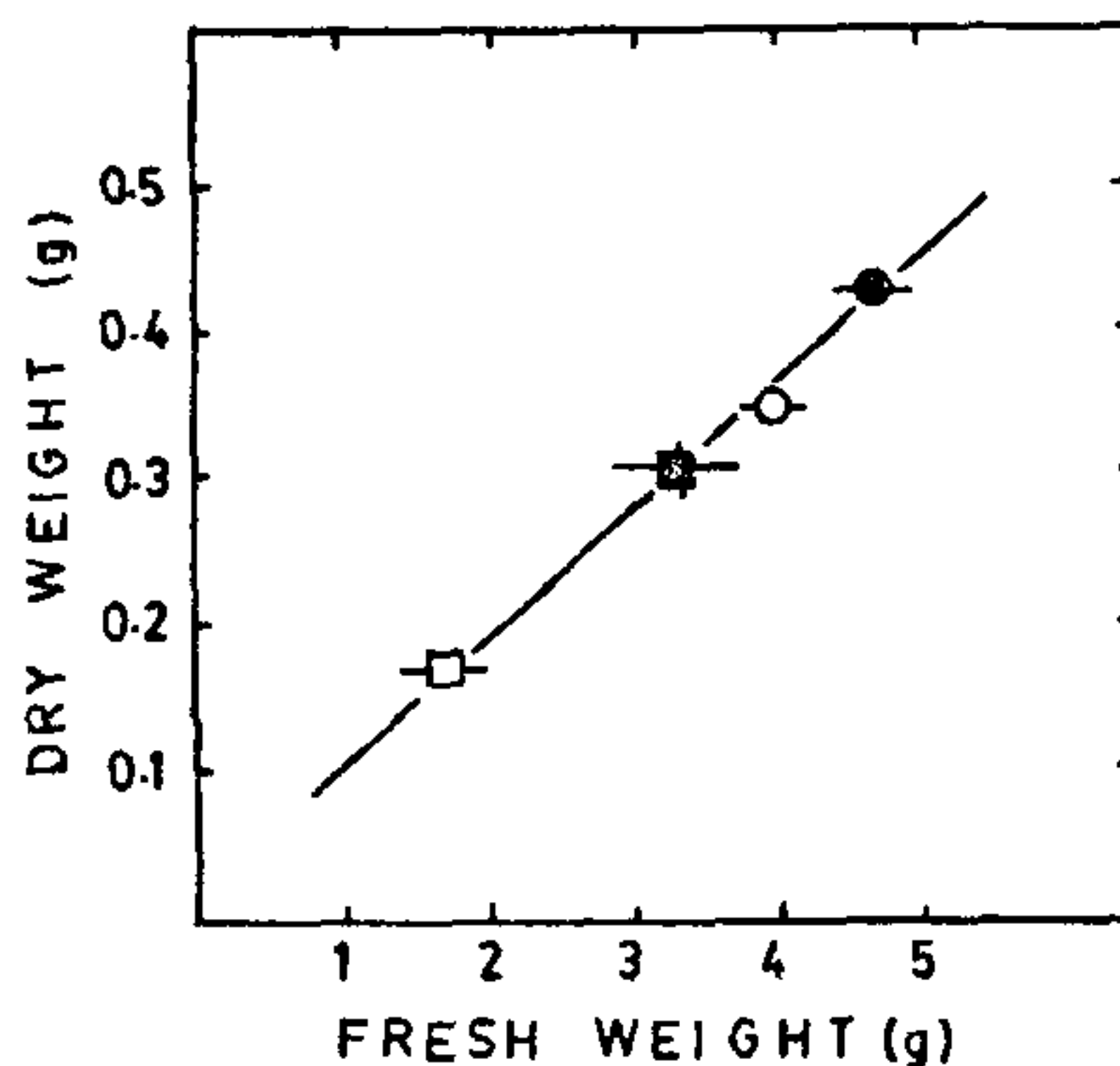
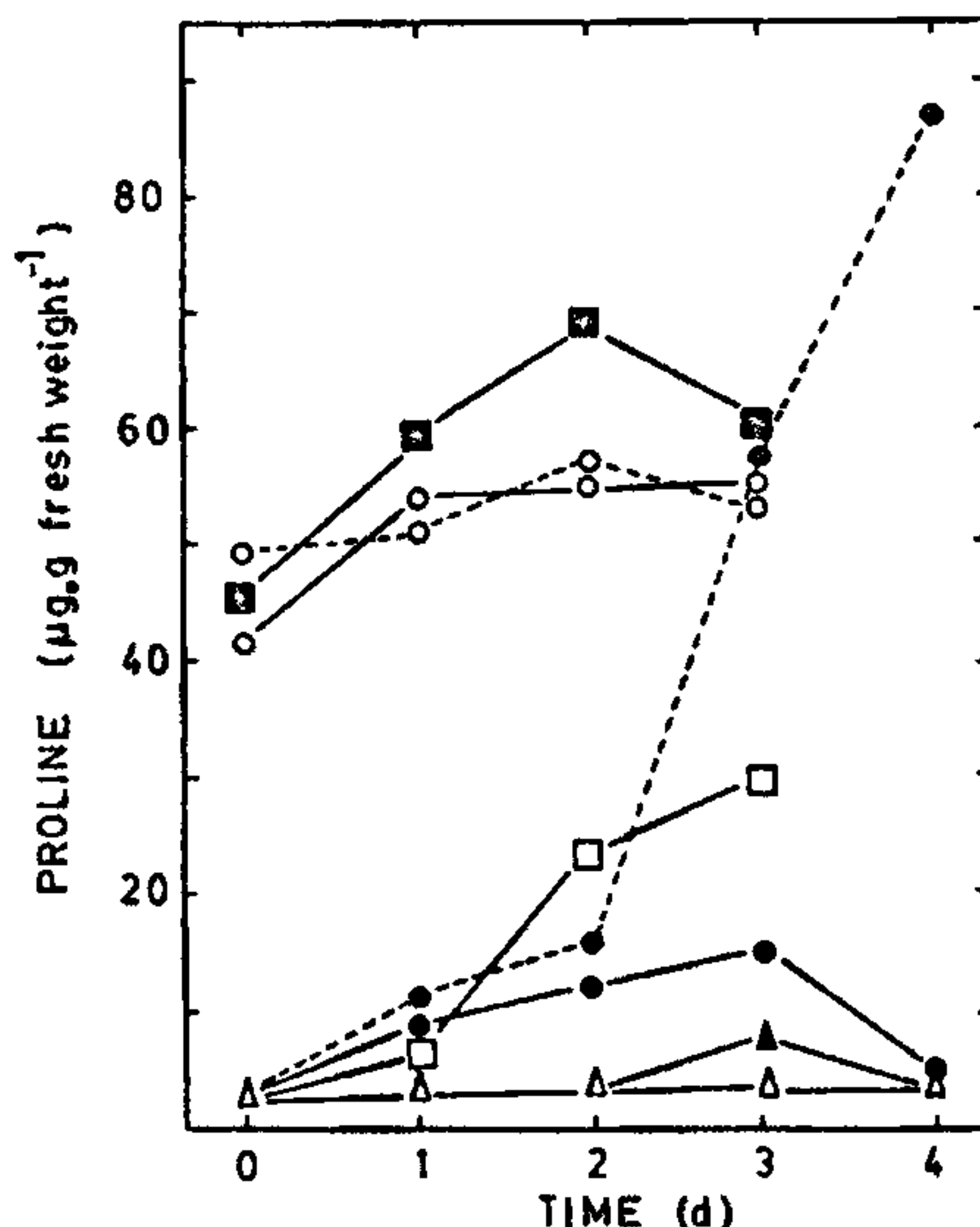


Figure 1. Kinetics of growth inhibition of sorghum seedlings exposed to methylparathion. [● Untreated; ○ 100 ppm; ■ 200 ppm and □ 400 ppm methylparathion treated seedlings. The horizontal and vertical bars represent the standard error of the mean values of fresh and dry weights respectively.]

of seedling growth as the plants required watering to continue the experiment.

Figure 1 represents the kinetics of growth of the seedlings when the seeds are exposed to methylparathion of different concentrations for 1 hr before germination. The results show a linear and steep reduction in the growth rate caused by increasing levels of the pesticide. These results also indicated that the water content of the tissue remains unchanged (90%) on treatment with methylparathion. Figure 2 shows a constant and low level of proline in untreated seedlings. When the water potential was reduced to a level very near the threshold level ( $-2.5$  MPa) for the induction of proline accumulation in sorghum<sup>1</sup> as a response to drought, by the addition of mannitol into the soil there was a slight increase in the proline content after one day and subsequently a steep increase was observed. Interestingly, in the case of seeds exposed to the pesticide (both in pesticide presoaked seeds and the ones in the pesticide pretreated soil) there was a pronounced increase in the levels of proline up to 100% over the levels of the water-stressed seedlings at the threshold level. By contrast, in the seedlings sprayed with twice (1000 ppm) that concentration of methylparathion only a limited and insignificant increase in proline was observed on the third day of spraying and this dropped down to the normal level on the fourth day. This drop in the proline level was due to the redistribution of the N which occurs at the end of the stressed condition<sup>16</sup>. There was no significant difference in the levels of proline in the plants grown in the soil pretreated with  $20 \mu\text{g}$  and  $50 \mu\text{g}$  of methylparathion per gram soil. However, in cases where the soil was treated with  $20 \mu\text{g}$  of the pesticide per gram soil after 5 days of initial growth of the seedlings there was only a marginal increase in the proline levels up to the third day of treatment and it came down to the normal level on the fourth day. A higher level of proline was found to accumulate on the third and fourth day of treatment in the seedlings treated with  $50 \mu\text{g/g}$  soil after 5 days of initial growth. The increase in the proline levels in the latter could be correlated with the degree of wilting; a high degree of wilting was observed on the fourth day of treatment. No wilting was observed in any other pesticide treatments. However, there was wilting in the seedlings grown in soil with low water potential.

It has been suggested that proline accumulation in response to high temperature and salinity could be due to a disturbance in tissue water status compared to that observed during simple water deficits<sup>3,5</sup>. But



**Figure 2.** Proline accumulation in sorghum seedlings in response to methylparathion and water stress.  $\Delta-\Delta$  Untreated seedlings;  $\blacktriangle-\blacktriangle$  Methylparathion sprayed (1000 ppm) after 5 days of seedling growth;  $\bullet-\bullet$  Soil treated with methylparathion ( $20 \mu\text{g/g}$  soil) after 5 days of growth;  $\bullet-\bullet$  Soil treated with methylparathion ( $50 \mu\text{g/g}$  soil) after 5 days of growth;  $\circ-\circ$  Soil pretreated with methylparathion ( $20 \mu\text{g/g}$  soil) before sowing the seeds;  $\circ-\circ$  Soil pretreated with methylparathion ( $50 \mu\text{g/g}$  soil) before sowing the seeds;  $\square-\square$  Soil treated with mannitol (1 M) after 5 days of growth;  $\blacksquare-\blacksquare$  Seeds treated with methylparathion (500 ppm) for 1 hr before sowing. All the estimations represent the mean of 3 sets of experiments. The standard deviation of the mean was  $< 0.8 \mu\text{g}$  proline per gram fresh weight of the tissue.

the fact that the seeds of sorghum exposed to methylparathion for 1 hr before germination could sustain high levels of proline (figure 2) for more than a week in the plants without any significant change in the water content of the tissue indicates that this physiological response is not exclusively expressed against water stress. In addition, proline accumulation as a response to nutrient deficiencies<sup>8,9</sup> waterlogging<sup>10</sup>, low temperature<sup>4,7</sup>, air pollutants<sup>12-14</sup> and the alterations of this response caused by plant

growth regulators<sup>21-23</sup> also question the argument explaining the accumulation caused by all these factors via a disturbance in tissue water status<sup>1</sup>. The vast difference in the proline levels between the plants exposed to methylparathion before seed germination and those after 5 days of germination (figure 2) indicates that the pesticide requires a suitable physiological condition to induce proline accumulation. This difference in the response was exhibited even in the continuing presence of the pesticide during the seedling growth until the harvest was made for proline estimations. Therefore, these results warrant a search for the common mechanism of the induction of proline accumulation in plants caused by various stress factors.

From the agronomic point of view, these results would further convey that methylparathion is relatively nontoxic to crop plants as a spray, while as residue in the soil it seems to exert a greater influence on the seed germination, seedling growth and metabolism.

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#### VIABILITY OF *CERCOSPORA CANESCENS* CONIDIA UNDER SIMULATED AIRBORNE CONDITIONS

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BLACK gram (*Vigna mungo* (L.) Hepper) is an economically important and highly priced pulse crop, cultivated throughout India, under a wide variety of edaphic and climatic conditions. Among the foliar fungal diseases of black gram leaf spot caused by *Cercospora canescens* Ell. & Mart. brings heavy destruction to the photosynthetic area. The conidia of this pathogen are aeri ally dispersed<sup>1</sup>. But for how long they retain viability under airborne conditions is not known. Since it is very difficult to bring the spores released into air back to a substratum after a period of time, the effect of aerial environment on conidial viability in *C. canescens* was studied in simulated airborne conditions.

Such a condition was created using fine threads drawn from the silkworm cocoon. The threads were wound round to two arms of a special metal fork and the threads were coated with dry conidia from freshly sporulating leaf spot. Twenty-four such units were prepared and exposed to natural conditions near the field. Two units were brought back to the laboratory at each hourly intervals, placed on slides