The growth rate studies supported the above observation. The control cells showed (figure 1) very short duration of lag and plateau phases whereas cells with 1%, 0.5%, 0.25%, and 0.1% honey showed three days lag phase and 18 days plateau phase. When honey was reduced to 0.5%, 0.25%, and 0.1%, the growth rate remained the same as the controls (figure 2). Replacement of MEM with Parker's medium 199 further prolonged the plateau phase.

Effect of honey at different concentrations (10%, 5%, 2.5%, 1.2%) was also studied on other cell cultures (like HeLa, HEP-2, McCoy, PS, CHO, MFS-8, etc primary chick embryo fibroblasts and human erythrocyte cultures). It was found that 1% honey in the medium is optimum for these cells except chick embryo fibroblasts. For chick embryo fibroblasts 0.5% honey in the medium was optimum (unpublished data).

Prolongation of plateau phase of LM cells in suspension by supplementation of glucose was shown by Eidman and Merchant.

In conclusion the natural honey at the concentration of 1% in the medium helps to maintain the cells in healthy condition, prolongs the plateau phase and controls the cell proliferation.

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EFFECT OF ETHYL-METHANE SULPHONATE ON TISSUE CULTURE OF GARLIC (ALLIUM SATIVUM L.)

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Tissue culture studies of Allium sativum L. (Garlic) have been carried out by several workers. Karyological studies by Novák show that variations may be possible in cultures. Since garlic does not reproduce sexually, it was important to induce variation through
mutation. The present study deals with the effect of the mutagen ethyl-methane sulphonate (EMS) on the physiology and the cytology of the garlic callus.

Garlic cloves were subjected to EMS treatment for 2 hr by soaking\textsuperscript{11}. The three concentrations of the mutagen (0.025\%, 0.05\% and 0.1\%) were prepared using phosphate buffer (pH 7) and then sterilized by passing through sintered glass bacterial filter (no. 5). For the 'control', garlic cloves were soaked in distilled water for the same time interval. The treated cloves were washed thoroughly and surface-sterilized using 5\% chlorogen. Thick slices (1–2 mm) were made and inoculated on Murashige and Skoog's basal medium\textsuperscript{6} supplemented with 4 mg/l IAA (indole acetic acid), 2 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid), 4 mg/l kinetin and 15\% coconut milk.

In mutagen-treated explants, the callusing response was rather poor as compared to control; and browning of explants was observed in some cases. The callus obtained was straw-coloured loose mass of globules. At the end of 60 days the callus was analysed for protein and RNA content using standard procedures\textsuperscript{5,10}.

It was observed that EMS brought about a reduction in protein content as well as in RNA content. This reduction increased with the increasing concentrations of mutagen (table 1).

Acetocarnine squashes were used for cytological studies. An increase in the percentage of polyploid cells was observed with EMS treatment. Characteristic effects of EMS such as chromosome breakage or occurrence of fragmentation were not observed (figures 1, 2 and 3, table 1).

As, the normally expected mutagenic effect is not

\begin{table}[h!]
\centering
\caption{Effect of EMS on protein and RNA content and on cytology of garlic callus.}
\begin{tabular}{lcccc}
\hline
Treatment & Dry wt (mg) & Proteins (mg/g dry wt) & RNA (mg/g dry wt) & Mitotic index (%) & Polyploid (%) \\
\hline
Control & 103 & 26.32 ± 0.47 & 15.71 ± 0.18 & 2.20 & 1.25 \\
0.025\% EMS & 98 & 20.5 ± 0.82\textsuperscript{*} & 15.15 ± 0.26 & 1.91 & 6.70 \\
0.05\% EMS & 85 & 18.95 ± 0.58\textsuperscript{*} & 6.67 ± 0.64\textsuperscript{*} & 2.00 & 7.19 \\
0.1\% EMS & 79 & 17.86 ± 0.63\textsuperscript{*} & 4.88 ± 0.18\textsuperscript{*} & 1.82 & 4.52 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{*} ± standard error of mean; \textsuperscript{*} \textit{t} values significant at 95\% confidence limits. 24 explants/treatment. Results expressed as average of 3 replicates.
observed and there is an increase in the number of polyplid cells accompanied by changes in the physiological patterns of the cell, it is felt that such changes could be well exploited in following up the differentiating pattern of these polyplid cells in culture, so as to obtain better variation and desirable characters.

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Table 1 Inhibitory effects of stigmatic exudates of sterile C. malabaricum on in vivo germination of viable pollen of different species of Chlorophytm.

<table>
<thead>
<tr>
<th>Species</th>
<th>% of germination</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. heyneanum</td>
<td>61.7</td>
<td>40.5</td>
</tr>
<tr>
<td>C. tuberosum</td>
<td>68.1</td>
<td>50.3</td>
</tr>
<tr>
<td>C. elatum</td>
<td>65.3</td>
<td>48.3</td>
</tr>
<tr>
<td>C. attenuatum</td>
<td>61.6</td>
<td>42.9</td>
</tr>
</tbody>
</table>

* Average of counts from ten stigmas.

These in vivo studies were supplemented by tests on the effect of exudates of sterile species, especially of C. malabaricum, on pollen inhibition in vivo. The stigmas of the fertile C. heyneanum were suitable for these tests since they supported pollen germination of all related species although intraspecific pollinations rarely led to fruit set. Fertile C. heyneanum stigmas were treated as follows. Forty stigmas of this species were dipped in the exudates of sterile C. malabaricum for 1 hr. Pollen grains of the four fertile species listed in table 1 were separately collected and pollens of each species were dusted on to ten of these treated stigmas. Normal in vivo germination was only around 60% and treated stigmas of four species showed a reduction in the range of 17-21%. Considering that the natural variation in