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% 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[8] 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 For chick embryo fibroblasts 0.5% honey in the medium was optimum (unpublished data).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[9,10].

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.

The author wishes to thank the Director, Central Bee Research Institute, Pune, for the supply of honey and the Director, National Institute of Virology, for his interest in this work.

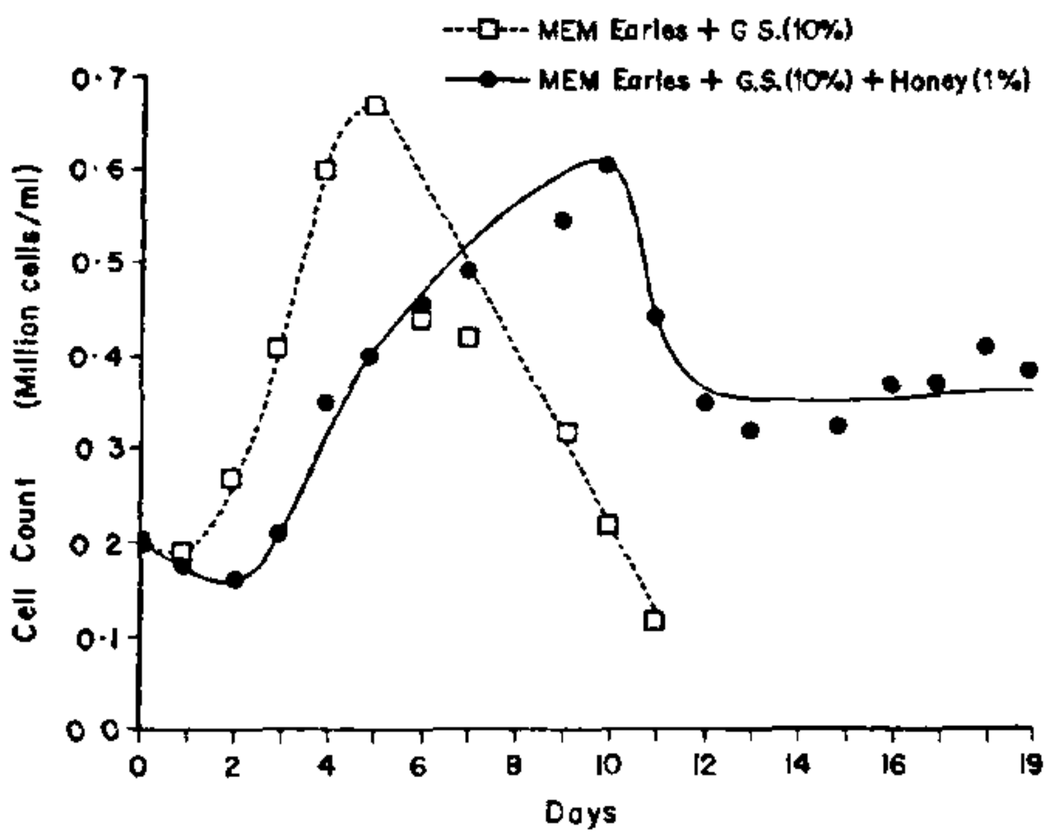

Figure 1. Growth rate of Vero cells in the presence and absence of one percent honey.

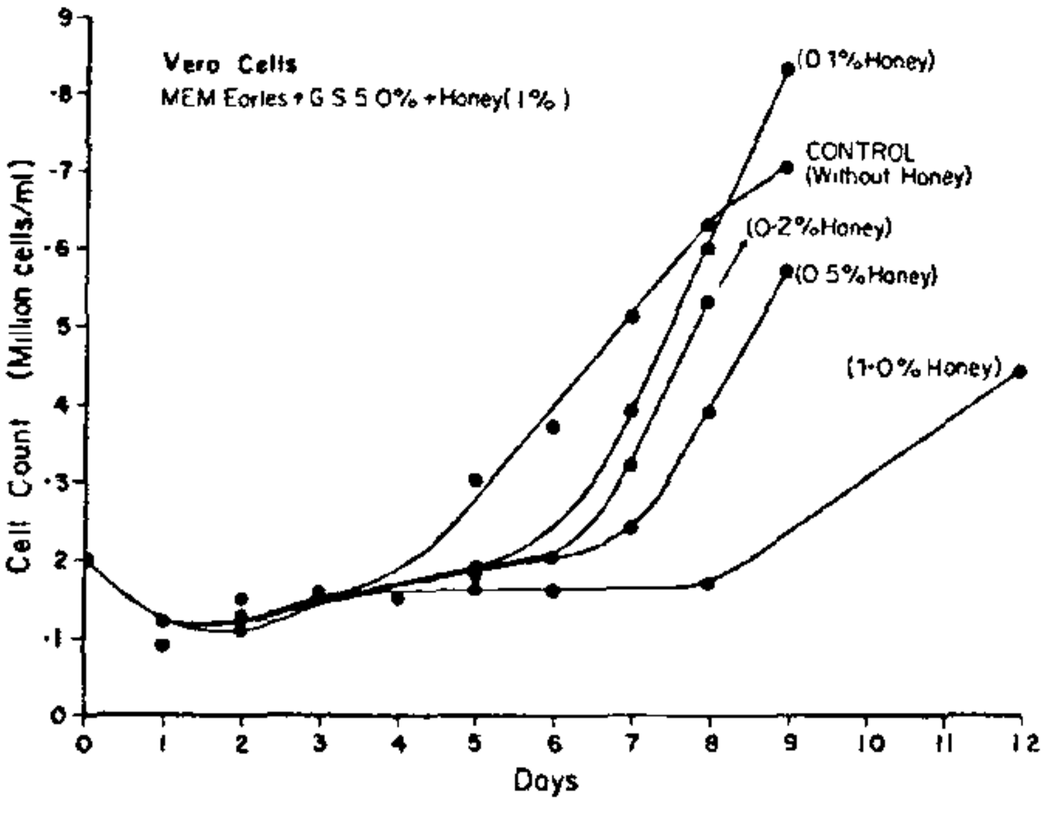

Figure 2. Effect of different concentrations of honey on growth rate of Vero cells.

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

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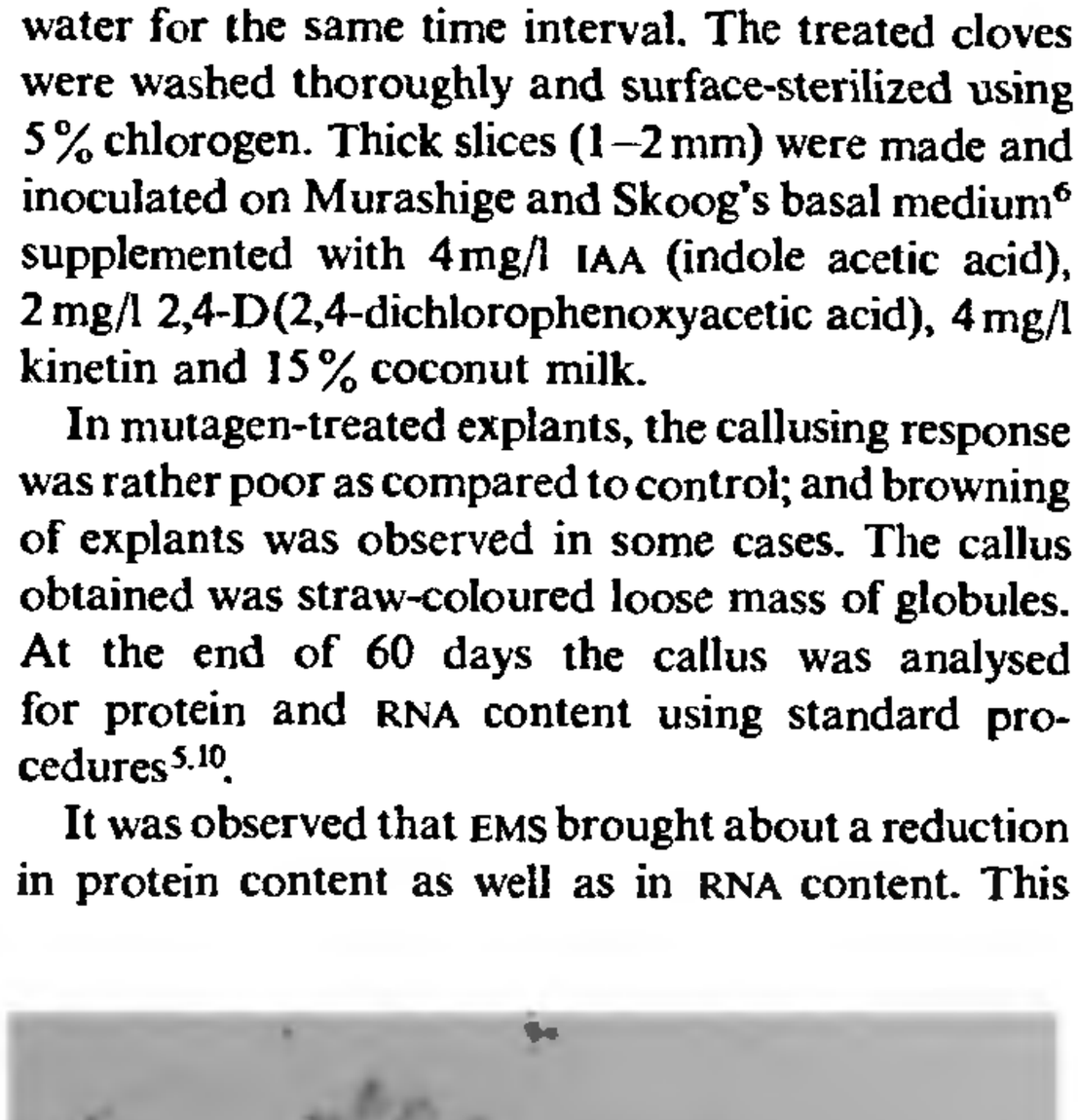
TISSUE culture studies of *Allium sativum* L. (Garlic) have been carried out by several workers[1–4]. Karyological studies by Novák[7–9] show that variations may be possible in cultures. Since garlic does not reproduce sexually, it was important to induce variation through

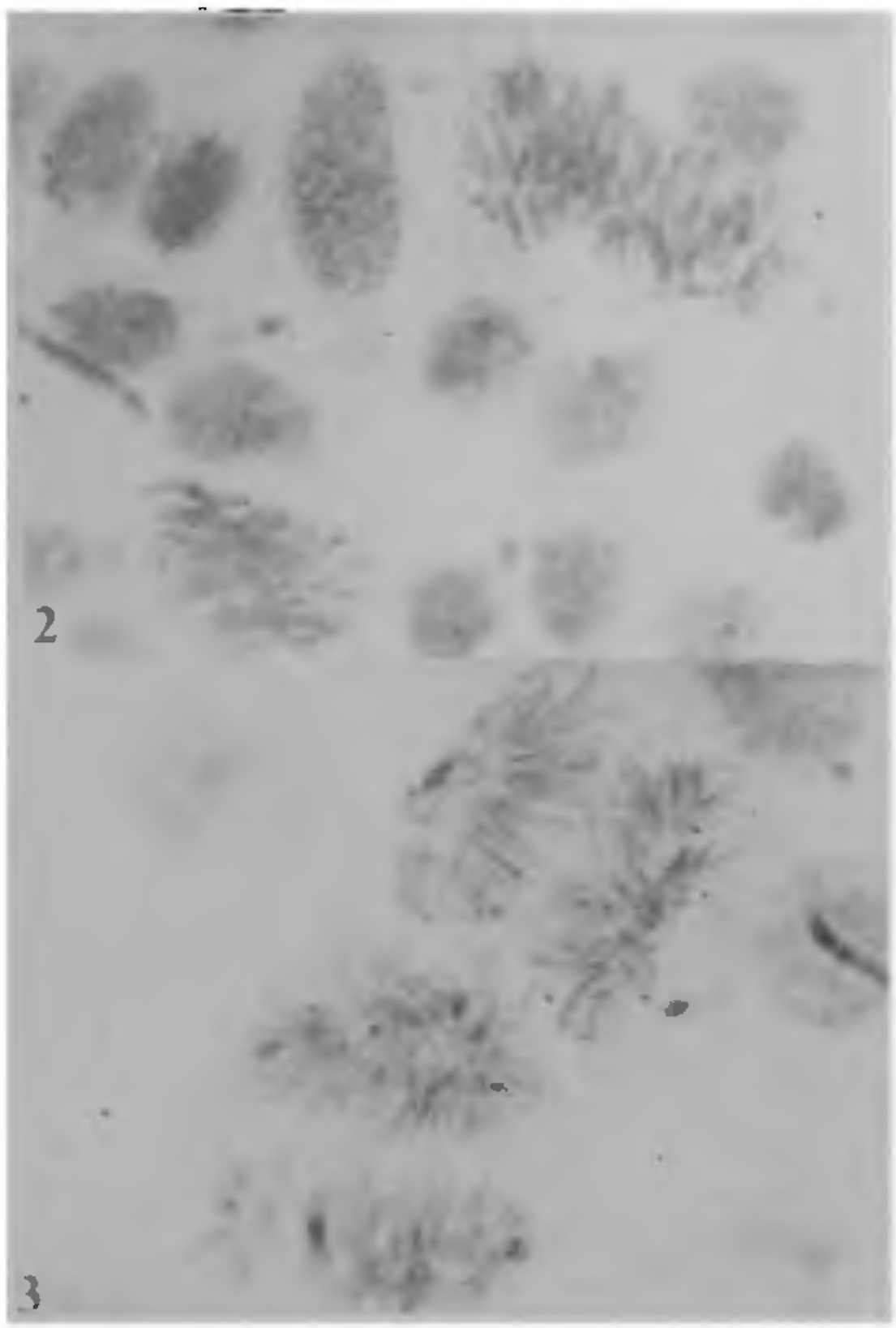
mutation. The present study deals with the effect of the mutagen ethyl-methane sulphonate (EMS) on the physiology and the cytology of the garlic callus.

Garlic cloves were subjected to EMS treatment for 2 hr by soaking[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[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[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).





Figures 1–3. EMS treatment. Polyploid cells **1 & 2.** in metaphase and **3.** in anaphase (magnification × 1200)

Acetocarmine 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

Table 1 *Effect of EMS on protein and RNA content and on cytology of garlic callus.*

Treatment	Dry wt (mg)	Proteins (mg/g) dry wt	RNA (mg/g) dry wt	Mitotic index (%)	Polyploid (%)
Control	103	26.32 ± 0.47	15.71 ± 0.18	2.20	1.25
0.025% EMS	98	20.5 ± 0.82*	15.15 ± 0.26	1.91	6.70
0.05% EMS	85	18.95 ± 0.58*	6.67 ± 0.64*	2.00	7.19
0.1% EMS	79	17.86 ± 0.63*	4.88 ± 0.18*	1.82	4.52

± standard error of mean; * *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 polyploid 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 polyploid cells in culture, so as to obtain better variation and desirable characters.

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NON-SELECTIVE INHIBITION OF POLLEN GERMINATION BY STIGMATIC EXUDATES OF *CHLOROPHYTUM* AND *DIPCADI* SPECIES (LILIACEAE)

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FERRARI and Wallace[1] suggested that water soluble substances in the stigmatic fluid of cabbage selectively inhibited self but not cross pollen. Other reports[2] also indicated the involvement of stigmatic surface compounds in the suppression of germination of self pollen. By contrast, stigmatic exudates of three members of Liliaceae we examined were characterised by indiscriminate inhibition, *i.e.* inhibition of self, cross and alien pollen. The nature of the inhibitory components of the stigmatic exudates of these plants was studied by chromatography and spectrophotometry and a summary of the preliminary results is presented in this report.

Stigmas of two species of *Chlorophytum*, *C. malabaricum* and *C. elatum variegatum* and an yet unidentified species of *Dipcadi* do not support germination of pollen from any source. In addition, stigmatic exudates or even 20 sec extracts of the stigmas of these species can induce *in vitro* and *in vivo* inhibition of development of otherwise normal pollen.

Induction of *in vitro* inhibition was tested on the pollens of four fertile species of *Chlorophytum* tabulated in table 1. In standard 10% sucrose culture solutions of Brewbaker and Kwack[3], pollen grains of all these fertile species recorded a germination rate of around 90%. Stigmatic extracts or exudates of the sterile species, when added to the culture solution in the ratio of 1:1, reduced the pollen germination of various fertile species examined here by a margin of 40–50%.

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

Species	% of germination Control*	Trial*	% of inhibition
C. heyneanum	61.7	40.5	21.2
C. tuberosum	68.1	50.3	17.8
C. elatum	65 3	48.3	17.0
C. attenuatum	61.6	42.9	18.7

* Average of counts from ten stigmas.

These *in vitro* 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