

Table 1 Larvicidal activity of different plant oils against fourth instar larvae of *Anopheles stephensi*

Name of plant	Percentage mortality (ppm)									Control	LC ₅₀ Values ppm
	25	50	75	100	125	150	175	200	250		
<i>C. deodara</i>	16	32	56	64	72	90	100	100	100	0	63.2
<i>C. nardus</i>	2	10	20	48	62	80	90	94	100	0	105.4
<i>C. flexuosus</i>	4	16	36	56	66	82	92	100	100	0	91.4
<i>C. martinii</i>	3	12	28	50	62	78	94	96	100	0	100.0
<i>L. officinalis</i>	10	20	42	58	70	88	90	100	100	0	83.6
<i>M. arvensis</i>	8	22	42	60	74	90	98	100	100	0	83.8
<i>R. communis</i>	7	18	31	47	58	69	86	93	100	0	113.0
<i>E. globulus</i>	11	24	35	51	62	72	88	99	100	0	98.5
<i>E. caryophyllus</i>	10	31	40	51	70	78	97	100	100	0	96.5
<i>M. azadirachta</i>	21	93	41	54	70	81	97	100	100	0	88.5

these oils may provide an extremely effective vector control measure at a rather low cost.

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IN VITRO REGENERATION FROM CALLUS CULTURES OF *SCILLA INDICA* (ROXB.) BAKER

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SCILLA INDICA (Roxb.) Baker of Liliaceae is cultivated in India for its commercial products^{1,2}. The ornamental leaves add to its horticultural value.

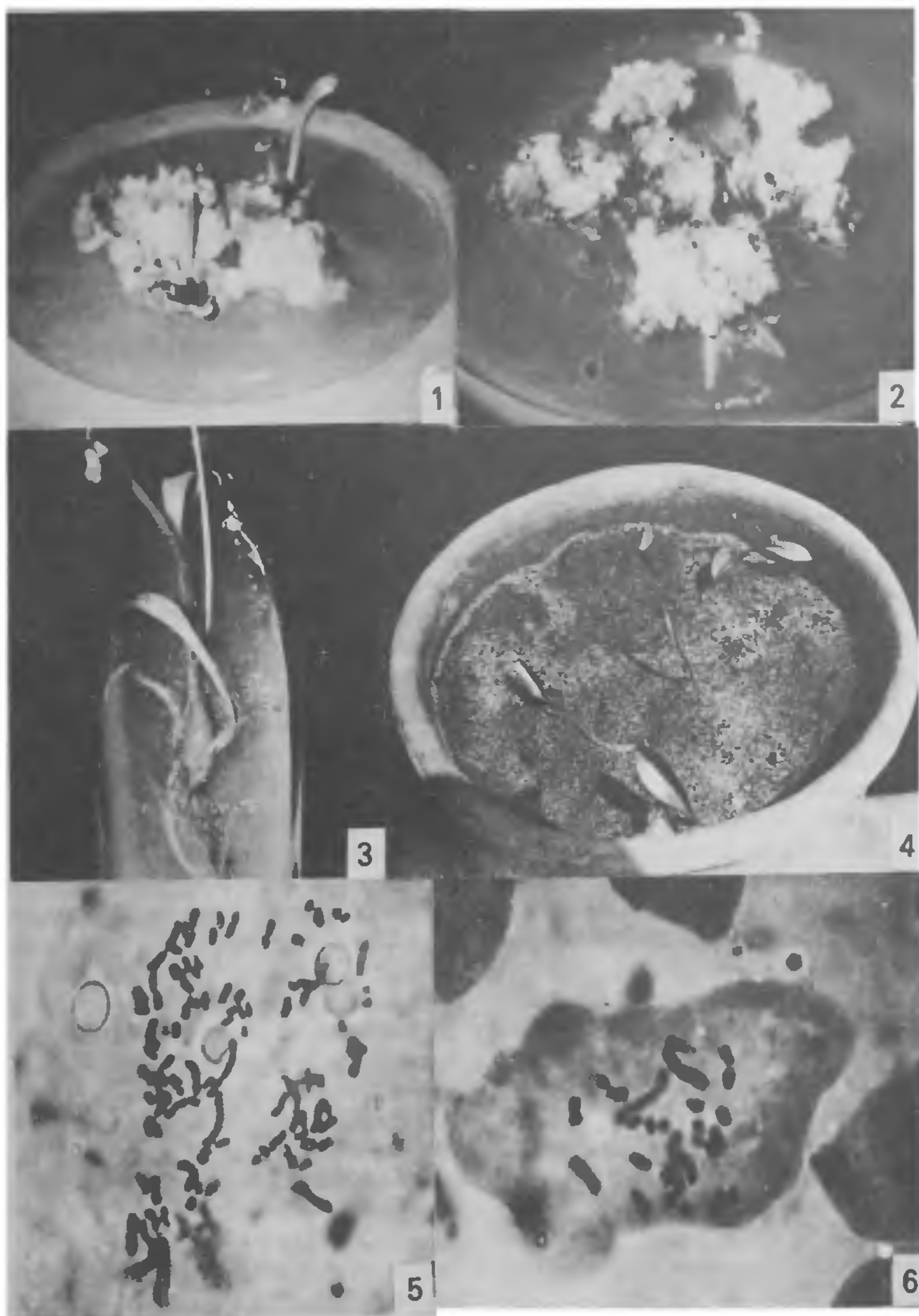
S. indica responds readily to artificial media unlike most monocots^{3,4} and is characterized by a slow rate of natural propagation. The objective of this work was to explore the possibility of rapid propagation through *in vitro* regeneration from callus cultures, if possible, without resorting to the long process of embryo development. The effects of two auxins on the callus and the chromosome analysis of the callus and regenerated plants have also been worked out.

Diploid plants of *S. indica* ($2n=30$) were collected from Pune and Mahabaleshwar and replanted in the college garden. Young leaf-tips and scale-leaves of sprouting bulbs were sterilized in 0.1% HgCl_2 solution for 15 and 25 min respectively. Explants of about 1.5×1 cm were excised out from them aseptically, and callus cultures were initiated on the basal medium of Murashige and Skoog⁵ (MS) with an addition of 2 mg/l 2,4-D and 15% (V/V) coconut milk. The pH of the medium was adjusted to 5.6, solidified by 0.5% Agar and cultures were incubated at a temperature of $25 \pm 1^\circ\text{C}$ for 16/8 hr light/dark period.

For cytological investigation, callus pieces were fixed in Carnoy's fluid and stained in 2% aceto-orcin : (N) HCL (9:1). Young root-tips and shoot-tips from regenerated plants were also similarly fixed and stained.

Calli were initiated on the surface of the explants after 30 days of growth in the medium containing 2,4-D. The callus was subcultured onto fresh medium after every 30 days.

Regeneration of shoots and roots was observed when a 60-day-old callus was transferred to MS medium devoid of 2,4-D but containing 15% coconut milk and 2 mg/l NAA (figure 1). The regenerat-



Figures 1-6. 1. 60-day-old callus showing initiation of shoots in MS media containing 15% coconut milk and 2 mg/l NAA; 2. 240-day-old callus showing rhizogenesis in the same medium; 3. 120-day-old regenerated plantlet showing rooting and bulb initiation in 1/2 MS medium; 4. 5-month-old plantlet transferred to potted soil; 5. Metaphase plate from callus cells showing polyploidy; 6. Metaphase plate from regenerated plant root-tip showing diploid chromosome number ($2n=30$).

ing potential of the callus depended on the age of callus in culture. A 120-day-old callus showed maximum ability for organogenesis in terms of number of shoot-buds/callus and the organogenetic potency gradually decreased thereafter. A 240-day-old callus showed only rhizogenesis with very little shoot-forming ability (figure 2).

Young shoot-buds developed into leafy shoots after about 30 days growth in the same medium and these were transferred to MS media devoid of hormones and containing half concentration of salts. On transferring to fresh media, rooting and complete regeneration of plantlets were observed after 120 days (figure 3). Plantlets (4-5 month old) showing bulb formation were transferred to potted soil (figure 4). Regenerated plants were observed to be morphologically similar to plants growing in the field.

Cytological study of the callus tissue showed only diploid cell populations after 30 days of growth. After 90 days growth, diploid cells were 85% and polyploid cells were 15%. In a 120-day-old callus about 78% diploid cells and 22% polyploid cells were found, and in a 240-day-old callus the diploid cells were 28% and the polyploid cells were 72%. The study of root-tips and shoot-tips of regenerated plants revealed only the diploid number ($2n=30$) in plants regenerated from calli of all ages.

Thus it was noted that 2,4-D was an essential factor in inducing callus formation in the medium containing coconut milk. The omission of 2,4-D and the presence of NAA in the medium influenced the origin of shoots and roots and this resembles another monocot, *Gladiolus* sp.⁶

The callus tissue derived from explants showed heterogeneous cell populations containing both diploid and polyploid cells (figure 5) but the plants regenerating from these calli, showed only diploid cells (figure 6).

In older cultures, where the percentage of polyploid cells is high, the number of plants that can be regenerated decreases. Such a behaviour has also been reported in other species^{7,8}. Thus, the karyologic state of a callus culture affects the *in vitro* regeneration of plants.

Therefore, the present study involving *in vitro* culture and cytological analysis of tissues grown *in vitro* has demonstrated the scope of utilizing this technique for rapid propagation of genetically stable plants in *S. indica*. The instability in callus offers further scope of utilization of the different genotypes.

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LEAF BUD ORGANOGENESIS *IN VITRO* IN A FERN—*AMPELOPTERIS PROLIFERA* (RETZ.) COPEL.

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Of the associated organs in fern organography, the shoot buds have attracted attention more extensively than the root and leaf buds¹. The latter and their role in vegetative propagation have been described in several ferns²⁻⁴ but the *in vitro* studies dealing with control mechanisms are very few⁵.

Ampelopteris prolifera forms thickets by prolific buds borne on fronds of two distinct growth patterns viz determinate and indeterminate (= runner fronds). The bud organogenesis *in vivo*^{6,7} is depicted in figure 1. Our present study is aimed at assessing the organogenetic potentials of buds in response to interacting effects of sucrose (S), a combination of indole-3-acetic acid (IAA) and kinetin (KN) and the age of buds.

Bud-bearing rachis segments were cultured on modified Knudson's 'C' basal medium (= BM) under 12 hr duration in white light (1000 lux). Three replicates of each of the following culture media were tested. (i) On sucrose-free BM, the buds were activated 4 days after inoculation (ii) On 4% sucrose-containing BM, the response was delayed by about 48 hr. The delayed response was followed by differentiation of fronds with diminutive pinnae and markedly reduced extension growth of petioles.