acid water (4 1:1 v/v) ascendingly. The chromatogram was divided into 8 equal parts, eluted in water and placed on needle punctures on the abaxial side of the young coconut leaves. Only the eluate from a yellow band (Rf 0.75) was effective in inducing visible symptoms *i.e.* necrosis by 24-48 hr and chlorosis by 72 hr with longer incubation the chlorotic area extended longitudinally. Culture medium processed and tested similarly served as control. Toxic activity was not evident from any of the control fractions. With the toxic substance the symptoms appeared only when placed on pin-pricks and not on intact surfaces.

On spray tests with chemical reagents the yellow compound reacted positively to iodine vapour, folin-phenol, sulphanilic acid, *p*-nitroaniline-sodium hydroxide[2]; it absorbed light when viewed under long-UV. The yellow substance was found to be dialyzable and thermostable. It was soluble in water, chloroform, ethylacetate, butanol, ethanol and acetone but not in benzene. It had absorption maxima at 253, 260 and 270 nm. The toxin appears to be a low molecular weight phenolic compound with simple molecular structure.

Helpful suggestions of Dr R. N. Swamy, Reader, University Botany Laboratory, Madras, are gratefully acknowledged.

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1. Menon, K. P. V. and Pandalai, K. M., *The Coconut Palm-a manograph*, Indian Central Coconut Committee, Ernakulam, Kerala.
2. Stahl, P., *Thin layer chromatography*, Academic Press, London, 1969, p. 1041.

## EFFECTS OF NIA 23509, A JUVENILE HORMONE ANALOGUE ON *DYSDERCUS CINGULATUS* (RED COTTON BUG)

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THE morphological, physiological and biochemical responses to the action of juvenoids, have been well studied: Slama[1], Bafhr[2] and Martin[3] have estimated the qualitative and quantitative changes in haemolymph proteins (HP) at different developmental stages of insects. Certain JHA and JH mimica also cause failure in reproduction when immature stages of insects are treated.

This paper presents the effect caused by NIA 23509 on reproductive system, and on protein content in the haemolymph of a supernumerary nymphal instar (sixth instar) of *Dysdercus*.

The colony of *Dysdercus cingulatus* was reared in the insectary as described by Geering[4]. Dilutions of NIA 23509 were prepared in acetone and 1 $\mu$l of the solution was applied topically to freshly molted fifth instar nymphs using an agla micrometer syringe. Treated insects were held in observation cages until final molt and were fed on cotton seeds soaked in water. Percent inhibition of growth was calculated from the graph drawn between the log dose and probit inhibition. Insect haemolymph was collected by centrifugation and proteins from the collected haemolymph samples were precipitated using 10% and 5% trichloracetic acid (TCA). The precipitate (PPT) was first washed with alcohol saturated with sodium acetate, with alcohol and ether (1:1) and finally with ethyl ether. The dry PPT was dissolved in 0.6 N KOH and the total haemolymph proteins were estimated by Lowry's method[5]. Bovine albumin serum was employed as a reference standard.

Reproductive systems of supernumerary nymphs, male and female were dissected out in insect Ringor solution. The reproductive organs were examined under binocular microscope and compared with a normal one.

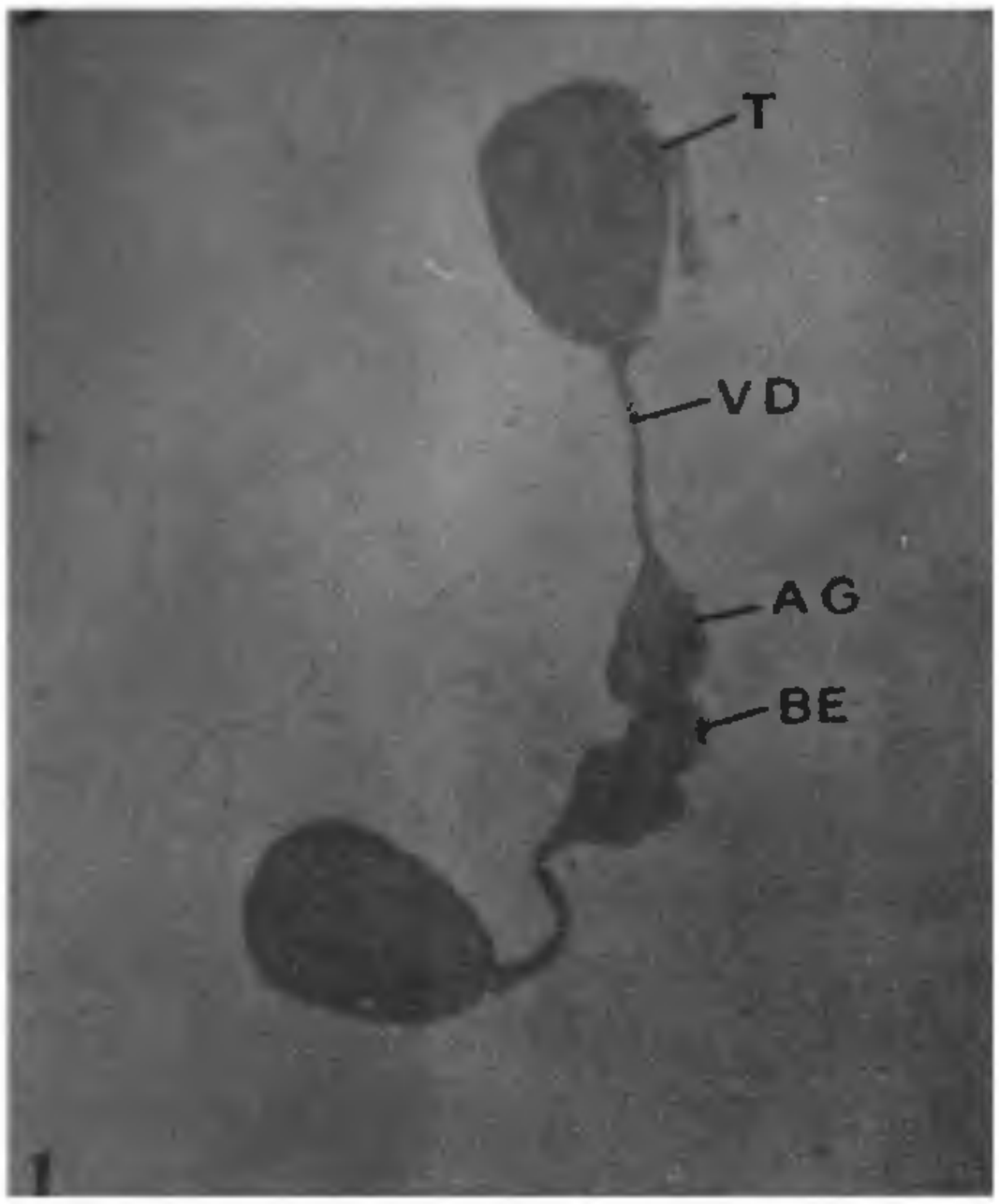
The dissected material was fixed in Bovin's fixative, passed through alcoholic grades, stained with Borax carmine and mounted in Canada balsam.

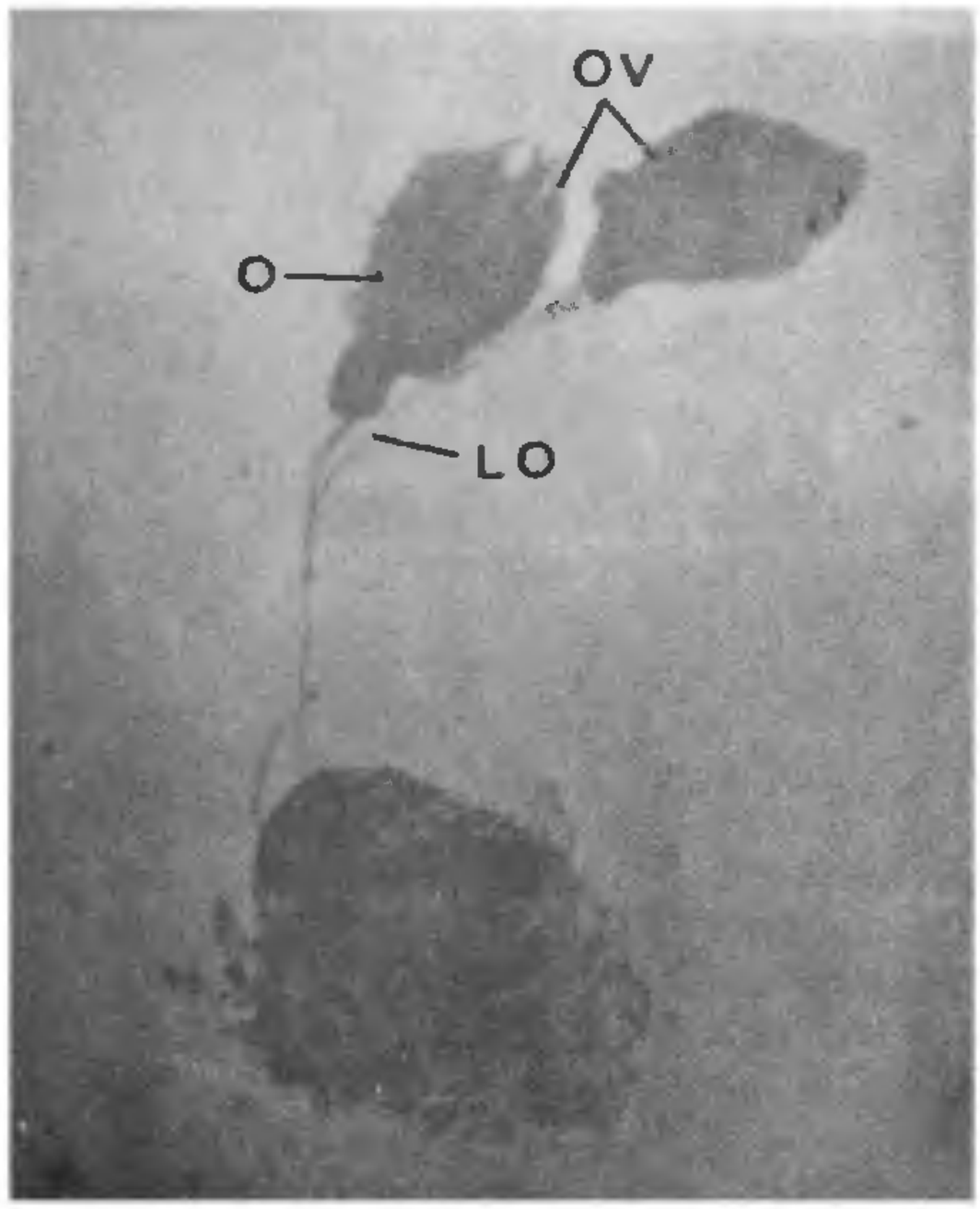
The log dose for 50% and 90% inhibition, calculated from the graph were 0.0208 $\mu$g and 0.0794 $\mu$g/ nymph. The extra larval instars and adultoids emerged after treatment were incapable of reproduction. Blood proteins of such instars when estimated were very low compared to normal adults and fifth instars (table 1). This drastic decline in total HP is the effect of JHA treatment. Moreover, there was a difference in the amounts of HP of the two sexes. Males had more HP

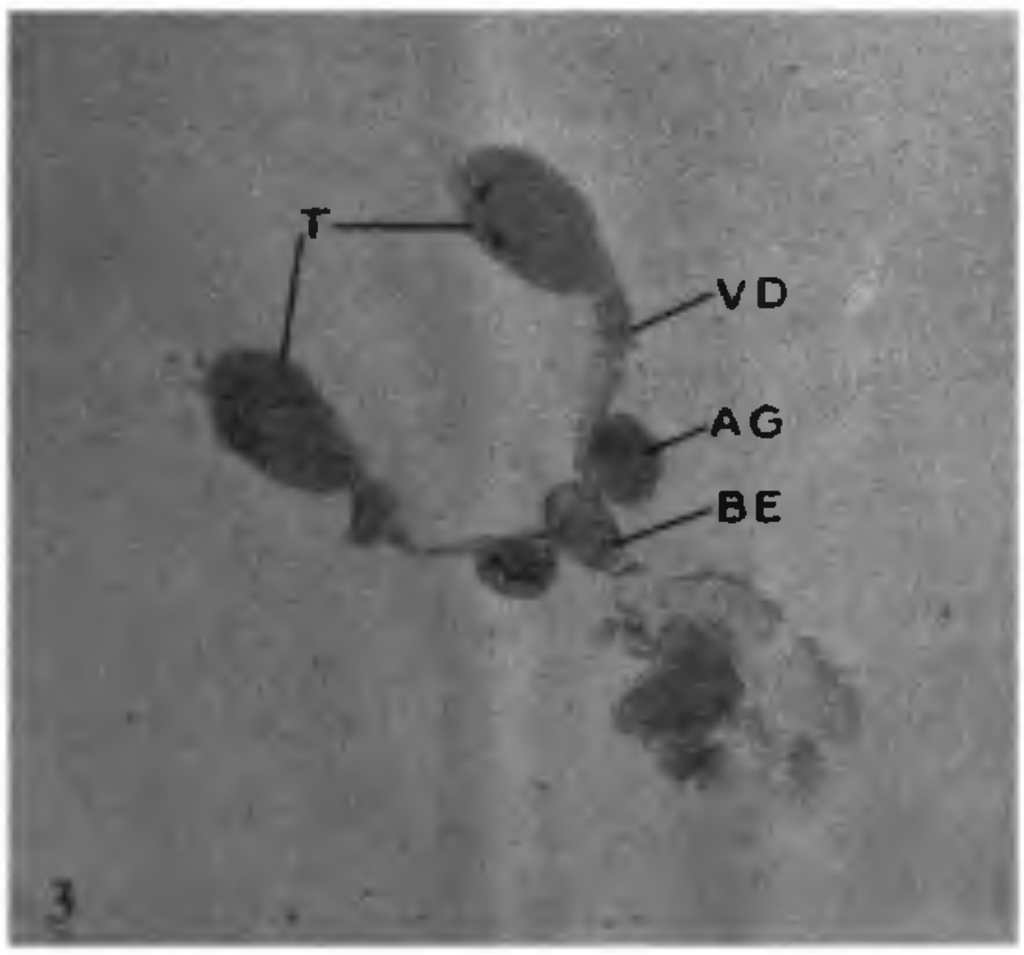
TABLE 1

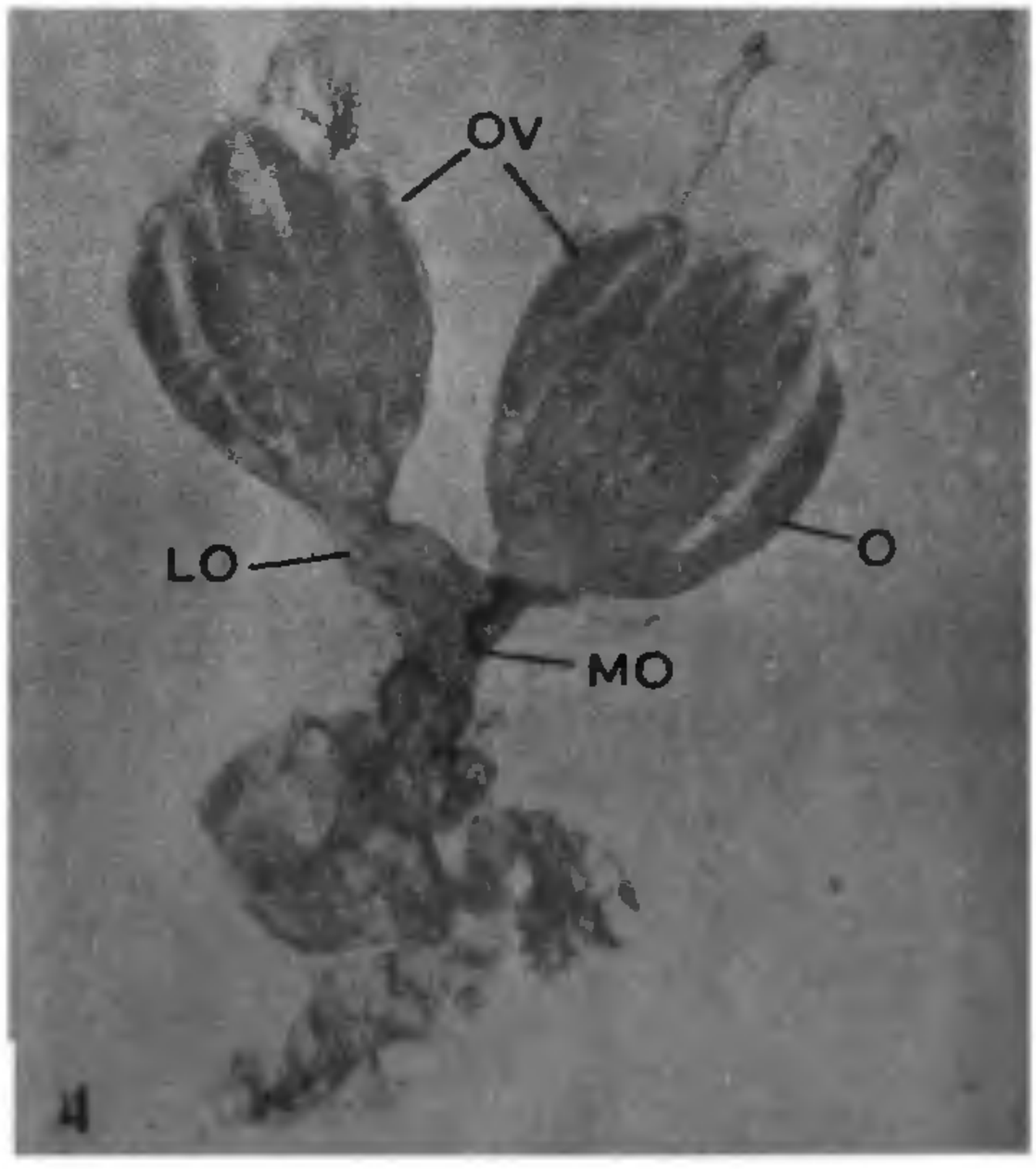
*Total haemolymph proteins of normal (fifth instar, adults) and treated (supernumerary nymphs).*

| Stage of insect | Total proteins in mg/ml of haemolymph | |
|---|---|---|
| | Male | Female |
| 1. Supernumerary nymph (sixth instar) | 35 mg. | 20 mg. |
| 2. Normal adult | 57 mg. | 78 mg. |
| 3. Fifth instar nymph (normal) | 61 mg. | |




**Figures 1–4. 1 & 2.** Effect of NIA 23509 on reproductive systems of supernumerary instars of *Dysdercus* **1.** Male **2.** Female; **3 & 4.** Reproductive systems of normal adults of *Dysdercus* (freshly molted) **3.** Male, **4.**Female; T = testis V. D. = vas deference; A. G. = accessory gland; B. E. = bulbus ejaculatorious; Ov = ovary; O = ovarioles; LO = lateral oviduct; MO = median oviduct.

compared to females, whereas normal insects followed the opposite pattern. The results are in agreement with Maya Menon[6]. According to her, there was a difference in protein constitution in the blood of the two sexes; it was greater in female cockroaches when a growth is taking place.

NIA 23509 had also exhibited an atrophy of the reproductive system in the treated insects. Sixth instar males when dissected out had deformed testes and the most affected part was vas deference. They were thin and elongated compared to normal. Accessory glands were reduced in size considerably (figures 1 and 3).

The female reproductive system was equally affected with poorly developed ovaries. The germarium and ovarioles looked as if the content inside were all disintegrated. The oviduct was very thin and elongated compared to a normal one (figures 2 and 4). This type of suppressed gonadal growth in *Blatella germanica* was reported by Pincus[7] which could be attributed to the action of JHA.

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1. Slama, K., *J. Insect. Physiol.*, 1964, **10**, 773.
2. Bafhr, J. C. and Porcheron, P. J., *Insect Physiol.*, 1979, **18**, 689.
3. Martin, M. D., *Aust. J. Biol. Sci.*, 1969, **22**, 935.
4. Geering, Q. A., *Bull. Entomol. Res.*, 1956, **46**, 743.
5. Lowry, O., *J. Biol. Chem.*, 1951, **193**, 265.
6. Maya Menon, *J. Anim. Morphol. Physiol.*, 1965, **12**, 76.
7. Pincus, D. S., *J. Insect. Physiol.*, 1977, **23**, 73.

## REGENERATION OF A TREE— *LEUCOSCEPTRUM CANUM* SM. THROUGH MERISTEM CULTURE.

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THE application of tissue culture for rapid propagation of trees offers forest biologists unique method for increasing forest productivity. In contrast, conventional vegetative propagation through cuttings in tree species generally fails because of the difficulties in rooting. Moreover, from tissue culture experiments one can probably get an insight of the factors, especially of auxin requirement, for root initiation and such knowledge can also be employed to stock or scion, for the development of roots, when propagation through grafting is aimed.

So far, a number of reviews have appeared where success has been achieved to regenerate whole plants from angiosperm trees[1-4]. However, the number of such reports is meagre in comparison to the existing tree angiosperms. Most of the achievements are from the embryonic or seedling tissue rather than mature trees. Methods for clonal propagation from mature trees would be more useful in forestry. Furthermore, multiplication of plant through meristem culture is the most common method for conservation of existing genotype[5]. During the present study an attempt has been made to propagate *Leucosceptrum canum*, a Labiatae tree through apical and axillary meristems of mature trees.

Buds from a 40 ft *Leucosceptrum* plant in Gantok, Sikkim, were collected by courtsey of late Dr V. N. Gadgil. Apical portion (1–1.5 cm) and small (ca. 1.5 cm) stem segments at nodes with axillary meristems of young twigs were washed thoroughly with water and then treated for 10 min with 5% Teepol solution. After washing the Teepol completely, plant materials were sterilized with 0.1% (w/v) mercuric chloride solution for 3 min followed by five washings in sterile distilled water. The apical meristems (ca. 0.5 mm) and axillary meristems were dissected aseptically and placed (one per culture tube) on different media with the cut end in contact with the medium. MS basal medium[6] supplemented with different concentrations (*i.e.* 0.1, 0.5, 1.0, 2.5 and 5.0 mg/l) of 6-banzylaminopurine (BAP) or kinetin alone and in combination with indole-3-acetic acid and indolebutyric acid (IBA) at 0.1 and 0.5 mg/l was tested. The cultures were incubated at 25 ± 2° C, ca. 55% humidity, under a photoperiod of 16 hr light (2000 lux).

Of the different supplements tried so far, rapid response was obtained in BAP–2.5 mg/l and BAP–0.5 + IBA–0.1 mg/l where leafy shoot-buds developed within 7 days of incubation. In the latter medium a prominent apical dome with two leaves appeared from the apical shoot meristem; which after excision of the two leaves sub-cultured on the same medium produces multiple shoot-buds (*i.e.* 3–5 per explant). Thus, from a single meristem, over 50 shoot-buds could be obtained within a month by 3 subcultures of 7 days each, keeping a few shoot-buds intact. Shoot-bud formation was delayed by 3 or 4 days when axillary meristems were taken as explants. No roots were formed when shoots with 6–10 leaves (figure 1) were transplanted to MS basal medium without any growth regulators. However, when transferred on to MS consisting of half strength macrosalts and supplemented