EVALUATION OF SEEDLINGS AND CLONAL PROGENIES OF KATTUPATAVALAM (*Trichosanthes cucumerina* L.) FOR YIELD AND QUALITY

By

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THESIS

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DECLARATION

I, Sreerekha M.V (2004-12-06) hereby declare that this thesis entitled **'Evaluation of seedlings and clonal progenies of Kattupatavalam (*Trichosanthes cucumerina* L.) for yield and quality'** is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Certified that this thesis, entitled **'Evaluation of seedlings and clonal progenies of Kattupatavalam (*Trichosanthes cucumerina* L.) for yield and quality'** is a record of research work done independently by **Ms. Sreerekha M.V** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Affectionately dedicated to

My

Beloved Family

INTRODUCTION

1. INTRODUCTION

Medicinal plants and their derivatives are looked upon not only as sources of affordable health care, but also as important commodity items of international trade and commerce. The increasing acceptance of traditional herbal systems of medicine like Ayurveda, within India and outside has resulted in the revival of ancient traditions of medicine. As per the estimates of WHO, plant based drugs cater to the health needs of nearly 80 per cent of world population (Kamboj, 2000). In view of the phenomenal increase in demand of herbal drugs, medicinal plants have been indiscriminately overexploited leading to rarity and extinct conditions of many valuable plant species. In Kerala, more than 90 per cent of plant species utilized by the user industry are collected from the wild, posing immense threat to their genetic pool. Hence, a national policy to preserve endangered medicinal species and promote cultivation of remunerative plants has been evolved. Keeping in tune with this national policy, in the state of Kerala also, steps to encourage domestication and cultivation of medicinal plants as pure crop, as intercrop in existing cropping systems and as home garden component are gaining momentum, wherein non-availability of quality planting materials is a major constraint.

The medicinal plant, *Trichosanthes cucumerina* (Kattupatavalam), occupy a place of prominence in the Ayurvedic drug industry in terms of profitability and market feasibility. It is regarded as a laxative and a blood purifier. The plant is considered to be beneficial in the treatment of dermatic and intestinal disorders (Sivarajan and Balachandran, 1997). Presently, its entire supply is met from the wild and unscrupulous exploitation has led to erosion of the genetic resources of this crop. Also, since efforts are on to popularize the cultivation of this crop, in the homesteads of Kerala, considerable shortage of seed supply is being felt by the farming community. The seed germination of the species is erratic as well. Hence, to address this constraint, attempts were made at the Department of Plantation Crops and Spices to standardize protocols for clonal

multiplication of this species from axillary buds through *in vitro* techniques, as a part of a DBT funded collaborative project with Kottakkal Arya Vaidyasala. As a result, a feasible protocol to multiply and for short term conservation of the crop *in vitro* was developed and transferred to Kottakkal Arya Vaidyasala, to generate propagules for distribution to the farming community.

In the course of these attempts, feedback from a few recipient farmers indicated instances of considerably less biomass yield, when *in vitro* derived plantlets were used as propagules. Also, queries as to the suitability of the crop to be raised as an intercrop in coconut plantation, came up, since generally cucurbitaceous crops are grown in the open. Lack of scientifically validated literature in this regard, necessitated taking up studies to assess the comparative field performance of seedling derived and clonal progenies of *Trichosanthes cucumerina* and to determine the feasibility of raising the species as an intercrop in the coconut gardens of Kerala.

In this context, the present study entitled "Evaluation of seedlings and clonal progenies of Kattupatavalam (*Trichosanthes cucumerina* L.) for yield and quality", was taken up at the Department of Plantation Crops and Spices with the following objectives.

1. To study the feasibility of domestication of the target species in open and under shade through investigations on growth, yield and quality attributes.
2. To assess the comparative performance of seedlings and clonal progenies of *Trichosanthes cucumerina* in open and under shade, with respect to growth, yield and quality.
3. To compare the drug quality of domesticated samples and market samples through studies of biochemical parameters and anatomical features.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Written records on the use of medicinal plants for curing human and animal diseases in India can be traced back to Vedic ages (3500-1800 BC). Now the emergence of new infections, chronic and drug resistant diseases have prompted scientists to look towards medicinal plants as potential agents in distinct health care management systems. The medicinal species, *Trichosanthes cucumerina* is a reputed laxative, entering into major Ayurvedic formulations like Patavaladikashayam. The plant is listed among the ten most remunerative medicinal species of Kerala, in terms of unit value (Joseph, 2001).

Efforts to domesticate the crop necessitates generation of quality planting materials including perusal of *in vitro* techniques. Investigations on *in vitro* response of the crop have yielded a feasible protocol for raising clonal plants through axillary bud culture. The field performance of the resultant plantlets in comparison to the conventionally propagated seedlings, as well as their suitability as intercrops in coconut gardens need to be studied to render them as a prospective medicinal plant worthy of domestication. Keeping in view of the above facts, the present review is focused on the general features of the target plant with emphasis on its medicinal properties, the major active ingredient, its pharmacological action and other secondary metabolites in the species as well as in the related species. Influence of growth conditions *viz.*, shade and open on the performance of medicinal plants in general and cucurbitaceous species, as well as the performance of tissue culture derived plants in comparison to conventionally propagated ones, in various crop species are also dealt with.

2.1 Habit and distribution of *Trichosanthes cucumerina*

Trichosanthes cucumerina L. belonging to the family Cucurbitaceae, is a slender, annual, monoecious climber with furrowed stem and bifid or trifid tendrils. Leaves simple, orbicular, reniform or broadly ovate, more or less deeply

5 lobbed. Flowers are monoecious, white coloured and pollinated by insects. Male flowers are present on axillary racemes and females are axillary and solitary. Fruits are ovoid, fusiform, indehiscent berries, tapering at both ends, striped with white when immature and scarlet red when ripe. Seeds are semi ellipsoid when ripe, compressed and surround with red pulp (Chakravarty, 1959 and Nayar and Singh, 1998).

The plant is distributed throughout India. It prefers light to medium heavy soils and requires well-drained soils. It enjoys a tropical climate. Some ayurvedic texts recognize two kinds of patola, namely a small-fruited bitter variety called 'Patola' and a large fruited sweetish edible variety called 'Svadupatola' (Mooss, 1976). The former is highly bitter and is locally called Kaipanpatavalam or Kattupatavalam. This is the one commonly used as medicine by the physicians of Kerala and is equated with *Trichosanthes cucumerina* of Cucurbitaceae family (Rheede, 1688). Many authors have conceded the use of both *T. dioica* and *T. cucumerina* as Patola (Kirtikar and Basu, 1918; Nadkarni, 1954 and Chopra *et al.*, 1956)

2.2 Biochemical constituents of cucurbitaceous plants

Literature pertaining to biochemical constituents in the target species, *Trichosanthes cucumerina* is scanty. Hence, it is attempted to review the biochemical constituents of cucurbitaceous plants and cucurbitacin, the main active ingredient of the species under study. Majority of the species in cucurbitaceae family contain bitter principles in some portion of the plant at some stage of crop development. Investigation on the triterpenes of plants belonging to the family cucurbitaceae revealed the presence of cucurbitacins, a group of tetracyclic compounds. Biochemically, the cucurbits are characterized by bitter principles called cucurbitacins. Chemically, cucurbitacins are tetracyclic triterpenes, having extensive oxidation level. They occur in nature, free as glycosides or in complicated mixtures (Seshadri, 1986).

The distribution of the bitter principles, known as cucurbitacins, was studied in a total of fortyfive species belonging to 18 genera (Rehm, 1958). It was also reported that the leaves of both bitter and sweet strains of cultured cucumber contain approximately 10 mg cucurbitacin C / kg fresh weight (Rehm, 1960). Of the 19 cucurbitacins (A-S), cucurbitacins B and E constitute the primary components. Cucurbitacin A is found only in *Cucumis* and generally in association with cucurbitacin B. Biogenetically, cucurbitacin B may be the precursor of cucurbitacins A, C, D and E. Cucurbitacin E may give rise to cucurbitacins C, D, F, G, H, I, J and L. All the 19 cucurbitacins have been reported from the subfamily Cucurbitoidae. In the subtribe Luffineae, cucurbitacin B alone occurs in four of the six species investigated so far. Cucurbitacin B is of rare occurrence in the tribe Trichosantheae (Pasha and Sen, 1998).

Occurrence of many cucurbitacins as glycoside was reported by Enslin and Rivette (1957) in cucurbitaceous plants. Whitaker and Davis (1962) mentioned that the highest concentrations of bitter principles are usually found in fruits and roots. Leaves and stems are normally non-bitter or only slightly bitter. The bitter principles presented in the seedlings are cucurbitacins B and E.

Chambliss *et al.* (1968) did not find cucurbitacin E glycoside in the watermelon fruits of the non bitter cultivar Hawksbury. Murty *et al.* (1970) isolated several triterpenoids from the cucurbitaceae family. Among them the most important are cucurbitacins, which are wide spread in this family. At least 19 cucurbitacins (A-S) have been isolated and characterized so far.

The antigibberellin activity of cucurbitacins was examined by Guha and Sen (1973). About nine µg of cucurbitacin B, from cucurbits, reduces 50 percent of the promoting effect of 5 µg GA_3/plant. Cucurbitacin B also counters the induction of α-amylase in cereal endosperm. However, it has no effect on auxin-induced growth of coleoptile tissues.

From the rind and pulp of the fruits of *Trichosanthes palmata*, a bitter principle resembling colocynthin, called trichosanthin has been isolated (Dey and Mair, 1973). Guha (1974) also reported that cucurbitacin B antagonizes cytokinin action in senescence. Thus, the growth and physiological function of any organ are determined by the balance of cucurbitacins, gibberellins and cytokinins in cucurbits.

Guha and Sen (1975) reported cucurbitacin C in fruits of a bitter cucumber variety named Hanzil. They also found cucurbitacin E in fruits of sweet cucumber variety. Kano *et al.* (1997) reported that young vigorous *Cucumis sativus* plants of a Japanese variety synthesized larger amounts of cucurbitacin C than older less vigorous plants. Keulen (1981) observed that mature top leaves of 10 different cucumber varieties contain between 130-1130 mg cucurbitacin C /kg fresh weight. Herrington *et al.* (1986) reported that the hybridization resulted in a plant producing bitter red fleshed watermelons with a cucurbitacin E glycoside content of 480mg/kg fresh fruit.

From the traditional medicinal herb *Trichosanthes*, saponins were isolated by Wu and Zhu (1986). Kitajima and Tanaka (1989) isolated methyl palmitate, palmitic acid, suberic acid, cucurbitacin B, cucurbitacin D and D-glucose from the fresh roots of *Trichosanthes kirilowii* Maxim. var. *japonicum* Kitam. They also reported that the bitter taste of *Trichosanthes* root is due to cucurbitacins B and D.

From the fresh roots of *Trichosanthes bracteata* Voigt., Kitajima *et al.* (1989) identified palmitic acid, suberic acid, cucurbitacin B, isocucurbitacin B, 3-epi-isocucurbitacin B, 23, 24- dihydrocucurbitacin B, 23, 24- dihydroiso-cucurbitacin B, 23,24- dihydro-3-epi-isocucurbitacin B, cucurbitacin D, isocucurbitacin D and D-glucose.

Chandra and Sastry (1990) identified and isolated hentriacontane, ceryl alcohol, beta sitosterol, alpha amyrin, taraxerone and sucrose from the fresh fruits of *Trichosanthes anguina.* Toyokawa *et al.* (1991) isolated a new abortifacient protein, named karasurin from fresh roots of *Trichosanthes kirilowii* var *japonicum* by acetone fractionation and ion exchange chromatography.

Halaweish (1993) reported that fruits of *Cucurbita texana* contain high concentrations of cucurbitacins E and I. In addition to these compounds cucurbitacin D, 3-epi-isocucurbitacin-D and cucurbitacin E were isolated for the first time from this species. Four new cucurbitacins and the known dihydrocucurbitacins B and E, dihydro iso cucurbitacin B and E, dihydro iso cucurbitacin B, cucurbitacins B and D, E, G, H, P and 22-deoxy cucurbitacin D were isolated by Farias *et al.* (1993) from the methanolic extracts of roots of *Wilbrandia bracteata.*

Isokarounidol was isolated by Akihisa *et al.* (1993) from the saponified seed extract of *Trichosanthes kirilowii.* Two new cucurbitane triperpenes were isolated by Konoshima *et al.* (1993) from the branches and leaves of *Cowania execana.* They are 15-oxo-cucurbitacin F and 15-oxo-23, 24-dihydro cucurbitacin F along with some known compounds like cucurbitacin F and 23,24-dihydro cucurbitacin F. Trichosanthin (TCS) is a protein isolated from the root tuber of the Chinese medicinal herb *Trichosanthes kirilowii* Maxim. as reported by Shaw *et al.* (1994). Mulholland *et al.* (1997) isolated cucurbitane triterpenoids from the leaves of *Momordica foetida.*

Cucurbitacin B, isocucurbitacin B, dihydro cucurbitacin B, Cucurbitacin E, dihydrocucurbitacin E, isocucurbitacin D, dihydro iso cucurbitacin D, Cucurbitacin I, dihydro cucurbitacin I, Cucurbitacin Q1 and dihydro cucurbitacin Q1 were identified from *Cucumis prophetarum* L. by Afifi *et al.* (1999). Cucurbitacin B, Cucurbitacin O, Cucurbitacin P, Cucurbitacin Q1,

dihydrocucurbitacin Q1, isocucurbitacin E and dihydroisocucurbitacin E also were isolated from *Cucumis prophetarum* Jusl. ssp. *dissectus*.

From the dried fruits of *Trichosanthes kirilowii* Waxia. and *Trichosanthes uniflora* Hao, Huang (1999) extracted saponins, organic acids and resins. An alkaloid trichosanatine has been isolated from the fruit as well. A novel cucurbitacin glycoside was isolated from aerial parts of *Kageneckia oblonga* by Munoz *et al.* (2000). Its structure was elucidated as 3-beta-(beta-D-glucosyloxy)-16-alpha, 23-alpha epoxy cucurbita-5, 24-dien-11-one. From the fruits of *Luffa operculata*, Kawahara *et al.* (2001) isolated 2 noval cucurbitacins called neocucurbitacin A and neo cucurbitacin B.

From *Trichosanthes tricuspidata* fruits, four cucurbitaine glycosides (Khekadaengosides A-J, M-N, Cucurbitacin J 2-0-beta-glucopyranoside and cucurbitacin K 2-0-beta-glucopyranoside), a hexanor cucurbitane glycoside (Khekadaengoside K) and octanor cucurbitane (Khekadaengoside L) were isolated along with two known cucurbitane glycosides (Cucurbitacin 2-0-glycopyranoside and 25-0-acetyl-cucurbitacin 2-0-beta glucopyranoside (Kanchanapoom *et al.*, 2002).

From the fruits of *Luffa operculata*, Monte *et al.* (2003) discovered five known triterpenoids namely Cucurbitacin B, Cucurbitacin D, 3-epi-isocucurbitacin B, isocucurbitacin B and iso cucurbitacin D, having the 19 (10 > 9 beta) abeo-10-alpha-lanostane (Cucurbitane) skeleton. Ekam (2003) studied the seed oil composition of *Trichosanthes cucurmerina*. It has high oil content of 46.3 ± 4. Ester value and saponification value are also high.

The natural cucurbitacins constitute a group of triterpenoid substances which are well known for their bitterness and toxicity. Structurally, they are characterized by the tetracyclic cucurbitane nucleus skeleton, namely 19-(10 – 9 beta)-abeo 10 alpha-lanost-5-ene (also known as 9 beta-methyl-19-norlanosta-5-

ene), with a variety of oxygen substitutions at different positions (Chen *et al.*, 2005). *Trichosanthes cucumerina* L. seed oil gave punicic acid and other fatty acids by extraction. Leaves contain sitosterol and luteolin glucoside. *Trichosanthes tricuspidata* Lour. roots contains trichosterol (Farooq, 2005).

2.3 Pharmacological properties of cucurbitacins

A crystalline mixture obtained from ethrel extract of the fruit juice of *Trichosanthes cucumerina* L. possessed very strong cytotoxic action *in vitro* against human carcinoma cells of the floor of the mouth. Thin layer chromatography and mass- spectrometry indicated that cucurbitacin B was predominant in the mixture (Archa *et al.*, 1981).

Pharmacological effects of cucurbitacins were studied by Miro (1995). He listed the pharmacological properties of cucurbitacins which included gastro intestinal effects, cytotoxic and antineoplastic properties, hepatoprotective and hepatocurative properties, anti-inflammatory properties, contraceptive properties, cardiovascular effects, effects on central nervous system, antimicrobial and anthelmintic properties, antigibberellin activity and insecticidal properties. The effect of cucurbitacin and of *Ecballium* extract on the formation of mRNA coding for laccase was examined by Gonen *et al.* (1996) in the cultures of *Botrytis cinerea*. From the analysis, it was evident that cucurbitacin I and *Ecballium* extract specifically repress the amount of mRNA coding for laccase.

Cucurbitain B and E were isolated from a medicinal plant, *Wilbrandia ebracteata* and investigated by Peters *et al.* (1997). They reported that these two compounds inhibited carrageenan induced oedema.

Cheol *et al.* (2002) isolated cucurbitacins 1 and 2 from the roots of *Trichosanthes kirilowii* and spectroscopic analysis revealed that compounds 1 and 2 were cucurbitacin D ant 23, 24-dihydro cucurbitacin D respectively. These

compounds effectively inhibited the activity of tyrosinase and the synthesis of melanin in B16/F10 melanoma cells.

From the bark of *Elaeocarpus mastersic* cucurbitacin D and cucurbitacin F as cytotoxic principles, together with 2 ellagic acid derivatives were isolated by Ito *et al.* (2002) who evaluated them against a panel of human tumour cell lines. Mai *et al.* (2002) isolated a series of cucurbitacins from the fruits of *Trichosanthes tricuspedata* of which two are new, Tricuspedatin and 2-0-glucocucurbitacin J. It was found that, they are cytotoxic against cancer cells.

Jayaprakasam *et al.* (2003) reported the anticancer and anti inflammatory activities of cucurbitacins, obtained from a cucurbit called *Cucurbita andreana.* The cucurbitacins are highly oxygenated triterpenoid compounds found in several botanical families that show high toxicity and varied biological activities (Valente, 2004). Cucurbitacin E, like other cucurbitacins is a potential antineoplastic agent. Cucurbitacin E did not produce any significant cytotoxic effects on the lymphocytes. But it had a stimulatory effect and was capable of inducing and maintaining high proliferation rates in lymphocytes (Attard *et al.*, 2004).

Attard *et al.* (2005) studied the effects of cucurbitacin E, extracted from *Ecballium elaterium* fruits on ovarian cancer cell lines *in vitro.* Human ovarian cancer cells and human lymphocytes were treated with cucurbitacin E. The results obtained indicate that cucurbitacin E is toxic to ovarian cancer cells but not to normal peripheral lymphocytes.

From the tubers of *Hemsleya jinfushanensis* L.T. Shen four new cucurbitane glycosides were isolated by Chen *et al.* (2005). They are Jinfushanosides A-D, as well as four other known compounds of cucurbitacin 5-8, which have been tested for bioactivity against rabbit platelet aggregation. Sun *et*

al. (2005) reported that cucurbitacin Q inhibits selectively the action of STAT 3 and shows anti tumour activity in human.

2.4 Medicinal properties of *Trichosanthes cucumerina*

Aiyer and Kolammal (1963) reported the important preparations of *Trichosanthes cucumerina* as Gulgulutiktakam Kashayam, Mahatiktaka Gritham, Vajrakam Kashayam and Mahatiktakam Kashayam. They also reported that the fruits are light and bitter, promote digestion and sexual vigour and dispel the pathogenic organisms. The stem cures diseases caused by vitiation of kapha. Roots are good purgatives. The plant as a whole along with roots are used medicinally.

Blatter *et al.* (1975) reported that *Trichosanthes cucumerina* is used in Tridosha and that cures bronchitis. It is a febrifuge (given in decoction with ginger, chiretta and honey). The leaf juice is emetic and the stalk decoction is expectorant. *Trichosanthes cucumerina* as whole is regarded as a general and cardiac tonic with laxative, antipyretic and emetic properties as reported by Chatterjee and Chandraprakashi (1977). The plant source of the drug in the northern provinces of India is said to be *Trichosanthes dioica* Roxb. (Dey, 1980, Chunekar, 1982 and Sharma , 1983)

Khory and Katrak (1981) listed actions and uses of *T. cucumerina* as a laxative. They reported that it is also used against syphilis and rheumatism. The roots of *T. cucumerina* are used in bronchits. The fruits as well as roots are cathartic. *Trichosanthes cucumerina* L. is used as cardiac tonic, alternative and antipyretic useful for boils and intestinal worms. *Trichosanthes dioica* leaves made into decoction with equal parts of coriander given in bilious fever as a febrifuge and laxative (Singh and Khan, 1990).

Patola is regarded as a good blood purifier and hence beneficial in the treatment of skin diseases. It is bitter, acrid in taste, hot in action, appetizer, digestive, germicidal, laxative and aphrodisiac. It aids digestion, cures haematemesis, dermatosis, fever, cough and ulcers. It overcomes itching sensation, excessive thirst, intestinal disorders, concocted poisons, eye diseases and morbidity of tridoshas. Leaves are easily digested and cure diseases caused by pitta (Sivarajan and Balachandran, 1997). Leaves are prescribed in biliousness and their juice is applied to bald patches of alopecia. Seeds are anthelmintic and antifebrile (Ambasta, 1994).

Yadava and Syeda (1994) reported the anthelmintic properties of seeds of *T. cucumerina* that are used to treat syphilis. The hot aqueous extract of root tubers of *Trichosanthes cucumerina* L. exhibited significant anti-inflammatory activity against carrageenin induced mouse's hind paw oedema (Kolte *et al.*, 1997). Mooi *et al.* (1999) examined the ethanolic extracts of *Trichosanthes cucumerina* that showed antitumour promoting activity. Kapoor *et al.* (2001) evaluated the alcoholic extract of entire plant of *T. cucumerina* for its antihepatotoxic activity against carbon tetrachloride induced hepatotoxicity in rats. Kar *et al.* (2003) examined the blood glucose lowering activity of *Trichosanthes cucumerina* and obtained positive results.

2.5 Medicinal properties of related species of *Trichosanthes cucumerina*

Stuart (1911) mentioned that *Trichosanthes multiloba* is a thirst relieving, tonic and astringent in fluxes. They are also administered in jaundice, suppression of urine, relaxation of the mucous membranes, retained placenta and syphilitic ulcers. Gupta (1984) mentioned some of the important medicinal uses of *Trichosanthes* spp. The decoction of the leaves of *Trichosanthes dioica*, barley and coriander seeds administered with honey and sugar allays diarrhoea.

Decoction made by the following ingredients like leaves of *Trichosanthes dioica, Tinospora cordifolia,* the tubers of *Cyperus rotundus*, the bark of *Justicia adhatoda*, *Hedysarum alhagi*, *Agathotes cheyrata*, bark of *Melia azadirachta, Picrorrhiza kurrooa* and *Oldenlandia biflora* administered internally, causes the immature pox to dry up. It is beneficial in fevers caused by malignant boils and also cures diseases of the mouth.

The ingredients of the Patolacunthi ghrita are the paste of the leaves of *Trichosanthes dioica* and ginger or the paste of only the leaves of *Trichosanthes dioica,* boiled with ghee. This ghee is alleviative of phlegm and bile. The decoction of *Trichosanthes dioica,* belleric myrobalans, emblic myrobalans, chebulic myrobalans, *Melia azadirachta* and turmeric taken internally cures sores, boils and fever of children.

Lee *et al.* (1986) observed that ether and petroleum fractions of *Trichosanthes kirilowii* showed potential antitumour activity and cytotoxic activity. Patients of non-ulcer dyspepsia and peptic ulcer have been successfully treated with herbal preparations with *Trichosanthes dioica*. The species also possesses additional beneficial effects on body systems (Singh and Singh, 1987).

Dagar and Chaghtai (1989) reported that Onges and Ranchi tribals of Andaman and Nicobar islands used *Trichosanthes bracteata* against many skin diseases. Ethanolic extract of *Trichosanthes dioica* plant caused a significant lowering of blood sugar in fasted rats and depressed the peak value in glucose loaded single and long term fed groups of rats. The ethanolic extract of the aerial part of *T. dioica* also induced significant depression in the peak values in the glucose loaded models (Chandrasekhar *et al.*, 1989).

Sakai *et al.* (1989) reported that the water extracts of *Aloe ferox, Trichosanthes kirilowii, Ginkgo biloba, Crataegus cuneata, Momordica cochinchinensis* and *Smilax glabra* have a capacity of faster elimination of ethanol

from blood of normal rats when administered orally 30 minutes before oral administration of ethanol.

Sharma *et al.* (1989) found that whole fruit and pulp of *Trichosanthes dioica* show significant hypocholesterolemic, hypotriglycerdemic and hyperphospholipidemic effects in the normal as well as mild diabetic subjects. High density lipoproptien cholesterol increased whereas low density lipoprotein cholesterol levels decreased significantly in normal as well as mild diabetic human subjects.

Anti ulcer activities of the fruits of *Trichosanthes kirilowii* var *japonicum* were investigated, wherein, it was observed that at a dose of 100-1000 mg/kg showed potent protection against experimental gastric lesions and acetic acid induced gastric ulcer (Takano *et al.*, 1990). The polysaccharide fraction from the rhizome of *Trichosanthes kirilowii* showed antitumour and cytotoxic activity with immuno potentiating activity (Chung *et al.*, 1990). Sreedharan (1991) reported that bitter fruits such as *Trichosanthes dioica, Chenopodium album* and *Azadirachta indica* were effective against arthritis (sandivata).

Vlietinck *et al.* (1998) reported that *Trichosanthes kirilowii* is useful against Human Immuno Deficiency virus (HIV). Its plant extract contains Trichosanthin, a protein, which is having the medicinal property. Pumpkins and squashes contain cholin esterase inhibitors (Decoteau, 2000). Atul *et al.* (2002) listed the medicinal uses of *Trichosanthes cordata* Roxb. and stated that its tubers are used as tonic and dried powder is given in enlarged spleen or liver.

2.6 Antimicrobial and insecticidal properties of cucurbitacins

Chambliss *et al.* (1968) examined that certain squash varieties lack cucurbitacin and consequently they attract beetles for feeding. The bitter principle elaternide gives watermelon resistance to insect pests. Sharma and Halls (1971)

found that external application of cucurbitacins on seedlings of different cucurbits induced preference for diabroticite beetles. Action of cucurbitacins as kairomone for cucumber beetle *Diabrotica undecipunctata* and *D. balteata* was reported by Ferguson *et al.* (1982). Chandravardana and Pal (1983) reported the phenomenon of non-preference or antibiosis that provides resistance to pests is governed by naturally occurring biochemical constituents in plant tissues.

The effect of cucurbitacins A, B, C and E on the feeding activity of *Aulacophora foevicollis* was reported by Mehta and Sandhu (1992). Cucurbitacin B elicited the strongest feeding activity followed by cucurbitacin E, A and C. The report confirmed that cucurbitacins play a major role in host plant acceptance. Kolte *et al.* (1996) investigated that the chloroform extract of roots of *T. cucumerina* Linn. showed significant antibacterial activity against *Pseudomonas eruginosa* which is comparable with that of gentamicin but its activity against *Staphylococcus aureus* was not significant.

In the case of pumpkin and bottle gourd, low content of cucurbitacin, a tetracyclic triterpenoid is the cause of their resistance to red pumpkin beetle (Sinha and Krishna, 1970; Pal *et al.*, 1998). Agarwal *et al.* (2002) mentioned that cucurbitacins produced by *Cucumis sativus* plants were less attractive to predatory mites than plants that lack cucurbitacins and predators were also half as fecund on those bitter plants.

Martin *et al.* (2002) noticed that cucurbitacins are feeding stimulants for diabrotice beetles, including corn rootworms and cucumber beetles. One of them, cucurbitacin E glycoside is water soluble and easily processed from mutant bitter Hawkesbury watermelons that express elevated levels of cucurbitacins. Cucurbitacin content in *Cucumis sativus* was significantly correlated with spider mite resistance (*Tetranychus urticae*) (Boomstra *et al.*, 2003).

2.7 Performance of medicinal species under shade and in the open condition

Studies on domestication of *Trichosanthes cucumerina* have not been attempted so far. Hence, literature pertaining to performance of other medicinal species and cucurbitaceous plants under domesticated conditions is reviewed here under.

In turmeric no significant difference was observed between cultivars at different shade levels, but all cultivars gave highest rhizome yield at 50 per cent shade. Dry matter production and harvest index were also more at 50 per cent and 75 per cent respectively (Paul, 1992).

Menon (1994) observed that in *Plumbago rosea* flower initiation was comparatively early in the open as compared to shade. From January onwards the plants under open were taller as compared to that under shade. Plant spread was more in east-west direction in open while in shade north-south spread was more. A sudden increase in internodal length was noticed during January in the case of plants grown under shade. In the case of plants under open, a progressive increase in the length was recorded upto March which declined during April.

Kurian (1999) observed that asparagus performed well as an intercrop in coconut plantation recording high root yield and high BCR (1.25), when compared to pure crop (0.88)

In kalmegh plants grown under increasing light intensity, chlorophyll content per unit leaf area decreased. Also the total dry matter production of the plants grown under full light was less, as compared to plants grown under 15 percent light (Kapur and Kapur, 1999). Menon (1999) studied the performance of *Plumbago indica* in open and under shade. The plant comes up well under shade and can be successfully raised as an intercrop in coconut gardens, but therapeutic

principles of *Plumbago indica* did not significantly vary between pure crop and intercrop.

In Clocimum (*Ocimum gratissimum* Linn.), Pillai (1990) observed that the effect of shade on plant height and spread was positive upto intermediate shade level whereas its effect on number of branches, number of flowering shoots, length of inflorescence and leaf area was negative. The flowering and attainment of maturity were delayed progressively with increasing intensities of shade. Total herbage yield, highest values of oil content and oil yield were also recorded by the plants grown in the open.

Nair *et al.* (2000) conducted experiments on intercropping of cucurbitaceous vegetables in 25 years old coconut plantations at Sipighat. *Laginaria siceraria, Cucurbita moschata* and *Trichosanthes anguina* failed to flower and set fruits in the first two seasons. Neerakkal *et al.* (2001), in their investigation on leaf anatomy of medicinal species under different light regimes observed a significant decrease in leaf thickness for *Plumbago indica* grown under shade due to decrease in intercellular space and cell number in palisade layer. They further reported that in *Plumbago indica* maximum photosynthesis was noticed only for a short period in open sunlight, whereas, peak photosynthesis under shade continued for 3-4 hours even at mid day.

Gangadharan (2003) reported that lower shade level promoted higher rhizome yields in kacholam, while increased shading intensity lead to increase in qualitative contents in rhizomes. Provision of open conditions during the initial stages of growth followed by imposition of shade during the rhizome development phase, resulted in production of more quantity of officinal part with increased quality. Kurian *et al.* (2003) observed that *Plumbago rosea, Kaempferia galanga* and *Asparagus racemosus* gained high benefit cost ratio, when these crops are raised as intercrops, which showed the better adaptability of those crops for intercropping.

In safed musli (*Chlorophytum borivilianum*), rate of plantlet survival was 87 per cent and 90 per cent under open field and shade net conditions, respectively. Plantlets grown *ex vitro* under shade net and field conditions produced tuberous roots which could be grown in the next season as a secondary propagule (Dave *et al.*, 2003).

Joy *et al.* (2004) found that *Curculigo orchioides* plants yield high dry matter production at 25 per cent shade. Also the uptake of nutrients was higher under shaded condition. In *Cephaelis ipecacaunha*, Kurian and Shankar (2007) mentioned that planting in open fields gives higher content of alkaloids but lesser root yield. Growing the plants under the shade of nitrogen fixing plants gave encouraging results.

2.8 Performance of tissue culture derived plants

Since reviews pertaining to performance of tissue culture plants in the experimental species are scanty, performance of micropropagated plants in indigenous medicinal plants and other cucurbitaceous species are reviewed hereunder.

Tissue culture techniques are widely employed in various aspects of farming. By far, the best commercial application of tissue culture technology has been in the production of clonal plants at a very rapid rate compared to the conventional methods. Such plants are reported to grow faster and to mature earlier than seed propagated plants (Vasil and Vasil, 1980).

Investigation conducted by Ahuja *et al.* (1982) on *Ocimum* spp observed that plantlets propagated by *in vitro* method were successfully transferred to the field with only 10-25% mortality. CIMAP (1992) released two improved clones of *Mentha arvensis* generated by tissue culture techniques and

those two were characterized by higher herbage yield, better regeneration of foliage after first cut and better harvesting index.

In vitro produced plantlets of *Tylophora* showed 90-100% survival in soilrite. After 6-8 weeks they could be successfully transferred to the field as reported by Sharma and Chandel (1992). Bhat *et al.* (1992) reported that rooted plants of long pepper in MS media supplemented with IAA 0.1mg/l were successfully established in soil. In *Woodfordia fruticosa* (L.)Kurz., Krishnan and Seeni (1994) attempted *in vitro* shoot tip cultures and obtained regenerated plantlets with uniform morphological growth and flower characters.

Sharma and Singh (1997) reported that *in vitro*-derived ginger plants performed well under field conditions, were morphologically identical to the mother plants and were free from ginger yellows caused by *Fusarium oxysporum* f. sp. *zingiberi*. Well-developed rhizomes obtained from the tissue-cultured plants did not rot during storage upto 6 months, thus indicating that the method is also effective in checking storage rot caused by *F. oxysporum* f. sp. *zingiberi*.

In *Kaempferia galanga*, Joseph (1997) studied different parameters of clonal progenies and seedlings in the field. Percentage survival of plants was maximum for conventionally propagated plants (100%), followed by clonal progenies (90%). There was no difference in leaf orientations between these two. Conventionally propagated and clonally propagated plants had horizontally oriented leaves. Maximum leaf area per leaf was recorded in conventionally propagated plants as compared to clonally propagated ones.

Sudharshan *et al.* (1997) investigated that tissue culture derived clones showed variations in the type of panicle, capsule shape and size in cardamom. Borthakur *et al.* (1998) reported that regenerated plantlets of *Alpinia galanga* in MS medium were successfully transferred to soil where they grew well within10-12 weeks with 80% survival.

A rapid clonal propagation system for *Kaempferia galanga* (Zingiberaceae), a rare folk medicinal herb has been developed. Hardened plantlets produced normal storage roots as the parent plants. Around 1,000 plantlets have been produced successfully for field transfer (Shirin *et al.*, 2000).

Successful acclimatization and transplantation of *in vitro* derived plantlets of *Acorus calamus* was reported by Rani *et al.* (2000). Similar studies were also reported by Saini and Jaiwal (2000) in an important medicinal plant, *Peganum harmala.* Successful transplantation of micropropagated *Lawsonia inermis* was reported by Rout *et al.* (2001), Bhattacharyya and Bhattacharya (2001) in *Phyllanthus amarus* and Ahmed *et al.* (2001) in *Holarrhena antidysenterica* Similar results were also reported by Jadhav and Hegde (2001) in *Gloriosa superba* , Pandey and Jaiswal (2002) in *Terminalia arjuna* and Ananthakrishnan *et al.* (2003) in *Cucurbita pepo*.

Four months after establishment, the micropropagated plants of *Hemidesmus indicus* were stable and showed uniform morphological and growth characteristics. After 12 months of cultivation in the field, on an average, a micropropagated plant consisted of 4–5 shoots, 5–8 branches per shoot and increased root biomass (13.5 g) compared to the poor growth (single shoot and 2–3 branches) and root production (4.6 g) values obtained with plants raised from conventional rooted stem cuttings (Sreekumar *et al.*, 2000).

A protocol for the micropropagation of *Lilium nepalense* D.Don, a highly prized medicinal plant of Nepal, has been developed. After rooting the cloned plantlets were successfully hardened under *ex vitro* conditions. Preliminary trials in Nepal showed that the method can be successfully applied for the production of plants suitable for field cultivation (Anon, 2001).

The micropropagated turmeric plants showed a significant increase in shoot length, number of tillers, number and length of leaves, number of fingers

and total fresh rhizome weight per plant when compared with conventionally propagated plants (Salvi *et al.*, 2002). Martin (2003) reported that shoots of *Rotula aquatica* Lour. were successfully transferred to field conditions and 80% of the plantlets survived. A rare, threatened and near-endemic medicinal herb *Saussurea obvallata* (DC.) Edgew. was propagated by *in vitro* techniques and 66.7% of the plantlets has established in the field (Joshi and Dhar, 2003).

The micropropagated plants of *Plumbago rosea* were transferred to experimental plots and cultivated for 10 months to obtain a significantly higher number (18.0 ± 0.5) of larger tuberous roots (137.4 ± 3.4 g fw/plant) compared to conventional rooted cuttings (14.0 ± 1.7, 47.9 ± 1.6 g fw/plant). During this period, the concentration of the root-specific compound, plumbagin recorded per g dw (1.5 %), was higher than that of conventionally propagated plants (0.9 - 1.0 %) (Satheeshkumar and Seeni, 2003).

Muthukumar and Arockiasamy (2003) mentioned that *in vitro* plants from *Datura metel* were successfully transferred to the field. The regenerated plantlets showed 90% of survival and resumed growth in the field. The content of active ingredients in tissue cultured cells are less than those in conventionally raised saffron pistils. However, the quantity of crocin A, which showed good anticancerous activity was 2-3 times more than that in conventionally raised saffron pistils (Sun *et al.*, 2004).

In thathiri (*Woodfordia fruticosa*), plantlets produced by *in vitro* techniques were free from any morphological abnormalities. However, chlorophyll mutations were observed during four months after transplantation (Gayathri, 2005).

Samples of the Himalayan medicinal plant *Swertia chirata* (Gentianaceae) obtained from a local market in Nepal, from a micropropagated field cultivated clone and from two *in vitro* clones were compared by means of

HPLC. The substance patterns of methanolic and dichloromethane extracts of the *in vivo* grown materials showed good conformity while in the samples from tissue culture, major compounds were missing. Results confirm that the secondary metabolites of *in vitro* cultivated plants normally differs from that of plants in their natural environment (Wawrosch *et al.*, 2005).

2.9 Analytical techniques for estimation of secondary metabolites in medicinal species, with special reference to cucurbitacin

Using a semi-quantitative analytical method for cucurbitacins, Rehm and Wessels (1957) measured the cucurbitacin content of radicles and cotyledons of ordinary food plants. Lisoba (1969) and Neher (1969) suggested the use if Libermann- Burchard reagent for detection of triterpenoids. Sinsheimer *et al.* (1970) used techniques like thin layer chromatography (TLC), partition chromatography and column chromatography to separate acidic glycosides from *Gymnema sylvestre.*

The concentrated methanol extracts are used directly for the examination of triterpenoids and argentative TLC is employed for separating triterpenoids (Harborne, 1973). A hexahydroxy triterpene was isolated by Chakravarthi and Debnath (1981) from *Gymnema* by successive extraction with chloroform, ethyl acetate and ethanol followed by chromatographic separation of acid hydrolysed products of the extracts.

A quantitative method for the determination of cucurbitacin-C in *Cucumis sativus* was presented by Keulen (1981). Crude chloroform extracts of the leaves were subjected to thin layer chromatography (TLC). After treatment with antimony trichloride and heating, the fluorescence of the spots was measured with a flying spot densitometer.

Qualitative tests like Dragendorf test, Mayers test and Wagners test for alkaloids, tests for phenols, terpenes, tannins and saponins were described by

Harborne (1973) and Chatwal (1988). Lin and Cordell (1989) extracted triterpenoids from the roots of *Salvia prionitis* by ethanol and partitioned them as chloroform and water extracts. The organic layer was further subjected to column chromatography and preparative TLC.

A colorimetric method was used to determine the total cucurbitacin levels and a TLC densitometric assay was employed for estimating dihydrocucurbitacin F and its acetate, two active principles in *Hemsleya dolichocarpa* plants. Cucurbitacin contents rise progressively from May to November, the month of November is the most desirable time for harvesting plant materials for medicinal uses. It was noticed that the spoiled plant samples gave an intense color reaction while dihydrocucurbitacin F and its acetate levels were not significantly altered. The accuracy of the assays is discussed (Yang *et al.*, 1991).

Raj (1997) estimated crude extractables present in various medicinal plants by performing soxhlet extraction with petroleum ether as solvent. To estimate crude extractables present in *Sida* spp. Sankar (1998) used soxhlet extraction with methanol as solvent.

Aruna *et al.* (1999) evolved a procedure for thin layer chromatography of *Tephrosia procumbens*. Stem and leaf materials were collected and powdered coarsely. It was extracted with chloroform and methanol in order of polarity to get their residue. The chloroform extract residue showed a number of spots on TLC.

From *Jatropha gossipifolia*, Madhumati *et al.* (1999) extracted essential oils using general thin layer chromatography method, which has antifungal property against *Candida albicans*, *Aspergillus terreus*, *Fusarium oxysporum* and *Pestalotiopsis palmarum*. Stum and Stuppner (2000) applied high pressure liquid chromatography- Mass spectrometry for the analysis of cucurbitacin in medicinal plants. High molecular weight plant constituents were

separated from cucurbitacins by gel chromatography of the crude methanolic extracts.

Manjusha (2001) performed qualitative tests for secondary metabolites (phenols, flavanoids, terpenoids and alkaloids) present in *Adhatoda zeylanica and Adhatoda beddomei*, using thin layer chromatography. She also employed soxhlet extraction method using methanol as solvent to estimate alkaloid content of *Adhatoda* spp. Dinen *et al.* (2001) inferred that the predominant methods for isolation of compounds like cucurbitacins and steroids are solvent extraction or partition followed by column chromatography and thin layer chromatography/HPLC.

Cucurbitacin E was quantified by chromatographic studies with UV absorption at 229 nm, from the callus material of *Ecballium elaterium* L. and also by spectrophotometric method with optical density at 492 nm (Attard, 2002). From an oriental medicinal plant, *Cucurbita moschata* Yang *et al.* (2002) isolated anticomplementary substances from chloroform and ethyl extracts, using thin layer chromatography.

Biological and spectrophotomeric methods still being used for saponin determination provide, to some extent, valuable results on saponin concentrations in plant material. Thin-layer chromatography on normal and reversed-phases (TLC, HPTLC, 2D-TLC) provides excellent qualitative information and in combination with on-line coupling of a computer with dual-wavelength flying-spot scanner and two-dimensional analytical software can be used for routine determination of saponins in plant material. The densitometry of saponins has been very sensitive (Oleszek, 2002).

Estimation of aloe emodin, chrysaphanol, emodin and rhein in free and combined forms of *Cassia pumila* in the plant parts like root, stem, leaf, pericarp

and seed except flower has been carried out by Shingare *et al.* (2003) using thin layer chromatography- colorimetric method.

Yakovishin (2003) reported that colour reactions are used to detect triterpene glycosides on thin layer chromatography plates because pure triterpene glycosides are colourless compounds. Bo *et al.* (2004) developed a method for analysis of saponins from *Panax pseudoginseng* var. *notoginseng* using pressurized solvent extraction coupled with high performance thin layer chromatography. A simple and sensitive spectrophotometric method in ultraviolet region has been developed for the determination of phyllanthin in different parts of *Phyllanthus niruri*. Phyllanthin shows maximum absorbance at 280 nm (Venkatesh *et al.*, 2004).

Coarse powder of *Benincasa cerrifera* root was extracted with different solvents, each solvent extact was passed through qualitative tests. Each solvent was further subjected to thin layer chromatography. The qualitative tests and TLC indicated the presence of two steroids in the roots. Alkaloids were found to be absent. Further study was performed by UV and IR spectrophotometer (Chatterjee *et al.*, 2004).

For quantification of saponins in *Gymnema sylvestre*, Nair (2005) used thin layer chromatography using chloroform:acetone:methanol as the solvent system. The study was conducted by Hamdah *et al.* (2005) adopted thin layer chromatography to detect adulterants from the powdered medicinal plant materials.

Extraction of cucurbitacin I from rat plasma was performed by using liquid chromatography-mass spectrometry. The developed assay is sensitive, specific, reproducible and reliable for quantitative analysis of cucurbitacin I (Molavi *et al.*, 2006). Soxhlet extraction was performed by Shankar (2006) to estimate crude extractables from *Coleus amboinicus* Lour. using ethyl acetate as solvent.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled "Evaluation of seedlings and clonal progenies of Kattupatavalam (*Trichosanthes cucumerina* L.) for yield and quality" was carried out at the Department of Plantation Crops and Spices and the biochemical analyses were done in the Laboratories of Plantation Crops and Spices and Plant Biochemistry at College of Horticulture, Vellanikkara, during 2005-2006. The materials used and methodologies adopted for the study are described in this chapter.

3.1 DETAILS OF EXPERIMENTAL FIELD

3.1.1 Area

Field experiment was conducted in an adult coconut garden under 35 per cent shade and in an open field.

3.1.2 Location

The experimental field is situated at 10^0 30' N and 76^0 13' E longitudes with an altitude of 22.25m above Mean Sea Level.

3.1.3 Soil

The soil of the experimental plot is deep laterite with clay loam texture of pH 5.6.

3.1.4 Climate

The area enjoys a warm humid tropical climate. The weather situation during the period under study was normal and in tune with the annual cycle without any significant variation. The details of the meteorological observations for the period of the experiment are presented in Appendix 1.

3.2 EXPERIMENT I

Field evaluation of seedlings and clonal progenies of *Trichosanthes cucumerina* in the open and under shade in an adult coconut garden for growth and yield attributes.

3.2.1 Materials

3.2.1.1 Raising of source plants

The tissue culture plants employed in the study were hardened plantlets, generated at Kottakkal Aryavaidya Sala, Malappuram, utilizing the standardized protocol for nodal bud culture developed at the Department of Plantation Crops and Spices, College of Horticulture. Seeds procured from *Trichosanthes cucumerina* plants at the Department of Plantation Crops and Spices were used to raise seedlings.

3.2.2 Methodology

The study was carried out as a split plot experiment in RBD with two main treatments, S_1 (in mature coconut plantations) and S_2 (in open) and two subplot treatments, T_1 (seedlings) and T_2 (clonal progenies).

3.2.2.1 Table 1. Details of experiment

Total number of treatments	4
Number of replications	5
Number of plants per treatment	3
Plant to plant distance	2m x 2m
Design	RBD

3.2.2.2 Main field planting

Seedlings and clonal progenies were raised in the Gokhale block of the Department of Plantation Crops and Spices in February 2005-2006, in the open and in mature coconut plantation. Pits were prepared at 2m x 2m spacing. At the

Plate 1a. General field view of *Trichosanthes cucumerina* plants raised in the open

Plate 1b. Seedling progeny of *T. cucumerina* in the open

Plate 1c. Tissue culture derived progeny of *T. cucumerina* in the open

Plate 2a. General field view of *Trichosanthes cucumerina* plants raised in an adult coconut garden

Plate 2b. Seedling progeny of *T. cucumerina* raised in the adult coconut garden

Plate 2c. Tissue culture derived progeny of *T. cucumerina* raised in the adult coconut garden

time of field preparation farmyard manure was applied @ 10 t/ha. In one pit, one plant was retained (Plate 1 and Plate 2).

Irrigation was done daily for 2 weeks, immediately after planting. Subsequently, plants were irrigated on alternate days. Channel watering was done. After one month, weeding was done and farmyard manure was top-dressed @ 10 t/ha. Mounds were taken to strengthen the base of the plant. The crop was raised on pandals to facilitate vining. Observations were taken at an interval of two weeks from planting onwards to assess important growth characters of seedlings and clonal progenies under shade and in the open.

3.2.2.3 Harvesting

The experimental plants were harvested four months after planting. Mature vines were uprooted and roots were washed thoroughly in running water to remove adhering soil particles. Fresh herbage yield was recorded. Morphological variations, if any, between seedlings and clonal progenies with respect to shape of leaves and fruits were also observed.

3.2.2.4 Drying

Plants were then kept under shade for drying. During drying, the herbage was frequently turned over to ensure proper drying and dry weight was taken till the weights reached a constant value. After thorough drying, the materials were finely ground which was used for subsequent biochemical analyses.

3.2.2.5 Morphological characters observed

a. Main vine length (cm)
b. Primary branches per plant
c. Days to flowering
d. Days to fruit maturity
e. Number of fruits per plant

f. Fruit yield per plant (g)

g. Total fresh yield of whole plant (shoots, fruits and roots) (g)

h. Total dry yield of whole plant (shoots, fruits and roots) (g)

i) Total crop duration

3.3 EXPERIMENT II

Biochemical analyses for evaluation of seedlings and clonal progenies of *Trichosanthes cucumerina* in the open and under shade in an adult coconut garden for quality.

Biochemical analyses of experimental samples and market samples were carried out in the Plant Biochemistry Laboratory of College of Horticulture, Vellanikkara.

3.3.1 Soxhlet extractables

Two grams of dried and finely powdered sample was taken in a filter paper thimble and soxhletted for 2 hours using 150 ml of petroleum ether (40-60^0 C) in a water bath. After extraction, the solvent was removed by vacuum drying and the amount of the crude extractables calculated.

3.3.2 Hot water extractables

To estimate the quantity of crude extractables, by hot water extraction, ten grams of fresh sample was taken in a beaker with 200 ml water. The initial weight of the beaker was noted. The beaker was closed with a lid and heated on a water bath. Temperature was maintained at 60^{0}C for one hour. After one hour the beaker was weighed again to record final weight and the difference between final and initial weights was recorded.

3.3.3 Estimation of cucurbitacin

3.3.3.1 Thin layer chromatography

To identify the important cucurbitacins present in seedlings and clonal progenies of *Trichosanthes cucumerina*, thin layer chromatography was performed.

3.3.3.1.1 Preparation of crude extract

Three grams of oven dried powdered sample was extracted with 20 ml chloroform. This chloroform extract was used for spotting on TLC plates.

3.3.3.1.2 Preparation of TLC plates

Sixty grams of Silica Gel GF 254 of 160 to 250 mesh size was taken in a round bottomed flask, mixed with 120 ml of 2.5% silver nitrate solution. The slurry was spread on a gel plate of 20cm x 10cm size with an applicator to provide 0.25mm thick gel layer. The plates were allowed to set for 10 minutes at room temperature and then placed in a chromatographic chamber at 110^0 C for an hour to activate the silica gel.

3.3.3.1.3 Spotting on TLC plates

By using capillary tubes, 10µl sample was taken for spotting on TLC plates. Spotting was done at a distance of 2cm from the lower side and also from right and left sides of the plate, maintaining a distance of 2cm between each spot. To avoid the excessive spreading of samples, intermitent application of small doses of sample was done. After application of each small dose, the solvent was evaporated from the point of application by giving hot air from a hair dryer. This was repeated till all the 10µl volume of the sample was completely applied.

3.3.3.1.4 Running solvent system

The solvent system, chloroform-acetic acid in the ratio 1:1 was poured into the tank and closed tightly with a lid. After 30 minutes, the spotted plates were placed in the tank such that the spots were above the surface of solvent

system. When solvent had run two third length of the plate, the plate was taken out and placed under an exhaust flow of air to evaporate the solvents from the gel plate.

The plates were then kept for 15 minutes to evaporate the remaining solvents from plates. These plates were examined under UV light (366 nm) and the Rf values were noted.

$$\text{Rf value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by solvent}}$$

3.3.4 Quantification of cucurbitacin

To quantify the content of cucurbitacin from seedlings and tissue culture plants of *T. cucumerina* grown in open and under shade conditions, quantitative TLC was performed. To prepare standard curves, the following steps were carried out.

3.3.4.1 Preparation of TLC plates

As mentioned earlier, by using homogenous slurry of silica gel GF 254 and silver nitrate (2.5%), glass plates were coated and oven dried for one hour at 110^0 C.

3.3.4.2 Preparation of crude extract

1.5 g each of the dry sample of seedlings raised in open, tissue culture plants raised in open, seedlings grown under shade, tissue culture plants grown under shade and market samples were powdered and extracted with 15 ml chloroform. The extracts were filtered thoroughly through Whatmann no: 42 filter paper and filtrates were collected in test tubes.

3.3.4.3 Spotting on TLC plates

Varying concentrations of 10µl, 15 µl, 20 µl, 25 µl, and 30 µl were taken from the extract and spotted on TLC plates, 2 cm apart. Hair dryer was used to minimize the spreading of spot.

3.3.4.4 Running solvent system

Two hundred and fifty millilitre of chloroform-acetic acid in the ratio of 1:1 was transferred to the tank and closed tightly with the lid. After 30 minutes, the sample applied plates were placed in the tank such that the spots were above the surface of solvent system. In about one hour elution of spots was achieved when the solvent had run two third length of the plate. The plates were then placed under an exhaust flow of air to evaporate the solvents from the gel plate.

The spots present on the TLC plates were scrapped gently and transferred into test tubes, 5ml of chloroform was poured into each tube and shaken vigorously. These solutions were centrifuged at 10000 rpm for 10 minutes. The supernatant was taken and read in a UV spectrophotometer at 660 nm against the reagent blank (chloroform). The absorbance of cucurbitacin was recorded for each tube and prepared the graph for quantification.

3.3.5 Qualitative analysis for other secondary metabolites in experimental samples

To identify the other secondary metabolites present in experimental samples and market samples, the following tests were carried out.

3.3.5.1 Alkaloids

3.3.5.1.1 Mayer's test

3.3.5.1.1.1 Preparation of Mayer's reagent

Solution A was prepared by dissolving 0.36g of mercuric chloride in 60ml distilled water. By dissolving 5g potassium-iodide in 10ml distilled water,

solution B was prepared. Both these solutions were mixed thoroughly and made up to 100ml to make Mayer's reagent solution.

3.3.5.1.1.2 Test for alkaloids

Five hundred milligram of dry powdered sample was extracted with 5ml chloroform. The extract was filtered through Whatmann no: 1 filter paper to a get clear solution. 5ml of Mayer's reagent was added to 2ml of sample extract and shaken gently for few minutes and observed the colour.

3.3.5.1.2 Wagner's test

3.3.5.1.2.1 Preparation of Wagner's reagent

Wagner's reagent was prepared by dissolving 2g of potassium iodide in to 5ml distilled water. This solution was added to 100ml 0.1N iodine solution.

3.3.5.1.2.2 Test for alkaloids

Dried and finely ground sample of 0.5g was extracted with 5 ml chloroform. The extract was filtered. To 2ml aliquot, 5ml of Wagner's reagent was added. After shaking this solution, colour developed was noted.

3.3.5.1.3 Dragendorff test

3.3.5.1.2.3 Preparation of Dragendorff reagent

Solution A and solution B were prepared by adding 8g bismuth subnitrate in 20ml conc. nitric acid and by dissolving 27.2g potassium iodide in 50 ml of distilled water, respectively. These two solutions were mixed well and allowed to precipitate. The supernatant was made up to 100ml.

3.3.5.1.2.4 Test for alkaloids

0.5 g of dry powdered sample was extracted with 5ml chloroform. This extract was filtered through Whatmann no: 1 filter paper to get a clear solution.

5ml of Dragendorff reagent was added into 2 ml of sample extract and shaken gently for few minutes and developed colour was noted.

3.3.5.2 Phenols

3.3.5.2.1 Preparation of crude extract

1.5 g dry powdered sample was extracted with 15 ml of methanol and evaporated under an exhaust chamber to reduce the volume to 3 ml. This was used for spotting on TLC plates.

3.3.5.2.2 Preparation of TLC plates

Cleaned glass plates of 20cm x 10cm were used for TLC. The plates were coated with homogenous slurry of silica gel G. For one glass plate 10 g of silica gel and 20 ml distilled water were sufficient. Silica gel and water were taken in a conical flask and shaken vigorously to get a homogenous mixture and poured into the TLC plate gel applicator of GCME type. The plates were passed one by one through the slit quickly to avoid setting of the silica within the applicator itself. The plates were allowed to dry for 15 minutes, after which they were kept on TLC stand and placed in chromatography oven at 110^0 C for one hour. These activated plates were used for spotting samples.

3.3.5.2.3 Spotting on TLC plates

By using capillary tubes, 10µl sample was spotted on TLC plates as described in 3.3.3.1.3.

3.3.5.2.4 Running solvent system

Different proportions of chloroform-acetic acid solvent system were tried to get clear spots of phenols. Chloroform-acetic acid of 10:1 ratio gave better elution of sample on TLC plates. Running of TLC plates in the solvent system was carried out as detailed in 3.3.3.1.4.

3.3.5.2.5 Preparation of spray reagent

Folin-Ciocalteau phenol reagent was used as the spray reagent for phenol. Uniform spraying was done with fine droplets of spray reagent by using TLC sprayer. After spraying, the plates were kept at 110^0 C in a chromatography oven to develop coloured spots.

3.3.5.3 Tannins

One gram each dry sample of experimental plants and market sample were hydrolysed separately with 10ml of 2M hydrochloric acid for 30 min. Filtered them through ordinary filter paper and the filtrates were heated in a water bath at 100^0C for 30 minutes and then allowed to cool and observed the colour.

3.3.5.4 Saponins

Aqueous alcoholic plant extract was prepared in a test tube and shaken well to detect the presence of saponins.

3.3.5.5 Terpenes

Detection of terpene was done by using Liebermann-Burchard reagent. This reagent was prepared by adding 1ml concentrated sulphuric acid to a mixture of 20ml acetic anhydride and 50ml chloroform.

Finely ground, 0.5g sample was extracted with 10ml chloroform. From this, 0.2ml was taken to which Liebermann-Burchard reagent was added and shaken well.

3.3.5.6 Total terpene estimation

For quantification of terpenes, colorimetry was employed. Twenty gram fresh sample (leaf, stem, fruit, root and whole plant) of experimental materials were extracted with 20 ml saturated lead acetate and 20 ml methanol. For obtaining a clear extract, centrifugation was done at 10,000 rpm for 10 min. To this, 25% potassium dihydrogen ortho phosphate was added drop by drop till

the complete precipitation of excess lead. The above solution was filtered through filter paper and filtrate was collected in a conical flask. The filtrate was again extracted with 100 ml chloroform for 3 times using a separating funnel. The chloroform extract was evaporated at 60^0C in a water bath to obtain the white crystalline residue. Dissolved the crystals in 10 ml distilled water and only two ml of this solution was taken for further analysis. To this solution, 2.5 ml of Folin-Ciocalteau reagent and 10 ml of 20% sodium carbonate were added carefully and the volume made up to 100 ml. This was used for taking reading in spectrophotometer at 650 nm. Standard curve was prepared by taking different concentrations of extract, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml and 3.0 ml.

3.4 EXPERIMENT III

Anatomical investigations for evaluation of domesticated samples and market samples.

Thin cross sections of stem, leaf and root of *T. cucumerina* were prepared by placing the specimen between two layers of thermocol tightly. The fine sections were placed on a clean slide to which DPX mountant was added and placed a cover slip over the sections to make the slides permanent.

The slides were observed under stereomicroscope to examine anatomical differences if any, between seedlings and clonal progenies raised in open and under shade and between experimental samples and market samples.

3.5 Statistical analysis

Data were analysed using the analysis of variance for split plot design (Gomez and Gomez, 1984).

RESULTS

4. RESULTS

The present study was conducted at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara to evaluate seedlings and tissue culture derived progenies of *Trichosanthes cucumerina*, in open and under shade conditions with respect to growth, yield and quality. The results of this study are presented in this chapter.

4.1 EXPERIMENT I

Field evaluation of seedlings and clonal progenies of *Trichosanthes cucumerina* in open and under shade for growth and yield attributes

4.1.1 Growth characters

Data on the morphological characters of seedlings and tissue culture derived plantlets of *Trichosanthes cucumerina* are presented below.

4.1.1.1 Main vine length

Results of the study with respect to main vine length of experimental plants are presented in Table 2.

It was observed that experimental plants exhibited no significant variation in vine length, in shade (S_1) and in the open (S_2). But a slight increase in vine length was recorded in the open (306.18 cm) while a mean vine length of 304.44 cm was recorded under shade.

Significant variation was recorded between subplot treatments. Seedling progenies were superior with respect to mean vine length, registering a mean value of 363. 04cm. For clonal progenies, the mean vine length was 247.58cm.

Seedlings and clonal progenies expressed significant variation in mean vine length, under shade as well as in the open conditions. Seedlings under shade recorded maximum vine length (363.87 cm) as compared to clonal progenies (245.05 cm). Similar result was obtained in the open as well. Maximum vine length was noticed for seedlings under open condition (362.25cm) followed by clonal progenies (250.10cm).

Main vine length did not differ significantly in open and under shade, for seedlings as well as for tissue culture derived plants. Seedlings recorded a mean vine length of 363.87cm in shade and 362.25cm in the open. Clonal progenies registered 245.05cm vine length in shade and 250.10cm in open conditions.

4.1.1.2 Primary branches per plant

There was no significant variation in primary branches per plant between main plot treatments while significant variation was observed in mean number of primary branches between seedlings and clonal progenies. Mean number of primary branches for seedlings was 9.63 while clonal progenies recorded 7.90 branches (Table 3).

In the case of interactions between main plot and sub plot treatments, T_1 recorded more number of branches in shade (9.53) as compared to T_2, the values being significantly different. Superior performance of seedlings with respect to mean number of primary branches per plant was evident in open conditions as well, wherein seedlings recorded 9.73 branches per plant while clonal progenies recorded 8.00 branches per plant.

In shade and open conditions, homogenous performance was observed for seedlings and clonal progenies. T_1 recorded 9.53 primary branches in shade and 9.73 in the open situation. Clonal progenies also showed similar trends registering no significant difference with respect to primary branches per plant under shade (7.80) and in open (8.00).

Table 2. Effect of shade and open conditions on main vine length of seedlings and clonal progenies of *Trichosanthes cucumerina*

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	363.87 $^{a\,A}$	362.25 $^{a\,A}$	363.04 *
Clonal progenies (T_2)	245.05 $^{b\,A}$	250.10 $^{b\,A}$	247.58 *
Mean	304.44 NS	306.18 NS	
CD for comparison = 10.80			

Table 3. Effect of shade and open conditions on primary branches of seedlings and clonal progenies of *Trichosanthes cucumerina*

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	9.53 $^{a\,A}$	9.73 $^{a\,A}$	9.63 *
Clonal progenies (T_2)	7.80 $^{b\,A}$	8.00 $^{b\,A}$	7.90 *
Mean	8.67 NS	8.87 NS	
CD for comparison = 0.69			

* → Significance at 1% level

** → Significance at 5% level

NS → non- significance

a and b → column wise interactions

A and B → row wise interactions

4.1.1.3 Days to flower

Effects of shade and open conditions on flowering of seedlings and clonal progenies are presented in Table 4.

Significant variation was observed in mean number of days to flower between plants raised in open and under shade. Plants raised in open took 66.53 days to flower as compared to plants raised under shade (70.33 days).

Seedlings registered a mean value of 72.53 days for flowering while clonal progenies flowered in 64.33 days, the values being significantly different (Table 4).

As to interactions, seedlings and clonal progenies were statistically heterogenous in open and shaded conditions, registering significant variation in the parameter studied. In shaded condition, T_1 recorded more number of days for flowering (74.80 days) while T_2 took 65.86 days to flower. In the open, mean number of days for flowering was 70.27 days for T_1 whereas for T_2, the value was 62.80 days.

Both seedlings and clonal progenies, considered singly, showed significant variation in days to flower under shade and in the open. T_1 recorded 74.80 days for flowering in shade and 70.27 days in the open. T_2 recorded 62.80 days for flowering in open condition while under shade, 65.86 days were required for flowering of clonal progenies, the values being significantly different.

4.1.1.4 Days to fruit maturity

Data on the effect of shade and open conditions on seedlings and clonal progenies with respect to days taken for fruit maturity are presented in Table 5.

Table 4. Effect of shade and open conditions on days for flowering of seedlings and clonal progenies of *Trichosanthes cucumerina*

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	74.80 ^{aA}	70.27 ^{aB}	72.53 *
Clonal progenies (T_2)	65.86 ^{bA}	62.80 ^{bB}	64.33 *
Mean	70.33 *	66.53 *	
CD for comparison = 2.05			

Table 5. Effect of shade and open conditions on days to fruit maturity of seedlings and clonal progenies of *Trichosanthes cucumerina*

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	27.40 ^{aA}	23.87 ^{aB}	25.63 NS
Clonal progenies (T_2)	27.20 ^{aA}	24.13 ^{aB}	25.67 NS
Mean	27.30 *	24.00 *	
CD for comparison = 0.86			

Between main plot treatments, significant difference was observed in days taken for fruit maturity. Mean number of days of 27.30 was recorded for fruit maturity of experimental plants under shade as compared to open (24.00 days).

Between subplot treatments, no significant variation was observed. Seedlings recorded 25.63 days to attain fruit maturity while clonal progenies took 25.67 days for fruit maturity.

In shaded condition, T_1 recorded 27.40 days to attain fruit maturity, which was statistically on par with T_2, which registered 27.20 days, for fruit maturity. In open condition also the respective values were on par (23.87 days for seedlings and 24.13 days for clonal progenies).

Significant variation was recorded in the *per se* performance of T_1 and T_2 with respect to days to fruit maturity in shade and the open. Seedlings recorded 27.40 days and 23.87 days for fruit maturity, in shade and in open conditions respectively. In the open, T_2 attained fruit maturity earlier (24.13 days) as compared to shade (27.20 days), the difference being significant.

4.1.1.5 Number of fruits per plant

Number of fruits per plant varied significantly in main plot and subplot treatments. Data pertaining to the number of fruits per plant are presented in Table 6.

Fruit number was maximum in plants under shade (43.07) followed by plants in the open (37.70). In subplot treatments, clonal progenies recorded more number of fruits (45.47) while for seedlings fruit number was only 35.30.

T_1 and T_2 were statistically heterogenous in number of fruits per plant under shade. T_2 recorded significantly more number of fruits (49.00) as compared to seedlings (37.13). In the open, T_1 recorded 33.47 fruits which was on par with T_2 (41.93).

Fruit number recorded for seedlings under shade (37.13) and in the open (33.47) did not exhibit significant differences between themselves. Clonal progenies also registered values, which were on par in open (41.93) condition as well as under shade (49.00).

4.1.1.6 Fruit yield per plant

Results obtained for fruit yield per plant in seedlings and clonal progenies in open and under shade are presented in Table 7.

There was no significant difference in mean fruit yield per plant between main plot treatments, wherein plants raised under shade recorded a mean fruit yield of 275g/plant, while plants raised in open recorded 244.64g/plant.

Subplot treatments did not vary significantly. For clonal progenies 270.96g of fruits was obtained while for seedlings, the respective value was 248.68g, the difference in mean fruit yield being non significant.

In shaded condition, T_1 recorded a fruit yield of 248.19g, which was on par with T_2 (301.82g). Similar results were obtained in the open as well, wherein, seedlings recorded a mean fruit yield of 249.17g per plant and clonal progenies recorded a mean fruit yield of 240.10g, the differences being non-significant.

Per se performance of experimental plants was homogenous in shade and in open conditions. Seedlings recorded values on par with each other in open (249.17g) and in shade (248.19g). Similar trends were noticed in the case of clonal progenies as well, where the respective values were 240.10g and 301.82g.

4.1.1.7 Fresh yield of whole plant

No significant difference was observed between main plot and subplot treatments with respect to fresh yield of whole plant (Table 8). S_1 recorded higher mean total fresh yield of whole plant (654.73g) as compared to S_2 (613.00g).

Table 6. Number of fruits per plant in seedlings and clonal progenies of *Trichosanthes cucumerina* in open and under shade

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	37.13 ^{bA}	33.47 ^{aA}	35.30 *
Clonal progenies (T_2)	49.00 ^{aA}	41.93 ^{aA}	45.47 *
Mean	43.07 *	37.70 *	
CD for comparison = 9.22			

Table 7. Fruit yield per plant in seedlings and clonal progenies of *Trichosanthes cucumerina* in open and under shade

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	248.19 ^{aA}	249.17 ^{aA}	248.68 NS
Clonal progenies (T_2)	301.82 ^{aA}	240.10 ^{aA}	270.96 NS
Mean	275.00 NS	244.64 NS	
CD for comparison = 96.59			

Between sub plot treatments, T_2 recorded higher fresh yield (655.25g) than T_1 (612.49g), though the differences were not significant.

Results of main plot and subplot interactions revealed that T_2 recorded higher fresh yield (694.80g), which was on par with T_1 (614.66g) under shade. In open condition also, T_2 recorded fresh yield (615.69g) which was on par with T_1 (610.31g).

Per se performance of seedlings (T_1) was on par in both open and under shade. Seedlings under shade, recorded an average yield of 614.66g while in open, the average yield was 610.31g. Clonal progenies recorded an yield of 694.80g under shade and 615.69g in the open, the values being on par with each other.

4.1.1.8 Dry yield of whole plant

Table 9 shows the results obtained for dry yield of whole plant in the experimental plots.

No significant difference was obtained in main plot and subplot treatments with respect to total dry yield per plant. Between main plot treatments, mean dry yield was more in plants grown under shade (157.15g) as compared to plants grown in the open (145.65g). Between subplot treatments, T_2 recorded a mean dry yield of 156.20g and T_1 recorded a yield of 146.59g, the values being not significantly different.

In shade, T_1 recorded a dry yield of 148.25g which was on par with T_2 (166.04g). T_1 (144.93g) and T_2 (146.37g) registered no significant difference in their total dry yield per plant in the open as well.

In shade and in open conditions, T_1 performed similarly yielding 148.25g of dry yield under shade and 144.93g in the open. Under shade and in

Table 8. Total fresh yield of whole plant in seedlings and clonal progenies of *Trichosanthes cucumerina* in open and under shade

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	614.66 ^{aA}	610.31 ^{aA}	612.49 NS
Clonal progenies (T_2)	694.80 ^{aA}	615.69 ^{aA}	655.25 NS
Mean	654.73 NS	613.00 NS	
CD for comparison = 126.17			

Table 9. Total dry yield of whole plant in seedlings and clonal progenies of *Trichosanthes cucumerina* in open and under shade

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	148.25 ^{aA}	144.93 ^{aA}	146.59 NS
Clonal progenies (T_2)	166.04 ^{aA}	146.37 ^{aA}	156.20 NS
Mean	157.15 NS	145.65 NS	
CD for comparison = 33.30			

open, T_2 also performed homogenously with a mean dry yield of 166.04g in shade and 146.37g in the open.

4.1.1.9 Total crop duration

Significant difference in total crop duration was observed in main plot and sub plot treatments. The results obtained are presented in Table 10.

Experimental plants grown in open and under shade exhibited significant differences in total crop duration. Highest mean crop duration was observed in plants, which were grown in the open (115.13 days) as compared to crops grown in shade (111.07 days). Clonal progenies recorded a mean crop duration of 106.30 days whereas seedlings registered a mean crop duration of 119.90 days, the difference in crop duration being significant.

Significant difference was observed in total crop duration of seedlings and clonal progenies, both under shade and in open conditions. In shade, T_2 recorded a crop duration (102.67 days) significantly lower than that of T_1 (119.47 days). In open condition also, T_2 recorded a shorter crop duration (109.93 days) as compared to T_1 (120.33 days).

Per se performance of seedlings was evaluated in open and under shade, wherein it was observed that there was no significant variation in total crop duration between shade (119.47 days) and open (120.33 days) conditions. But clonal progenies showed significant difference in total crop duration under shade (102.67 days) and in open (109.93 days).

4.1.1.10 Incidence of pests and diseases

Major pests observed in the field were fruit fly (*Dacus cucurbitae*) and snake gourd semilooper (*Plusia peponis*). Extent of damage observed was more in plants grown under shade. No difference was observed in the extent of damage

Table 10. Total crop duration in seedlings and clonal progenies of *Trichosanthes cucumerina* in open and under shade

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	119.47 $^{a\,A}$	120.33 $^{a\,A}$	119.90 *
Clonal progenies (T_2)	102.67 $^{b\,B}$	109.93 $^{b\,A}$	106.30 *
Mean	111.07 *	115.13 *	
CD for comparison = 2.12			

between seedling plants and tissue culture derived plants, in terms of pest incidence. No major disease was noticed during the entire crop period.

4.2 EXPERIMENT II

Biochemical analyses for evaluation of seedlings and clonal progenies of *Trichosanthes cucumerina* in the open and under shade for quality.

4.2.1 Weight of soxhlet extractables

Data pertaining to the results of soxhlet extraction of field grown samples and market samples for obtaining crude extractables are presented in Table 11.

Significant difference in fresh weight of crude extractables obtained by soxhlet extraction was observed in main plot and sub plot treatments. The plants grown under shade yielded higher mean amount of crude extractables (0.312g) as compared to plants grown in the open (0.241g). T_2 registered superior performance with a mean yield of 0.358g while T_1 yielded 0.195g, the results being significantly different.

In shaded condition, T_1 and T_2 differed significantly with respect to weight of soxhlet extractables. Fresh weight of crude extractables was more for T_2 (0.408g) than T_1 (0.216g) in shade. In open condition also, T_2 performed better (0.307g) as compared to T_1 (0.175g), with respect to fresh weight of crude extractables by soxhlet extraction.

Weight of crude extractables obtained *per se* from seedlings and clonal progenies grown under shade and in the open also varied significantly. T_1 recorded higher amount of extractables (0.216g) under shade than in the open (0.175g). T_2 also yielded high extractables in shaded condition (0.408g) as compared to open (0.307g).

Market sample recorded a mean weight of 0.213g of crude extractables by soxhlet extraction, a value which was superior to seedlings grown in the open.

4.2.2 Weight of hot water extractables

Data pertaining to weight of crude extractables obtained by hot water extraction is presented in Table12.

There was no significant difference between main plot treatments with respect to weight of crude extractables obtained by hot water extraction. Plants grown under shade recorded a mean yield of 0.903g of crude extractables, while in open, the value was 0.881g.

Significant variation was observed between sub plot treatments. T_2 recorded a higher mean amount of crude extractables (0.929g) than T_1 (0.855g).

In shade, T_1 and T_2 showed significant variation between themselves with respect to fresh weight of crude extractables. T_2 yielded higher amount of extractables (0.960g) under shade, followed by T_1 (0.845g). But in the open, T_1 recorded 0.865g of extractables which was on par with T_2 (0.898g).

Per se performance of seedling and clonal progenies in open (0.865g for seedlings and 0.898g for clonal progenies) and in shade (0.845g for seedlings and 0.960g for clonal progenies) did not vary significantly with respect to weight of crude extractables. Crude extractables from market sample recorded the lowest mean yield of 0.718g when extracted with hot water.

Table 11. Fresh weight of soxhlet extractables

Growing conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	0.216 ^{bA}	0.175 ^{bB}	0.195 *
Clonal progenies (T_2)	0.408 ^{aA}	0.307 ^{aB}	0.358 *
Mean	0.312 **	0.241 **	
Market sample	0.213		
CD for comparison = 0.04			

Table 12. Fresh weight of hot water extractables

Growing Conditions / Progenies	Shade (S_1)	Open (S_2)	Mean
Seedlings (T_1)	0.845 ^{bA}	0.865 ^{aA}	0.855 **
Clonal progenies (T_2)	0.960 ^{aA}	0.898 ^{aA}	0.929 **
Mean	0.903 NS	0.881 NS	
Market sample	0.718		
CD for comparison = 0.09			

4.2.3 Detection of cucurbitacin

4.2.3.1 Thin layer chromatography

The spotted chromatographic plates when visualized under UV light at 366nm gave a blue fluorescence, which was identified as cucurbitacin (Plate 3a-3e).

Under UV flourescence, cucurbitacin was detected both in the test samples (Plate 3a) and market samples (Plate 3b). While observing the spotted TLC plates, tissue culture derived plants and seedlings grown in open and under shade as well as market samples expressed single spots with varying Rf values. It is inferred that lower the Rf value, higher the molecular size of cucurbitacin. So the TLC analysis recorded low molecular size cucurbitacin in clonal progenies grown under shade with a mean Rf value of 0.615, whereas seedlings grown in open showed high molecular weight cucurbitacin registering a mean Rf value of 0.473. Seedlings grown under shade and tissue culture derived plantlets raised in open recorded intermediate mean Rf values of 0.526 and 0.528 respectively. Market samples recorded a mean Rf value of 0.563 (Table 13).

4.2.3.2 Quantitative TLC for estimation of cucurbitacin

Quantitative TLC of cucurbitacin was performed in different samples. A standard curve was prepared by plotting absorbance values against different concentrations of experimental samples.

Cucurbitacin content was quantified from the standard curve for varying ranges of absorbance and indicated in units and the data are presented in Table 14a. The absorbance values and the corresponding units for mean absorbance values of the test samples read spectrophotometrically, are presented in Table 14 b.

Plate 3a. Thin layer chromatogram for detection of cucurbitacin in seedlings and clonal progenies of *Trichosanthes cucumerina* grown in open and under shade

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Plate 3b. Thin layer chromatogram of market sample of *Trichosanthes cucumerina* for detection of cucurbitacin

Table 13. Rf values of cucurbitacin detected in test samples and in market sample

Shade (S_1)			Open (S_2)	
Seedlings (T_1)		TC (T_2)	Seedlings (T_1)	TC (T_2)
0.541		0.620	0.472	0.584
0.521		0.620	0,497	0.513
0.522		0.624	0.467	0.534
0.498		0.599	0.515	0.535
0.549		0.611	0.416	0.474
Mean	0.526	0.615	0.473	0.528
Market sample		0.563		

Table 14 a. Units of cucurbitacin from standard curve

Values	Units	Values	Units
0.008-0.012	1	0.033-0.037	6
0.013-0.017	2	0.038-0.042	7
0.018-0.022	3	0.043-0.047	8
0.023-0.027	4	0.048-0.052	9
0.028-0.032	5	0.053-0.057	10

Table 14 b. Spectrophotometric readings of cucurbitacin

Shade (S_1)			Open (S_2)	
Seedlings (T_1)		TC (T_2)	Seedlings (T_1)	TC (T_2)
0.059		0.044	0.040	0.050
0.028		0.056	0.021	0.038
0.029		0.056	0.028	0.029
0.027		0.048	0.022	0.032
0.030		0.049	0.022	0.028
Mean	0.035 (6 units)	0.051 (9 units)	0.027 (4 units)	0.035 (6 units)
Market sample		0.032 (5 units)		

Maximum cucurbitacin content was recorded for clonal progenies grown under shade (9 units) and minimum for seedlings raised in the open (4 units). Seedlings under shade and tissue culture plants in the open recorded same units each (6 units). For market sample, the value recorded was 5 units.

4.2.4 Detection of other secondary metabolites

4.2.4.1 Alkaloids

Seedlings and clonal progenies grown under shade and in open conditions gave positive results for Dragendorff, Mayer's and Wagner's tests. Test samples showed an orange red precipitate for Dragendorff reagent, white colour for Mayer's reagent and brown precipitate for Wagner's reagent, confirming the presence of alkaloids in experimental samples. Market sample also responded positively to all these tests, indicating the presence of alkaloids.

4.2.4.2 Phenols

All the test samples were subjected to TLC to identify the presence of phenols. All domesticated samples as well as the market samples revealed one spot of same Rf value on TLC plates, identified as phenol, with the solvent system, chloroform-acetic acid. The Rf value for the identified spot in all the field and market samples were identical (0.36) (Plate 4).

4.2.4.3 Tannins

All test samples gave positive results with slight red colour during the detection of tannins.

4.2.4.4 Saponins

Saponins were identified in seedlings, clonal progenies and market samples. All test samples including market sample gave permanent foam, when shaken with alcohol.

Plate 4. Thin layer chromatogram of *Trichosanthes cucumerina* plants for detection of phenols

4.2.4.5 Terpenes

With Liebermann- Burchard reagent, all samples gave red colour, indicating the presence of terpenes in seedlings and clonal progenies grown in open and under shade, as well as in market samples.

4.2.4.6 Terpene estimation

Spectophotometry was performed to quantify the terpene in test samples as well as market samples. Standard curve was prepared by plotting values against different concentrations of sample. Terpene content was quantified from the standard curve for varying ranges of absorbance and indicated in units. The data are presented in Table 15 a. The absorbance readings for the test samples were recorded spectrophotmetrically and the data are presented in Table 15 b, along with the corresponding mean unit values.

It was revealed that, clonal progenies grown under shade performed best, with maximum units (13 units) of terpenes, followed by seedlings under shade (11 units), tissue culture plants in open field (10 units) and seedlings in open conditions (9 units). Market samples also recorded 9 units of terpenes.

4.3 EXPERIMENT III

4.3.1 Anatomical investigations for evaluation of domesticated samples and market samples

Anatomical features of stem, leaves and roots of experimental plants and market samples are presented below.

4.3.1.1 Stem

Stem section revealed a hollow cavity with five prominent ridges alternating with equal number of furrows with shoot hairs, epidermis, hypodermis,

15 a. Units of terpene from standard curve

Values	Units	Values	Units
0.09-0.12	1	0.37-0.40	8
0.13-0.16	2	0.41-0.44	9
0.17-0.20	3	0.45-0.48	10
0.21-0.24	4	0.49-0.52	11
0.25-0.28	5	0.53-0.56	12
0.29-0.32	6	0.57-0.60	13
0.33-0.36	7	0.61-0.64	14

15 b. Spectrophotometric reading of terpenes

Shade (S_1)			Open (S_2)	
Seedlings (T_1)		TC (T_2)	Seedlings (T_1)	TC (T_2)
0.515		0.600	0.384	0.522
0.509		0.618	0.391	0.489
0.528		0.608	0.484	0.482
0.473		0.587	0.535	0.483
0.503		0.597	0.361	0.426
Mean	0.506 (11 units)	0.602 (13 units)	0.431 (9 units)	0.480 (10 units)
Market sample		0.426 (9 units)		

cortex, endodermis, pericycle, pith and bicollateral vascular bundle. Clonal progenies, seedlings and market sample revealed similar characters (Plate 5a)

4.3.1.2 Leaf

Leaf section presented the normal features of a dicotyledonous leaf like cuticle, upper epidermis, palisade tissue, bundle sheath, xylem, phloem, air space, spongy tissue and lower epidermis. Seedlings, clonal progenies and market sample recorded similar features (Plate 5b).

4.3.1.3 Root

Root sections of all samples were similar to a dicot root possessing cork, cork cambium, primary phloem, secondary phloem, secondary xylem, primary xylem and medullary rays (Plate 5c).

Experimental plants raised in open and under shade, as well as market samples revealed identical anatomical features of stems, leaves and roots.

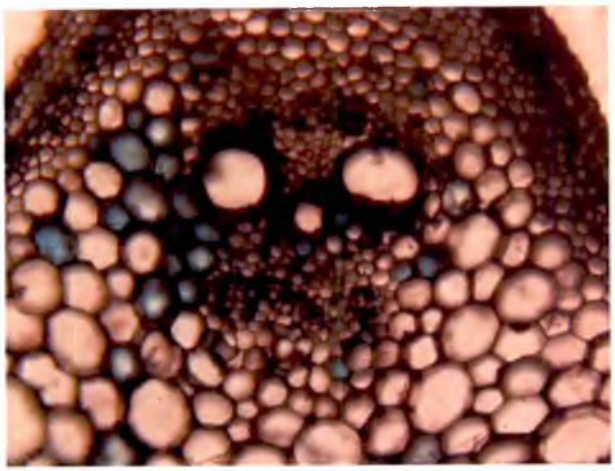


Plate 5a. C.S of *Trichosanthes cucumerina* stem

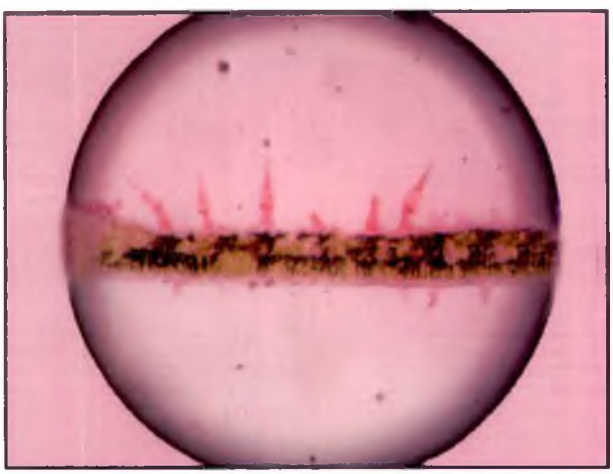


Plate 5b. C.S of *Trichosanthes cucumerina* leaf

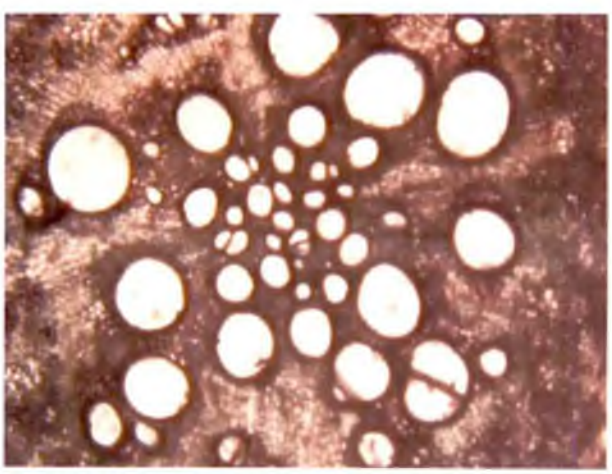


Plate 5c. C.S of *Trichosanthes cucumerina* root

DISCUSSION

5. DISCUSSION

The results of this study entitled "Evaluation of seedlings and clonal progenies of Kattupatavalam (*Trichosanthes cucumerina* L.) for yield and quality" are discussed in this chapter. This study was conducted to evaluate the seedlings and clonal progenies of *Trichosanthes cucumerina* for growth, yield and quality attributes under open and shade conditions.

5.1 EXPERIMENT I

Field evaluation of seedlings and clonal progenies of *Trichosanthes cucumerina* under open and shade conditions for growth and yield attributes

In the present study, growth and yield attributes of seedlings and tissue culture derived plantlets of *T. cucumerina* were evaluated and the results obtained are discussed below.

5.1.1 Main vine length

Seedlings and clonal progenies exhibited no significant variation in mean vine length under shade and in open. But a slight increase in mean vine length was recorded in open (306.18cm) as compared to the plants grown under shade (304.44cm) (Fig. 1a). Seedlings recorded a mean vine length of 363.87cm under shade which was on par with plants grown in open (362.25cm). Clonal progenies also recorded similar results with 245.05cm of vine length in shade and 250.10cm in open. The suggested reasons for this difference are the decreased photosynthetic rate, stomatal conductance, transpiration rate, stomatal index and stomatal frequency in shaded condition as reported by Ajithkumar *et al.* (2002).

Paul (1992) revealed that, in turmeric, there was no significant difference in plant height observed between cultivars at different shade levels. Gangadharan (2003) also reported similar results in *Kaempferia galanga* grown under shade and in open. However, Menon (1994) observed that in *Plumbago*

rosea, plants grown in open condition were significantly taller when compared to plants grown under shade. A study conducted by Pillai (1990) in *Ocimum gratissimum*, revealed that the effect of shade on plant height and spread was positive upto intermediate shade level. Similar results were reported in soyabean as well. The growth attributes recorded lower values under shade as compared to open condition (Sharma and Chauhan, 2003).

Seedlings and clonal progenies registered significant variation in mean main vine length (Fig. 1b). Seedlings recorded a higher mean vine length of 363.04cm than clonal progenies (247.58cm).

Among interactions, seedlings recorded maximum vine length of 363.87cm and 245.05cm respectively under shade and in open, which was significantly superior to clonal progenies grown under shade and in open (Fig. 1c). The results are in conformity with the findings of Hazarika *et al.* (2002) who also observed similar performance in tissue culture derived plants. The suggested reasons for the same are abnormal leaf morphology, poor photosynthetic efficiency, malfunctioning of stomata and marked decrease in epicuticular waxes in *in vitro* derived plantlets. However, in the present study, seedlings and clonal progenies recorded similar leaf morphology. Hence, decrease in the vine length of clonal progenies may be attributed to poor photosynthetic efficiency when field transplanted from *in vitro* conditions.

Anon *et al.* (1993) has also reported that the stomata in *in vitro* raised plants seldom become functional, probably as a result of physiological adaptation to conditions of high relative humidity which occur *in vitro*. Reports supporting the experimental result in the present study regarding decreased vine length of tissue culture derived plantlets include the observation by Shylaraj (1988) who revealed that in lemon grass, tissue culture derived plantlets recorded lower plant height as compared to seedlings. Similar results were reported by Misra (1996) in patchouli, wherein tissue culture derived plantlets were shorter than seedlings. However, Pradeep *et al.* (1992) have obtained contradictory results, which

Fig.1a. Effect of shade and open conditions on main vine length in *Trichosanthes cucumerina*

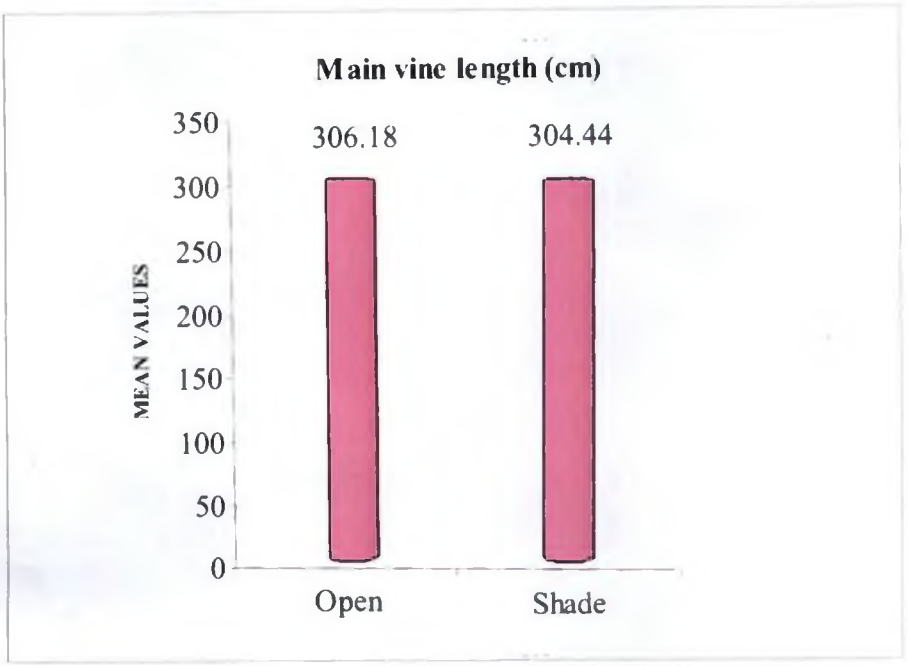


Fig.1b. Main vine length in seedlings and clonal progenies of *Trichosanthes cucumerina*

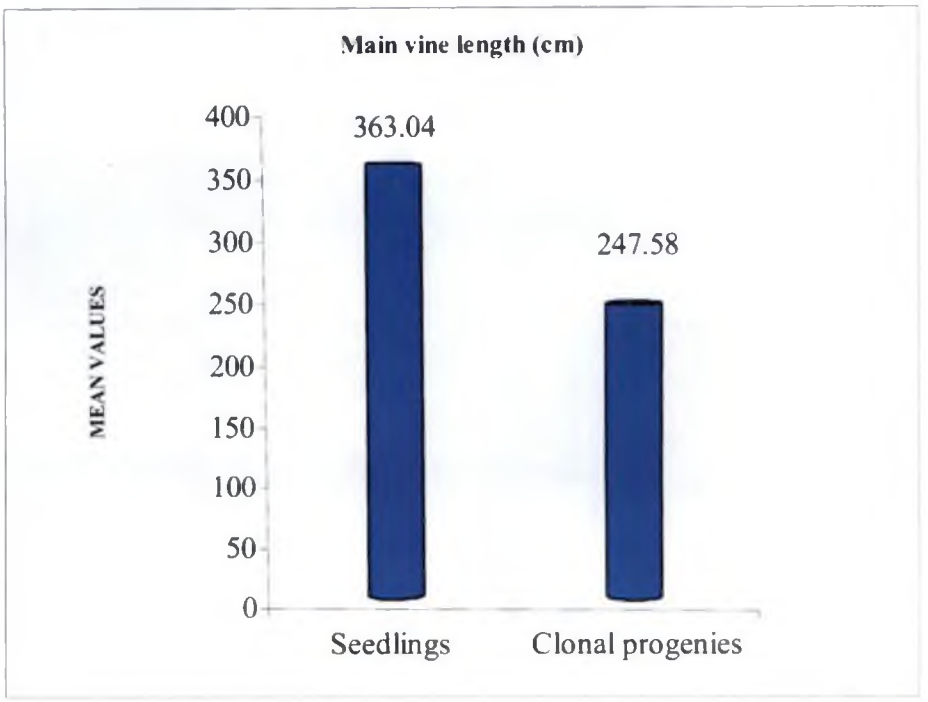
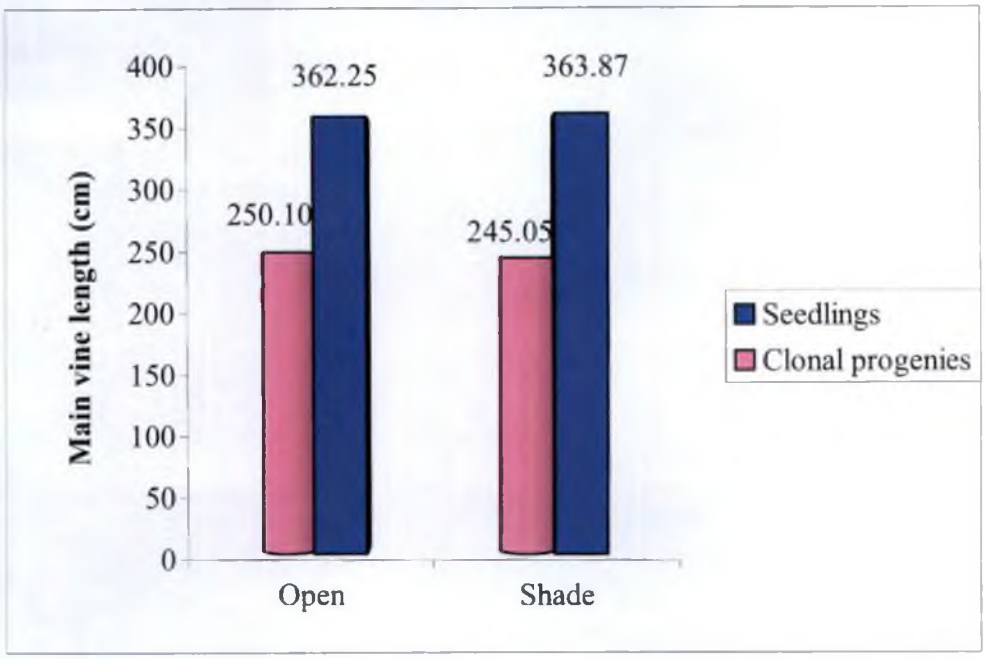


Fig.1c. Interaction effect in seedlings and clonal progenies of *T. cucumerina* on main vine length under shade and in open



indicate that tissue culture derived banana plants recorded no significant difference in height at flowering stage as compared to normal plants.

5.1.2 Primary branches per plant

In the present study, primary branches per plant did not differ significantly in shade and in open. Taken individually, seedlings and clonal progenies did not register any difference in primary branches per plant when grown under shade and in open conditions. Seedlings recorded a mean branch number of 9.53 under shade and 9.73 in the open. Clonal progenies registered a mean number of primary branches of 7.80 under shade and 8.00 primary branches in open condition. Plants grown under shade recorded 8.67 primary branches while a slight increase was noticed in plants grown in open though the difference was not significant (Fig. 2a).

The reasons for the same may be the low photosynthetic rate, stomatal conductance and transpiration rate of plants grown under shade as suggested by Ajithkumar *et al.* (2002). Similar results were observed in ginger wherein lower number of tillers per clump was recorded in the crop grown as intercrop in an arecanut plantation (Hegde *et al.*, 2000). Pillai (1990) also reported that, in *Ocimum gratissimum*, the number of branches was more in open condition and was superior to the rest of the treatments. Similar results were reported by Bai (1981) in sweet potato and coleus and Varughese (1989) in ginger and turmeric.

Seedlings registered significant superiority with respect to primary branches per plant as compared to clonal progenies. Seedlings recorded 9.63 mean primary branches whereas clonal progenies registered only 7.90 number of primary branches per plant (Fig. 2b). In shaded condition, seedlings recorded higher number of branches (9.53) as compared to clonal progenies (7.80). In open condition also, similar results were obtained (Fig 2c).

Fig. 2a. Effect of shade and open conditions on primary branches per plant in *Trichosanthes cucumerina*

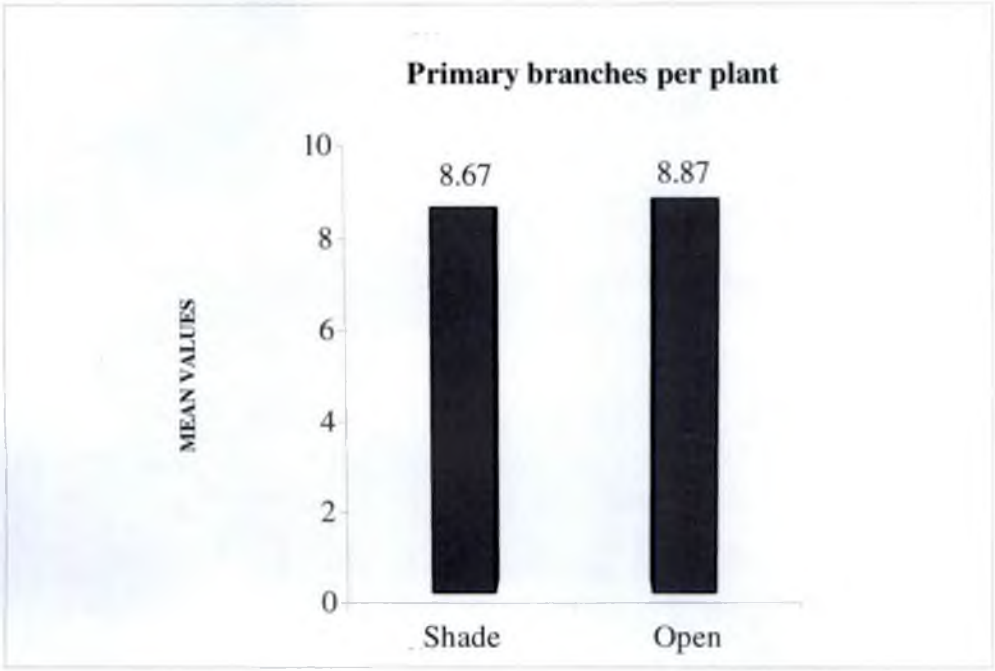


Fig. 2b. Primary branches per plant in seedlings and clonal progenies of *Trichosanthes cucumerina*

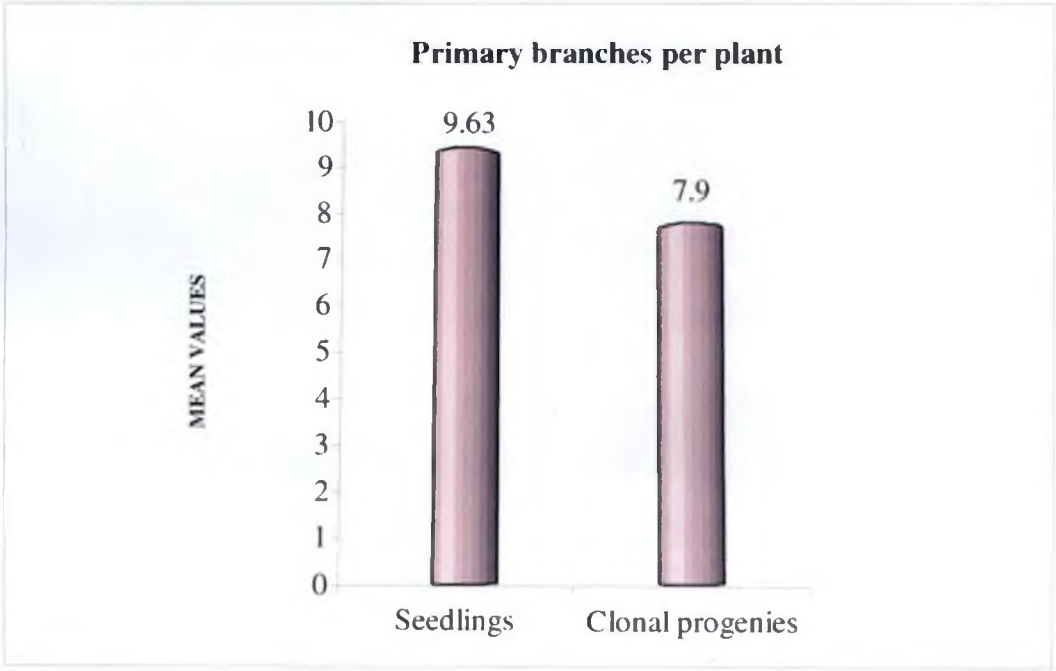
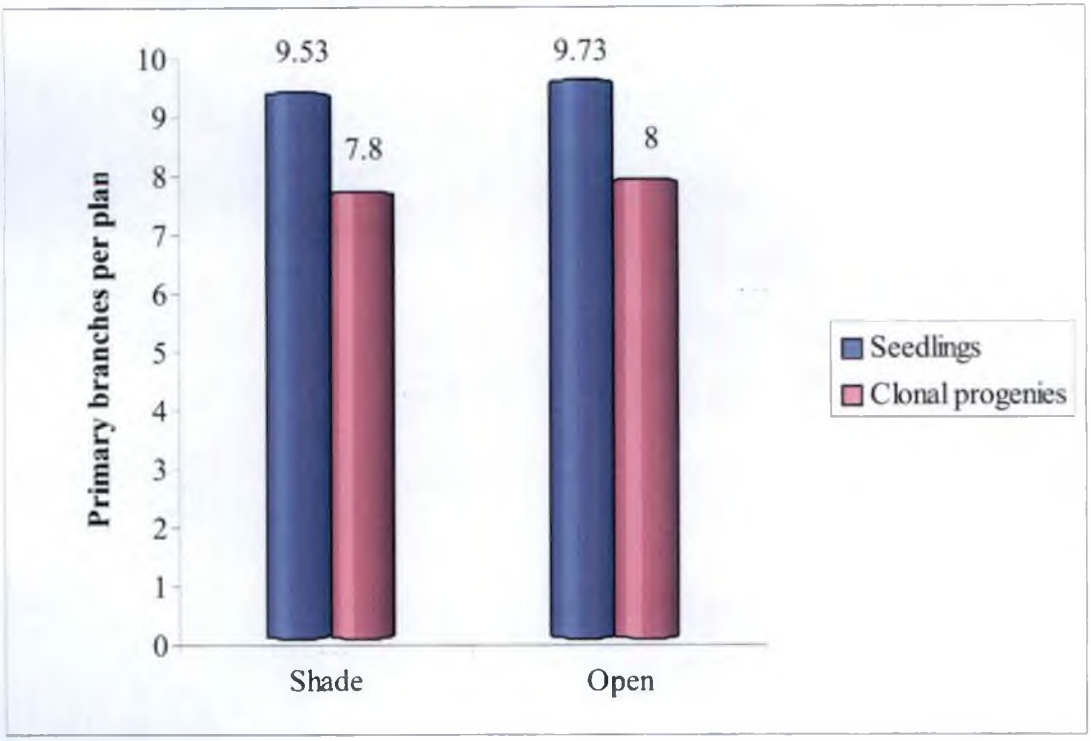


Fig. 2c. Interaction effect in seedlings and clonal progenies of *T.cucumerina* on primary branches per plant under shade and in open



Anon *et al.* (1993) and Hazarika *et al.* (2002) described that tissue culture derived plants often exhibited malfunctioning of stomata, lower photosynthetic rate and transpiration rate which accounted for the poor performance of clonal progenies as compared to seedlings. However, Hwa *et al.* (2002) reported that, in *Cinnamomum kanehirae*, tissue culture derived plants recorded better tree forms in terms of an erect stem and forked stem branches.

5.1.3 Days to flower

Plants raised in open and under shade differed significantly in mean number of days to flowering (Fig. 3a). Plants raised in shade took 70.33 days to flower whereas plants grown in open, flowered in 66.53 days. Seedlings as well as clonal progenies showed significant variation in days to flower under open and in shade. Seedlings registered 74.80 days to flower under shade and 70.27 days in open. Clonal progenies also recorded earlier flowering in open (62.80 days) as compared to shaded condition (65.86 days). This may be due to the faster growth of vines and high photosynthetic rate in the open condition.

Results in the present study are in agreement with the reports of Menon (1994) in *Plumbago rosea*, who observed that flower initiation in *P. rosea* was comparatively early in the open as compared to shade. Pillai (1990) also reported that flowering and attainment of maturity delayed progressively with increasing intensities of shade in *Ocimum gratissimum*. However, reports contrary to the above have been put forth by Graz (2003) in *Schinziophyton rautanenii*, wherein it was observed that, seedlings grew faster under moderate shade than in the open, implying that extreme open condition may not favour early flowering.

The present study revealed that clonal progenies flowered comparatively earlier (64.33 days) as compared to seedlings (72.53 days) (Fig. 3b). This result is in conformity with the findings of Vasil and Vasil (1980) who reported that, in general, tissue culture derived plants attain maturity earlier than

Fig. 3a. Effect of shade and open conditions on days to flower in *Trichosanthes cucumerina*

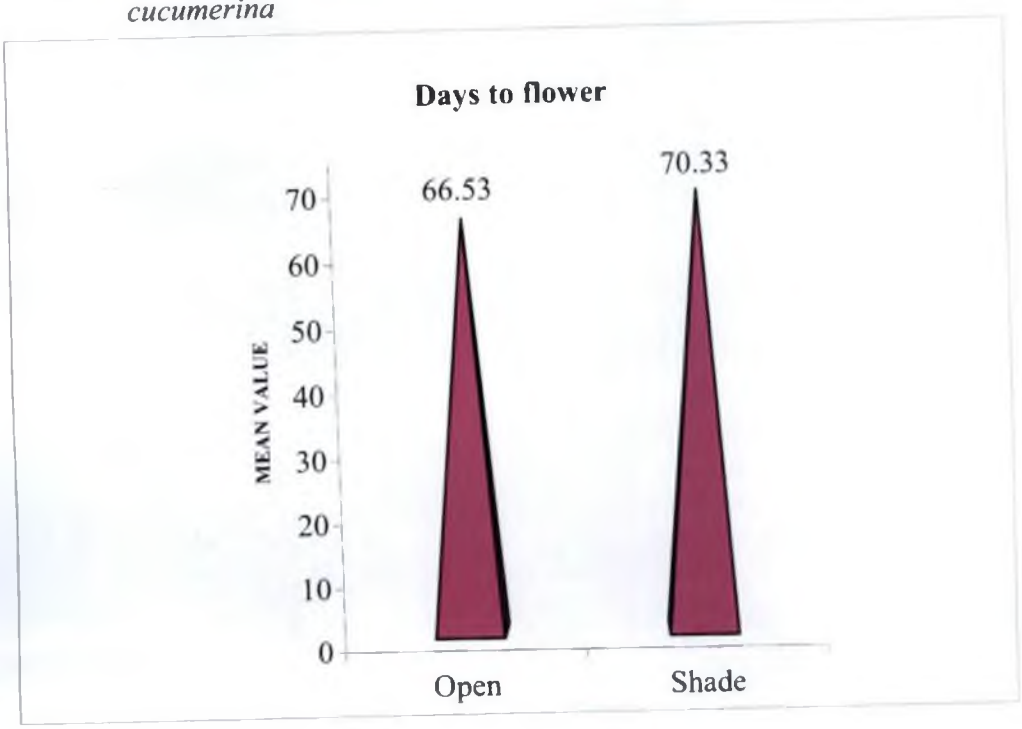


Fig 3b. Days to flower in seedlings and clonal progenies of *Trichosanthes cucumerina*

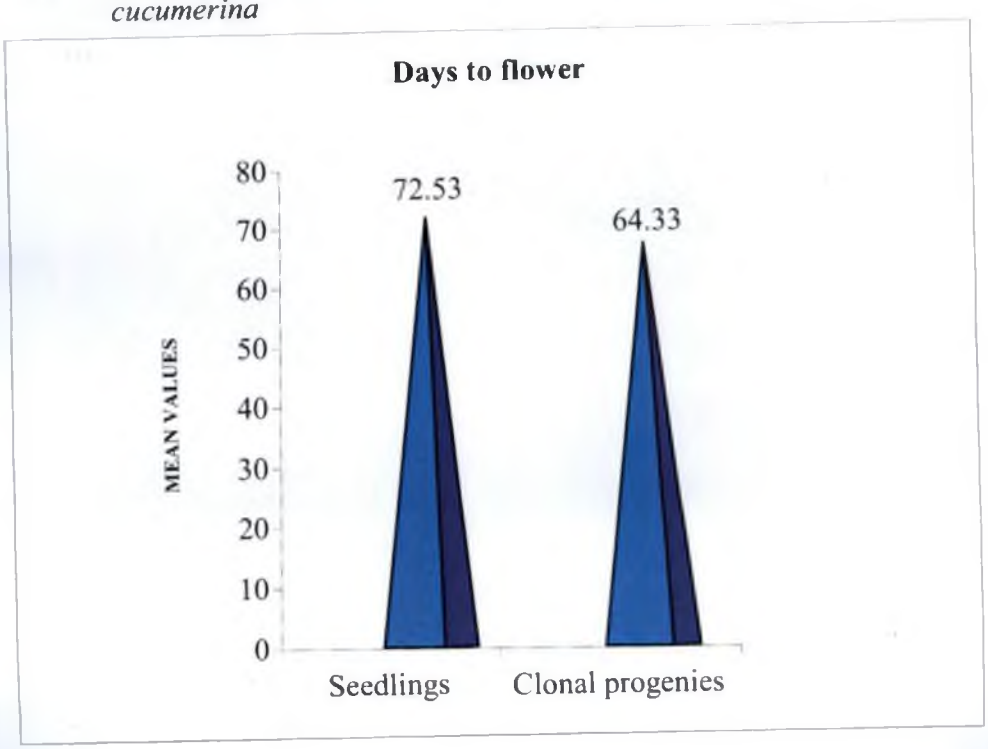
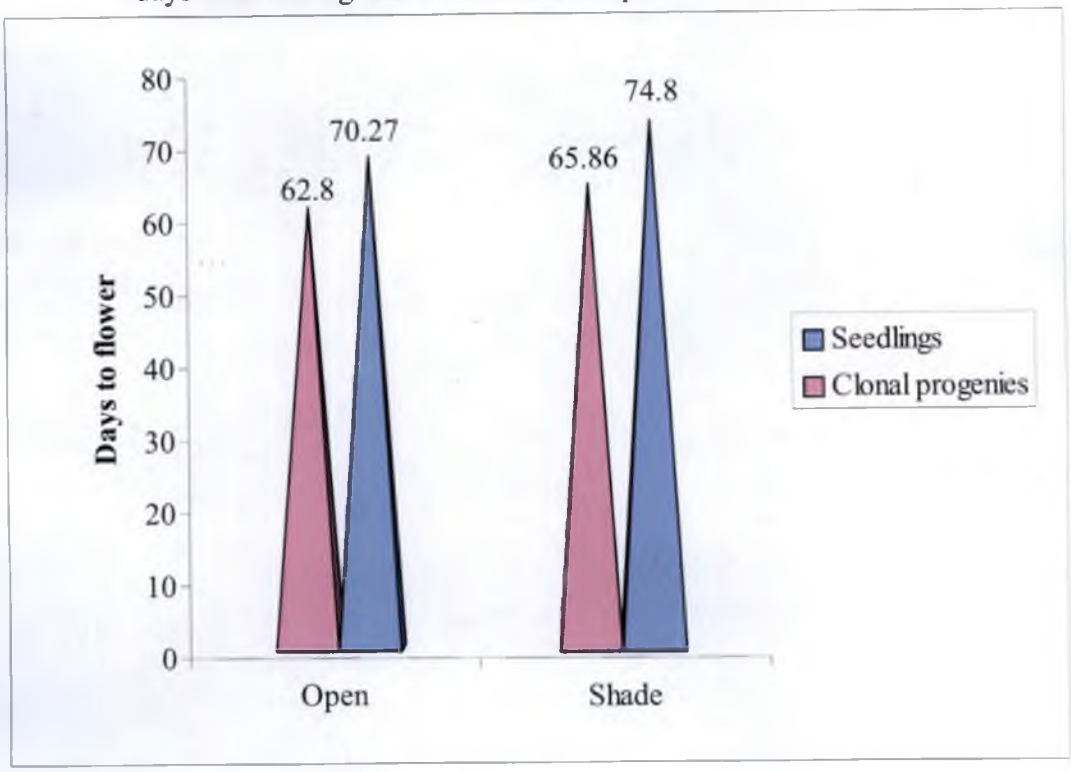


Fig. 3c. Interaction effect of seedlings and clonal progenies of *T. cucumerina* on days to flowering under shade and in open



seed propagated plants. A probable reason for the same is that the influence of various plant growth hormones present in the basal growth media in the *in vitro* phase may be extended to the hardening and plant out stages of the tissue culture derived plantlets, leading to early flowering.

Under shade, seedlings took 74.80 days to flower whereas clonal progenies put forth flowers in 65.86 days. In open condition also similar trend was exhibited by seedlings and clonal progenies. Clonal progenies started flowering earlier (62.80 days) than seedlings (70.27 days) in open (Fig. 3c).

Onokpise *et al.* (1992) reported that in *Xanthosoma sagittifolium*, flowering of tissue culture derived plantlets occurred 20-30 days earlier than sucker derived plants. Similar results were reported by Kumar *et al.* (2003) in *Tagetus erecta* and Dhananjaya and Sulladmath (2003) in *Anthurium andreanum*. Kumar *et al.* (2004) have further reported that tissue culture derived *Tagetus erecta* plants flowered 2-3 weeks earlier. However, earliness in the flowering exhibited by tissue culture derived plantlets was genotype dependent as well, as observed by Sheela and Nair (2001) who revealed that tissue culture banana plantlets did not flower earlier, as compared to sucker derived plants.

5.1.4 Days to fruit maturity

Plants grown under shade attained fruit maturity in 27.30 days, while in the open, fruit maturity was attained in 24.00 days, the values being significantly different (Fig. 4a). Seedlings attained fruit maturity earlier in open condition (23.87 days) as compared to shade (27.40 days). Clonal progenies also took more number of days to attain fruit maturity in shade (27.20 days) than in open.

Seedlings and clonal progenies were on par with each other in days to fruit maturity (Fig. 4b). Seedlings recorded 25.63 days to attain fruit maturity and for clonal progenies, it was 25.67 days. Similarity in days to fruit maturity

Fig. 4a. Effect of shade and open conditions on days to fruit maturity in *Trichosanthes cucumerina*

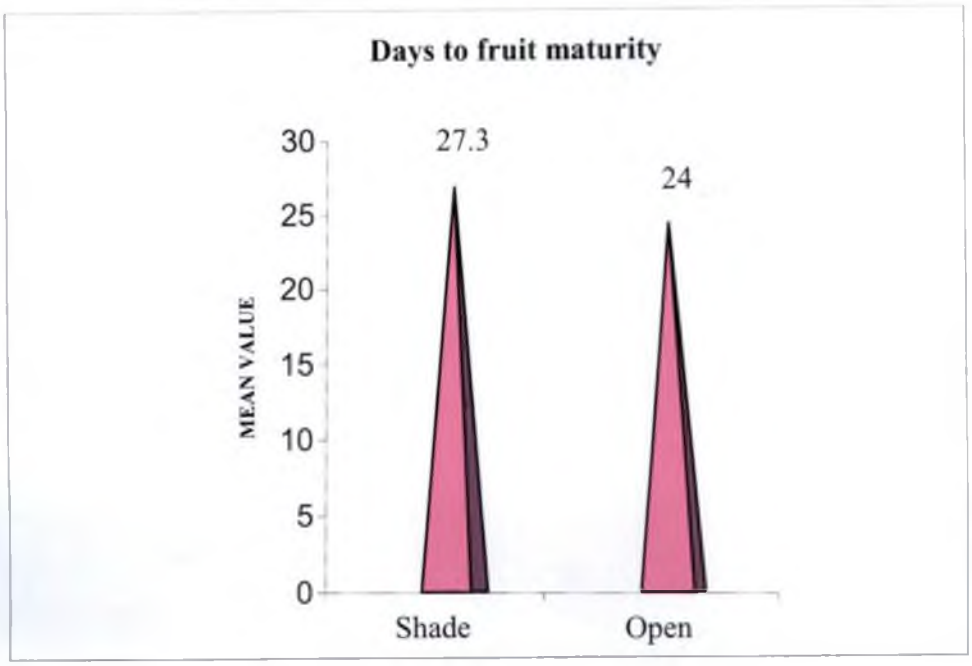


Fig. 4b. Days to fruit maturity in seedlings and clonal progenies of *Trichosanthes cucumerina*

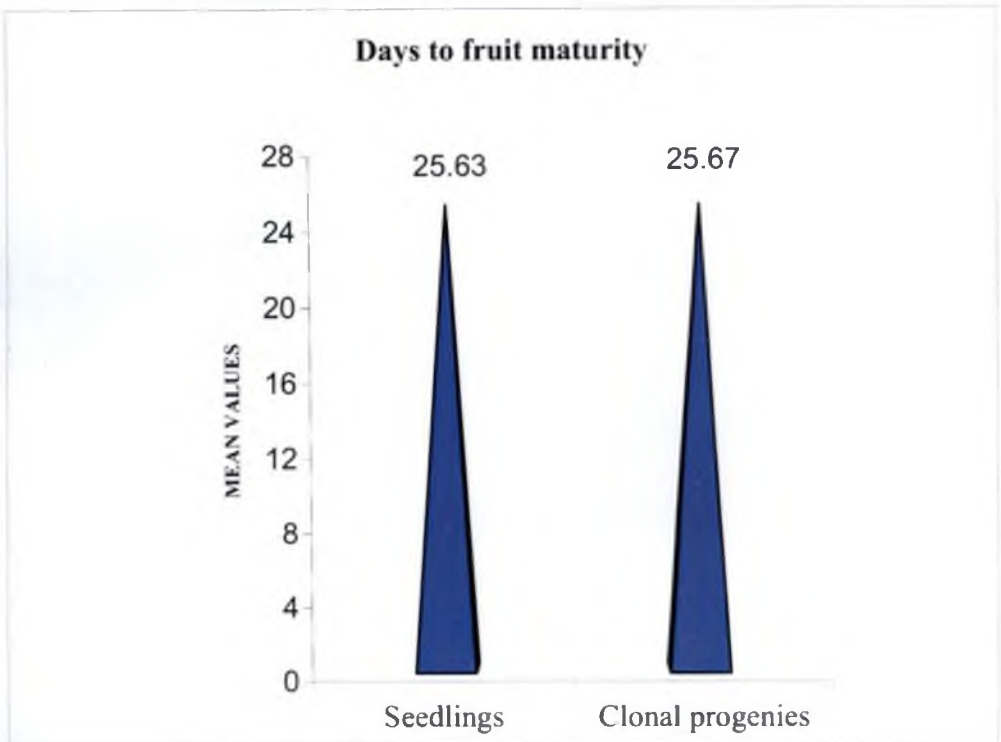
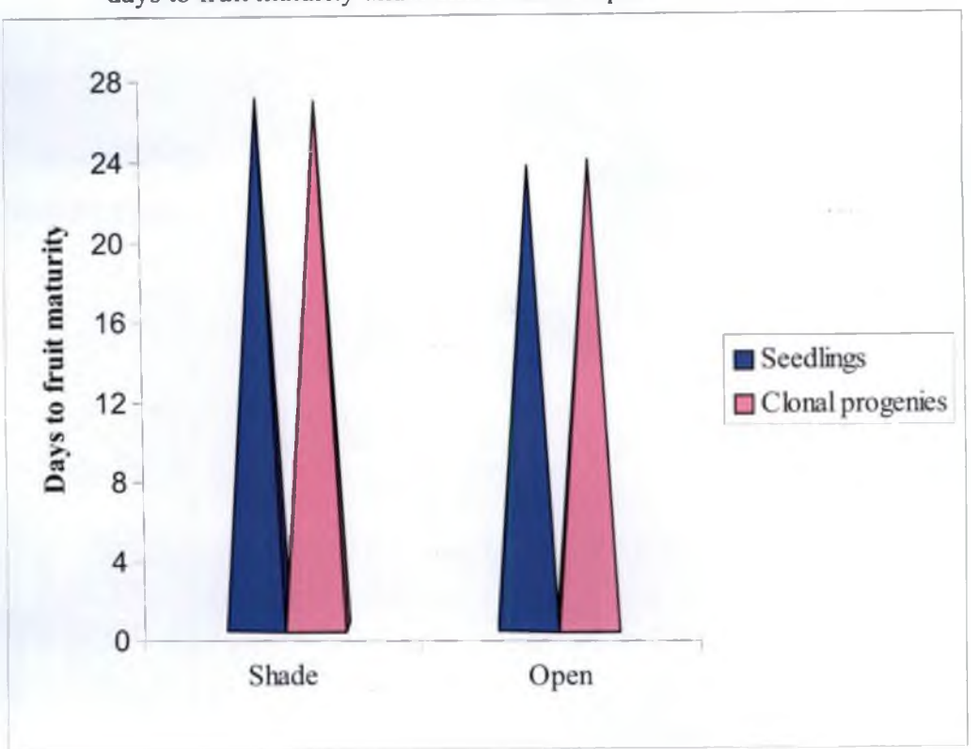


Fig. 4c. Interaction effect in seedlings and clonal progenies of *T. cucumerina* on days to fruit maturity under shade and in open



recorded by seedlings and clonal progenies was evident both under shade and in open (Fig. 4c).

In this context, it is worthwhile to recall the absence of growth hormones like ethylene in the nutrient media of tissue culture derived plantlets in the *in vitro* phase, which could explain the similarity exhibited by seedlings and tissue culture derived plantlets with respect to fruit maturity, the supplemented growth hormones in *in vitro* phase having exerted no influence on early attainment of fruit maturity. It is assumed that faster growth of vines and high photosynthetic rate that contributed to early flowering may also have reduced the number of days to fruit maturity in the open.

5.1.5 Number of fruits per plant

Significant variation was observed with respect to number of fruits per plant, in plants grown in open and in shade (Fig. 5a). Number of fruits per plant was highest for plants grown under shade (43.07) as compared to plants grown in open. Performance of seedlings and clonal progenies showed no variation with respect to number of fruits per plant under shade and in open conditions. Seedlings recorded a mean number of 37.13 fruits under shade and 33.47 fruits in open. Clonal progenies registered 49.00 fruits under shade and 41.93 fruits in the open.

There is a marked influence of temperature on initiation of flowers and total number of flowers per plant, which are major components of number of fruits per plant. As suggested by Heuvelink and Dorais (2005), temperature affects initiation, fruit set and fruit growth and their effects are closely associated with light. Hence reduced temperature and light under shaded conditon, in mature coconut plantation may have favourably influenced the partitioning of biomass into reproductive component, at the expense of vegetative growth as evident from the results of the present study, wherein, plants grown under shade, recorded more number of fruits.

In gladiolus, Rana *et al.* (2002) reported that number of florets per spike was higher under 75 per cent shade than in open. Another study conducted by Alam *et al.* (2002) in rubber, explained that overall growth was more in plants grown under partial shade due to the maintenance of photosynthesis for longer duration than in open conditions.

Seedlings and clonal progenies exhibited significant variation in number of fruits per plant. Clonal progenies recorded significantly more number of fruits (45.47) than seedlings (35.30) (Fig. 5b). When interaction effects are considered, though clonal progenies recorded maximum number of fruits than seedlings under shade, in the open, both clonal progenies and seedlings were on par with each other with respect to number of fruits per plant (Fig. 5c). Influence of growth hormones like BA that had been supplemented to the growth media in the *in vitro* phase of tissue culture derived plants account for increased number of flowers, which in turn may have resulted in more number of fruits per plant.

Similar results were recorded by Hendre *et al.* (1998). They reported that tissue culture derived plants of paddy cultivars recorded increase in number of spikelets per panicle. Also in *Tagetus erecta*, Kumar *et al.* (2004) revealed that tissue culture derived plants recorded higher number of flowers per plant than seed derived plants. Shivasankara *et al.* (2001) explained the physiology of tissue culture derived plants in banana which had higher photosynthetic rate, stomatal conductance and mesophyll efficiency. Hence, number of fingers and yield in tissue culture banana were slightly higher than that in normal plants. Sheela and Nair (2001) reported that in tissue culture banana, number of fingers was more as compared to plants from suckers. Other reports supporting the experimental result in the present study include the findings put forth by Sood *et al.* (2002) who indicated that the growth performance of tissue culture derived bamboo plants was relatively better.

Fig. 5a. Effect of shade and open conditions on number of fruits per plant in *Trichosanthes cucumerina*

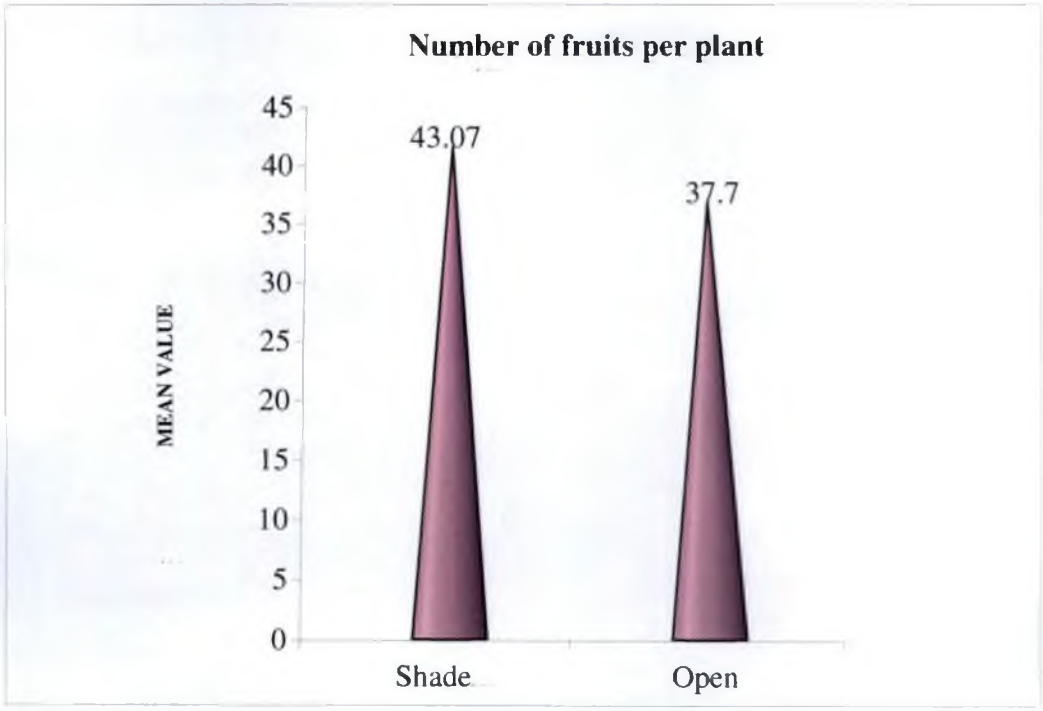


Fig. 5b. Number of fruits per plant in seedlings and clonal progenies of *Trichosanthes cucumerina*

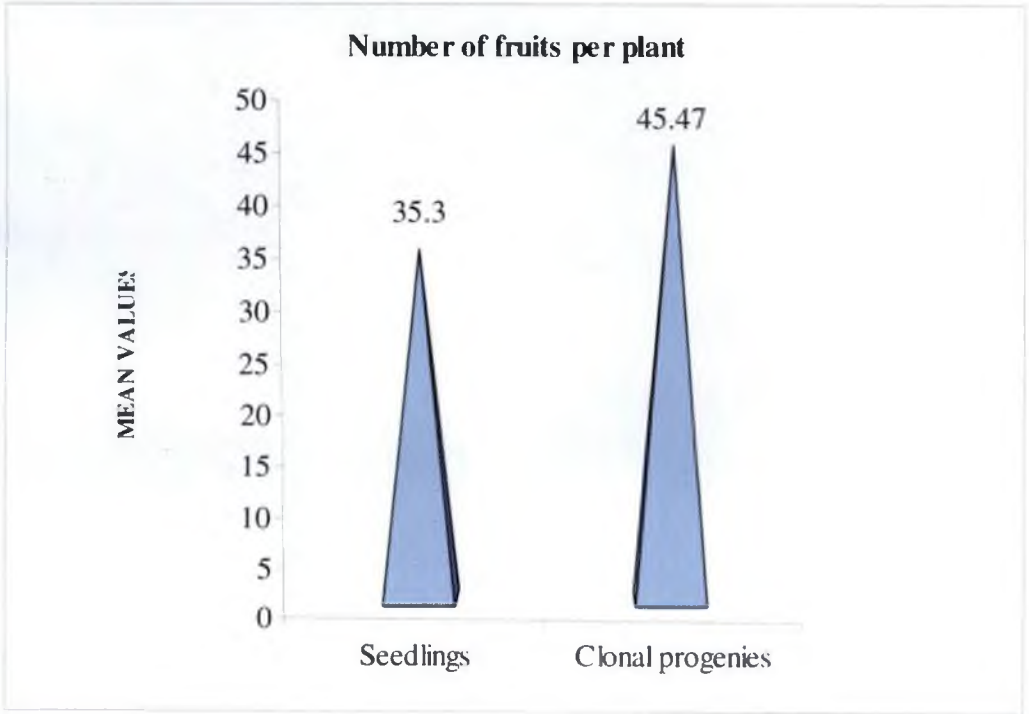
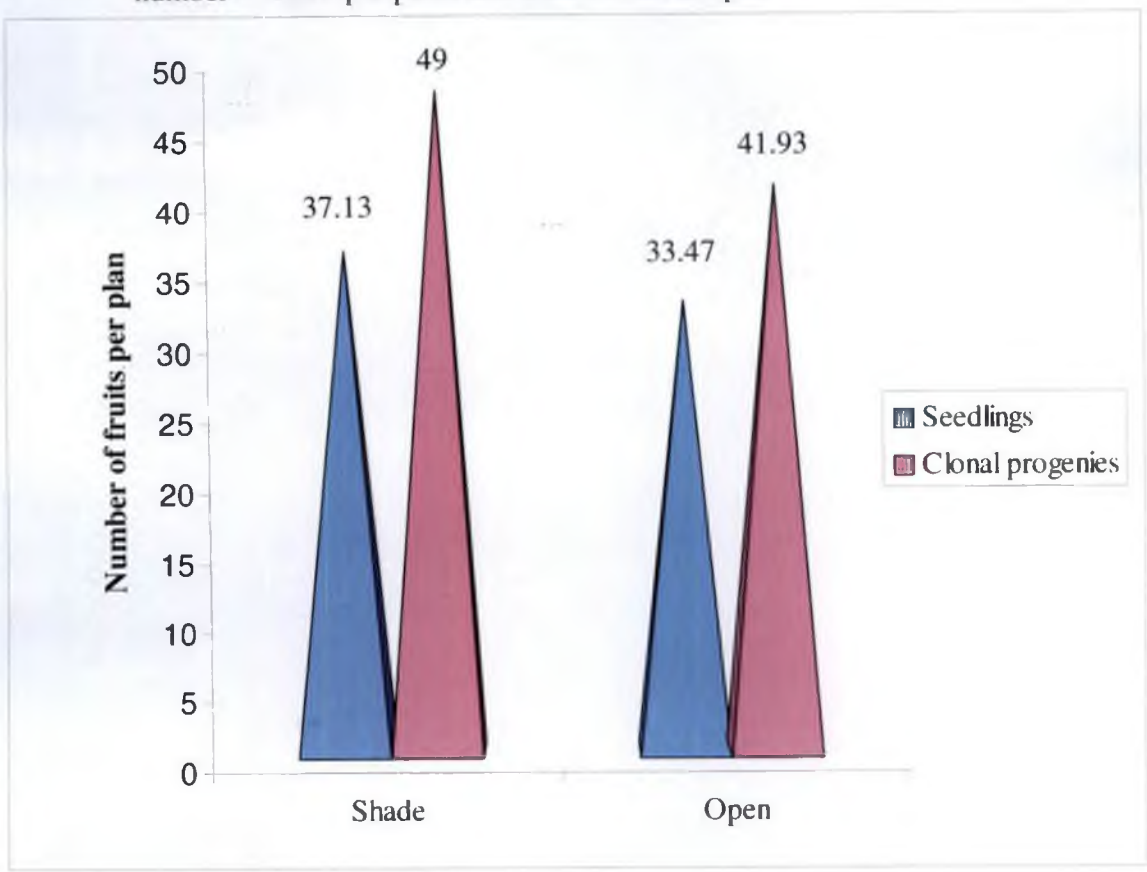


Fig. 5c. Interaction effect in seedlings and clonal progenies of *T. cucumerina* on number of fruits per plant under shade and in open



5.1.6 Fruit yield per plant

Fruit yield per plant did not vary between shade and open conditions (Fig. 6a). In the present study, though significantly higher fruit number was recorded by plants under shade, the superior performance was not evident in fruit yield per plant, in shaded condition. Here, higher incidence of fruit fly (*Dacus cucurbitae*) and snake gourd semilooper (*Plusia peponis*) under shade, during the later phases of fruit maturity was the major reason for the same. However, a marginal increase in fruit yield was registered by plants grown under shade. Plants grown under shade recorded a mean fruit yield of 275.00g and those grown in open recorded a mean fruit yield of 244.64g.

These results are supported by the findings of Sharma and Chauhan (2003). They reported that in soyabean, number of grains per pod and hundred grain weight under shade and in open, were on par. However, Priya *et al.* (2002) reported that the fruit yield in capsicum was highest for plants grown in coconut plantation than those grown in the open. Similar results were reported by Jayachandran *et al.* (1998) and Hegde *et al.* (2000) in ginger.

Seedlings and clonal progenies performed similarly, registering values on par with each other, with respect to fruit yield per plant (Fig. 6b). This finding points to the fact that a slight increase in fruit size and seed content of fruits of seedlings as compared to clonal progenies (Plate 6a and 6b), may have offset the superiority of the clonal progenies exhibited with respect to fruit number, thereby registering no significant difference in fruit yield per plant between seedlings and clonal progenies. This result is in conformity with the findings of Naumann and Seipp (1986) who observed that tissue culture derived plants grew vigorously, but yield did not differ from conventionally propagated plants. Shylaraj (1988) also revealed that lemon grass seedlings and clonal progenies did not differ significantly in yield attributes. Similar observations were made by Smith and Aynsley (1995) in date palm and Vuylsteke and Ortiz (1996) in banana.

Fig. 6a. Effect of shade and open conditions on fruit yield per plant in *Trichosanthes cucumerina*

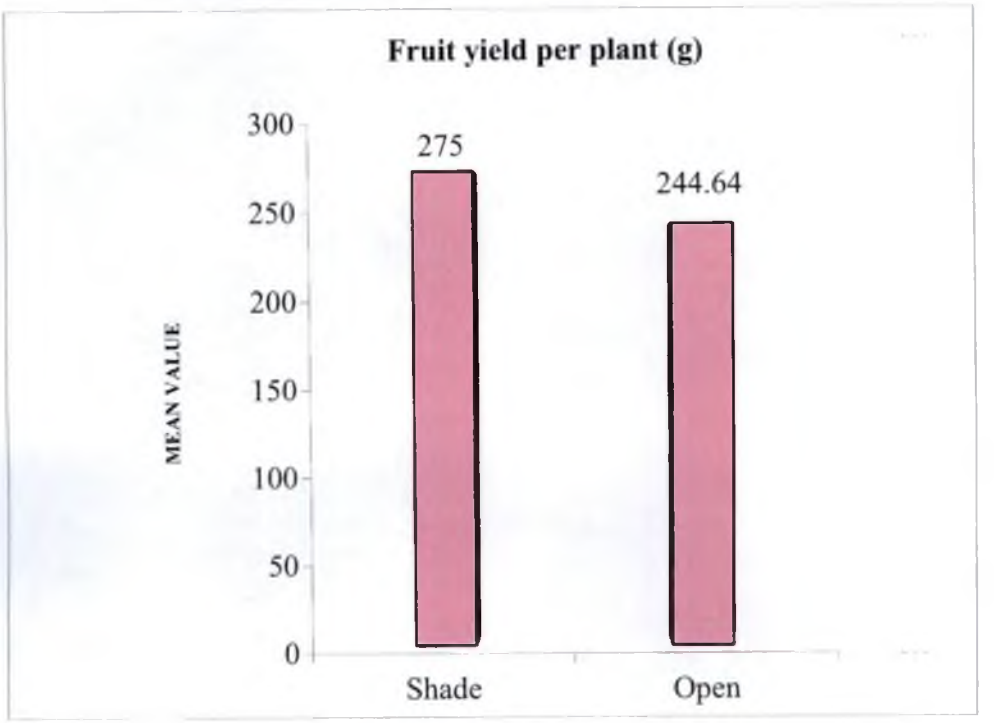


Fig. 6b. Fruit yield per plant in seedlings and clonal progenies of *Trichosanthes cucumerina*

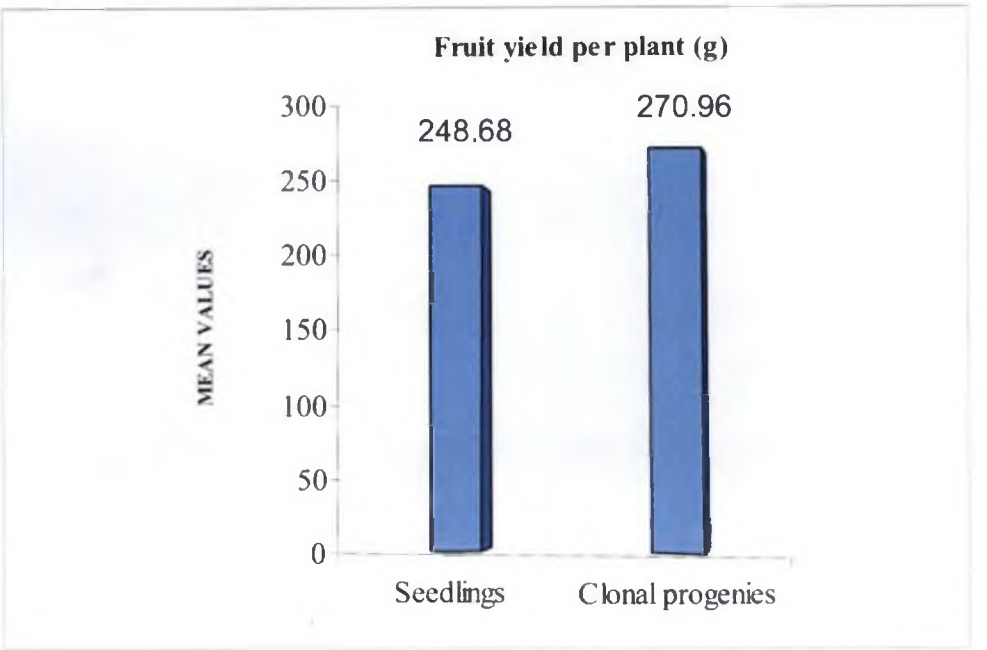
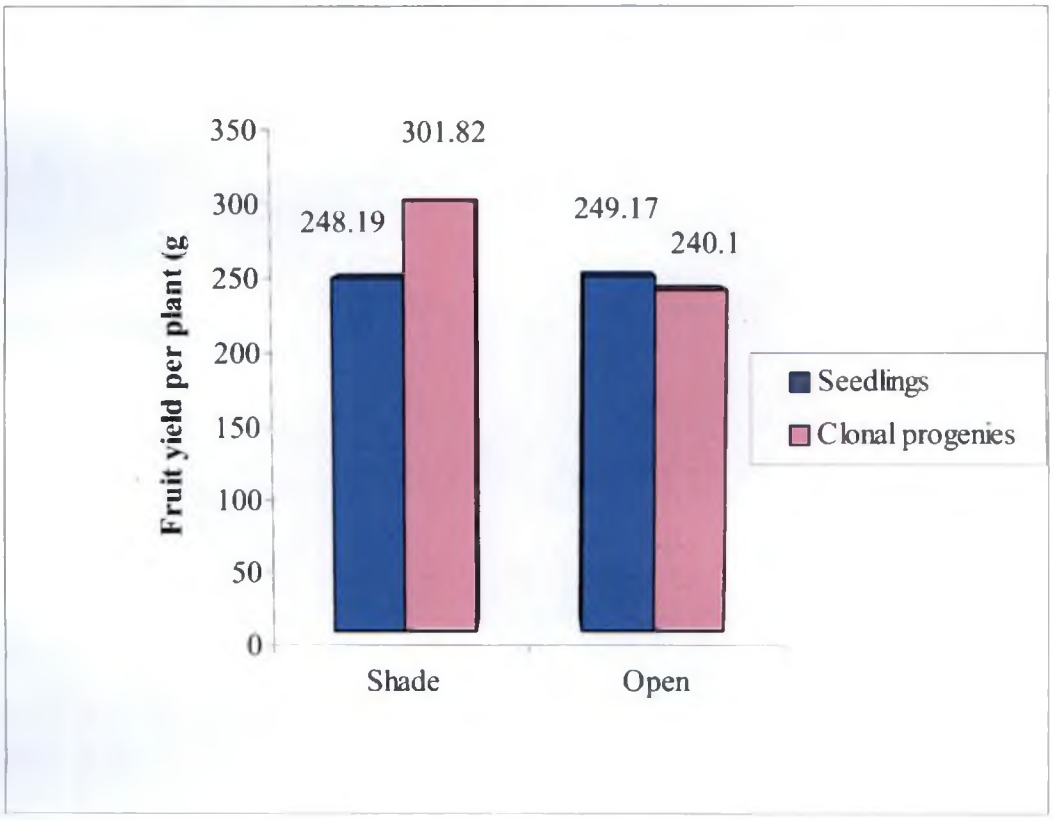


Plate 6a. L.S of a fruit of seed derived progeny of *Trichosanthes cucumerina*

Plate 6b. L.S of a fruit of tissue culture derived progeny of *Trichosanthes cucumerina*

Fig. 6c. Interaction effect of seedlings and clonal progenies of *T.cucumerina* on fruit yield per plant under shade and in open



However, though not statistically significant, a slight increase was noticed in fruit yield in tissue culture derived plants. Pattanshetti and Sulikeri (1982) also reported that clonal progenies had higher cumulative average yield per clump in cardamom. Findings of Pradeep *et al.* (1992) in Nendran banana, Robinson *et al.* (1993) in banana, Hae *et al.* (1997) in passion fruit and Johnston *et al.* (1997) in taro also support the above results.

5.1.7 Fresh yield of whole plant

In the present study, total fresh yield of whole plant did not differ significantly under shade and in open conditions (Fig. 7a). But a slight increase in fresh yield was recorded in plants grown under shade (654.73g) than in open. Absence of significant variation in total fresh yield per plant between open and shade may be expected by the fact that the vegetative biomass was more in plants grown in the open as represented by greater main vine length and more number of primary branches, whereas plants under shade, recorded more number of fruits and fruit yield per plant. So, when total fresh plant yield inclusive of fruits was considered, plants in both open and shade conditions performed similarly. This finding is of significance in the Ayurvedic user industry, where the crude drug is prepared from the entire vegetative biomass, along with fruits.

Hardy (1958) explained that the better performance of some crops under shade is due to the presence of threshold illumination intensity beyond which the stomata of shade loving plants tend to close. Yadav *et al.* (1982), Dey and Choudhary (1984) and Pareek and Gupta (1985) also reported that many medicinal plants recorded more yield in shade as compared to open. Similar results were reported by Jayachandran *et al.* (1991) who observed that under 25 per cent shaded condition, fresh rhizome yield in ginger was high, as compared to other treatments. Latha *et al.* (1995) also reported similar results in *Curcuma longa*.

Kurian (1999) observed that asparagus performed well as an intercrop in coconut plantation recording high root yield. The result of the present study also could be considered advantageous to cropping situation of Kerala, facilitating the raising of *T. cucumerina* in coconut plantations as an income generating intercrop, without compromising on yield. It is especially noteworthy that this performance is contrary to what is expected of cucurbitaceous plants which normally perform well only in the open. But an inverse relationship was reported in *Ocimum gratissimum* by Pillai (1990) who explained that total herbage yield and highest oil content were recorded in open condition.

Seedlings and clonal progenies did not differ significantly with respect to total fresh yield of whole plant. But clonal progenies recorded a slight increase in total fresh yield (655.24g) as compared to seedlings (612.49g) (Fig. 7b). This may be due to increased number of fruits and fruit yield in clonal progenies. So with respect to overall fresh plant biomass inclusive of fruits, clonal progenies did not prove inferior. This finding refutes the views expressed by certain farmers who rated tissue culture derived plants supplied to them as inferior to seedling progenies. Their contention was that, since *in vitro* derived plants flowered earlier, and had shorter crop duration, their total biomass yield was comparatively less than seed derived progenies. However, the present study indicates that greater fruit yield per plant of tissue culture derived plants compensates for the decreased vegetative biomass, rendering them as feasible planting materials. Superior performance of tissue culture derived plants with respect to yield have also been substantiated by Grabner and Grabner (1971) in a Hungarian plant. Similar results were reported in patchouli by Misra (1996) and in taro by Zok *et al.* (1998). Salvi *et al.* (2003) also obtained comparable results in turmeric.

5.1.8 Dry yield of whole plant

Performance of seedlings and clonal progenies under shade and in open conditions with respect to total dry yield of the whole plant were on par (Fig. 8a). This experimental result was the expected outcome of the trend obtained in

Fig. 7a. Effect of shade and open conditions on total fresh yield of whole plant in *Trichosanthes cucumerina*

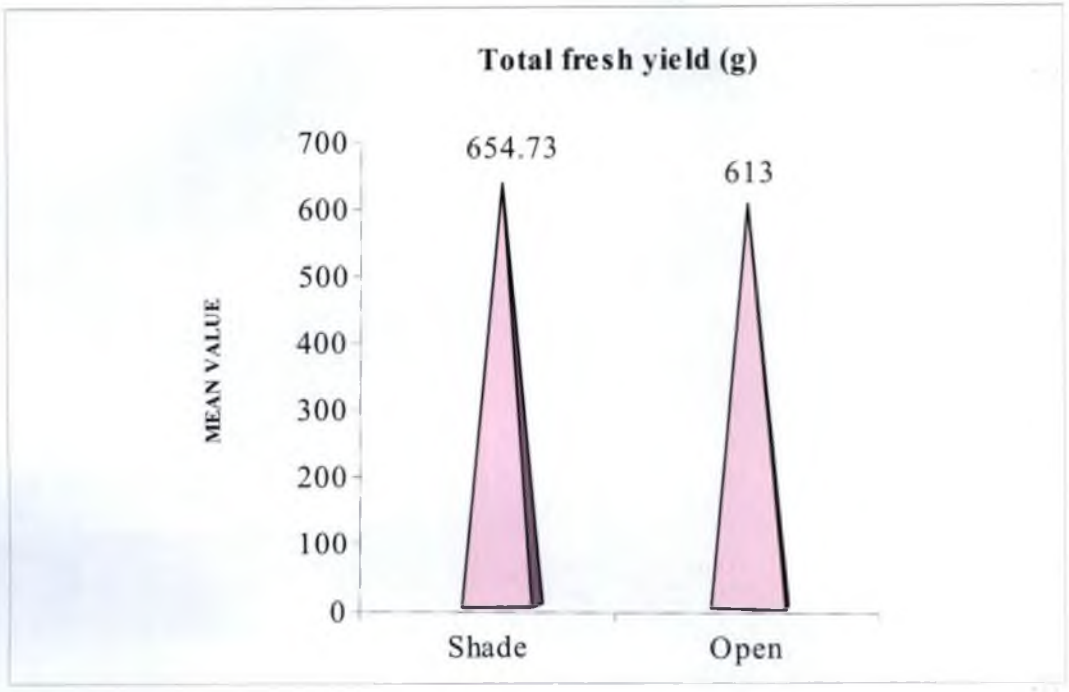
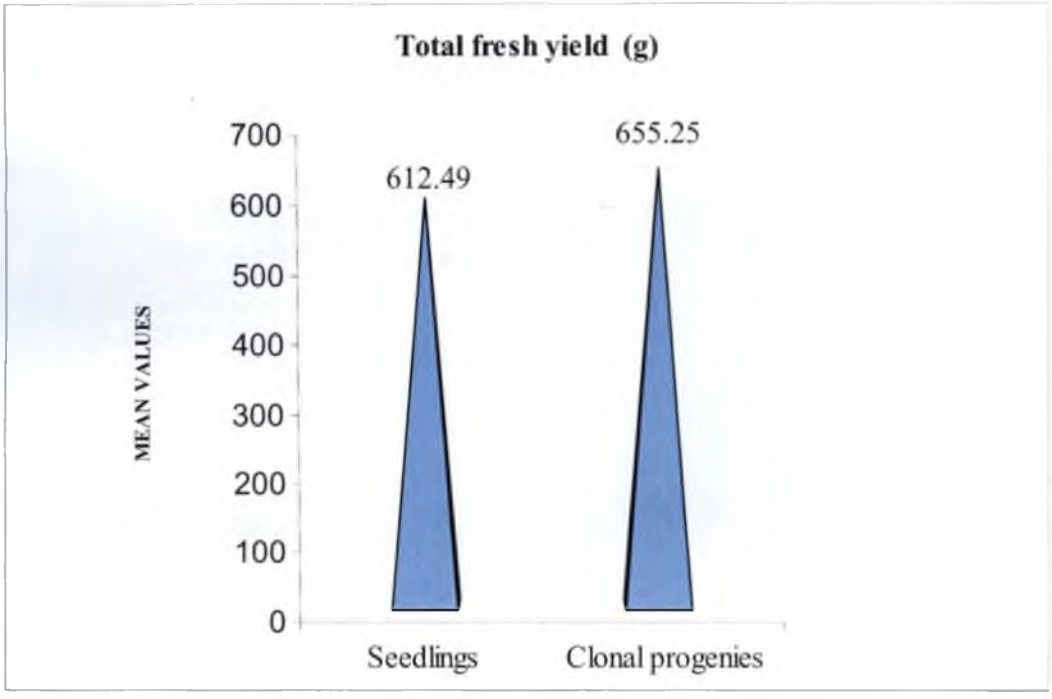


Fig. 7b. Total fresh yield per plant in seedlings and clonal progenies of *Trichosanthes cucumerina*



fresh yield per plant in both open and under shade, wherein, no significant variation is observed between plants in open and under shade, provided no difference in driage occurs between plants raised in open and under shade. However, Jayachandran *et al.* (1991) reported that dry ginger recovery was highest under shade. Similar results were registered by Kapur and Kapur (1999), Ajithkumar *et al.* (2002) and Joy *et al.* (2004) in kalmegh, ginger and *Curculigo orchioides* respectively. In the present study also, a slight increase was noted in dry yield per plant in plants raised under shade (157.15g) as compared to those in open (145.65g), a trend which was reflected in total fresh yield per plant as well.

Seedlings and clonal progenies did not show significant difference in total dry yield (Fig. 8b). However, clonal progenies recorded a slightly higher mean dry yield (156.20g) as compared to seedlings (146.59g). Seedlings recorded 148.25g mean dry yield under shade and clonal progenies, 166.04g. In shade and in open, seedlings performed similarly with a mean yield of 148.25g under shade and 144.93g in open. Clonal progenies registered 166.04g of dry yield in shade and 146.37g in open (Fig. 8c). The result is repetitive of the trend obtained in fresh yield per plant, wherein tissue culture derived plantlets exhibited higher values as compared to seedlings, though the differences were not significant.

High biomass of tissue culture derived plants after drying has been reported in several medicinal species. For instance, Roja and Heble (1996) reported that in *Rauvolfia serpentina*, biomass of tissue culture derived plants was higher than that of normal plants on a dry weight basis. This finding again confirms the suitability of tissue culture derived plantlets of *T.cucumerina* as planting materials, since the target plant enters into several Ayurvedic formulations, after being dried.

5.1.9 Total crop duration

Total crop duration of seedlings and clonal progenies of *T.cucumerina* varied significantly under shade and in open (Fig. 9a). Shorter crop duration was

Fig. 8a. Effect of shade and open conditions on total dry yield of whole plant in *Trichosanthes cucumerina*

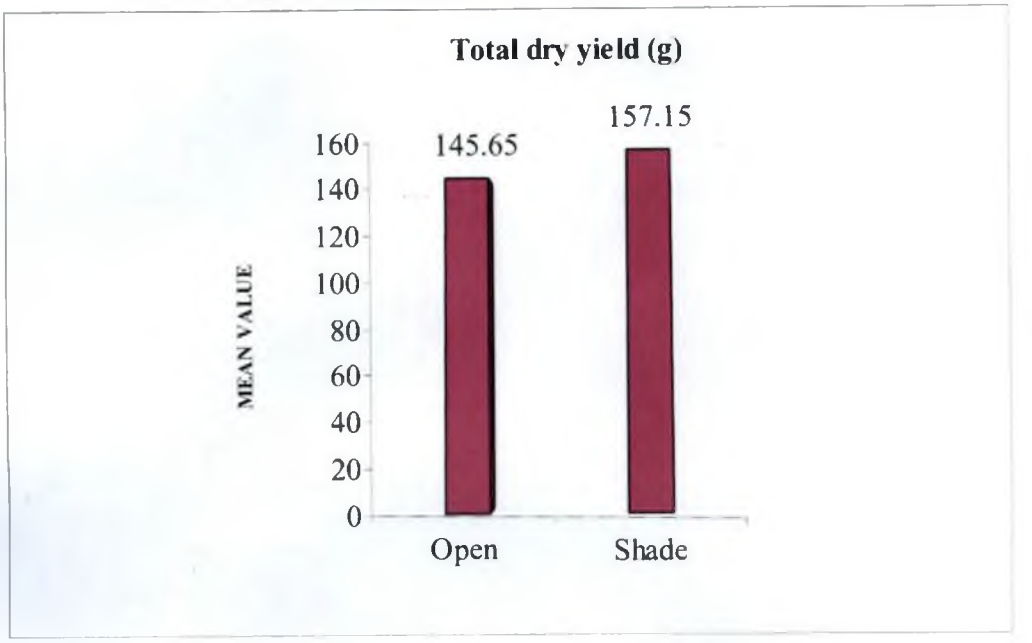


Fig. 8b. Total dry yield per plant in seedlings and clonal progenies of *Trichosanthes cucumerina*

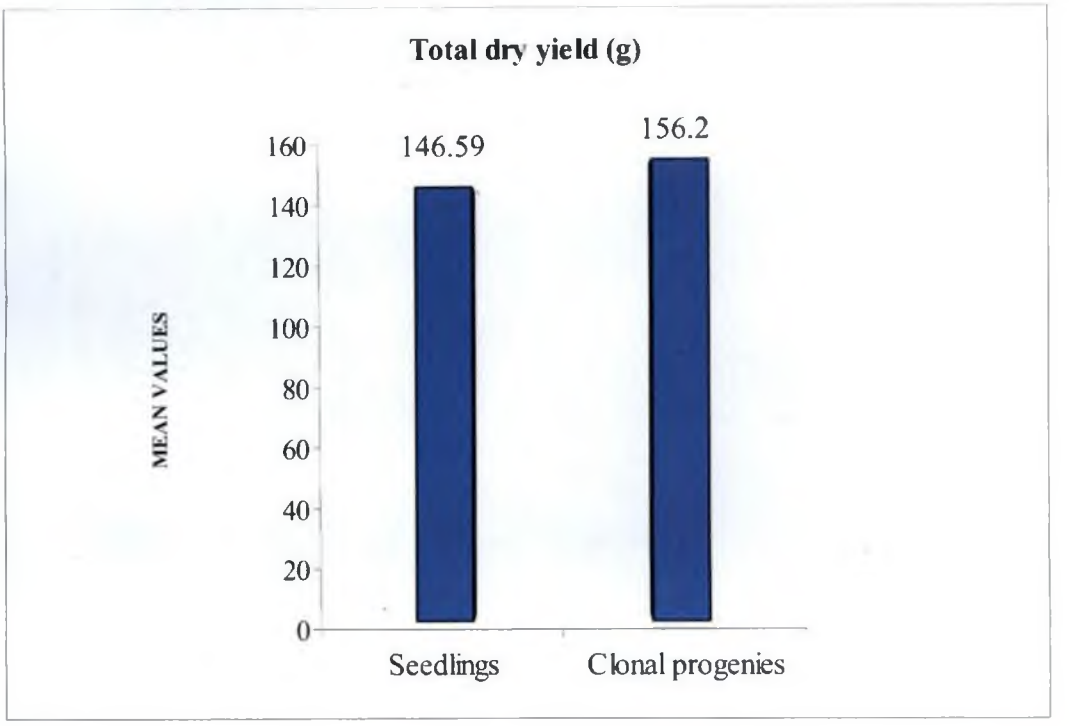
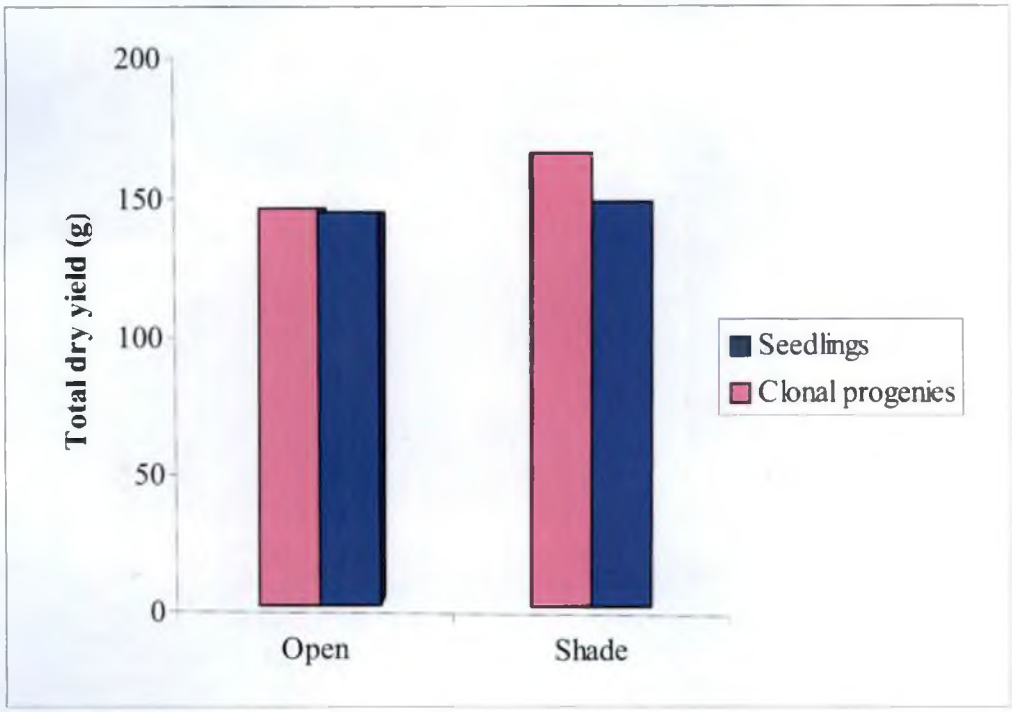


Fig. 8c. Interaction effect in seedlings and clonal progenies of *T. cucumerina* on total dry yield of whole plant under shade and in open



observed under shade (111.07 days) as compared to open (115.13 days). Also clonal progenies recorded shorter crop duration (106.30 days) than seedlings (119.90 days) (Fig. 9b).

The reasons for shorter crop duration under shade may be, the decreased photosynthetic rate, stomatal conductance, transpiration rate, stomatal index and stomatal frequency in shaded condition (Ajithkumar *et al.*, 2002). Accumulation of photosynthetic products was less in shaded plants due to the above reasons and the crop completed its life cycle earlier. It may be brought to notice that, in the present study, shorter crop duration of plants grown under shade did not in any way lead to a total yield decrease. In fact, owing to greater fruit number produced by plants grown under shade, a slight yield increase was noticed in them, as compared to plants grown in open, though the differences were not significant.

While considering the differences in crop duration between seedlings and tissue culture plants, the decreased crop duration of tissue culture derived plantlets may be attributed to early flowering witnessed in them, probably due to the influence of growth regulators supplemented in the *in vitro* phase. In the case of clonal progenies, Anon *et al.* (1993) and Hazarika *et al.* (2002) reported that due to poor photosynthetic efficiency of tissue culture derived plantlets, photosynthetic products will be exhausted within a few days and the plants try to complete their life cycles earlier.

5.2 EXPERIMENT II

Biochemical analyses for evaluation of seedlings and clonal progenies of *Trichosanthes cucumerina* under open and shade conditions, for drug quality.

Analytical results with respect to varied biochemical parameters of the experimental plants and market samples of *T. cucumerina* are discussed below.

Fig. 9a. Effect of shade and open conditions on total crop duration in *Trichosanthes cucumerina*

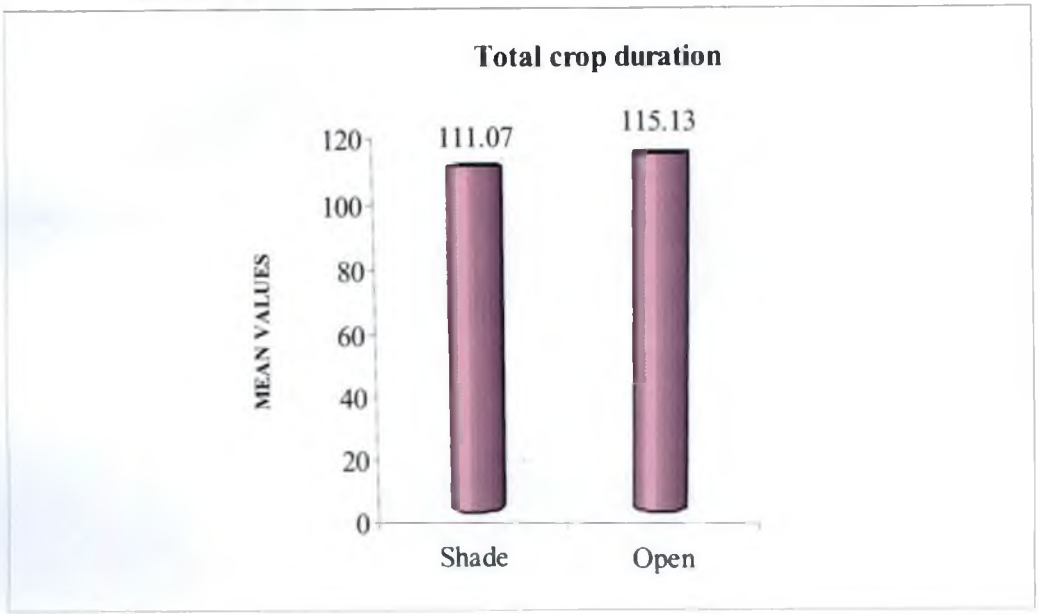
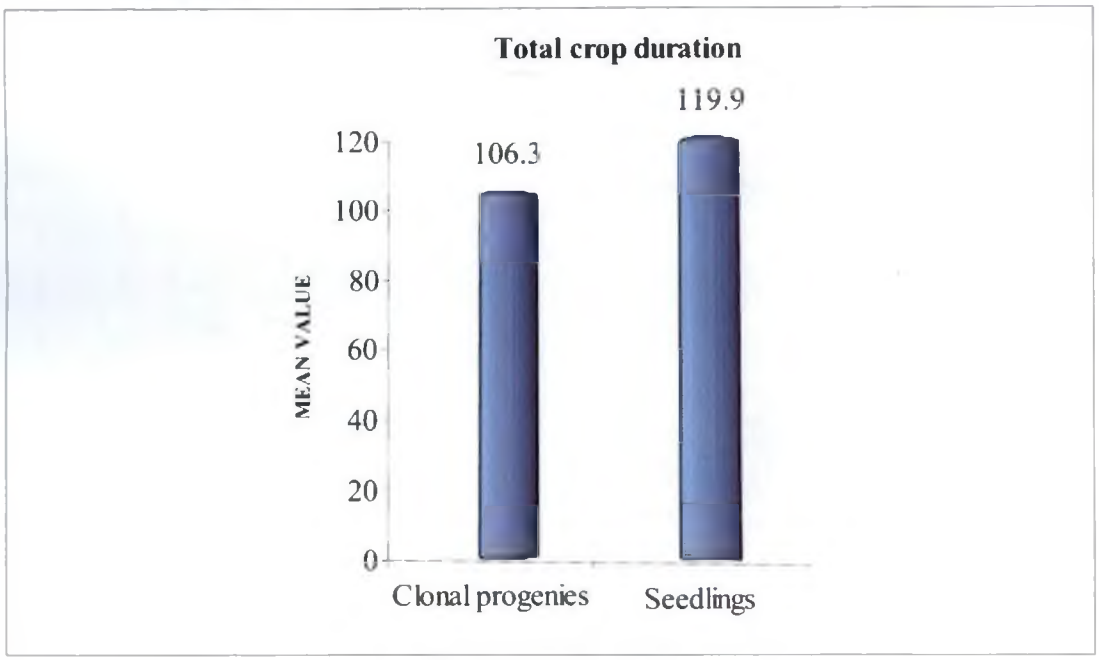


Fig. 9b. Total crop duration in seedlings and clonal progenies of *Trichosanthes cucumerina*



5.2.1 Fresh weight of crude extractables

5.2.1.1 Soxhlet extractables

Significantly higher yield of crude soxhlet extractables was recorded in plants grown under shade (0.312g) as compared to plants grown in open (0.241 g) (Fig. 10a). This may be due to the production of primary as well as secondary metabolites at a higher rate in shade, which may have contributed to a greater amount of total extractable matter. This trend was exhibited by both seedlings as well as clonal progenies. Also, clonal progenies recorded a mean yield of soxhlet extractables of 0.358 g which was significantly superior to that obtained from seedlings (0.198 g) (Fig. 10b).

Higher quantity of crude soxhlet extractables is indicative of the superiority of the drug prepared out of the sample. Biotechnological interventions in generating propagules of medicinal plants, will offer great advantages since it will be possible to obtain uniform, high quality planting materials (Calixto, 2000). Tissue culture plants being grown in controlled culture conditions of temperature and light with optimum inputs, register better quality attributes as indicated by higher amount of crude extractables in the experimental samples of tissue culture derived plantlets.

5.2.1.2 Hot water extractables

Shade and open conditions did not bring about any difference in hot water extractables in the experimental samples as against the soxhlet extractables (Fig. 11a). The reason may be that the influence of shade was marked in solvent soluble extractables than water soluble extractables. But seedlings and clonal progenies recorded significant difference in water soluble extracts (Fig. 11b). The influence of better growing environment of tissue culture plants may have exerted a positive influence on the water extractables as in the case of soxhlet extractables. Also clonal progenies recorded higher water extractables due to the influence of inputs supplied to the culture media in the *in vitro* phase. This

Fig. 10a. Effect of shade and open conditions on total soxhlet extractables in *Trichosanthes cucumerina*

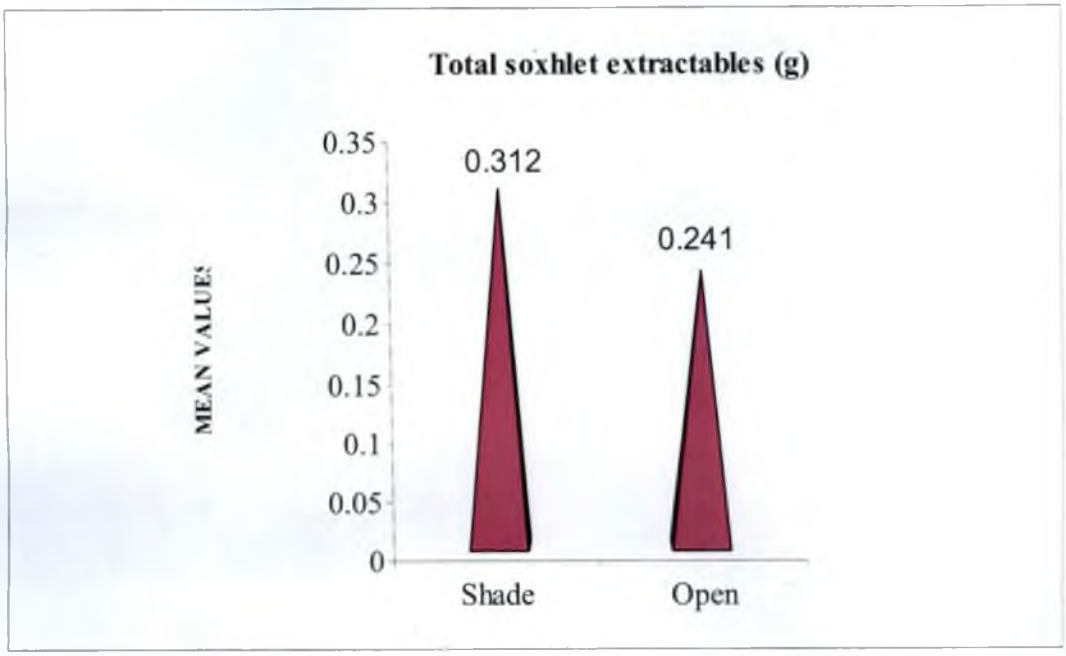
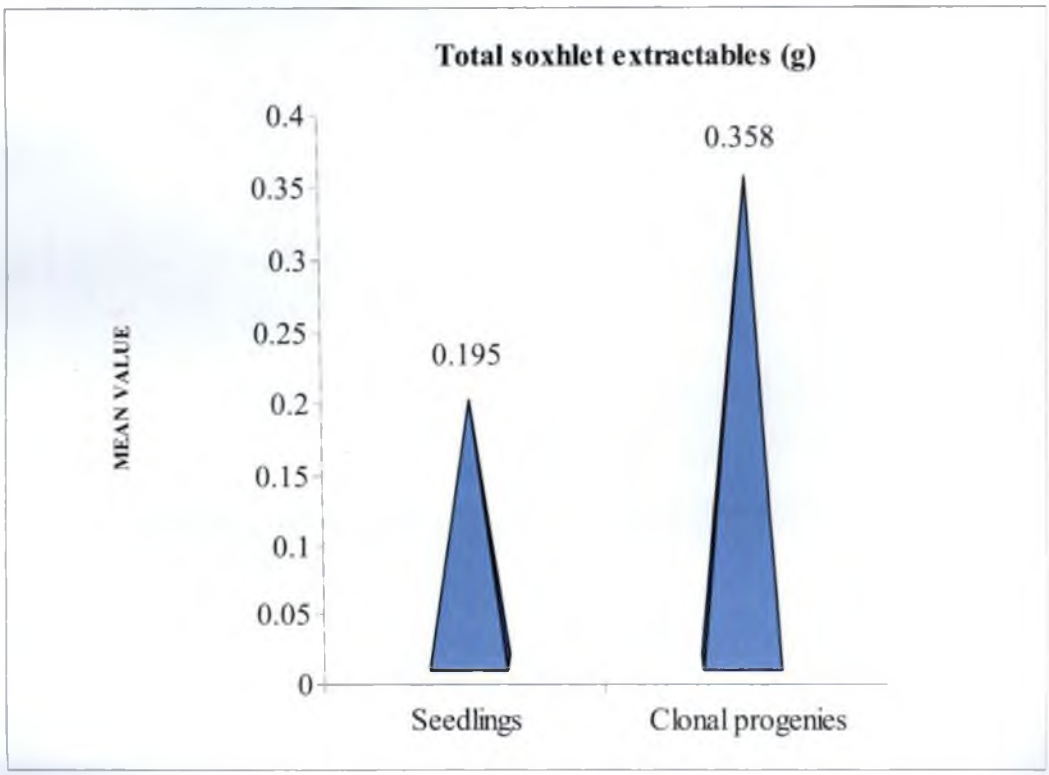


Fig. 10b. Total soxhlet extractables in seedlings and clonal progenies of *Trichosanthes cucumerina*



difference was more evident in plants grown in shade, where the respective values varied significantly, while in open the differences were not significant.

Adaptation of tissue culture plants to shade facilitating better acclimatization may have enhanced the quantity of water extractables in tissue culture plants under shade, that was significantly superior to those from seed derived plants. In shade, clonal progenies yielded 0.960 g of water extractables and in open, the respective value was 0.898g. That the overall performance and quality of tissue culture plants was superior to the conventional seedlings, has been reported by Sudharshan *et al* (1998) in cardamom, as well.

With respect to fresh weight of soxhlet extractables, market samples compared well with domesticated plants, registering values superior to those obtained from seedlings grown in the open. However, market samples were inferior to domesticated samples with respect to fresh weight of hot water extractables. The source plants, from which market samples of crude drug are prepared, are collected from the forests, where extreme shaded situations prevail. As is evident from the results of the present study, unlike in the case of soxhlet extractables, influence of shade was not that beneficial in enhancing hot water extractables. This may be the reason for the comparable performance of market samples with respect to soxhlet extractables and their inferior performance with respect to hot water extractables, as compared to domesticated samples.

5.2.2 Estimation of cucurbitacin

Analytical results in the course of identification and quantification of cucurbitacin, the important secondary metabolite in the target species and in the market samples are discussed below.

Rf values indicating the presence of cucurbitacin in seedlings and clonal progenies grown under shade and open conditions and market samples showed variation. High Rf value represents low molecular size cucurbitacins.

Fig. 11a. Effect of shade and open conditions on total hot water extractables in *Trichosanthes cucumerina*

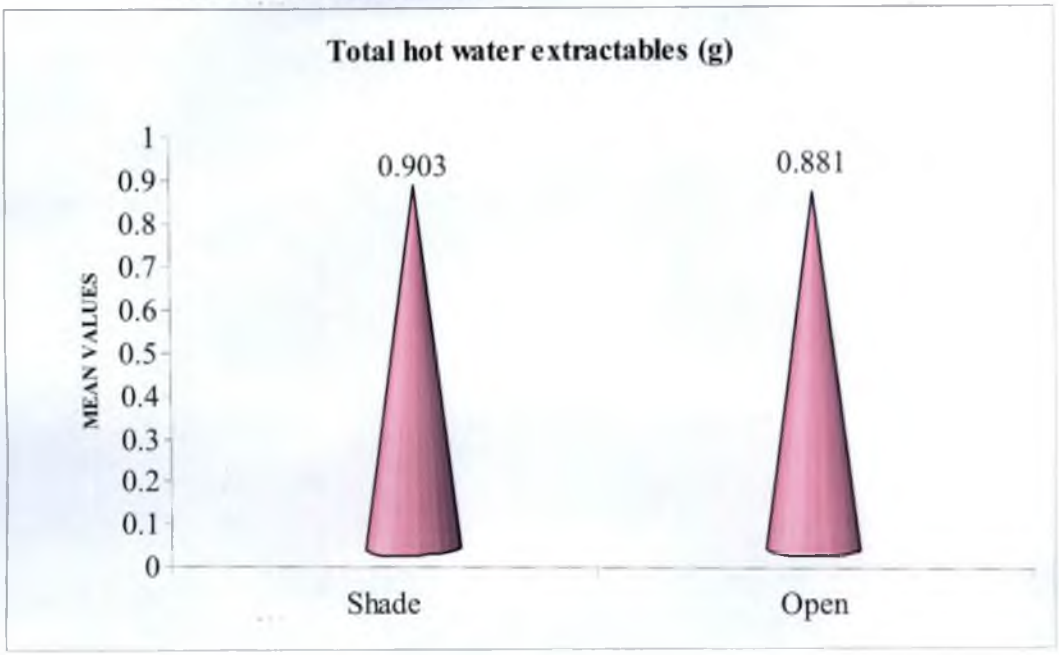
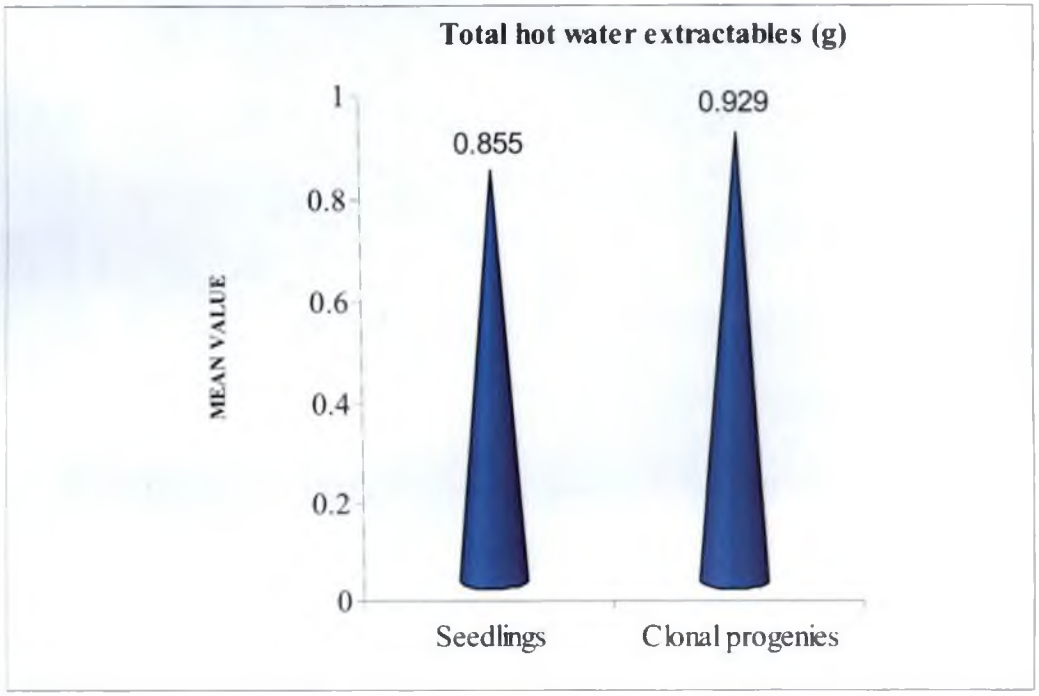


Fig. 11b. Total hot water extractables (g) in seedlings and clonal progenies of *Trichosanthes cucumerina*



Tissue culture derived plants grown under shade, recorded high Rf value (0.615). Hence, it can be inferred that tissue culture derived plants grown under shade contain cucurbitacins of low molecular weight. Seedlings grown in open registered a low Rf value of 0.473 indicating the presence of cucurbitacins of high molecular weight. Clonal progenies in open, seedlings grown under shade and market sample registered intermediate Rf values of 0.588, 0.526 and 0.563 respectively.

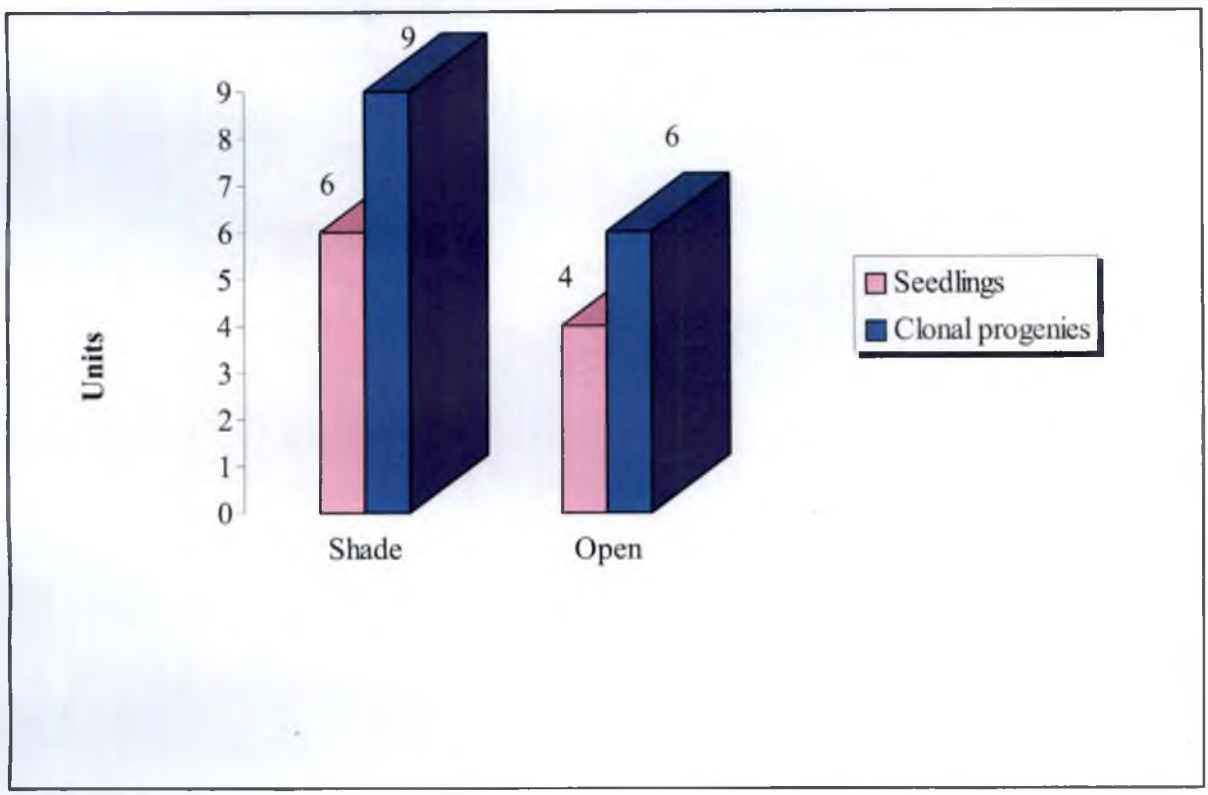
Murty *et al.* (1970) has isolated 19 cucurbitacins (A-S) from the members of cucurbitaceae family. Cucurbitacin B and E were isolated from *T. cucumerina* by Mallavarapu and Row (1979). Kitajima and Tanaka (1989) isolated cucurbitacin B and D from a related species, *T. kirilowii* Maxim. var *japonicum* Kitam. From another species, *Trichosanthes bracteata* Voigt., Kitajima *et al.* (1989) identified cucurbiatcin B. From the fruits of *Trichosanthes tricuspidata*, Mai *et al.* (2002) isolated a series of cucurbitacins.

Variability in the Rf values of cucurbitacin in the samples of open, shade and user industry indicated the influence of environmental conditions on growth, development and storage. In general, basic properties of cucurbitacins are not much affected due to the presence of unaltered constitutional structure of the compound.

5.2.3 Quantification of cucurbitacin

Quantitative TLC was performed to quantify cucurbitacin present in experimental samples. Cucurbitacin content was quantified from standard curve of sample and expressed as units. Clonal progenies grown under shade established marked superiority with respect to cucurbitacin content recording 9 units of cucurbitacin. This was followed by clonal progenies grown in open and seedlings grown under shade, which registered 6 units each. Seedlings grown in the open was inferior, recording only 4 units of cucurbitacin (Fig. 12). Market sample recorded 5 units of cucurbitacin.

Fig. 12. Cucurbitacin content (units) in seedlings and clonal progenies of *T. cucumerina* under shade and in open conditions



The above result indicated the superiority of tissue culture derived plantlets and favourable influence of shade on the content of cucurbitacin. Suggested reasons are that, shade has a positive influence on the production of secondary metabolites including cucurbitacin. Here also, analysis of market samples gave promising results revealing the presence of five units of cucurbitacin. Though the cucurbitacin content was less than that obtained from the clonal progenies employed in the study, presence of the major active ingredient in the market sample in comparable amount confirms the identity of the crude drug.

The experimental results are in agreement with the findings of Toth *et al.* (1991) in belladonna. They reported that content of secondary metabolite was more in tissue culture regenerated plants as compared to normal plants. Satheeshkumar and Seeni (2003) also reported that in *Plumbago rosea*, plumbagin content was higher in micropropagated plants than that of conventionally propagated plants.

Sun *et al.* (2004) reported that the quantity of active ingredient, crocin A of saffron pistils is 2-3 times more in tissue culture derived plantlets than that in cultivated saffron pistils. Wawrosch *et al.* (2005) indicated that *in vitro* propagated *Swertia chirata* plantlets normally differs from conventional plants in their natural environment with respect to secondary metabolite content. Gangadharan (2003) also reported that in kacholam rhizomes, quality attributes are increasing with increased levels of shade intensity. Similar results were reported by Priya *et al.* (2002) in paprika.

The method employed in the present study, for estimating cucurbitacin, has been attempted with reliable results in several crops. Yang *et al.* (1991) performed colorimetric method to determine the total cucurbitacin content of *Hemsleya dolichocarpa*. He also employed a TLC- densitometric assay for estimation of dihydrocucurbitacin F from this crop. A similar study was conducted by Attard (2002) for quantifying cucurbitacin E from *Ecballium elaterium* L. He quantified Cucurbitacin E by chromatographic studies with UV

absorption at 229 nm and also by spectrophotometric method with optical density at 492 nm. Mathew (2005) also used this method to quantify cucurbitacin from the fruits of *Cucumis melo* var *conomon*.

5.2.4 Qualitative estimation of minor secondary metabolites

Qualitative tests were done to identify the presence of alkaloids, phenols, tannins and saponins present in domesticated samples and market samples. Except in the case of phenols, simple colour tests were performed. The result showed that only one type of phenol was present in all samples. All samples including market samples gave positive results with respect to alkaloids, tannins and saponins, further confirming the true identity of the drug in the market samples. These results are in agreement to the findings of Wu and Zhu (1986) who isolated saponins from a traditional medicinal herb, *Trichosanthes* sp. From another species, *Trichosanthes uniflora* Hao., Huang (1999) extracted saponins and an alkaloid, trichosanatine.

5.2.5 Estimation of terpenes

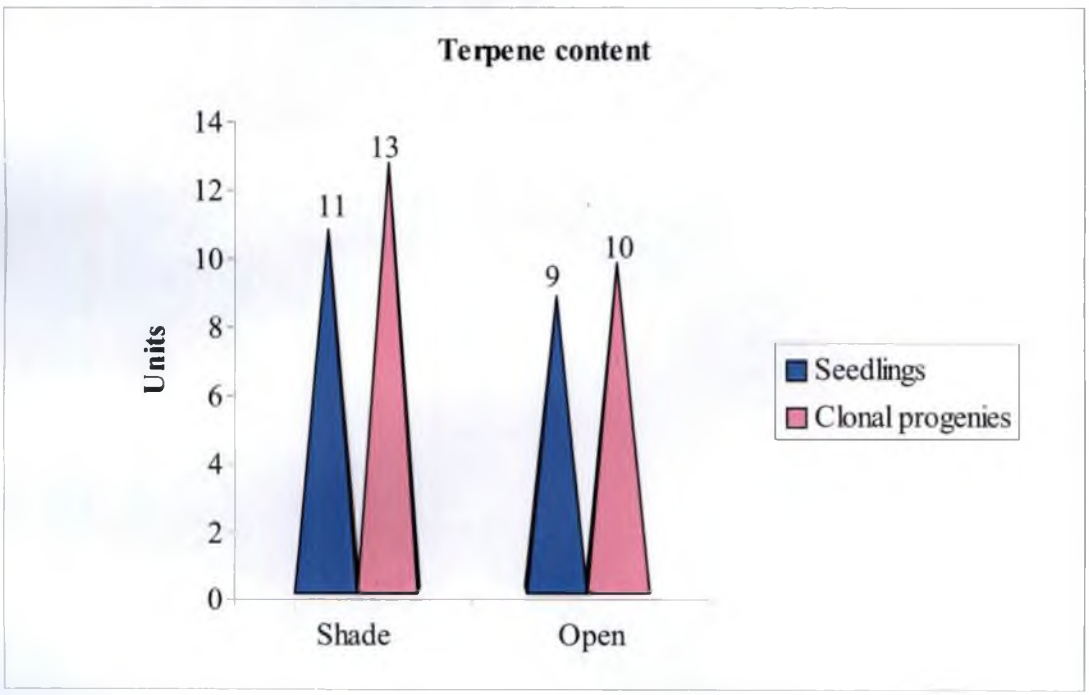
Cucurbitacin, being a triterpenoid, estimation of other terpenes present in the samples was also carried out. The trend of the presence of terpenes was similar to that of cucurbitacin content. Tissue culture derived plantlets grown under shade recorded 13 units, which was superior to the rest, followed by seedlings grown under shade (11 units), clonal progenies in the open (10 units), seedlings in the open and market sample (9 units) (Fig. 13). But unlike in the case of cucurbitacins, market sample recorded lowest value with respect to terpene content implying the need for better quality control of crude drugs in the Ayurvedic sector and the necessity for domesticating medicinal species.

5.3 EXPERIMENT III

Anatomical investigations for evaluation of domesticated samples and market samples

The results of anatomical investigations (stem, root and leaf) conducted with the experimental samples and market sample are discussed hereunder.

Fig. 13. Terpene content (units) in seedlings and clonal progenies of *T. cucumerina* under shade and in open conditions



Anatomical study especially in medicinal species is an effective tool to confirm the true identity of a species for preparation of crude drug and to detect adulterants and substitutes.

Stem sections of experimental samples revealed the presence of outermost epidermis with multicellular shoot hairs. Below the epidermis, collenchymatous hypodermis and parenchymatous cortex were observed. Endodermis and sclerenchymatous pericycle were also seen. These features are typical of a dicot stem. Vascular bundles were bicollaterally arranged. A vascular bundle in which the intraxylery phloem is in close contact with the xylem is termed as bicollateral vascular bundle. This feature is typical of cucurbitaceous plants, which further confirms its taxonomic status. Also, this characteristic may be employed as a diagnostic feature to detect adulteration in the fresh crude drug sample by non-cucurbitaceous plants.

Shade and open conditions did not exert any variation in the stem anatomy of the experimental samples. Also seedlings and clonal progenies displayed similar features. Market sample also had identical features, dispensing with the popular notion that many of the market specimens of crude drugs are adulterated and do not confirm to the true identity of the medicinal species.

Leaf anatomy was identical for plants raised in the open and under shade, seedlings, clonal progenies and market samples. Leaf section of the species revealed characters of a typical dicot leaf, possessing single layer of epidermis, parenchymatous mesophyll consisting palisade and spongy tissue, vascular bundle, midrib region and the lower epidermis.

Root sections of both seedlings and clonal progenies grown under shade as well as in the open revealed the existence of outermost piliferous layer with unicellular root hairs, many layered parenchymatous cortex, endodermis, parenchymatous tissue and vascular bundles.

The above observations support the findings of Tayal (1972), Esau (1972) and Abraham (1994) who studied the histology of cucurbitaceous species which revealed similar features like presence of phloem on the inside and outside of xylem in stem sections. Earlier Fakuda (1967) has reported that the internal phloem may be in close contact with inner sides of the xylem.

However, it is interesting to note that, the crude drug of *T. cucumerina* is dispensed in dried form as well and anatomical investigations to confirm the identity of the crude drug is feasible only in fresh samples. Hence, possibility of adulteration in dried market sample has to be investigated through biochemical characterization, to totally eliminate the possibility of adulteration in Kattupatavalam.

SUMMARY

6. SUMMARY

A study entitled "Evaluation of seedlings and clonal progenies of Kattupatavalam (*Trichosanthes cucumerina* L.) for yield and quality" was conducted at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2005-2006.

The experiment envisages to study the feasibility of domestication of seedlings and clonal progenies of *T. cucumerina* L. in open and shade through investigations on its growth, yield and qualitative characteristics by comparison with the drug source in the user industry, in terms of quality.

- With respect to main vine length, it was observed that seedlings and clonal progenies exhibited no significant differences in open and under shade. Between subplot treatments, seedlings recorded significantly higher mean main vine length (363.04 cm) than clonal progenies, both under shade and in open.

- In the case of primary branches per plant as well, shade and open conditions exerted no significant influence. An increased number of primary branches were noticed for seedlings (7.90) as compared to clonal progenies.

- Number of days to flowering was found significantly influenced by shade and open conditions in seedlings and clonal progenies. Plants grown in open recorded less number of days to flowering (66.53 days). Under shade and in open, clonal progenies registered lesser number of days to flowering.

- Experimental plants raised in open recorded fruit maturity earlier (24.00 days) than those grown under shade (27.30 days). Both seedlings (23.87 days) and clonal progenies (24.13 days) recorded earliest fruit maturity in open condition as compared to shade when their individual performances

were assessed. But seedlings and clonal progenies performed on par with respect to days to fruit maturity.

- The results of the study revealed that shade significantly influenced the number of fruits per plant in seedlings and clonal progenies. Highest mean number of fruits per plant was observed in plants grown under shade (43.07). Clonal progenies grown under shade yielded 49.00 fruits per plant. Clonal progenies recorded a mean number of 45.47 fruits per plant which was significantly superior to that of seedlings (35.30). Least number of fruits was obtained for seedlings grown in open.

- Fruit yield per plant did not differ significantly in shade and in open condition. Seedlings and clonal progenies also yielded results on par with each other, the respective values being 248.68 g and 270.96 g. But a slight increase in fruit yield was recorded in clonal progenies grown under shade, the differences being non significant.

- Shade and open conditions had no significant influence on total fresh yield as well as total dry yield of whole plant. Seedlings and clonal progenies also showed no significant variation in fresh and dry yield. However, maximum fresh (655.25 g) as well as dry yield per plant (166.04 g) was noticed in clonal progenies grown under shade though the differences were not significant.

- Plants grown in shade registered significantly shorter crop duration (111.07 days) as compared to plants grown in open (115.13 days). Between subplot treatments, clonal progenies recorded shorter crop duration (106.30 days). Least crop duration was registered by clonal progenies grown under shade (102.67 days) and maximum crop duration was observed in seedlings grown in open (120.33 days).

- Fresh weight of soxhlet extractables was significantly influenced by shade and open conditions. Shaded condition recorded a higher mean crude extractables of 0.312 g per plant in experimental plants as compared to open (0.241 g). Significant differences were observed in seedlings and clonal progenies also. Clonal progenies yielded high amount of crude extractables by soxhlet extraction (0.358 g). Clonal progenies registered superior performance with respect to quantity of crude extractables both in open as well as in shade. Market sample yielded 0.213g of soxhlet extracatables.

- Also, significant differences were observed between seedlings and tissue culture derived plantlets with respect to amount of hot water extractables. Clonal progenies yielded higher quantity of crude extractables (0.929g) than seedlings (0.855 g). Positive influence of shade on clonal progenies was noticed in the study, wherein higher quantity of water extractables (0.960 g) was obtained from clonal progenies grown under shade. However, amount of hot water extractables was not significantly influenced by growing environment *viz.*, shade or open. Market sample recorded a mean yield of 0.718 g of hot water extractables.

- During detection and estimation of cucurbitacin highest Rf value was recorded in clonal progenies grown under shade (0.615) indicating the presence of cucurbitacins of low molecular weight. Cucurbitacins of high molecular weight was identified in seedlings grown in the open (0.473). Market sample recorded a Rf value of 0.563.

- Shade had a significant role in enhancing the amount of cucurbitacin in seedlings and clonal progenies as is evident from the experimental results. Clonal progenies grown under shade recorded the highest amount of cucurbitacin (9 units), followed by seedlings grown in shade and tissue culture plants raised in the open (6 units). Biochemical analysis of

composite market sample yielded 5 units of cucurbitacin confirming their superiority to seedlings grown in open, with respect to cucurbitacin content.

- Variability in cucurbitacin content in the samples of open, shade and user industry indicated the influence of environmental conditions including storage.

- Qualitative analysis conducted in the present study revealed that other secondary metabolites like alkaloids, phenols, tannins, saponins and terpenes were also present in experimental samples. Market samples were also identical to experimental samples with respect to presence of the above secondary metabolites. Estimation of phenols revealed that only one type of phenol was present in all the samples. Analytical data of terpene quantification revealed that highest quantity of terpene was observed in clonal progenies grown under shade indicating that shade had a positive role in terpene production in plants.

- Experimental plants as well as the composite market samples were identical in the anatomic features of leaf, stem and root sections, revealing the typical features of a dicotyledonous plant. The investigation revealed the presence of bicollateral vascular bundles in all the experimental samples and market samples, which is typical of cucurbitaceous plants.

- From the study it could be inferred that, domesticating the species (*Trichosanthes cucumerina*) under shade was superior to growing in the open, with respect to yield and quality. Also, progenies derived through *in vitro* multiplication techniques performed better than seedlings with respect to various yield and quality parameters.

- Market samples compared well with the domesticated samples in various quality parameters including cucurbitacin content as well as anatomical features and were superior to seedlings raised in the open.

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* Originals not seen

APPENDICES

Appendix 1

Particulars	February	March	April	May
Mean maximum temperature (^{0} C)	34.3	34.8	33.4	31.8
Mean minimum temperature (^{0} C)	22.3	23.8	24.7	24.3
Highest maximum temperature (^{0} C)	36.6	36.8	35.4	34.8
Lowest minimum temperature (^{0} C)	19.4	20.4	21.8	22.2
Mean R H morning (%)	71	86	90	91
Mean R.H evening (%)	31	49	59	66
Mean R.H (%)	51	68	75	79
Rainfall (mm)	0.0	95.2	86.2	675.5
Rainy days	0	4	3	14
Evaporation (mm)	193.1	160.4	135.7	112.6
Sunshine (hrs)	267.8	236.5	211.3	179.9
Mean sunshine (hrs)	9.6	7.6	7.0	5.8

EVALUATION OF SEEDLINGS AND CLONAL PROGENIES OF KATTUPATAVALAM (*Trichosanthes cucumerina* L.) FOR YIELD AND QUALITY

By

SREEREKHA M. V.

ABSTRACT OF THE THESIS

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(PLANTATION CROPS AND SPICES)

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ABSTRACT

A study entitled "Evaluation of seedlings and clonal progenies of Kattupatavalam (*Trichosanthes cucumerina* L.) for yield and quality" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, during 2005-2006, to evaluate the seedlings and clonal progenies of Kattupatavalam for growth and yield in open and under shade. The study also aimed at assessing the quality attributes of seedlings and clonal progenies, grown under shade and in the open by comparison with the drug source in the user industry.

Results of the study revealed that shade and open conditions had a significant influence on various parameters of growth, yield and quality attributes of seedlings and clonal progenies. Seedlings and clonal progenies also showed significant difference in some growth parameters as well as in yield and quality attributes.

Considering main vine length and primary branches per plant, seedlings were superior as compared to clonal progenies. Growing in the open, conferred earliness in flowering, wherein the species registered 66.53 days for flowering. Between subplot treatments, earlier flowering was recorded for clonal progenies as compared to seedlings. Plants grown in the open attained fruit maturity earlier as compared to plants grown under shade. But seedlings and clonal progenies recorded values on par with each other.

Highest mean fruit number was recorded for plants grown under shade as compared to plants in the open. Clonal progenies recorded 45.47 mean number of fruits, which was superior to fruit number per plant in seedlings.

No significant difference was observed in fruit yield per plant, in plants grown under shade as well as in the open. Similar trend was exhibited by seedlings and clonal progenies as well. Total fresh yield and total dry yield of whole plant, did not differ significantly under shade and in the open. It was also revealed that, seedlings and clonal progenies registered values on par with each other, on the above two yield parameters. Shorter crop duration was recorded in

plants grown under shade (111.07 days) as compared to plants in the open. Clonal progenies recorded shorter crop duration as compared to seedlings.

Fresh weight of soxhlet extractables was highest for plants grown under shade (0.312g). Clonal progenies recorded 0.358 g of soxhlet extractables, which was superior to that of seedlings. It was found that, shade and open conditions had no influence on the fresh weight of hot water extractables. Considering subplot treatments, clonal progenies recorded higher mean weight of hot water extractables (0.929). Market samples yielded 0.718g of hot water extractables and 0.213 g of soxhlet extractables.

Tissue culture derived plants grown under shade, registered cucurbitacin of low molecular weight, while high molecular weight cucurbitacin was recorded in seedlings grown in the open. Intermediate types of cucurbitacin were expressed by clonal progenies in the open, seedlings under shade and market samples.

Cucurbitacin content was more for clonal progenies grown under shade, while least quantity was observed in seedlings grown in the open. Market sample yielded five units of cucurbitacin, which was superior to cucurbitacin content of seedlings grown in open. On further biochemical analyses, it was revealed that, other secondary metabolites like alkaloids, phenols, tannins and saponins were present in domesticated samples as well as in market samples. Quantification of terpene revealed that tissue culture derived plants grown under shade registered higher amount of terpenes (13 units) than others.

With regard to anatomical studies of experimental samples, no difference in anatomy of stem, root and leaf sections of domesticated samples and market samples was noted.

The study conclusively proved that, *Trichosanthes cucumerina* can be ideally raised as a profitable intercrop in the coconut gardens of Kerala and that performance of tissue culture derived plantlets was comparable to seedlings. Also, market sample did not reveal presence of adulterants, as indicated by qualitative as well as anatomical investigations.