CHARACTERIZATION OF BETEL VINE (*Piper betle* L.) TYPES OF MALAPPURAM DISTRICT

by
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(2012-11-118)

THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Characterization of betel vine (*Piper betle* L.) types of Malappuram district"** is a bonafide record of research done by me during the course of study and the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "**Characterization of betel vine (*Piper betle* L.) types of Malappuram district**" is a record of research work done independently by Preethy T.T. (2012 - 11 - 118) 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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ABBREVIATIONS

DAL : Days after lowering

GI : Geographical Indication

GCV : Genotypic Coefficient of Variation

PCV : Phenotypic Coefficient of Variation

PCC : Phenotypic Correlation Coefficient

GCC : Genotypic Correlation Coefficient

H : Heritability

WTO : World Trade Organization

DUS : Distinctiveness, Uniformity, Stability

Introduction

1. INTRODUCTION

Betel vine (*Piper betle* L.) is a dioecious, evergreen creeper belonging to the family Piperaceae. It grows in moist tropical and subtropical regions of different countries such as China, Thailand, Philippines, India, Bangladesh, Sri Lanka *etc.* It is native to Central and Eastern Malaysia (Chattopadhyay and Maity, 1967). In India, it is cultivated in an approximate area of 45,000 ha as cash crop. Everyday about 15 - 20 million people in the country consume betel leaves. Sixty six per cent of the total production of betel leaf in India is contributed by West Bengal (Guha, 2006).

Betel vine is an indigenous medicinal plant with glabrous, deep green, heart shaped leaves as economically important part. The betel vine leaves are popularly known as *Paan* and is also known in other names like *Tamalapaku, Tambul, Vettilai etc.* in different parts of India. Betel leaf is aromatic, commonly used as a masticator due to medicinal, nutritional and stimulating properties.

Betel leaves have many medicinal properties and are used in Indian system of medicine to cure indigestion, stomach ache, diarrhoea, flatulence and to heal wounds, scales, burns, swelling *etc.* The leaves are credited with wound healing property. In *Susruta Samhita,* it is mentioned as aromatic, sharp, hot, acrid and beneficial as laxative and appetizer (Pradhan *et al.*, 2013). In case of respiratory disorders, betel leaves are soaked in mustard oil, made warm and applied on chest for getting relief from cough and breathing problems. Fresh juice of betel leaves is used in many Ayurvedic preparations. The leaves are used by singers to improve their voice. The leaves are nutritive and contain anticarcinogens, showing future opportunities in anticancer drugs. The leaves have a sharp taste and appealing smell that improve taste and appetite, lessen the thirst, clear the throat and purify the blood. It is considered as tonic to brain, heart and liver. It is also used as anti-inflammatory and antimicrobial agent.

In Kerala, Tirur and nearby areas of Malappuram district are famous for betel vine cultivation with an area of 183 ha (FIB, 2014). In earlier days *Paan Bazar* in Tirur was an exclusive market for betel leaf. Best quality Tirur betel vine is exported to Pakistan *via* North India and second grade leaves are sold in local markets (Nair, 2010). Presently *Tirur betel leaf* is also exported to Pakistan *via* Arab countries. Majority of people from Tirur and nearby areas depend on betel vine cultivation and allied sectors for their livelihood. Betel vine cultivars from Tirur area possess some special morphological and biochemical characters like unique flavor and aroma because of geographical features, traditional cultural practices, specific genotypes, special soil characters and peculiar climatic features of area of production.

In spite of close association of betel leaf with Indian culture from time immemorial, information about morphological and biochemical characters of betel vine is very limited. Even though *Tirur betel vine* from Malappuram is a unique agricultural product of the country, studies on the variability of betel vine cultivars in Malappuram district are very scanty. Characterization of popular betel vine types is a prerequisite to reveal the existing variability within the crop in Malappuram area. Studies on morphological and biochemical characters are to be undertaken to identify the best cultivars for commercial cultivation and to use in both medicine and cosmetic production. Moreover, documentation of unique characters is necessary for the registration of *Tirur betel vine* as a Geographical Indication from Kerala. Presently an accepted crop descriptor for betel vine is lacking. In this background, the present study was undertaken to document and characterize the betel vine cultivars of Malappuram district based on

- morphological characters
- biochemical characters and
- organoleptic properties

Review of literature

2. REVIEW OF LITERATURE

Betel vine (*Piper betle L.*) is a dioecious perennial cash crop and its cultivation is referred as most distinctive agricultural industry. This crop had been referred in the ancient Indian literature dating back to AD 473 (Singh, 1994). Chewing of betel leaf 'or *paan*' as it is called, was an ancient habit among all classes of people. In India this crop is mainly cultivated in West Bengal, Kerala, Karnataka and Tamil Nadu. Major portion of the betel vine cultivation of Kerala is in Malappuram district (FIB, 2014).

The betel vine types grown in Malappuram district are generally known as *Tirur betel vine* and as indicated by the name, Tirur is the largest betel vine growing tract in Kerala. *Tirur betel vine* is one of the popular betel leaf exported from India to countries like Pakistan, Afghanistan, UAE and other Arab countries (Nair, 2010). However, the cultivation practices followed for *Tirur betel vine* are very conventional and developed by farmers themselves.

Betel vine types from Tirur of Malappuram district are known for their characteristic pungency and fetch premium price in international markets. Pungency is an accumulated effect of both biochemical and organoleptic characters. Consumer preference for *Tirur betel vine* is also due to superior morphological characters. Information on morphological, biochemical and organoleptic properties will lead to successful evaluation of genotypes, which could be used for general cultivation and export purpose. A brief review of literature of morphological and biochemical characters of betel vine along with relevant studies in related crops are presented under the following headings.

1. Variability
2. Morphological characters
3. Biochemical characters

4. Organoleptic property - pungency

2.1 VARIABILITY IN BETEL VINE

Betel leaf had an esteemed position in human society from the dawn of civilization. The origin of betel vine is believed to be in Malaysia (Chattopadhyay and Maity, 1967) or in surrounding East Asian region. Eight crore sq. km. area in the whole of the world is estimated to be under betel vine cultivation. The major betel vine growing countries are India, Bangladesh, Sri Lanka, China, Indonesia, Malaysia, Nepal, Pakistan, South Africa, Philippines, Burma, South East Asia and Papua New Guinea (Khoshoo, 1981; Singh, 1994; Samanta, 1994; Jana, 1996; Sharma *et al.*, 1996; Ramji *et al.*, 2002; Banerjee, 2012). It is believed that betel vine was introduced to Sri Lanka and other South Asian Countries by Chinese and Arab merchants (Department of Export Agriculture, Sri Lanka, 2012).

2.1.1 Betel vine Cultivars of Different Countries

According to Mabberley (1997), of the 2000 cultivars of *P. betle* distributed in the whole world, ten were available in Nepal. Akther (2004) reported three main groups of betel vine varieties from Pakistan namely *Sanchi, Bangla* and *Meetha* and sub varieties like *Nuntia – Bantual, Ujani, Magai* and *Karpurkath.* Nearly ten wild relatives of betel vine and a large number of local accessions were reported from Sri Lanka (Arembewela *et al.*, 2005). *Kudamaneru, Mohamaneru, Galdalu, Ratadalu, Nagawalli* and *Malabulath* were the common types of betel vine reported from Sri Lanka. Even though betel vine was grown all over Sri Lanka, the commercial production of export quality betel vine, with bigger leaves and dark green color combined with thickness, known as *Kalu bulath,* was significantly confined to few districts such as Kurunegala, Gampaha, Kegalle, Kalutara and Colombo (Sumanasena *et al.*, 2005). The other betel vine varieties with high export quality reported from Sri Lanka were *Maneru, Ratadalu* and *Galdalu.*

2.1.2 Betel vine Cultivars of India

Based on the morphological characters and essential oil content, Singh (1994) grouped betel vine varieties into five main groups *viz., Bangla, Desawari, Kapoori, Sanchi and Meetha. Bangla* had large thin leaves with nine main nerves and ovate lamina with cordate base. Leaf apex was pointed and short, not curved. Petiolar sinus of *Bangla* was more prominent than other varieties. *Desawari* had large thin leaves and cordate lamina with seven to nine nerves. Leaf of *Desawari* was pinkish in color and leaf apex was short, acuminate and curved. *Kapoori* leaves were more elliptical and lamina was thin with undulated margin. Leaf apex of *Kapoori* was acuminate and petiolar sinus was inconspicuous. Leaves of *Meetha* were large and lamina was cordate to broadly ovate and thick. *Meetha* leaf was waxy in texture with yellowish dots and three to five main nerves. Leaf apex of *Meetha* was short and pointed. It had prominent joint in the petiole. *Sanchi* had cordate leaf base with more elliptical lamina and long tapering apex. Normally seven nerves were seen in *Sanchi*. Among the above types, *Kapoori* and *Sanchi* were the principal cultivars in the peninsular India whereas *Bangla* and *Deswari* were common in North India. *Meetha* was grown on commercial scale in West Bengal only. The same classification was reported in an investigation carried out by Kumar (1999) with an additional group namely *Kasi*.

About 125 - 150 cultivars of betel vine were recognized by the cultivators and traders in India (Ranade *et al.*, 2002; Anjali *et al.*, 2004) and most of them were known by the name where they were cultivated. Ranade *et al.* (2002) reported that *Kapoori* cultivars were more heterogenous while the *Bangla* cultivars were mostly similar to each other. After evaluation of seven cultivars of betel vine in West Bengal, Sheet (2002) observed that cv. *Chandrakona* was superior with respect to most of the characters compared to other cultivars.

Guha (2006) reported that 15 - 20 million people in the country consume betel leaves every day. It is cultivated in an area of 45,000 ha with an annual turnover worth Rs. 9,000 million, providing livelihood to millions of people. Betel vine has separate male and female plants. Usually the male plants are cultivated throughout India for harvesting green leaves (Lakshmi and Naidu, 2010). Betel vine cultivation is distributed in Andhra Pradesh, Assam, Bihar, Madhya Pradesh, Maharashtra, Karnataka, Kerala, Orissa, Tamil Nadu, Uttar Pradesh and West Bengal (Sugumaran *et al.*, 2011). The *Maghai* variety (literally from the Magadha region) grown near Patna in Bihar, India was reported to be the best betel leaf (Rani and Ramamurthy, 2012). Sengupta (2014) reported *Maghai* as one of the major betel vine types of India. *Piper nigrum* and *P. betle* are the widely cultivated species of *Piper* and these have attained an excellent commercial status in India. The state wise distribution of different betel vine cultivars is provided in Table 1.

Table 1. Major betel vine cultivars reported from different states in India

States	**Betel vine types/cultivars**	**References**
Orissa	*Godi Bangla, Nova Cuttak, Sanchi,Birkoli, Bangla, Bihari, Deshi local, Dhob Mahata , Kala Mahata, Mitha.*	Singh, 1994.
Tamil Nadu	*Pachaikodi, Vellaikodi, Thulasi, Venmani, Arikodi, Kalkodi, Karilanchi, Karpuram, Chelanthikarpuram, Koottakkodinandan, Perumkodi, Amaravila, Pramuttan, Kallarkodi, Revesi, Karpuri, SGM 1, Sirugamani 1, Anthiyur kodi, Kanyur kodi, Sirugamani, Karpoori, Vellakodi, Karuppu pachaikodi, Vellai Pachai Kodi.*	Singh, 1994.
Uttar Pradesh	*Deswari, Kapoori, Maghai, Bangla, Bihari, Deshi Bengla, Desi Desawari, Culcuttia, Kaker, Kapoori, Maghai, Mahoba, Kalkattia bangla.*	Singh, 1994.
West	*Kali Bangla, Simurali Bangla, Maharashtra Kallipatti,*	Singh, 1994;

Bengal	*Kapoori, Bangla (Ramtek), Mitha, Sanchi, Gachapan, Simurali Sanchi, Simurali Deshi, Banarasi, Bhavan, Simurali Bhabna, Chamundai Bhabna, Chandrakona, Vishnupuri, Jaleswar, Ghanagette.*	Das *et al.*, 1995; Sheet, 2002; Pariari and Imam, 2012a; Pariari and Imam, 2012b; Sengupta, 2014.
Madhya Pradesh	*Desi Bangla, Calcutta, Deswari, Jabalpur.*	Singh, 1994.
Kerala	*Kalkodi, Puthukodi, Venmani, Arikodi, Kalkodi, Karilanchi Karpuram, Chelanthikarpuram - Red, Chilanthikarpooram Kootak – kodinadan, Perumkodi, Amaravila, Pramuttan, Local, Kootakodi, Nadan, Theekan, Attukkirazhi, Pedu koti, Pozhikodi, Thulasivettila, Tulasikodi, Mulamkodi, Chilenthivella, Chettankodi, Nadankodi, Naravallie, Alwaye, Venmani vettila, Machary, Mundakam, Naravalie.*	Abraham, 1986; Chandini, 1989; Singh, 1994; Joseph, 1990; Thomas, 2004; Nair, 2010; KAU, 2011.
Karnataka	*Kariyale or Karibally, Nagabally or Yalakkiyele or Khasayele, Mysoreale, Ambadiale, Ambadi, Gangeri, Gidagap, Kumbala bally, Kanigale, Dodgya, Janabally, Hosakali, Shedgar, Lakkaballi, Chikodi, Chandrakona, Tellaka chinthalapudi, Shirpurkala, Halishahar sanchi, Gachi, Sirugamani, Malvi, Khasi, Culcuttia Bangla.*	Nair *et al.*, 1986; Singh, 1994; Shivashankara *et al.*, 2012.
Bihar	*Desi Pan, Calcutta, Paton, Maghai, Bangla, Kaker, Kapoori, Sanchi.*	Singh, 1994.
Assam	*Assam Patti, Awani pan, Bangla, Khasi Pan, Assamia pan, Awani pan, Bangla pan, Khasi pan, Mitha pan, Sanchi pan, Godi Bangla, Kodwa bangla, Banaras, Local*	Singh, 1994; Saikia *et al.*, 1995.

	bangla, .	
Andhra Pradesh	*Karapaku, Chennor, Tellaku, Bangla, Kalli Patti, Gundu kammeri, Kapoori, Lawangi, Peddakammeri, Vasani Kapoori, Vuyyur kapoori, Sangli kapoori, Pedachapelli kapoori, Dodipatta kapoori, Chinachapelli kapoori, Bihar kapoori, Chuddappah kapoori, Simurali Jhal, Ghanagatte.*	Singh, 1994; Lakshmi and Naidu, 2010.
Andaman& Nikobar islands	CARI-2, CARI-6.	Pariari and Imam, 2012a.

2.1.3 Betel vine Cultivars of Kerala

Abraham (1986) reported three major cultivars *viz., Pozhikodi, Nadankodi* and *Thulasivettila* from Kerala. Out of the 42 taxa studied by Joseph (1999), five taxa were coming under *P. betle*, namely *P. betle* var. *aluva* from Kizhakambalam, *P. betle* var. *nadankodi* from Calicut, *P. betle* var. *salem* from Salem, *P. betle* var. *thekkankodi* from Idukki and *P. betle* var. *thulasikodi* from Thevera. Thomas (2004) reported *Chilanthikarpooram* as the most popular cultivated type of betel vine in Trivandrum.

Nair (2010) reported *Venmony vettila* from Venmony near Chengannur as a famous cultivar from Kerala. Betel vine is cultivated in all districts of Kerala except Idukki, with a total area of 349 ha, out of this 183 ha is in Malappuram. The annual leaf production in Kerala is 21029 tonnes, of which 14071 tonnes is from Malappuram (FIB, 2014).

2.2 MORPHOLOGICAL CHARACTERIZATION

Morphological characters play an important role in genetic analysis. They are easily observable and measurable than chemical characters. Wide variability was observed in betel vine for many morphological traits like plant vigour, leaf size, leaf shape, leaf color, internodal length and stem pigmentation (Sengupta, 2014).

2.2.1 Plant Characters

2.2.1.1 Plant Height/ Vine Length

In an investigation on management practices for betel vine conducted by Chandini (1989), it was found that *Chilanthikarpooram – Red, Tulasikodi, Mulamkodi, Chilenthivella, Chettankodi and Nadankodi* were the superior types with regard to plant height. *Chilanthikarpooram red* recorded a plant height of 386.83 cm at 12 months after planting (MAP). A study conducted at Kerala Agricultural University (KAU) on the yield and quality of betel vine revealed that vine length ranged from 0.31 - 0.57, 1.01 - 1.27, 2.05 -2.86, 3.0 – 3.64 and 4.12 - 4.94 m at 2, 4, 6, 8 and10 MAP respectively (Thomas, 2004).

In a study conducted to evaluate betel vine cultivars in the gangetic alluvial plains of West Bengal by Pariari and Imam (2012a), vine length showed significant variation among different cultivars. During December – February, vine length was very less mainly due to low temperature. Maximum vine length (48.40 cm) was recorded in *Simurali Sanchi* which was at par with *Simurali Deshi* (48.01 cm) and *Simurali Bhabna* (45.91 cm). Observations recorded during March – May indicated that significantly higher vine length (92.23 cm) was observed in *Simurali Sanchi.* During June – August, length of vine in all the cultivars was maximum and significantly higher vine length (145.37 cm) was observed in *Simurali Sanchi.* The variation in vine length was probably due to changes in temperature and atmospheric humidity during various seasons and genetic variation among the cultivars. High

humidity (84.70– 98.90%), moderate temperature (25.94^{0}C to 34.15^{0}C) and high rainfall (953.79 mm) prevailed during the period of investigation, influenced the growth of the vines.

In black pepper, the lowest and highest values on height of bearing column were 281 cm (Panniyur 1) and 416 cm (Panniyur 2) respectively. Compared to Panniyur 1, Panniyur 2 recorded higher value (with an average value of 334 cm against 305 cm in Panniyur 1) with respect to height of bearing column (Stephan, 2002). Panniyur 2 showed significant difference with respect to bearing column height.

2.2.1.2 Internodal Color and Internodal Length

Chaveerach *et al.* (2006) reported that betel vine stem was stout with pinkish-stripe along, node dilated with roots. Internodal length varied significantly among cultivars and shortest was recorded in cultivar *Jabalpur* (3.38 cm). Longer vine with shorter internode is a desirable character in betel vine. Such vines produced more number of leaves due to increased number of nodes (Pariari and Imam, 2012a).

In black pepper, variation in internodal length was noticed from 4.0 - 4.8 cm in Panniyur 1 with an average value of 4.5 cm. In Panniyur 2, the range was 4.2 - 4.9 cm. No marked difference could be noticed between varieties with respect to internodal length of lateral branches, the average values being 4.5 cm and 4.4 cm for Panniyur 1 and Panniyur 2 respectively (Stephen, 2002).

2.2.1.3 Total Number of Leaves

Seshadri (1983) found that nitrogen applied in the form of organic manure improved the quality and yield of betel leaves. In an experiment carried out at Dharward, Karnataka to evaluate different cultivars, the highest yield was recorded by

Lakkaballi followed by *Chikodi*. Acharya and Padhi (1987) reported that maximum number of marketable leaves was produced by the application of neem and sawdust. Das *et al.* (1995) evaluated eight cultivars of betel vine from Bengal and found that cv. *Ghanagette* produced highest number of leaves (88) per vine. Sheet (2002) recorded highest number of leaves (62.66 lakh/ha) in cv. *Chandrakona*. Number of marketable leaves in *Chilanthikarpooram* ranged from 12.28 – 30.87 lakh/ha during the first year of planting (Thomas, 2004). Guha (2006) reported that annual yield of a good crop of betel vine was 60 – 70 leaves/ plant and 6 – 7 millions/ha. Choudhary (2006) observed that *Simurali Sanchi* produced highest (46.73/vine) number of leaves followed by *Ghanagette* (41.70/vine) and *Simurali Jhal* (37.63/vine) among five cultivars. In a field experiment conducted by Hedge *et al.* (2012) to study the effect of different nutritional sources on growth, yield and quality of betel vine, less number of leaves were seen at the initial growth stage. Application of FYM (25 t/ha) along with recommended dose of fertilizer recorded higher growth and yield attributes resulting in significantly higher annual leaf yield (588.55 leaves/vine). The lowest yield (279.28 leaves/vine) was obtained in the treatment consisting of farmers' practice alone. The work conducted by Pariari and Imam (2012a) showed significant variation with respect to number of leaves/vine among different cultivars and *Simurali Deshi* produced maximum number of leaves (58.56/vine), which was statistically superior to other cultivars and minimum number of leaves (37.63/vine) was shown by *Simurali Jhal*.

2.2.1.4 Angle between Orthotropic Shoot and Leaf

As the pepper plant grew over a support tree, it produced two developmentally different types of branches (dimorphic branching). They were straight, upward growing, monopodial, orthotropic branches, and the sympodial, laterally growing, plagiotropic fruiting branches. The main stem or orthotropic shoot climbed up the support, had indefinite growth and produced fruiting lateral branches. From the axils

of leaves of orthotropic shoot, lateral plagiotropic branches developed and they had sympodial growth habit. As the shoot grew, the terminal bud got modified into a spike and growth was further continued by axillary bud (Ravindran, 2000).

The angle of insertion of laterals of black pepper to the main stem varied from variety to variety to the tune of 45 -130^0 (Pillai *et al.*, 1979). An attempt was made in Indian Institute of Spice Research, Calicut to describe the crop ideotype characteristics for black pepper. They proposed that the black pepper ideotype should have more leaf angle at the bottom (130^0 - 140^0) compared to the top (100^0 - 110^0) to harvest maximum light especially by the bottom canopy. It is desirable that within a branch, the bottom leaves have lengthier petioles than the top leaves for getting maximum sunlight. The leaf angle of the lateral leaves also varied among the cultivars/varieties. Among the studied cultivars, highest leaf angle of 135 to 140^0 was noticed in OPKM and IISR *Girimunda* while the lowest (115^0) was recorded in HP 1411(Krishnamurthy *et al.*, 2010).

2.2.1.5 Number of Lateral Branches

Chandini (1989) conducted a study on different varieties of betel vine and *Chilanthikarpooram red* recorded significant difference in number of lateral branches. The number of lateral branches were 0.60, 1.78, 2.69, 3.59, 4.37 and 4.73 when recorded from two MAP to 12 MAP in two months interval. Thomas (2004) reported that the number of branches of *Chilnthikarpooram* ranged from 0.21 – 0.75, 0.82 - 1.67, 0.80 - 2.28, 4.17- 6.84 and 7.92 - 10.09 at 2, 4, 6, 8 and 10 MAP respectively.

In black pepper, a study conducted by Mathew and Rema (2000) showed that production of laterals from top shoots were quick. Panniyur 1 had 15.10 - 20.80 lateral branches per 0.25 m^2. With respect to Panniyur 2, number of lateral branches per 0.25 m^2 ranged between 12.70 - 17.50. The number of laterals was higher in

Panniyur 1when compared to Panniyur 2, the average values being 17.30 and 14.80 respectively (Stephen, 2002).

2.2.2 Lateral Branch Characters

Pradhan *et al.* (2013) reported that stem of betel vine was dichotomous, articulate, swollen and rooted at nodes with 3 mm diameter.

In black pepper, Mathai (1983) found that the upper part of the canopy of black pepper, with a relatively higher leaf area during the spike development period and higher photosynthetic rate promoted the growth and development of productive laterals.

2.2.2.1 Days to Lateral Branching and Days between Lateral Branch Emergence

In black pepper, the total annual extension growth of laterals in the variety Panniyur 1 varied from 5.28 to 12.04 cm, of which 82.43 per cent of total growth was recorded in June –July (Menon *et al.*, 1982). The new growth in black pepper was found to initiate in late May and continued to mid August with the maximum growth in June – July (Nalini, 1983; Mathai and Nair, 1990). However, varieties put forth new flushes at any time of the year when significant rains were received after a dry spell. A second flush was commonly noticed in pepper cultivars during North East Monsoon period (Nambiar *et al.*, 1978; Kurien and Nair, 1988).

2.2.2.2 Number of Nodes per Lateral Branch

An experiment conducted by Sreedevi *et al.* (2005) on black pepper revealed that number of nodes per lateral branch ranged from 21- 44 cm.

2.2.2.3 Pattern of Lateral Branching

Pillai *et al.* (1979) classified lateral branches of black pepper into drooping, horizontal and erect, on the basis of angle between main stem and lateral branch. They also observed that the drooping, horizontal and erect nature of the laterals determined the photosynthetic efficiency of vine. The main lateral branch produced primary, secondary and tertiary branches and grew to a maximum length of 50 – 75 cm depending upon variety, soil and fertility.

2.2.2.4 Shoot Tip Color

In pepper, runner shoots, orthotropic climbing shoots and young shoot tips were protected by the sheathing petiole of the leaf while plagiotropic flowering shoots, shoot tip and spike emerged from within a cap like structure. These structures, called prophylls, were the modified first leaf of the axillary branch. Generally dicots had two prophylls, but *Piper* sp. had only one. Prophyll subtended the axillary branch and the emerging spike. Prophyll was associated with the sympodially growing, flowering nodes, while the leaf sheaths were characteristic of the vegetative node (Ravindran, 2000).

Black pepper varieties, Panniyur 1 and Panniyur 2 reported to have no anthocyanin pigmentation in the tender stem of shoot tips. The mature stem in Panniyur 1 and Panniyur 2 was thick, fleshy and light to dark green, later turning to dark green with brownish patches. In Panniyur 4 and Subhakara, the tender stem was purple with anthocyanin pigments, elongated and slender. When mature, brownish color appeared but the stem was not as stout as in other group (Sujatha, 2001). An experiment conducted by Sreedevi *et al.* (2005) on black pepper recorded light purple for young orthotropic shoot tip.

2.2.2.5 Number of Leaves per Lateral Branch

Number of leaves per lateral branch in black pepper was higher in Panniyur 2, when compared to Panniyur 1, with mean values of 2.2 and 1.6 respectively (Stephen, 2002).

2.2.3 Leaf Characters

2.2.3.1 Leaf Length

Rahaman *et al.* (1997) observed variation in leaf length from 6.20 to 15.30 cm among 27 genotypes of betel vine. Lakshmi and Naidu (2010) conducted a comparative morpho-anatomical study in ten common cutivars of *P. betle* namely *Ghazipur, Bangladeshi, Jaleswar, Vishnupuri, Kapoori, Saunfia pan, Culcuttia, Desipan, Desawari* and *Banarasi*. The minimum leaf length (7.50 cm) was observed for *Desipan* and maximum (15.00 cm) for *Vishnupuri* and *Jaleswar*. Significant variation in leaf length among different cultivars of betel vine was observed by Pariari and Imam (2012a). The longest leaf (16.73 cm) was recorded in *Ghanagette*, which was statistically at par with *Simurali Sanchi* (16.71 cm), *Simurali Jhal* (16.43 cm), CARI-2 (15.75 cm), CARI-6 (15.33 cm) and *Sanchi* (14.75 cm). A study was undertaken by Pariari and Imam (2012b) on physical and qualitative characters of leaves in betel vine (cv. *Simurali Deshi*) after application of different combination of organic manures. The results showed that leaf length ranged from 12.83 - 14.65 cm.

2.2.3.2 Leaf Width

Variation in leaf width between 4.20 cm and 11.60 cm was reported by Rahaman *et al.* (1997) from a study with 27 genotypes of betel vine. Herath and Rathnasoma (1998) indicated that the leaves with more than 16 cm length and 12 cm width were considered as large leaves. Sheet (2002) reported maximum leaf width (12.43 cm) in cv. *Chandrakona* among seven cultivars of betel vine. Nirambewela *et*

al. (2005) found that the parameters such as stomatal index and leaf length to width ratio were similar in *Kudamaneru*, *Mohamaneru*, *Galdalu*, *Ratadalu* and *Nagawalli*, but these were different in *Malabulth*. Lakshmi and Naidu (2010) reported that the leaf width showed very wide range, starting from 5 cm to 14 cm. Pariari and Imam (2012b) conducted an investigation on betel vine and the result indicated that leaf width ranged from 8.65 - 10.45 cm. Leaves with 20 cm length and 15 cm width were preferred for export purpose (DMI, 2013).

2.2.3.3 Leaf Area

Saikia *et al.* (1995) conducted a field experiment with betel vine cultivar, *Local Bangla* at Assam Agricultural University and obtained a maximum leaf area of 116.41 cm^2. Rahaman *et al.* (1997) reported significant variation in leaf area from 22 to 147.20 cm^2 among 27 genotypes of betel vine. Among seven cultivars, Sheet (2002) observed highest leaf area (123.56 cm^2) in *Chandrakona*. Pariari and Imam (2012a) evaluated betel vine cultivars in the gangetic alluvial plains of West Bengal and the highest leaf area (167.82 cm^2) was recorded in *Ghanagette*, which was at par with *Simurali Jhal* (166.45 cm^2) and *Chamundai Bhabna* (164.37 cm^2). They conducted a study on physical and qualitative characters of leaves in betel vine (*Simurali Deshi*) after application of different combination of organic manures. The result showed that leaf area ranged from 114.17 - 129.00 cm^2 (Pariari and Imam, 2012b). Hedge (2012) reported that the maximum leaf size (127.30 cm^2) in betel vine was obtained in the treatment consisting of farmers' practice with foliar spray of 25 per cent vermiwash and lowest leaf size (78.27 cm^2) was obtained in the treatment consisting of existing farmers' practice alone.

The leaf area was found to vary widely in the calliclones of pepper and ranged from 44.49 – 84.59 cm^2 with a coefficient of variation of 17.34 (Sanchu, 2000). A study conducted by Tanuja (2003) found that basin irrigation upto March resulted in

maximum leaf area in black pepper during 2001 and 2002 (213.15 cm^2 and 303.48 cm^2 respectively). The leaf area per lateral in black pepper varied with applications of different growth regulators. The treatments like kinetin (200 ppm), absolute control and water spray showed a leaf area of 293.83 cm^2, 201.41 cm^2 and 194.95 cm^2 respectively. Raj *et al.* (2007) reported that in black pepper, leaf area increased towards upper canopy level.

2.2.3.4 Leaf Margin

Pariari and Imam (2012a) reported that betel leaf had entire margin and undulated margin. According to Ravindran (2000), leaf margins of black pepper are either even (entire) or wavy.

There was significant difference between leaf margins of reproductive branches and vegetative branches of *P. longum*. The leaves from the vegetative branches of *Assam*, *Nilaambur*, *Maharshtra* and *Pattambi* accessions of *P. longum* had even margin. The margin was wavy for *Kanjur*, NL-84-68 and *Odkkali*, whereas Viswam (a variety released from Kerala Agricultural University) exhibited slightly wavy margin. In the case of reproductive branch, only *Pattambi* had even margin while *Kanjur*, *Maharashtra*, NL-84-68, Viswam and *Odakkali* had wavy margin. The margin was slightly wavy for *Assam* and *Nilambur* cultivars (Jaleel, 2006).

2.2.3.5 Leaf Brittleness

A material is brittle if, when subjected to stress, breaks without significant deformation (strain). Leaf condition of soft, but not too brittle was preferred for tendu leaf (leaves used to wrap around tobacco to create the Indian *beedi*) marketing (Ministry of Agriculture, India, 2013).

2.2.3.6 Leaf Color

The evaluation of six betel vine cultivars in Sri Lanka revealed that *Malabulath, Galdalu, Mohamaneru* and *Kudameneru* had yellowish green leaves while *Ratadalu* had green coloured leaves. Green coloured leaves with yellow patches were seen in *Nagawalli* (Arambewela *et al.*, 2005). Similar observations were reported in a study conducted by Joseph (1990) in Kerala. In a study conducted by Lakshmi and Naidu (2010), most of the varieties had dark green leaves except *Kapoori* which had light green leaf. *Culcuttia* and *Jaleswar* varieties had yellowish green leaves. Well matured dark green leaves with high pungency were preferred for export purpose in Sri Lanka (DMI, 2013).

2.2.3.7 Leaf Weight and Leaf Weight per Unit Area

Due to lack of enough data in leaf weight per unit area and leaf weight, data related to weight of 100 leaves of betel vine was also reviewed. Reddy (1996) observed that the fresh weight of 100 leaves was 300.5 g in *Ramtek Bangla* and 246.5 g in *Godi Bangla*. Herath and Rathnasoma (1998) investigated the effect of support plants on morphological characters of betel vine and maximum mean weight (625.87 g) of 100 leaves of betel vine was achieved with glyricidia and the recorded minimum (496.13 g) was with *Kooratiya*. Das *et al.* (1995) evaluated the maximum fresh weight and dry weight of 100 fresh leaves of eight cultivars from Bengal and found maximum fresh (380.75 g) and dry weight (44.60 g) for *Ghanagette*.

Leaf weight is considered as one of the important parameters, because the price of export leaves is determined by the leaf weight too. So the real quality of "Black betel" was reported to be correlated to weight (Sumanasena *et al.*, 2005).

Evaluation of 14 cultivars of betel vine in the gangetic alluvial plains of West Bengal indicated that fresh and dry weight of 100 depetiolated leaves of *Simurali Sanchi* were 364.38 g and 52.29 g respectively (Pariari and Imam, 2012a). The fresh weight of 100 betel vine leaves ranged from 307.17 - 328.83 g for various combinations of organic and inorganic manures (Pariari and Imam, 2012b). A significant variation was observed in fresh weight of 100 leaves and same trend was also seen in dry weight of leaves among the cultivars.

2.2.3.8 Petiole Length

The petiole of betel vine leaves was roughly triangular and outline had deep furrows and ridges with 5.5 - 6.5 cm length (Pariari and Imam, 2012a). Reddy (1996) reported that petiole length of betel leaves varied (5.2 - 6.6 cm) significantly among cultivars. Rahaman *et al.* (1997) reported variation in petiole length between 5.90 cm and 17.50 cm in 27 genotypes of betel vine. Chaveerach *et al.* (2006) reported that petiole had 2.0 - 2.5 cm length. Pariari and Imam (2012a) reported longest petiole (10.60 cm) for *Chamundai Bhabna.* Depetiolated betel leaves had better shelf life than leaves with petioles irrespective of seasons.

The petiole length in calliclones of black pepper ranged from 1.24 - 3.94 cm (Sanchu, 2000). The petiole length of the eight accessions of *P.longum* showed significant variation with respect to each other. *Nilambur* recorded the maximum petiole length of 7.81cm and minimum petiole length (4.29 cm) was recorded by the female accession, *Pattambi* (Jaleel, 2006).

2.2.3.9 Leaf Lamina Shape

Chaveerach *et al.* (2006) observed ovate lamina for betel vine leaves. According to Pariari and Imam (2012a), leaf lamina of betel vine was smooth and cordate with even surface.

The shape and size of leaf lamina are very variable in *Piper* sp. Mainly five lamina shapes *viz.*, ovate, ovate elliptic, ovate lanceolate, elliptic lanceolate and cordate are distinguishable (IPGRI, 1995). The lamina shape differed between the orthotropic and plagiotropic branches in most cases. Again in the juvenile shoot also the size and shape were distinctively different in certain species. The leaves on the plagiotropic fruiting laterals showed more stable characters and the description of varieties and species were based on such leaves (Ravindran, 2000). The shape of the younger leaf of black pepper was either cordate or ovate. Ovate leaf was observed in 46.66 per cent of calliclones, ovate lanceolate in 30 per cent, cordate ovate in 13.33 per cent and elliptic ovate in 10 per cent of clones (Sanchu, 2000). Sreedevi *et al.* (2005) reported that ovate lanceolate, ovate cordate, elliptic lanceolate and ovate elliptic lamina shapes were shown by black pepper cultivars.

The shape of the leaf lamina in vegetative branch of *P. longum* for all the eight accessions under study was cordate, whereas it was lanceolate for the leaves of reproductive branch (Jaleel, 2006).

2.2.3.10 Leaf Base Shape

Chaveerach *et al.* (2006) reported leaf base in *P. betle* as cordate. Leaf base was either cordate or round in black pepper (Sanchu, 2000).

There was no significant difference for lamina base shape between the male and female accessions of *P. longum*. Cordate shaped lamina base was observed for the leaves of vegetative branch whereas unequally cordate base shape was seen in the leaves of reproductive branch for all the accessions (Jaleel, 2006).

2.2.3.11 Leaf Apex Shape

Chaveerach *et al.* (2006) indicated that leaf apex was acuminate in betel vine. Lakshmi and Naidu (2010) indicated that among the 10 studied cultivars, seven cultivars showed acute type of leaf tip. Curved accuminate leaf tip was shown by *Desipan* and *Desawari* while *Saunfia pan* showed acuminate leaf tip. According to Mubeen *et al.* (2014), the apex of betel leaf was acuminate with often unequal base.

2.2.4 Spike Characters

2.2.4.1 Floral Morphology

The flowers of plants coming under *Piper* L. genus were many, sessile, naked and compactly arranged on the inflorescence axis. A bract subtended each flower. In some species there were two stamens occupying on either side of the ovary, whereas in others, there were three stamens. Ovary was single, sessile, and sub globose or flask shaped, one - ovuled, orthotropous and the stigma was usually sessile. The fruit was a small one-seeded drupe (Vinay *et al.*, 2012).

CSIR (1969) reported that there were female and male plants separately in *P. betle*. The flowering and fruit setting of *P.betle* were very rare in Indian climate. The inflorescence of betel vine was an axillary spike. The fruit was a drupe embedded on rachis. According to Chaveerach *et al.* (2006), each female floret of *P. betle* had 4 - 6 stigmas with pubescent texture. In male plants, anther was with two stamens. A study in Thailand reported year round flowering and fruiting (Chaveerach *et al.*, 2006). The investigation carried out by Sengupta (2014) in West Bengal showed that out of 70 collections of *P. betle*, flowering occurred in 16 female and 13 male clones. Among these, continuous and profuse flowering was observed in two cultivars *viz.*, *SGM-1* (female) and *Swarna Kapoori* (male).

In black pepper inflorescence was produced in lateral branches and was leaf opposite. As axillary bud formed, the inflorescence was pushed out so that it became

leaf opposed. Spike production increased towards upper canopy level (Raj *et al.*, 2007).

Spike of *P.longum* was cylindrical and pedunculate. Spike emerged as axillary or extra axillary. At the initial stage of spike emergence it was green in color, turning to yellow later. Flowers were minute and unisexual. Fruits were small drupe, dark red when ripe, ovoid in shape, yellowish orange and sunk in fleshy spike (Manoj *et al.*, 2004).

2.2.4.2 Spike Length

Chaveerach *et al.* (2006) reported that betel spikes were 0.5 to 5.5 cm long and male spikes were larger and slender.

Morphometrical studies on black pepper by Ravindran *et al.* (1997) reported that *Vokkalu* showed smallest spike length (3 to 4 cm) including the peduncle followed by *Nedumbanchola* (5 to 6 cm). Sreedevi *et al.* (2005) found that spike length of black pepper varied from 8.2 to 16.6 cm. Black pepper spikes attained maximum length in 31.67 and 29.26 days in *Panniyur* 1 and *Karimunda* respectively (Raj *et al.*, 2007).

Mature female spikes of *P. longum* were shorter and thicker than male spikes (Manoj *et al*, 2004). Zaveri *et al.* (2010) indicated that female flower was up to 2.50 cm long but the male flower was larger and slender. There was significant difference for spike length among accessions. The male accessions, *Nilambur* and *Odakkali* produced longer spikes with a mean length of 7.55 cm and 7.31cm respectively. Among the female accessions, NL - 84 - 68 recorded the longest spike length of 4.73 cm. Viswam recorded the shortest spike length of 2.40 cm (Jaleel, 2006).

2.2.4.3 Spike Diameter

Chaveerach *et al.* (2006) found that the diameter of betel vine spikes of male and female accessions were 0.50 cm. Pradhan *et al.* (2013) reported three millimeter diameter for betel vine spikes.

The male and female accessions of *P. longum* differed significantly for spike diameter. The female accession, NL - 84 - 68 recorded the maximum diameter of 3.59 cm. The male accessions *Nilambur* and *Odakkali* recorded the minimum spike diameter of 1.31 cm and 1.4 cm respectively. The female accession, Viswam recorded a spike diameter of 1.53 cm which was on par with the male accessions. The other female accessions *viz., Kanjur, Maharashtra, Pattambi* and *Assam* recorded a spike diameter of 2.41, 2.32, 2.30 and 2.23 cm respectively (Jaleel, 2006). Zaveri *et al.* (2010) indicated that female spike of *P. longum* was 4 - 5 mm in diameter.

2.2.4.4 Peduncle Length

Chaveerach *et al.* (2006) reported that peduncle length of betel vine varied from 2 to 3 cm. The peduncle length ranged from 0.5 cm in *Vokkalu* to 2.1 cm in *Karimunda* variety of black pepper (Ravindran, 2000). Length of peduncle in black pepper ranged from 0.97 -1.3 cm (Sreedevi *et al.*, 2005).

2.3 BIOCHEMICAL CHARACTERIZATION

Morphological characters are easily amenable to environmental characters due to their oligogenic character. So depiction of genotypes solitarily based on these characters is not reliable. Hence, biochemical characterization along with morphological characterization is essential for effective characterization of betel vine types. The betel leaves were reported to possess anticancerous activity particularly against the tobacco carcinogens (Padma *et al.*, 1989; Wu *et al.*, 2004; Chang *et al.*, 2002a) due to presence of ingredients like hydroxychavicol (Amonkar *et al.*, 1989) and chlorogenic acid without affecting the normal cells unlike the common anticancer

drugs, applicable against wide ranges of environmental carcinogens in both prokaryotes and eukaryotes.

Baliga *et al.* (2011) reported that the eugenol and hydroxychavicol in betel leaf were excellent antimutagens. Piperbetol, ethylpiperbetol, piperol A and piperol B, isolated from leaves, selectively inhibited platelet aggregation induced by platelet activating factor in a concentration dependent manner. It was concluded that betel leaf was a novel candidate for immunosuppressive activity. Betel leaf raised body temperature due to cholinergic responses. Leaves possessed broad spectrum of antimicrobial activity against various bacterial strains. Chewing of betel leaves not only accelerated the salivation, but also enhanced gastric juice secretion to aid digestion process. This might be the reason for chewing *pann* after food (Banerjee, 2012)

The chemo preventive potential of betel leaf was reported against liver fibrosis. Phytochemical investigation on leaf characters of betel vine revealed presence of biochemical molecules like eugenol, chavicol and amino acid. The betel leaf had moisture (85.4 per cent), protein (3.1 per cent), fat (0.8 per cent), carbohydrate (6.1 per cent), fibre (2.3 per cent), minerals (2.3 per cent), reducing sugars (0.38 to 1.46 per cent), all vitamins and iodine also (Pradhan *et al.*, 2013).

Betel leaf is considered as the best natural substance that contributed best oral hygiene to oral activity (Pradhan *et al.*, 2013). The medicinal importance of the herb as discussed above evidently proved that, betel leaf is one of the most promising commercial botanical with lot of therapeutic values. Biochemical compounds in betel leaf are separately reviewed below.

2.3.1 Essential Oil

Betel leaf is a very perishable commodity and therefore, is always subjected to wastage by quick spoilage due to dehydration, fungal infection, dechlorophyllation

etc. This caused a post harvest loss ranging from 35 to 70 per cent during transport and storage. The chief constituent of the leaves is a volatile oil known as betel oil varying in chemical composition in betel vine varieties growing in different countries. The flavor of the leaf is due to the presence of essential oil (Banerjee, 2012).

2.3.1.1 Yield of Essential Oil from Betel Leaf

Guha (2006) found that the *Mitha, Sanchi* and *Bangala* varieties of betel vine had about 2.0, 1.70, and 0.80 per cent essential oil respectively. The average percentage yield of volatile oil of *P. betle* was 1.44 (Caburian and Osi, 2010). Sugumaran *et al.* (2011) obtained an essential yield of 0.31 per cent in volume by weight basis. Rani and Ramamurthy (2012) obtained 0.08 to 0.2 per cent of essential oil from betel leaves. The highest yield (15.6 per cent w/w based on betel powder) and high content of hydroxychavicol and eugenol (58 and 62.8 per cent w/w respectively) were obtained using ethyl acetate refluxed extraction (Singtongratana *et al.*, 2013). Pradhan *et al.* (2013) reported that the fresh new leaves had much more amount of essential oil.

2.3.1.2 Physical Features

Tyler *et al.* (1988) stated that pure volatile oils are colorless or with yellowish tinge when freshly prepared. Their taste varied like sweet, mild, pungent, hot acrid, caustic or burning. They had a characteristic aroma or odor. Most volatile oils are miscible in organic solvents but sufficiently soluble to form a saturated solution and impart its odour to the water. Caburian and Osi (2010) reported that the essential oil in betel vine was colorless to pale yellow when freshly extracted but acquired a darker yellow to orange color on exposure to light and heat. It had a strong aromatic odour (Sugumaran *et al.*, 2011), pungent taste and was greasy to touch. *P. betle* volatile oil had characteristics of most volatile oils. The *P. betle* volatile oil was miscible in all proportions in organic solvents like ethyl alcohol, chloroform, anhydrous ether and

petroleum ether. It was immiscible in the water in the ratio of 0.1: 0.1 but was soluble in 50.0ml of water or no separation of phase was observed.

2.3.1.3 Chemical Composition

Chemical components of oil and their quantities varied in different varieties. The qualitative and quantitative variation in the essential oil might be due to different factors like variety, soil, season and agronomic practices followed during growth season and plant part used for oil extraction (Garg and Jain, 1996).

The oil of *Bangala* variety was constituted by a mixture of about twenty one different compounds, of which eugenol was the chief ingredient constituting about 29.5 per cent of the oil. Terpenyl acetate was the chief constituent of some of the varieties (Guha, 2003). The chemical composition of common betel oil of Sri Lanka appeared to be closer to that of cultivar *Deshwari* in India. The chemical studies on *P. betle* of India revealed that composition of volatile oils in the leaves could be used as markers for identification of different cultivars (Arambewela *et al.*, 2005).

The GC – MS analysis of essential oil from different parts of common betel vine indicated that composition of stalk was different from that of the other parts. The stalk did not contain detectable amount of allylpyrocatechol diacetate (Arembewela *et al.*, 2005). It was observed that the content of major compounds like safrole and chavibetole acetate in the leaf was highest at the harvesting stage.

Hydroxychavicol was the major component of essential oil. It would vary based on different extraction procedure. Yield of hydroxychavicol from fresh leaves, extracted in boiling water, was reported to be 0.096 per cent in w/w determined by GC –MS (Tawastin *et al.*, 2006) and 5 per cent in w/w when determined by HPLC (Pandey and Bani, 2010). The compounds with highest retention time were 5-(2-propenyl) -1, 3-benzodioxole, eugenol isomers and 3-careen (Caburian and Osi, 2010). In GC - MS analysis conducted by Sugumaran *et al.* (2011), the total ion

chromatogram retention time was about 34.10 minutes and most of the components of oil were isolated during the first 30 minutes of the analysis. The 5-1, 3-benzdioxole (25.67%) was identified as the major constituent in the betel oil. The hydroxychavicol content of dried leaf by ethanolic extraction was reported to be 0.9 per cent w/w (Bandopadhay *et al.*, 2011) when determined by HPLC.

According to Banerjee (2012), eugenol was present in betel leaves and flower. Isoeugenol and methyl eugenol were present in flower. A total of 65 components were identified by GC – MS, representing 100 per cent of the oil. Some of the major compounds identified in betel oil of Sri Lankan cultivars were β-phellandrene, 4-terpinol, eugenol, chavibitol acetate, safrole and allylpyrocatechol diacetate (DMI, 2013).

Singtongratana *et al.* (2013) obtained chromatograms of the standards of hydroxychavicol with concentration of 1000 mg per liter and eugenol with concenteration of 50 mg per liter with retention time of 6.6 and 8.6 minutes respectively. The chromatogram of hydroxychavicol and eugenol of extracted oil by liquid - liquid extraction had shown a retention time of 6.59 and 8.45 minutes respectively. Hydroxychavicol and eugenol were the major compounds belonging to the propenyl phenol group. Heat sensitive compounds like 5-(2-propenyl)-1 and 3-benzodiaxole (18.27 per cent w/w) from fresh betel leaves determined by GC - MS. Major essential oil components identified through the study by Pradhan *et al.* (2013) were safrole, allyl pyrocatechol monoacetate, terpinen-4-ol, eugenyl acetate and chavicol. Eugenol was identified as the antifungal principle in the oil and chavicol was four times potent as antiseptic agent, compared to carbolic acid.

Different potential uses had been reported for the biochemical molecules identified from the betel leaf. Isoeugenol has use in the manufacture of vanillin (Merck, 1996). Eugenol, a major constituent in betel oil, is used in perfumeries, flavorings and medicine as a local antiseptic and anesthetic. Eugenol could be

combined with zinc oxide to form a material known as zinc oxide eugenol which has restorative and prosthodontic applications in dentistry (Jadhav *et al.*, 2004). As a flavoring agent, isoeugenol is added to nonalcoholic drinks, baked foods and chewing gums (NTP, 2010). Methyl eugenol is used in aroma therapy, massage oils and alternative medium (Government of Canada, 2010). It is also widely used as a fragrance ingredient in perfumes, toiletries and detergents. Methyl eugenol has been used as an insect attractant in combination with insecticides (NTP, 2000; HSDB, 2010). Methyl isoeugenol is natural food flavor and used for treating mood disorders (Fajemiroye *et al.*, 2011).

2.3.2 Chlorophyll Content

Chlorophyll is beneficial in maintaining healthy teeth, clearing the mouth and throat and helping in digestion by encouraging salivation and neutralizing excess acid (Loranty *et al.*, 2010). Shivashankara *et al.* (2011) conducted a biochemical study on three types of betel vine. Among these, *Sweet* type and *Bengaluru local* type had different intensities of green color. The variation in color intensity was mainly due to the differences in the chlorophyll content. Leaves of *Sweet* type had higher total chlorophyll content, compared to *Bengaluru local* and *Madras* type. The higher chlorophyll content in *Simurali Sanchi* had given dark green color to the leaves which was preferred by customers and fetch higher price in comparison to other cultivars (Pariari and Imam, 2012a).

2.3.2.1 Total Chlorophyll

Different cultivars of betel vine showed significant variation (0.93 to 2.49 mg/g) in chlorophyll content (Balasubrahmanyam *et al.*, 1990; Guha, 2006; Pariari and Imam, 2012a). Guha (2006) reported 0.01 to 0.25 per cent chlorophyll in betel leaves. An investigation by Pariari and Imam (2012a) to find out the most suitable cultivar with higher leaf yield and better quality in the gangetic alluvial plains of West

Bengal, India revealed that the total chlorophyll content was highest (2.45 mg) in *Simurali Sanchi* among the cultivars.

2.3.2.2 Chlorophyll a

The investigation conducted by Pariari and Imam (2012b) concluded that chlorophyll a content in leaves varied significantly in various cultivars and maximum chlorophyll a content (1.61 mg per g) was found in *Sanchi.* Chlorophyll a content in leaves varied between 1.69 to 1.74 mg per g according to doses of applied nitrogen.

2.3.2.3 Chlorophyll b

In a study by Pariari and Imam (2012b) among different cultivars, significant variation was observed for chlorophyll b content and maximum chlorophyll b content (1.00 mg/g) was recorded in *Simurali Sanchi.* Source of organic manures had a significant effect on chlorophyll b content in leaves. Maximum amount (0.57mg/g) of chlorophyll b was recorded with the application of neem cake as nutrient source and minimum (0.48 mg/g) was in poultry manure. It was seen that nutrient supply through inorganic source or chemical fertilizer decreased chlorophyll b content in leaf.

2.3.3 Total Protein Content

Chandini (1989) found that nitrogen application at higher levels enhanced the protein content of marketable leaves and *Chilanthikarpooram red* contained 3.39 per cent protien. Guha and Jain (1997) reported that betel vine leaves contained significant amount of all the essential aminoacids except lysine, histidine and arginine, which were found only in traces. According to Guha (2000), six leaves of betel vine were comparable to about 300 ml of milk in relation to nutrient content.

Akther (2004) found that an aqueous diffuse of bleached leaves had lucien (18.3 mg per 100 ml), phenyl alanine (14.2 mg per100 ml), arginine (2.4 mg per 100 ml), threonine (12 mg per 100 ml), aspartic acid (23 mg per 100 ml), glutamic acid (29.7 mg per 100 ml), valine (3.8 mg per 100 ml), tyrosine (1.2 mg per100 ml) and gama amino butynic acid (20.2 mg per 100 ml). The amount of total protein in betel leaf was reported to be 3 to 3.5 percentage (Akther, 2004; Banerjee, 2012; Pradan *et al.*, 2013).

2.3.4 Total Phenol Content

Nair *et al.* (1986) stated that cultivar *Ambadi* had a higher disease index (anthracnose) and a lower phenolic content (7.76 mg/g) than the more resistant variety, *Kareyele* with a higher phenol content of 11.38 mg/g. Generally plants with significant therapeutic properties were found to be rich in phenols and had high antioxidant properties. Sazwi *et al.* (2013) found that total phenolic content of the betel leaf was 1.8 times less than betel quid without calcium hydroxide but 1.7 times higher than betel quid with calcium hydroxide. Balasubramanym and Rawat (1990) suggested that the characteristic clove like aroma of *Bangla* and *Sanchi* leaves was due to presence of phenolic compounds including eugenol (63.56 and 33.22 per cent respectively) and the sweet fennel like taste of *Meetha* leaves was due to anthole (19.13 per cent). The radical scavenging capacity of betel leaf was primarily due to its phenolic constituents. *Bengaluru local* recorded highest phenolics followed by *Madras* type. Sweet betel vine recorded lowest phenol and flavanoid content in leaf. The same study also indicated that the total phenol content of betel vine was comparable with that of tea powder (Shivshankara *et al.*, 2011). The major phenolic compounds found in betel leaf were terpenoids which included hydroxychavicol, eugenole, chavibetole, 1, 8 – cineole, cadinene, camphene, caryophyllene, limonene, pinene, chavicol, allyl pyrocatechol, carvacrol and safrole. Hydroxychavicol was said to possess antibacterial, antioxidant, anticarcinogenic activities whereas eugenol had been used as a local anesthetic for toothache (Pradhan *et al.*, 2013). The presence of

phenols and terpene like bodies were the cause of pungent smell of the betel vine leaves. Phenol content was directly proportional to the quality of leaf; leaves with high phenol quantity would also have good quality with respect to shelf life, nutrient content and resistance against pest and disease. The total phenol content varied with gender. Female plants had three times higher phenol than male plants. The middle part of the main vein had largest quantity of tannin.

2.3.5 Antioxidant Capacity

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.

Inhibition of low density lipoprotein oxidation to the extent of 94 per cent was reported for betel vine and this was higher than cashew, Japanese mint, chilli fruit, papaya shoot and rosella calyx (Salleh *et al.,* 2002). A study conducted by Santhakumari *et al.* (2003) revealed that betel leaves possessed very high antioxidant capacity and it was more than that of tea in female types. The extract also showed strong hydroxyl radical and superoxide anion radical scavenging property (Dasgupta, 2004; Arambewela *et al.,* 2006; Rathee *et al.,* 2006; Pin *et al.,* 2010).

It was also reported that the antioxidants could reduce mortality rate of cardiovascular disease (Devasagayam *et al.,* 2004; Agoramoorthy *et al.,* 2008) and protect against cancer and other chronic diseases (Anani *et al.,* 2005). Polyphenol compounds like catechol, allylpyrocatechol *etc.* in betel leaf extract inhibited the radiation induced lipid peroxidation process effectively. This could be attributed to its ability to scavenge free radicals (Anon, 2004). Betel leaf had great potency to act

as natural antioxidant (Guha, 2006). Ascorbic acid content in fresh betel leaves varied from 0.005 to 0.01 per cent.

The antioxidant capacity, cytoprotective activity and total phenol content were positively correlated (Kondo *et al.*, 2007). The consumption of antioxidant rich foods would help to neutralize the free radicals in the body, thus preventing or delaying the oxidative damage of lipids, proteins and nucleic acids (Lim *et al.*, 2007). The extracts reduced most of the Fe^{3+} ions and possessed strong reducing ability (Maniguha *et al.*, 2009).

Differences in the antioxidant capacity and radical scavenging ability were found to be related to the pungency level. Pungent female type found to have more antioxidant capacity (Sivashankara *et al.*, 2011). It was also reported that total phenols, flavanoids, total antioxidant capacity and radical scavenging ability were highest in *Bengaluru* local type of betel vine and lowest in *sweet* type.

The study by Pariari and Imam (2012a) showed a significant difference among cultivars for ascorbic acid content with maximum (3.20 mg/100 g) in *Simurali Bhabna*. Pariari and Imam (2012b) reported that ascorbic acid content in betel leaves grown with different sources and combinations of nitrogen varied significantly with maximum ascorbic acid content of 2.75 mg/100 g of fresh leaves and minimum of 2.02 mg/100 g of fresh leaves. Banerjee (2012) stated that antioxidant activity included free radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reducing capacity. The high antioxidant activity was contributed by several biochemical molecules like phenols and terpenoids that included b-carotene, quinic acid, allylpyrocatachol, p –hydroxybenzoic acid, hydroxychavicol, ascorbic acid and eugenol. The antioxidant property was correlated with different biological activities like hepatoprotective, antidiabetic, antiarthritis, antistroke and anticancer properties (Pradhan *et al.*, 2013).

Sazwi *et al.* (2013) conducted a study on antioxidant and cytoprotective activities of *P. betle*, *Areca catechu*, *Uncaria gambir* and betel quid with and without calcium hydroxide. This study showed highest antioxidants (DPPH - IC_{50} = 6.4 ± 0.8 microgram/ml, FRAP - 5717.8 ± 537.6 micromol Fe (II)/ mg) for gambir. Betel quid without calcium hydroxide compared with betel quid with calicium hydroxide had higher antioxidants, total phenolic content and cytoprotective activities. Quinic acid was the major compound of gambir and betel quid.

2.4 ORGANOLEPTIC PROPERTY – PUNGENCY

An experiment conducted at the Betel Vine Research Station, Utkur, Andhra Pradesh indicated that the cultivar *Tella alin* was non pungent and *Peddakammeri, Kavapari* and *Gundu verneri* of Andhra Pradesh, *Chanchipan* of West Bengal, *Kaker* and *Belhari* of Bihar and *Begala* of Gujarat were pungent types (Anon, 1984). Seshadri (1983) reported that vines receiving groundnut cake, super phosphate and ammonium sulphate produced leaves with slightly higher pungency. According to Duke (1985) the flavor of the leaf was due to the presence of essential oils.

Leaves growing under more favorable conditions were bigger in size and less pungent than those growing under less favorable conditions. Hundreds of betel vine varieties cater to a very wide range of organoleptic preferences of the users. Betel vine leaves had specific strong pungent smell. Among the major betel vine types *Sanchi* was more pungent followed by *Bengla* and *Kapoori*. *Deswari* was very less pungent and *Meetha* was sweet type (Singh, 1994). The leaves of the betel vine varied in taste from very pungent to mild and even sweet as in case of *Meetha* grown in very small region of Bengal (Kumar, 1999).

Arambewela *et al.* (2005) reported that variety *Salem* possessed light green leaves with a characteristic penetrating smell and a peppermint like taste. Shivshankara *et al.* (2011) reported that betel vine leaves from female (*Bengaluru local)*, male (*Madras* type) vines and sweet type, varied in their pungency levels with

female types being more pungent. Among the three betel vine types, sweet type was more sweet and had clove like flavor. On the other hand *Bengaluru* local type was more pungent. *Madras* type was mild pungent with good flavor. However, they had different intensities of green color. The consumption of *Madras* and *Sweet* types were more because of less pungency, compared to the female (*Bengaluru* local) type.

Banerjee (2012) reported that normal betel juice had a pungent taste, chiefly due to the presence of chlorophylls. *Paan* lovers usually preferred reduction or even substantial elimination of this pungency by alternate heating and cooling of betel leaves whereby chlorophyll a and b present in the green leaves are changed to carotenoids with consequent reduction in pungency. Pradhan *et al.* (2013) indicated that the leaves had bitter compounds to the extreme of about 0.7 - 2.6 per cent. The bitter compounds included phenol and terpene like bodies, which were the cause of specific strong pungent aromatic flavor in leaves.

Materials and methods

3. MATERIALS AND METHODS

The present study was undertaken in the Department of Plant Breeding & Genetics, College of Horticulture, Thrissur and Aromatic and Medicinal Plants Research Station, Odakkali during 2012 - 2014. Experimental field was raised in farmer's field at Tirur, Malappuram which lies on the geographical coordinates of 10° 54' 0" N and 75° 55' 0" E with an altitude of two meter above Mean Sea Level (MSL).

3.1 MATERIALS

Betel vine cultivars from Malappuram district, popular as *Tirur vettila*, possess special morphological and biochemical characters because of genotypes under cultivation, specific cultural practices and geographical features. Information regarding betel vine types grown in Malappuram district and their special features were collected through a preliminary survey conducted in seven Block Panchayaths located in the area of production of *Tirur vettila* (Table 2) based on the information provided from the Office of Principal Agricultural Officer (PAO) of Malappuram district.

Table 2. Details of Block Panchayaths selected for survey in Malappuram district

Sl. No.	Name of the Block Panchayath
1	Ponmundam
2	Parappanangadi
3	Valanchery
4	Malappuram
5	Vengara
6	Tirur
7	Kondotty

Puthukodi, Chelan, Karinadan and *Nadan* were the betel vine types grown in Malappuram District. Planting material of these four types were collected during survey. Planting material of *Muvattupuzha Local* was collected from Asamanoor, Ernakulum district.

3.2 METHODS

Puthukodi, Chelan and *Karinadan* along with *Nadan* (Local check variety) and *Muvattupuzha Local* type from Asamannoor as check variety (Table 3) were raised in farmer's field in Malappuram district during 2013 - 14.

Table 3. Details of betel vine cultivars used in the study

Sl. No.	Genotypes	Source	
		Locality	District
1.	*Puthukodi*	Tirur	Malappuram
2.	*Chelan*	Tirunavaya	Malappuram
3.	*Karinadan*	Tirunavaya	Malappuram
4.	*Nadan* (local check)	Tirur	Malappuram
5.	*Muvattupuzha Local* (check)	Asamannoor	Ernakulum

The five betel vine cultivars were grown in a Randomized Complete Block Design with four replications. Betel vines were planted in circular basins having four feet diameter. In each basin, eight shoot cuttings having 0.5 m length were planted with 20 cm spacing. The betel vines planted in one basin is usually called as *Koottam*

(cluster). The distance between two clusters was four feet. The crop was raised using organic method of cultivation. Cultural practices followed by farmers were adopted to raise the crop.

The five betel vine types were characterized based on morphological, biochemical and organoleptic properties.

3.2.1 Morphological Characterization

The betel vine types were characterized and evaluated based on morphological characters. Morphological characters were recorded from whole plant, lateral branch and leaf. Growth parameters were recorded from 15 days after lowering (DAL) up to 90 DAL at fifteen days interval. Leaf observations were recorded from fourth leaf from the tip of the lateral branch. Observation on days to lateral branching was recorded from the date of planting. As profuse flowering was observed, characters of spikes were recorded as and when spikes appeared in each cultivar.

Currently there is no approved descriptor for betel vine and hence the "Descriptor for Black Pepper" (IPGRI, 1995) and Guidelines for the conduct of test for distinctiveness, uniformity and stability on black pepper (PPV & FR Authority, 2009) were followed (with suitable modifications) for characterization. Observations were recorded from 10 randomly selected vines of each cultivar and the mean was worked out.

3.2.1.1 Qualitative Characters

The qualitative characters observed were internodal color, lateral branch pattern, shoot tip color, leaf margin, leaf brittleness, leaf color, leaf lamina shape, leaf base shape and leaf apex shape.

3.2.1.1.1 Internodal Color

The color of the outer surface of internode was recorded from orthotropic shoot and lateral branch (before and after spike formation) and classified as

a) Orthotropic shoot

Code	Guide
1	Light green
3	Green
5	Uniform purple green
7	Light green with purple tinge
9	Green with purple color at nodal region

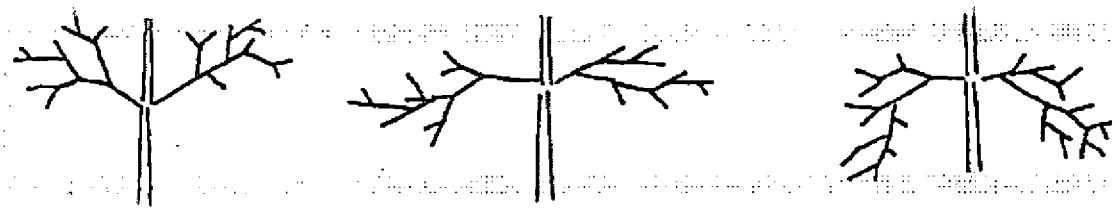
b) Lateral branch

Code	Guide
1	Light green
2	Green
3	Light purple with light green broken stripes
4	Purple with light green broken stripes
5	Dark purple

3.2.1.1.2 Lateral Branch Pattern

The visual appearance of lateral branch was recorded and classified (Fig. 1) as

Code	Guide
3	Semi erect
5	Horizontal
7	Hanging



a. Semi erect b. Horizontal c. Hanging

Fig.1. Lateral branch patterns in betel vine

3.2.1.1.3 Shoot Tip Color

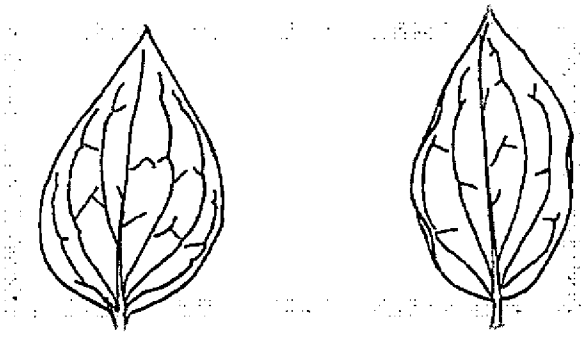
Shoot tip color in lateral branch before spike emergence was recorded and classified as

Code	Guide
1	Light green
3	Green
5	Light purple with light green broken stripes
7	Dark purple with light green broken stripes

3.2.1.1.4 Leaf Margin

Leaf margin was recorded and classified (Fig. 2) as

Code	Guide
3	Even
5	Wavy



a. Even b. Wavy

Fig.2. Leaf margin types in betel vine

3.2.1.1.5 Leaf Brittleness

The leaf was slightly pressed by hand and the tendency to break was recorded and classified as

Code	Guide
3	Low
5	Medium
7	High

3.2.1.1.6 Leaf Color

Leaf color and anthocyanin pigmentation at dorsal surface were recorded and classified as

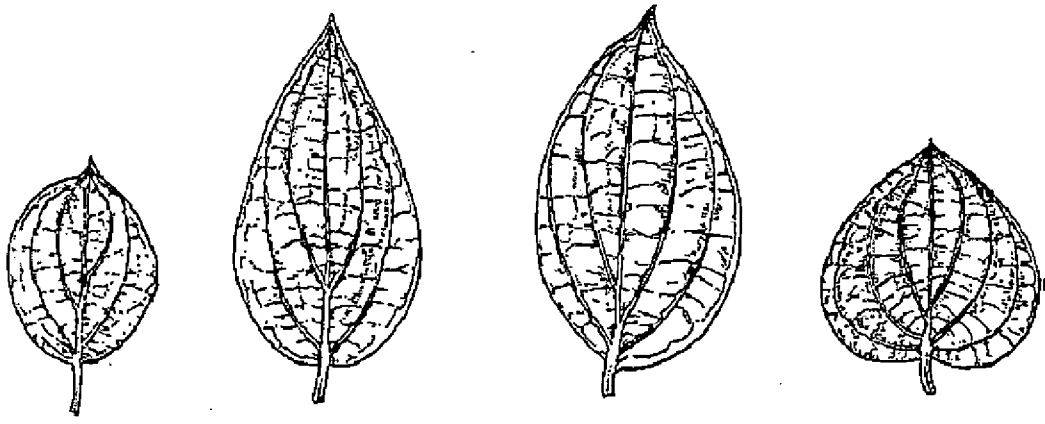
Code	Guide
3	Light green
5	Green

Dark green

3.2.1.1.7 Leaf Lamina Shape

Leaf lamina shape was recorded and classified (Fig. 3) as

Code	Guide
1	Ovate
3	Ovate – lanceolate
5	Ovate – elliptic
7	Cordate



a. Ovate b. Ovate - lanceolate c. Ovate - elliptic d. Cordate

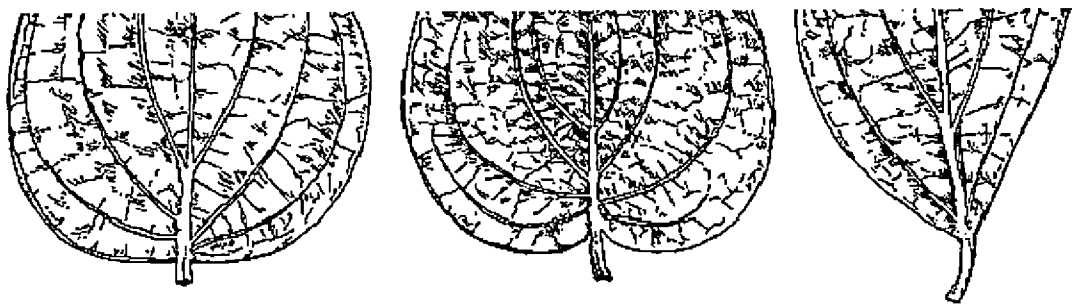
Fig.3. Leaf lamina shapes in betel vine

3.2.1.1.8 Leaf Base Shape

Leaf base shape was recorded and classified (Fig. 4) as

Code	Guide
3	Round

5	Cordate
7	Acute



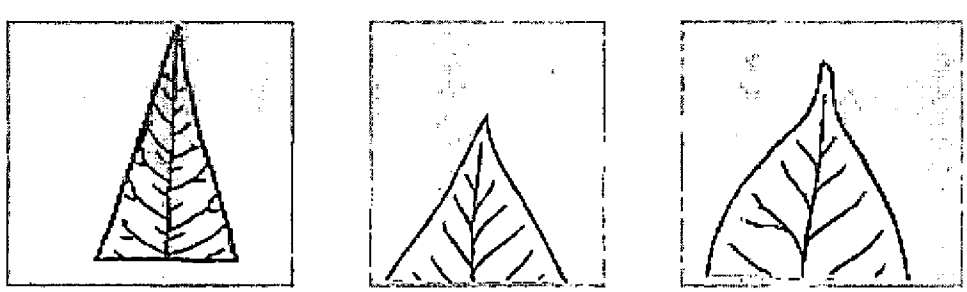
a. Round b. Cordate c. Acute

Fig.4. Leaf base shapes in betel vine

3.2.1.1.9 Leaf Apex Shape

Leaf apex shape was recorded and classified (Fig.5) as

Code	Guide
1	Accuminate
2	Aristulate
3	Apiculate



a. Accuminate b. Aristulate c. Apiculate

Fig.5. Leaf apex shapes in betel vine

3.2.1.2 Quantitative Characters

The quantitative characters observed were plant height, total number of leaves, angle between orthotropic shoot and leaf petiole, days to lateral branching, days between lateral branch emergence, number of lateral branches, number of nodes per lateral branch, number of leaves per lateral branch, leaf length, leaf width, leaf area, leaf weight, leaf weight per unit area, leaf petiole length, leaf tip angle, spike length, spike diameter and spike peduncle length.

3.2.1..2.1 Plant Height

Plant height was recorded in centimeters from the base of the vine to the tip of the orthotropic shoot.

3.2.1.2.2 Total Number of Leaves

The number of fully opened leaves were counted and expressed in number.

3.2.1.2.3 Angle between Orthotropic Shoot and Leaf Petiole

Angle between orthotropic shoot and leaf petiole was measured and expressed in degree. The observations were classified as

Code	Guide
3	Narrow (< 60°)
5	Medium (60 - 75°)
7	Wide (>75°)

3.2.1.2.4 Days to Lateral Branching

Number of days from planting to lateral branching was counted and expressed in days.

3.2.1.2.5 Days between Lateral Branch Emergence

The days between the emergences of two consecutive lateral branches were counted and expressed in number.

3.2.1.2.. Number of Lateral Branches

Number of lateral branches were counted and expressed in number.

3.2.1.2.7 Number of Nodes per Lateral Branch

Number of nodes of the selected lateral branches were counted and expressed in number.

3.2.1.2.8 Number of Leaves per Lateral Branch

Number of leaves of the selected lateral branches were counted and expressed in number.

3.2.1.2.9 Leaf Length

Leaf length was measured in centimeters from the base of the midrib to the tip of the leaf and classified as

Code	Guide
3	Short (< 10 cm)
5	Medium (10 - 16 cm)
7	Long (> 16 cm)

3.2.1.2.10 Leaf Width

Leaf width was measured in centimeters at the widest portion of the leaf blade and classified as

Code	Guide
3	Narrow (< 10 cm)
5	Medium (10 - 13 cm)
7	Broad (> 13 cm)

3.2.1.2.11 Leaf Area

Leaf area was measured by using LI -3000 leaf area meter and expressed in centimeter squares. The observations were classified as

Code	Guide
3	Low (<150 cm^2)
5	Medium (150 – 180 cm^2)
7	High (>180 cm^2)

3.2.1.2.12 Leaf Weight

Leaf weight was measured using analytical laboratory digital weighing balance and expressed in grams.

3.2.1.2.13 Leaf Weight per Unit Area

Leaf weight per unit area of the leaf was calculated using the following formula and expressed in gram per centimeter square.

$$\text{Leaf weight per unit area} = \frac{\text{Weight of the leaf (g)}}{\text{Area of the leaf (cm}^2\text{)}}$$

3.2.1.2.14 Leaf Petiole Length

Leaf petiole length was measured in centimeters from the petiole base to the insertion with the leaf lamina and classified as

Code	Guide
3	Short (< 3 cm)
5	Medium (3 – 4 cm)
7	Long (> 4 cm)

3.2.1.2.15 Leaf Tip Angle

Leaf tip angle was measured and expressed in degree. Observations were classified as

Code	Guide
3	Narrow ($< 40^0$)
5	Medium ($40 - 50^0$)
7	Wide ($> 50^0$)

3.2.1.2.16 Spike Length

Spike length was measured from the tip of the spike to the base of the spike in centimeters and grouped as

Code	Guide
3	Short (< 1 cm)
5	Medium (1-3 cm)
7	Long (> 3 cm)

3.2.1.2.17 Spike Diameter

Spike diameter was calculated and expressed in centimeters. The observations were grouped as

Code	Guide
3	Slender (<0.5 cm)
5	Medium (0.5 - 0.7 cm)
7	Thick (>0.7 cm)

3.2.1.2.18 Spike Peduncle Length

Spike peduncle length was measured in centimeters from the base of the peduncle to the base of the spike. The observations were grouped as

Code	Guide
3	Short (<3.5 cm)
5	Medium (3.5 – 4.5cm)
7	Long (> 4.5 cm)

3.2.2 Biochemical Characterization

The betel vine genotypes were characterized based on the following five biochemical parameters *viz.,* content and components of essential oil, chlorophyll content, total protein content, total phenol content and antioxidant capacity.

3.2.2.1 Essential Oil

3.2.2.1.1 Essential Oil Content

Essential oil was extracted from fresh leaves by hydro distillation using Clevenger apparatus (Augustin, 1998) adopting the following procedure.

One fifty grams of fresh leaves were taken in the round bottom flask of Clevenger apparatus. Water was added into it up to 60 per cent of its capacity. Glass beads were added to avoid bumping. Continuous cold water supply was provided. Fresh leaves were steam distilled in Clevenger apparatus for four hours to obtain essential oil. The oil being light in weight was collected over water in the delivery tube of the condenser. However oil was mixed with water and no separate layer of oil was seen. The procedure was again repeated twice for one sample. Once the distillation was over the mixture of oil and water collected in the delivery tube was transferred to a test tube containing small amount of sodium chloride to separate oil and water layers. Density of water was increased due to sodium chloride and oil became upper layer. The oil layer was separated carefully and dried over anhydrous

sodium sulfate and kept in sterile appendorf tubes. The volume of essential oil was measured and expressed as oil per cent (v/w) in the leaf tissue. The oil was stored in the refrigerator at 4^0C for further analysis using gas chromatography. Between the extractions of oil from two different samples, the condenser was washed with ether in order to remove any sticking oil.

3.2.2.1.2 Identification and Quantification of Components of Oil Using GC

Essential oil samples obtained were subjected to gas chromatography adopting following conditions; Gas chromatograph: DANI (Italy) make GC nodal Master with capillary column and FID detector; Column dimensions: length of 30mm, inner diameter of 0.25mm and film thickness of 0.25microns; Carrier gas: N_2 at flow rate of 1.2ml/min, H_2 at flow rate of 40ml/min, air at flow rate 280 ml/min; Injection volume: 1ml. The column was programmed as follows: held at 100°C for 1 minute, heated to 150°C @ 2.5°C/minute and held at 0.5 minute, heated to 225°C (@ 50°C/minute), held at 0.5 minute. The injector temperature was 250°C and detector temperature was 220°C. The sample was injected in a split ratio of 1:99. Data on the chromatogram and peak were integrated on to the computer based programme, CLARITY (DANI INPUTS, Italy, 2005). Eugenol, isoeugenol, methyl eugenol and methyl isoeugenol were used as standards. Relative amounts of individual components were worked out using GC peaks of standards.

The other possible components of essential oil were identified by comparing the values of retention time and area of corresponding peaks with literature data. Compounds with a relative amount of more than two per cent were identified as predominant chemical components of the specific oil sample.

3.2.2.2 Chlorophyll Content

Chlorophyll content was estimated by DMSO (Dimethyl Sulfoxide) method (Hiscox and Israelstam, 1979). 0.25 g of chopped betel vine leaves dipped in 25 ml DMSO and incubated at 60^0C for 30 minutes. After incubation, centrifuged at 4000 rpm for 10 minutes and supernatant was collected. The solution was made up to 25 ml. The optical density (OD) of the sample was read at 645 nm and 663 nm in a UV – Vis spectrophotometer.

The amount of total chlorophyll, chlorophyll a and chlorophyll b were calculated using the following formulas.

$$\text{Total chlorophyll} = [20.2\ (A645)+8.02\ (A663)]\frac{V}{1000\times W\times a}$$

$$\text{Chlorophyll a} = [12.7\ (A663)-2.69\ (A645)]\frac{V}{1000\times W\times a}$$

$$\text{Chlorophyll b} = [22.9\ (A645)+4.68(A663)]\frac{V}{1000\times W\times a}$$

Where A = Absorbance at specific wavelength

V = Final volume of chlorophyll extract in DMSO

W = Fresh weight of betel vine leaves used for extraction

a = Path length = 1

3.2.2.3 Total Soluble Protein Content

Total soluble protein content was estimated by Lowry's method (Sadasivam and Manickam, 1996). 500 mg of chopped betel vine leaves were homogenized in 25

ml phosphate buffer (pH – 7.4) by means of pestle and mortar. The supernatant was collected after centrifugation. This was used as sample. 0.2 ml of sample was pipetted out into a test tube and made up to 1 ml by adding distilled water. A blank was set up with 0.2 ml distilled water. Then 5 ml of alkaline copper sulphate (50 ml of 2 per cent sodium carbonate in 0.1 N sodium hydroxide, mixed with 1 ml of 0.5 per cent copper sulfphate in 1 per cent potassium sodium tartarate) reagent was added to each tube and mixed well, incubated at room temperature for 10 minutes. Then added Folin Ciocalteau reagent and kept in dark for 30 minutes. Protein standard used was 0.2 mg Bovine Serum Albumin (BSA) in 1 ml distilled water. Blue color developed was read at 660 nm in a UV – Vis spectrophotometer. The amount of protein was calculated using the following formula and expressed in mg per g of sample.

$$\text{Protein content(mg/g)} = \frac{(\text{OD of sample} \times \text{concentration of standard} \times \text{total volume})}{(\text{OD of standard} \times \text{volume used} \times \text{weight of sample})}$$

3.2.2.4 Total Phenol Content

Total phenol content of the leaf extract was determined using Folin Ciocalteau method (Sadasivam and Manickam, 1996). One gram chopped betel vine leaves was macerated with 10 ml of 80 per cent alcohol using mortar and pestle. This homogenate was centrifuged at 1000 rpm for half an hour. The supernatant was collected in a test tube and evaporated to dryness in water bath. The residue was dissolved and made up to 4ml in distilled water by using vortex mixer. 0.5ml of Folin Ciocalteau reagent was added to this mix. After 3 minutes, 2 ml of 20 per cent Na_2CO_3 was added to each test tube. The solution of each tube was mixed thoroughly and kept in boiling water for exactly one minute, cooled and measured the absorbance at 650 nm against a reagent blank. The standard was catechol 0.1 mg in 1 ml of distilled water and processed in the same way. The absorbance of standard was also

measured at 650 nm in a UV – Vis spectrophotometer. The phenol content was calculated using the following formula;

$$\text{Phenol}\left(\frac{g}{100g}\right)=\frac{T\times \text{concentration of standard}\times \text{total volume}}{S\times \text{volume used}\times \text{weight of sample}}\times 100$$

Where,

S = Absorbance of standard

T = Absorbance of sample

3.2.2.5 Antioxidant Capacity

Antioxidant capacity was determined by Phosphomolybdenum method (Prieto *et al.*, 1999). One g of chopped betel vine leaves was made into a homogenate by using pestle and mortar with 20 ml methanol. From this, 1 ml methanol extract was pipetted out into test tube. 9 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate mixed in a ratio of 1:1:1) was added into the extract and thoroughly mixed. Test tubes were kept in water bath at 90°C for 60 minutes. The cooled samples were read at 695 nm in a UV – Vis spectrophotometer against reagent blank. The standard used was 100 microgram of ascorbic acid in 1ml distilled water. Standard was also processed with same way and OD read at 695 nm. Antioxidant capacity was calculated as per the following formula and expressed as ascorbic acid equivalents.

$$\text{Antioxidant capacity (µg of ascorbic acid/gm)}=\frac{T\times 100\times 20}{S\times 1\times 1}$$

Where T = OD of test

S = OD of standard

3.2.3 Organoleptic Property – Leaf Pungency

A panel of twenty five judges, who were not regular chewers of betel vine, assessed the pungency of sample leaves of each type using the score card as given below.

Score	Guide
>4	Highly pungent
3 - 4	Medium pungent
2 – 3	Less pungent
1 - 2	Non pungent

3.2.4 Statistical Analysis

Data collected from the experimental field with respect to the quantitative traits and chemical parameters, as mentioned above, were tabulated and subjected to analysis of variance and diversity analysis. The different covariance estimates were calculated by the method suggested by Fisher (1954).

3.2.4.1 Analysis of Variance

Analysis of variance was carried out using MSTAT package. Treatments were compared using Duncan's Multiple Range Test (DMRT).

3.2.4.2 Estimation of Genetic Parameters

The variance components were estimated using the method suggested by Lush (1940).

3.2.4.2.1 Phenotypic and genotypic variances

Phenotypic variance $(\sigma^2 p) = \sigma^2 g + \sigma^2 e$

Where;

$\sigma^2 g$ = Genotypic variance

$\sigma^2 e$ = Environmental variance

Genotypic variance $(\sigma^2 g) = \frac{(Mg - Me)}{N}$

Where;

Mg = Mean sum of squares due to treatments

Me = Mean sum of squares due to error

N = Number of replications

Me = Environmental variance $(\sigma^2 e)$

3.2.4.2.2 Phenotypic and Genotypic Coefficients of Variation

The phenotypic and genotypic coefficients of variation were calculated by the formula suggested by Burton (1952).

Phenotypic coefficients of variation (PCV) $= \frac{\sigma P}{Mean} \times 100$

Where;

σp = Phenotypic standard deviation

$$\text{Genotypic coefficients of variation (GCV)} = \frac{\sigma g}{\text{Mean}} \times 100$$

Where;

σg = Genotypic standard deviation

As suggested by Sivasubramanian and Mathavamenon (1973) the PCV and GCV were classified as;

Low	Less than 10 per cent
Moderate	10 to 20 per cent
High	More than 20 per cent

3.2.4.2.3 Heritability

Heritability in broad sense (H^2) was estimated by the following formula suggested by Lush (1940).

$$\text{Heritability } (H^2) = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

As suggested by Johnson *et al.* (1955) the heritability values were categorized as;

Low	Less than 30 per cent
Moderate	30 - 60 per cent
High	More than 60 per cent

3.2.4.2.4 Genetic advance

As suggested by Johnson *et al.* (1955), the expected genetic advance under selection was estimated by following method at five per cent selection intensity using the constant K as 2.06 given by Allard (1960).

$$\text{Genetic advance (GA)} = \frac{\sigma^2 g}{\sigma p} \times K$$

Where

K = Selection differential at a particular level of selection intensity.

3.2.4.2.5 Genetic Gain (Genetic advance as percentage of mean)

Genetic gain was calculated by the following formula

$$\text{Genetic gain} = \frac{GA}{\text{Mean}} \times 100$$

The range of genetic gain was classified as, suggested by Johnson *et al.* (1955).

Low	Less than 10 per cent
Moderate	10 – 20 per cent
High	More than 20 per cent

3.2.4.2.6 Phenotypic and Genotypic Correlation Coefficients

The phenotypic and genotypic correlation coefficients were worked out to study the extent of association between the yield and other morphological characters. The phenotypic and genotypic correlation coefficients among the various characters were worked out in all possible combinations according to the formula suggested by Johnson *et al.* (1955).

Phenotypic correlation coefficients between two characters x and y were calculated by the formula;

$$\text{Phenotypic correlation } (r_p) = \frac{\sigma p\,(x,y)}{\sqrt{\sigma^2 px \times \sigma^2 py}}$$

Where $\sigma^2 px$ = Phenotypic variance of character x

$\sigma^2 py$ = Phenotypic variance of character y

Genotypic correlation coefficients between two characters (x and y) were calculated by the following formula;

$$\text{Genotypic correlation } (r_g) = \frac{\sigma p\,(x,y)}{\sqrt{\sigma^2 gx \times \sigma^2 gy}}$$

Where $\sigma^2 gx$ = Genotypic variance of character x

$\sigma^2 gy$ = Genotypic variance of character y

3.2.4.2.7 Path Coefficient Analysis

In path coefficient analysis, the correlation among cause and effect are portioned into direct and indirect effects of causal factors on effect factor.

The direct and indirect effects were rated as suggested by Lenka and Mishra (1973).

0.0 – 0.09	Negligible
0.10 – 0.19	Low
0.20 – 0.29	Moderate
0.30 – 1.00	High
More than 1.00	Very high

3.2.4.2.8 Diversity Analysis

The Unweighed Pair Group Method of Arithmetic averages (UPGMA) of cluster analysis (Sneath and Sokal, 1973) was done by taking into consideration of chemical and morphological characters. The cluster analysis helped to produce hierarchical classification of entries based on proximities.

Results

4. RESULTS

4.1 SURVEY AND SAMPLE COLLECTION

Betel vine (*Piper betle* L.) was cultivated in an area of 183 ha in Malappuram district during 2013 (FIB, 2014). A preliminary survey was conducted in main betel vine growing Block Panchayaths of Malappuram district namely Ponmundam, Parappangadi, Valanchery, Malappuram, Vengara, Tirur and Kondotty. Details of betel vine cultivars recorded from each Block Panchayath are given in Table 4. *Puthukodi, Karinadan, Nadan* and *Chelan* were the cultivars recorded from Tirur Block Panchayath. Except *Chelan* all other cultivars were observed in Malappuram and Ponmundam Block Panchayaths. *Puthukodi* and *Nadan* cultivars were recorded from the remaining Block Panchayaths namely Parappanangadi, Valanchery, Vengara and Kondotty. From the survey, it was revealed that the most common type under cultivation was *Puthukodi* followed by *Nadan*. Commonly, *Puthukodi* was cultivated under *Koottakodi* system while *Nadan* was grown under *Ottakodi* system using arecanut and coconut palms as support trees. Cultivars like *Chelan* and *Karinadan* were conserved by few farmers.

Planting material of four betel vine cultivars, namely *Puthukodi, Chelan, Karinadan and Nadan* were collected from Tirur Block Panchyath. Planting material of *Muvattupuzha Local,* to be used as check variety, was collected from Muvattupuzha. High level of variability for leaf color, leaf size, internodal color, lateral branch pattern and petiole length were seen across the cultivars (Plate 1). These five cultivars were raised in farmer's field (Plate 2) at Tirur, Malappuram for further study. Evaluation of these cultivars in respect of morphological and biochemical characters was undertaken during 2012 - 14. The experimental data was analysed statistically and the results are presented below.

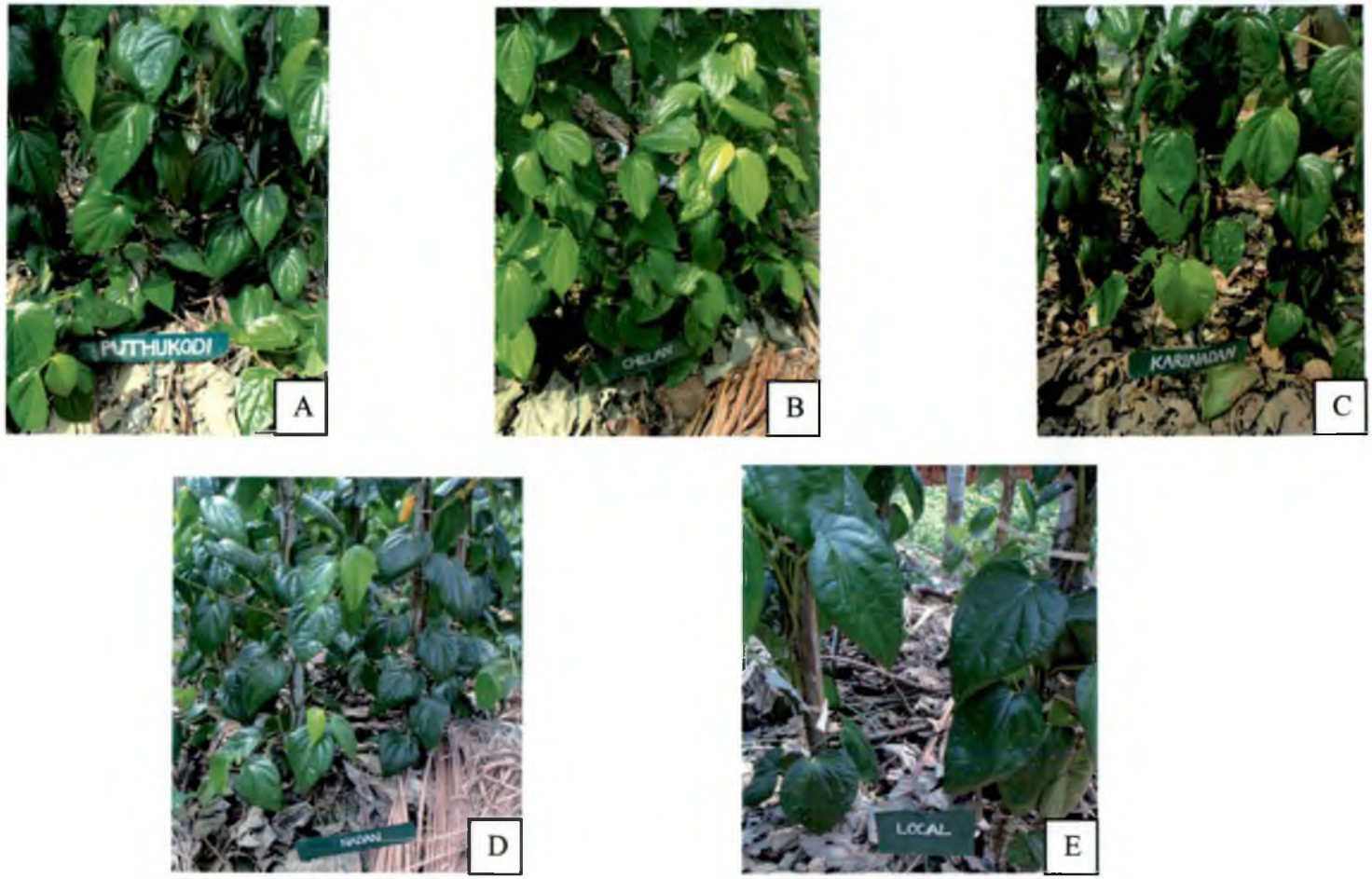


Plate 1. Betel vine cultivars used in the study: (A) *Puthukodi* (B) *Chelan* (C) *Karinadan* (D) *Nadan* (E) *Muvattupuzha Local*

Plate 2. Experimental field at Malappuram district during 2013 -14

Table 4. Results of survey conducted for identification of betel vine cultivars of Malappuram district during 2013 -14

Block Panchayaths	**Area (ha)**	**Krishibhavans**	**Betel vine cultivars recorded**	**System of cultivation**
Ponmundam	30.9	Ponmundam, Thanalur, Thanur Ozhur, Valavanur	*Puthukodi Chelan Nadan*	*Koottakodi Ottakodi*
Parappangadi	20.4	Nennambra, Tirurangadi, Parappanangadi Peruvallur, Thenhipalam	*Puthukodi Nadan*	*Koottakodi Ottakodi*
Valanchery	13.8	Kuttipuram , Valanchery Kalpakanchery, Athavanadu	*Puthukodi Nadan*	*Koottakodi Ottakodi*
Malappuram	36.7	Ponmala, Kottakkal	*Puthukodi Karinadan Nadan*	*Koottakodi Ottakodi*
Vengara	29.5	Oorakam, Thennala Edarikod, Vengara	*Puthukodi*	*Ottakodi Koottakodi*
Tirur	55.3	Tirunavaya, Tirur Thalakkad, Vettom, Purathur	*Puthukodi Chelan Karinadan Nadan*	*Koottakodi Ottakodi*
Kondotty	4.5	Vazhakadu , Kondotty, Pallikal	*Puthukodi Nadan*	*Koottakodi Ottakodi*

4.2 MORPHOLOGICAL CHARACTERIZATION

All the four betel vine cultivars of Malappuram were morphologically characterized, compared with *Muvattupuzha Local* and the data are presented as qualitative and quantitative characters.

4.2.1 Qualitative Characters

The qualitative characters of all five cultivars are presented in Table 5. These genotypes revealed variability with respect to most of the qualitative characters.

4.2.1.1 Internodal Color

Variability was noticed for internodal color in orthotropic shoot and lateral branch. The internodal color of orthotropic shoot in *Nadan, Puthukodi,* and *Muvattupuzha Local* were green with purple color at nodal region. *Chelan* showed internodal color of light green with purple tinge. *Karinadan* showed uniform purple green color for internode (Plate 3a).

Internodal color of lateral branch varied before and after spike formation. Purple pigmentation was present for all cultivars. *Karinadan, Puthukodi, Nadan* and *Muvattupuzha Local* recorded purple color with light green broken stripes whereas *Chelan* showed light purple with light green broken stripes at internodal region (Plate 3b). *Karinadan, Nadan, Puthukodi* and *Muvattupuzha Local* recorded green internodal color, whereas *Chelan* recorded light green color for lateral branch with spike (Plate 3c).

4.2.1.2 Lateral Branch Pattern

Lateral branches of *Chelan* showed semi erect pattern whereas *Puthukodi* and *Karinadan* showed hanging lateral branches. The check cultivars (*Nadan* and *Muvattupuzha Local*) showed both hanging and horizontal lateral branch pattern. However mostly hanging lateral branches were produced by both of them

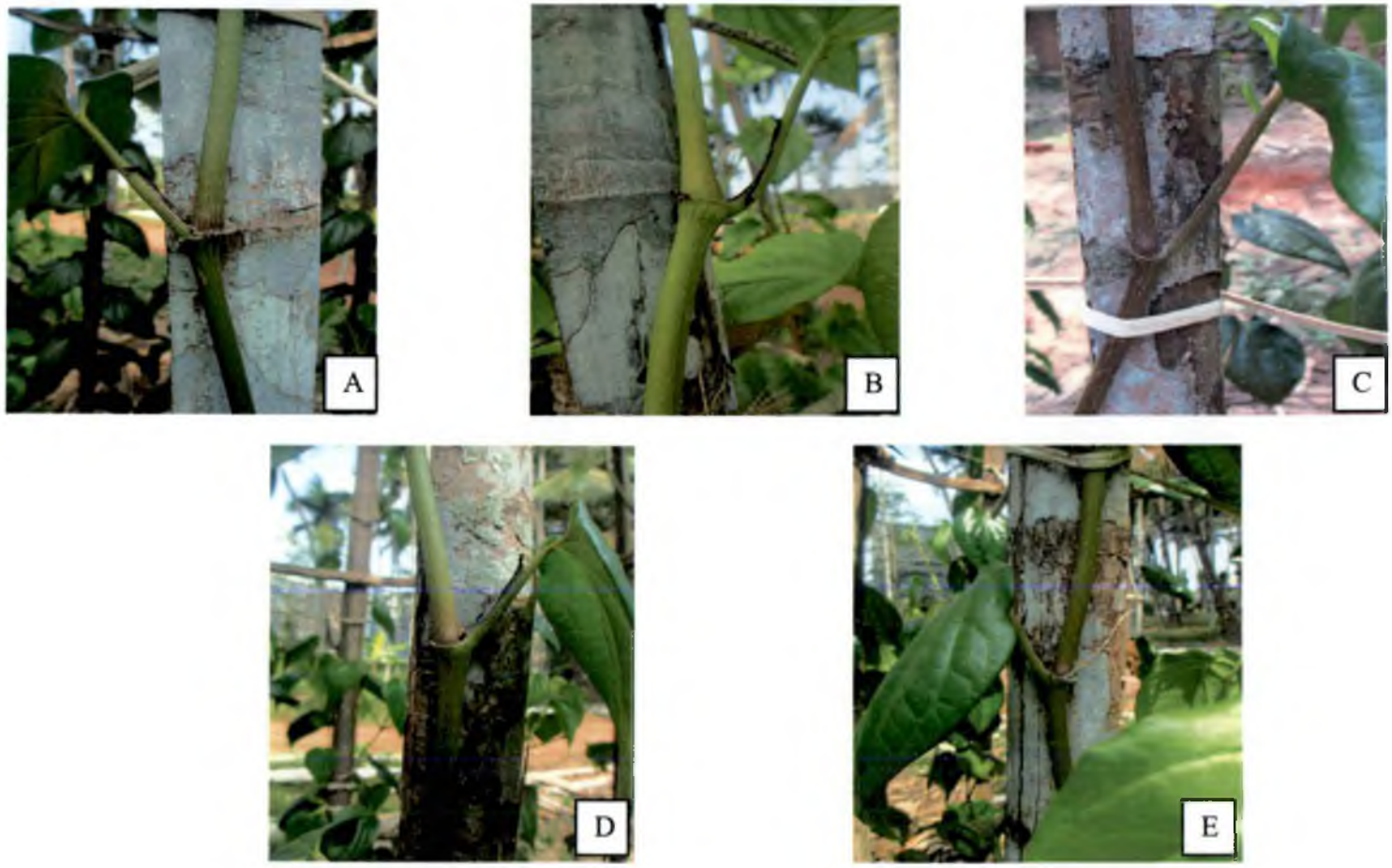


Plate 3a. Internodal color in orthotropic shoot of betel vine: (A) *Puthukodi* (B) *Chelan* (C) *Karinadan* (D) *Nadan* (E) *Muvattupuzha Local*

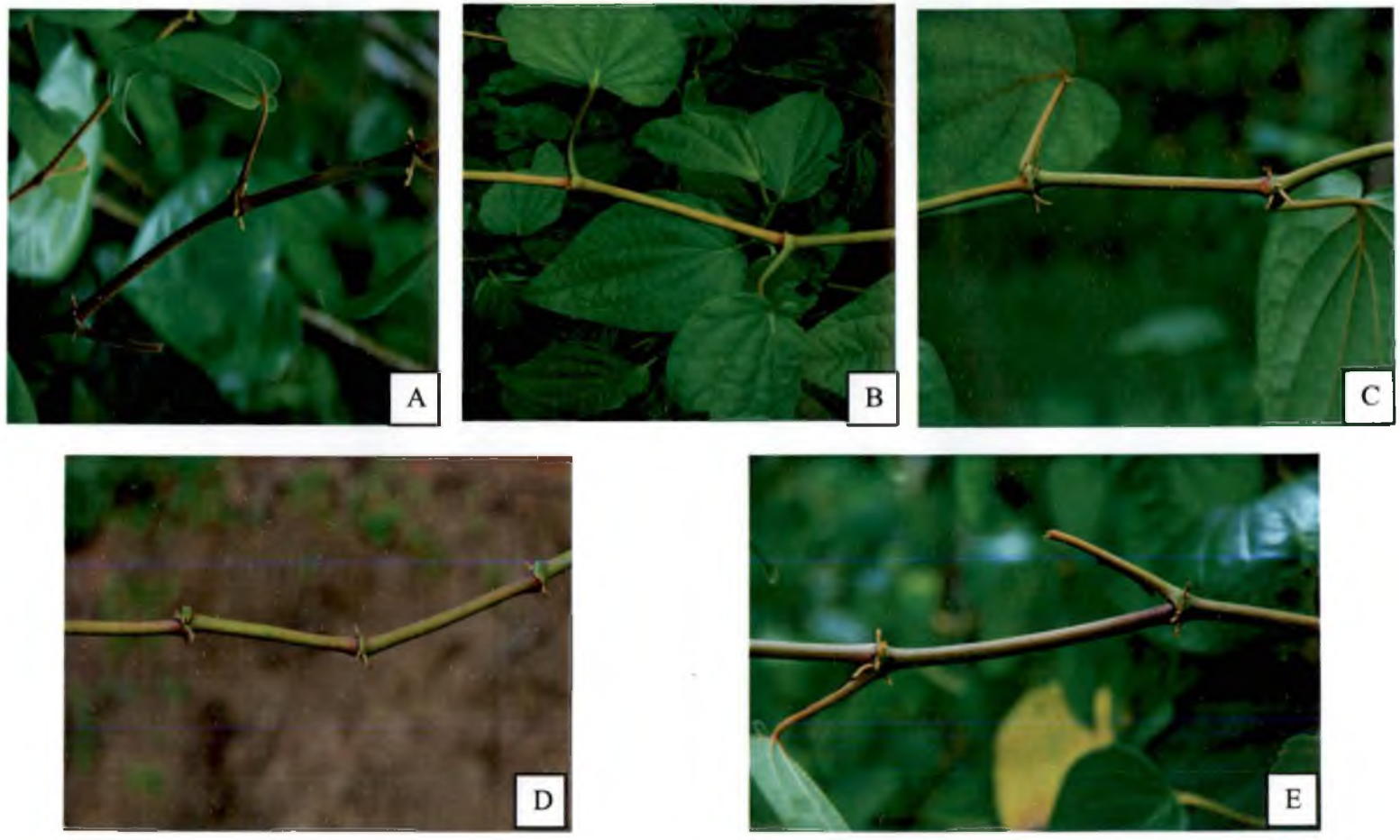


Plate 3b. Internodal color in lateral branch (without spike) of betel vine: (A) *Puthukodi* (B) *Chelan* (C) *Karinadan* (D) *Nadan* (E) *Muvattupuzha Local*

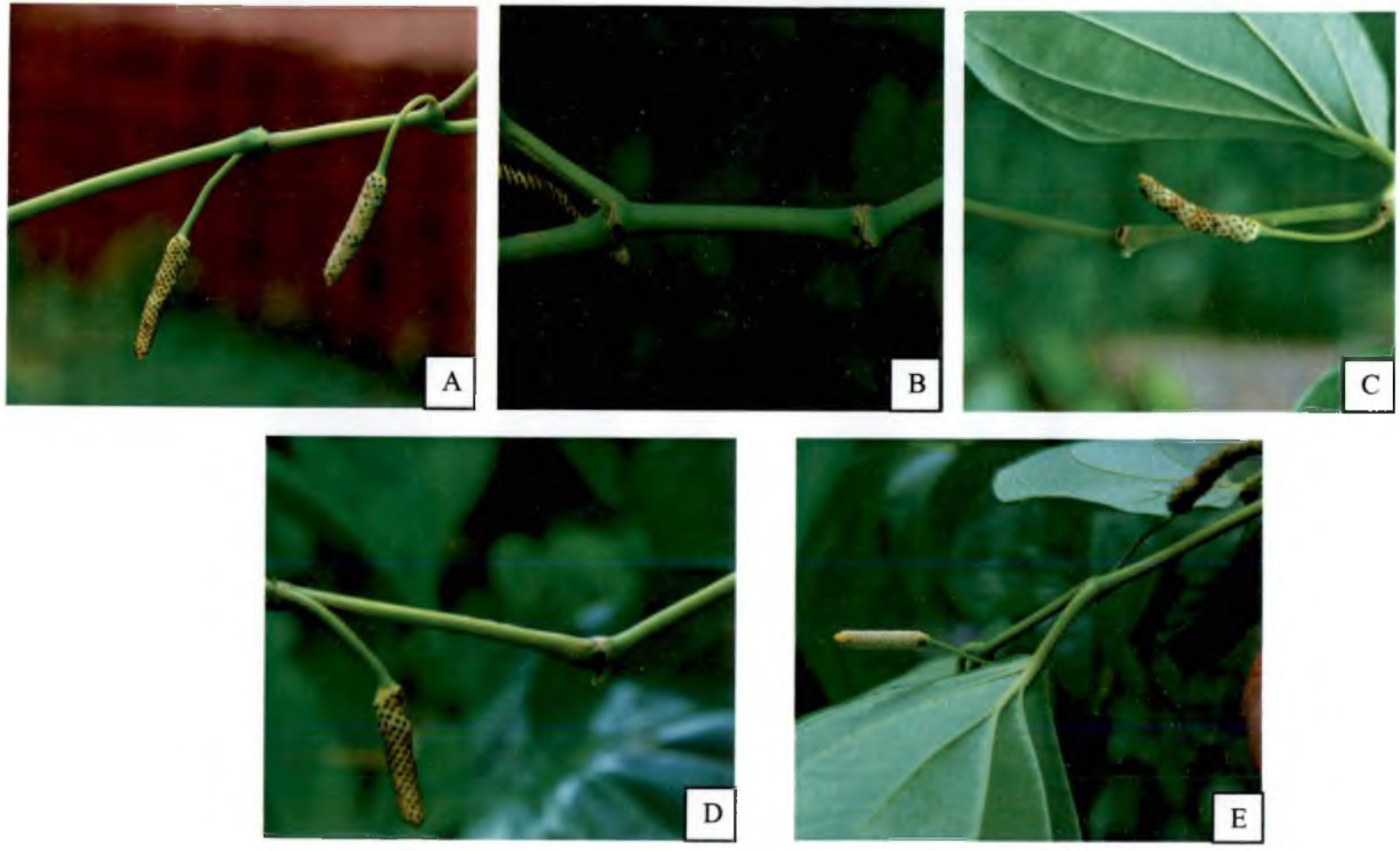


Plate 3c. Internodal color in laterl branch (with spike) of betel vine: (A) *Puthukodi* (B) *Chelan* (C) *Karinadan* (D) *Nadan* (E) *Muvattupuzha Local*

Table 5. Qualitative morphological characters of betel vine cultivars of Malappuram district during 2013 – 14

Sl. No.	Morphological Characters			Betel vine cultivars				
				Puthukodi	***Chelan***	***Karinadan***	***Nadan***	***Muvattupuzha Local***
1	**Internodal color in**	**Orthotropic shoot**		Green with purple color at nodal region	Light green with purple tinge	Uniform purple green color	Green with purple color at nodal region	Green with purple color at nodal region
		Lateral branch	**With spike**	Green	Light green	Green	Green	Green
			Without spike	Purple color with light green broken stripes	Light purple with light green broken stripes	Purple color with light green broken stripes	Purple color with light green broken stripes	Purple color with light green broken stripes
2	**Lateral branch pattern**			Hanging	Semierect	Hanging	Mostly hanging, rarely horizontal	Mostly hanging, rarely horizontal
3	**Shoot tip color**			Dark purple color with broken green stripes	Light purple with light green broken stripes	Dark purple color with broken green stripes	Dark purple color with broken green stripes	Dark purple color with broken green stripes
4	**Leaf margin**			Even	Wavy	Even	Even	Even
5	**Leaf brittleness**			Medium	Low	High	Medium	Medium
6	**Leaf color**			Green	Light green	Dark green	Green	Green
7	**Leaf lamina shape**			Mostly ovate elliptic, rarely ovate lanceolate	Ovate	Mostly ovate lanceolate, rarely ovate eliptic	Mostly ovate lanceolate, rarely cordate	Mosty ovate elliptic, rarely cordate
8	**Leaf base shape**			Cordate	Mostly round, rarely acute	Cordate	Mostly cordate, rarely round	Mostly cordate, rarely round
9	**Leaf apex shape**			Aristulate	Aristulate	Accuminate	Accuminate	Apiculate

4.2.1.3 Shoot Tip Color

Chelan had light purple shoot tips with light green broken stripes whereas *Nadan, Puthukodi, Karinadan* and *Muvattupuzha Local* showed dark purple color with broken green stripes for shoot tip.

4.2.1.4 Leaf Margin

Even leaf margin was seen in all betel vine cultivars except *Chelan. Chelan* exhibited wavy leaf margin (Plate 4). *Muvattupuzha Local* also showed even leaf margin.

4.2.1.5 Leaf Brittleness

Among the betel vine cultivars *Karinadan* showed high brittleness for leaves followed by medium brittleness in *Puthukodi* and *Nadan*. Leaves of *Chelan* showed low brittleness. *Muvattupuzha Local* cultivar also showed medium brittleness.

4.2.1.6 Leaf Color

Chelan had light green leaves while *Karinadan* had dark green leaves. The other betel vine cultivars had green colored leaves. The dorsal surface of leaf lamina of all cultivars showed slight anthocyanin pigmentation along main veins and at the point of insertion of petiole with leaf lamina.

4.2.1.7 Leaf Lamina Shape

Leaf lamina shape of *Puthukodi* and *Muvattupuzha Local* was mostly coming under ovate elliptic group. *Karinadan* and *Nadan* mostly produced ovate lanceolate leaves. *Chelan* always showed ovate leaf lamina. Rarely the leaves of *Muvattupuzha Local* and *Nadan* showed cordate leaf lamina (Plate 5). Rarely *Puthukodi* cultivar produced ovate lanceolate leaves whereas *Karinadan* rarely produced ovate elliptic leaves.

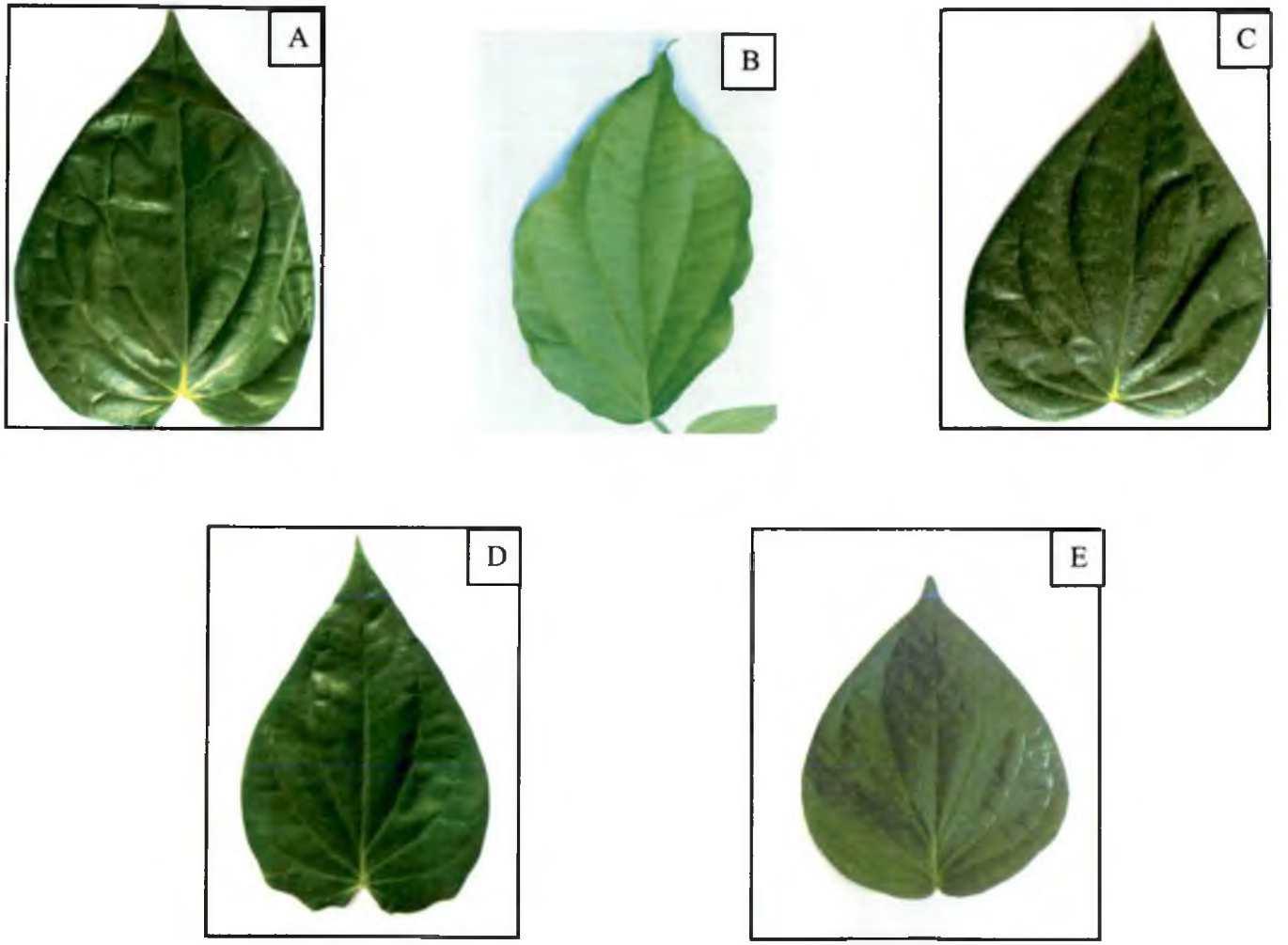


Plate 4. Leaf margin types in betel vine: (A) *Puthukodi* – Even (B) *Chelan* - Wavy (C) *Karinadan* - Even (D) *Nadan* – Even (E) *Muvattupuzha Local* - Even

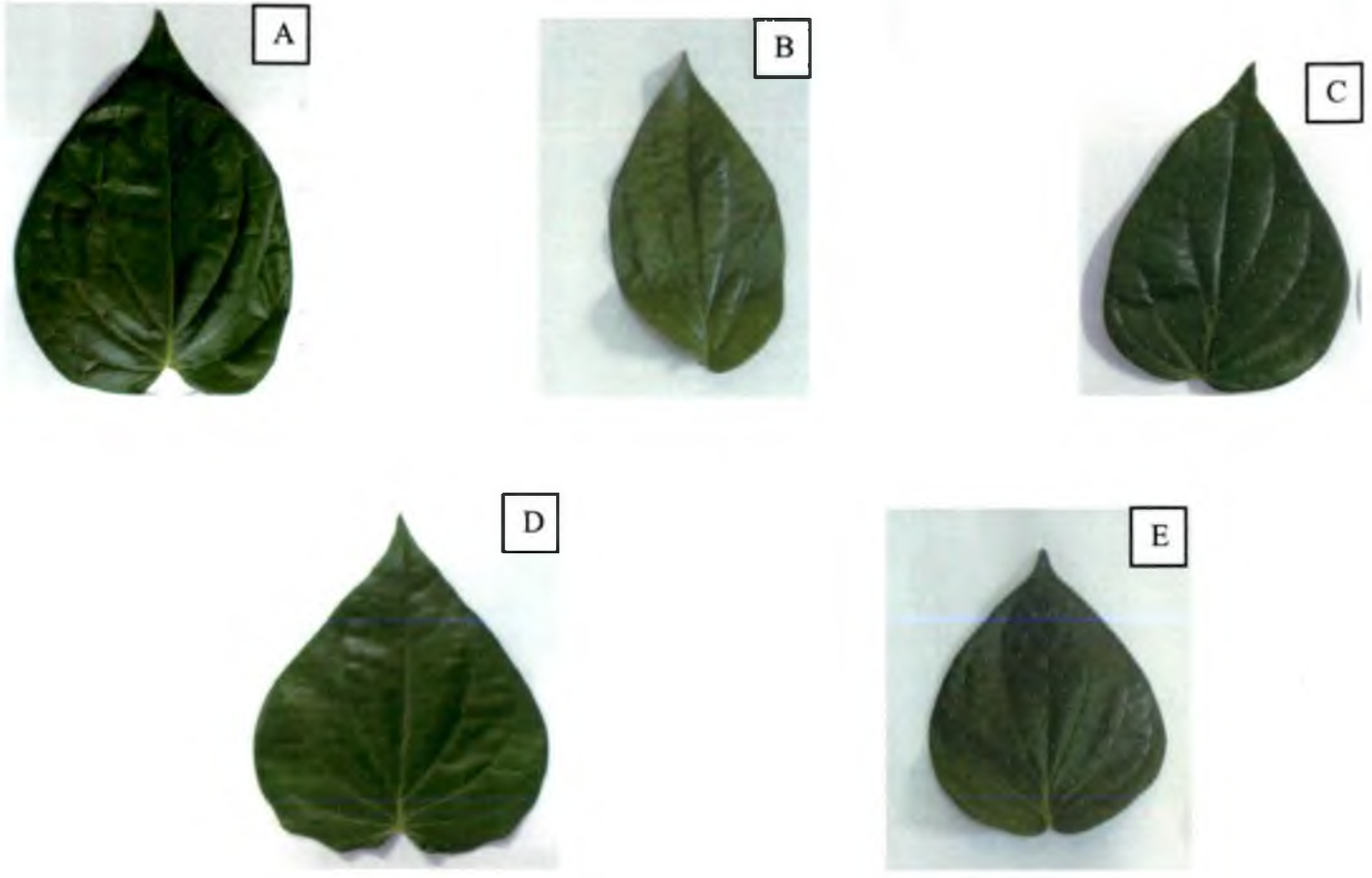


Plate 5. Leaf lamina shapes in betel vine: (A) *Puthukodi* – Ovate elliptic (B) *Chelan* - Ovate (C) *Karinadan* – Ovate lanceolate (D) *Nadan* – Ovate lanceolate (E) *Muvattupuzha Local* – Ovate elliptic

4.2.1.8 Leaf Base Shape

Commonly, cordate leaf base shape was seen in most of the betel vine cultivars except *Chelan*. However, *Nadan* and *Muvattupuzha Local* cultivars rarely produced leaves having round base. *Chelan* had round leaf base shape in most cases and acute leaf base in rare cases.

4.2.1.9 Leaf Apex Shape

Karinadan and *Nadan* cultivars showed acuminate shape for leaf apex whereas *Puthukodi* and *Chelan* showed aristulate leaf apex. Apiculate leaf apex was observed for *Muvattuuzha Local.*

4.2.2 Quantitative Characters

The quantitative characters of five betel vine cultivars were analyzed for significant difference using ANOVA. The growth parameters observed at fifteen days interval were plant height, total number of leaves, number of lateral branches, number of nodes per lateral branch and number of leaves per lateral branch.

4.2.2.1 Plant Height/ Vine Length

The data on height at different growth stages of betel vine cultivars are given in Table 6. *Chelan* recorded significantly high plant height (156.98, 183.15, 196.25, 269.78, 308.95 and 337.05 cm at 15, 30, 45, 60, 75 and 90 DAL respectively) at all stages of crop growth than other cultivars and *Muvattupuzha Local* cultivar. The plant height of *Muvattupuzha Local* cultivar was 138.25, 160.9, 187.18, 227.45, 263.28 and 292.55 cm at 15, 30, 45, 60, 75 and 90 DAL respectively. During crop growth, the vine length was lowest in *Karinadan* due to slow growth rate and it ranged from 126.75 – 264.28 cm at different growth stages.

The plant height of betel vine cultivars at 90 DAL varied significantly from 264.28 - 337.05 cm. Among these, *Karinadan* recorded significantly shorter plant

height (264.28 cm) followed by *Puthukodi* (284.33 cm), *Muvattupuzha Local* (292.55 cm) and *Nadan* (313.05 cm). The highest plant height (337.05 cm) was observed in *Chelan* (337.05 cm).

4.2.2.2 Total Number of Leaves per Plant

Significant variation was observed with respect to total number of leaves per vine among different cultivars (Table 7) during different stages of crop growth. Significantly higher leaf number (57.70, 81.68, 120.15, 182.93, 241.05 and 280.35 at 15, 30, 45, 60, 75 and 90 DAL respectively) was recorded from *Chelan* at all growth stages which was statistically superior over all other cultivars. Total number of leaves in *Muvattupuzha Local* ranged from 37.30 – 191.33 during the period of 15 – 90 DAL.

At 90 DAL, the total number of leaves was found to vary from 175.68 - 280.35. Significantly high leaf number (280.35) was observed in *Chelan* followed by *Nadan* (198.00). *Karinadan* had significantly less number of leaves (175.68) compared with other betel vine cultivars. *Muvattupuzha Local* recorded 191.33 number of leaves.

4.2.2.3 Number of Lateral Branches

There was significant difference in number of lateral branches among cultivars during 15 – 90 DAL (Table 8). Significantly higher number of lateral branches (3.70, 5.20, 9.38, 11.83, 14.00 and 16.88 at 15, 30, 45, 60, 75 and 90 DAL respectively) was produced by *Chelan* at all growth stages. Significantly lower number of lateral branches (1.75, 2.00, 3.08, 4.60, 8.10 and 10.88 at 15, 30, 45, 60, 75 and 90 DAL respectively) was produced by *Karinadan*. *Muvattupuzha Local* recorded 2.96, 3.65, 5.35, 7.33, 11.05 and 14.28 lateral branches at 15, 30, 45, 60, 75 and 90 DAL respectively. The observations recorded at 90 DAL revealed that significantly higher number of lateral branches was found in *Chelan* (16.88) and significantly minimum

Table 6. Plant height in different betel vine cultivars of Malappuram district at specific growth stages during 2013 - 14

Betel vine cultivars	Plant height (cm)					
	15 DAL	30 DAL	45 DAL	60 DAL	75 DAL	90 DAL
Puthukodi	137.50^{b}	155.2^{c}	175.20^{b}	216.95^{d}	247.35^{d}	284.33^{d}
Chelan	156.98^{a}	183.15^{a}	196.25^{a}	269.78^{a}	308.95^{a}	337.05^{a}
Karinadan	126.75^{c}	143.6^{d}	166.75^{b}	206.98^{e}	237.50^{e}	264.28^{e}
Nadan	155.28^{a}	177.9^{b}	177.90^{b}	242.65^{b}	283.38^{b}	313.05^{b}
Muvattupuzha Local	138.25^{b}	160.9^{c}	187.18^{b}	227.45^{c}	263.28^{c}	292.55^{c}

DAL – Days After Lowering

Table 7. Total number of leaves in different betel vine cultivars of Malappuram district at specific growth stages during 2013 - 14

Betel vine cultivars	Total number of leaves					
	15 DAL	30 DAL	45 DAL	60 DAL	75 DAL	90 DAL
Puthukodi	43.98^{c}	61.05^{c}	90.93^{b}	115.15^{d}	155.83^{c}	182.55^{d}
Chelan	57.70^{a}	81.68^{a}	120.15^{a}	182.93^{a}	241.05^{a}	280.35^{a}
Karinadan	32.50^{e}	54.38^{e}	90.65^{b}	109.8^{e}	138.78^{d}	175.68^{e}
Nadan	52.18^{b}	72.80^{b}	94.40^{b}	132.23^{b}	169.53^{b}	198.00^{b}
Muvattupuzha Local	37.30^{d}	47.4^{d}	96.20^{b}	124.23^{c}	158.68^{c}	191.33^{c}

DAL – Days After Lowering

was in *Karinadan* (10.88). *Muvattupuzha Local* recorded 14.28 number of lateral branches at this stage.

4.2.2.4 Number of Nodes per Lateral Branch

Significant variation was observed with respect to number of nodes per lateral branch among different cultivars during the period from 15 to 90 DAL (Table 9). *Karinadan, Nadan* and *Muvattupuzha Local* recorded significantly less number of nodes per lateral branch whereas significantly higher number of nodes per lateral branch was produced by *Chelan* followed by *Puthukodi* between 15 - 45 DAL. After 45 DAL, *Nadan* recorded faster growth rate with significantly higher number of nodes per lateral branch than *Puthukodi. Chelan* recorded highest number of nodes per lateral branch (43.95) whereas *Karinadan* recorded significantly lower number (26.28) of nodes per lateral branch at 90 DAL.

4.2.2.5 Number of Leaves per Lateral Branch

The data on number of leaves per lateral branch (Table 10) showed significant variation among different cultivars during the entire period of observation. Significantly high number of leaves per lateral branch (10.00, 17.45, 21.35, 29.13, 33.20 and 43.95 at 15, 30, 45, 60, 75 and 90 DAL respectively) was recorded in *Chelan* at all growth stages followed by *Puthukodi* up to 45 DAL. After 45 DAL, *Nadan* produced significantly high lateral branches after *Chelan*. Number of leaves per lateral branch recorded from *Muvattupuzha Local* and *Nadan* was same at 90 DAL.

Observations on other quantitative morphological characters *viz.,* days to lateral branching, days between lateral branch emergence, angle between orthotropic shoot and leaf petiole, leaf length, leaf width, leaf area, leaf weight, leaf weight per unit area, leaf petiole length, leaf tip angle, spike length, spike diameter and spike peduncle length are presented in Table 11.

Table 8. Number of lateral branches in different betel vine cultivars of Malappuram district at specific growth stages during 2013 - 14

Betel vine cultivars	Number of lateral branches					
	15 DAL	30 DAL	45 DAL	60 DAL	75 DAL	90 DAL
Puthukodi	2.73[b]	3.38[b]	5.58[b]	6.85[c]	10.78[c]	13.78[d]
Chelan	3.70[a]	5.20[a]	9.38[a]	11.83[a]	14.00[a]	16.88[a]
Karinadan	1.75[c]	2.00[c]	3.08[c]	4.60[d]	8.10[d]	10.88[e]
Nadan	2.48[b]	3.23[b]	5.45[b]	7.28[b]	10.38[c]	15.23[b]
Muvattupuzha Local	2.96[b]	3.65[b]	5.35[b]	7.33[b]	11.05[b]	14.28[c]

DAL – Days After Lowering

Table 9. Number of nodes per lateral branch in different betel vine cultivars of Malappuram district at specific growth stages during 2013 - 14

Betel vine cultivars	Number of nodes per lateral branch					
	15 DAL	30 DAL	45 DAL	60 DAL	75 DAL	90 DAL
Puthukodi	6.78[b]	10.08[b]	13.88[b]	15.85[c]	20.33[c]	26.20[c]
Chelan	10.00[a]	17.45[a]	21.35[a]	29.13[a]	33.20[a]	43.95[a]
Karinadan	4.53[c]	7.55[c]	10.63[c]	13.80[d]	17.13[d]	26.28[c]
Nadan	5.23[c]	10.08[b]	15.78[b]	18.78[b]	22.13[b]	36.75[b]
Muvattupuzha Local	4.68[c]	9.53[b]	13.55[b]	18.30[b]	21.00[bc]	36.73[b]

DAL – Days After Lowering

Table 10. Number of leaves per lateral branch in different betel vine cultivars of Malappuram district at specific growth stages during 2013 - 14

Betel vine cultivars	Number of leaves per lateral branch					
	15 DAL	30 DAL	45 DAL	60 DAL	75 DAL	90 DAL
Puthukodi	6.78^{b}	10.08^{b}	13.88^{b}	15.85^{c}	20.33^{c}	26.20^{c}
Chelan	10.00^{a}	17.45^{a}	21.35^{a}	29.13^{a}	33.20^{a}	43.95^{a}
Karinadan	4.53^{c}	7.55^{c}	10.63^{c}	13.80^{d}	17.13^{d}	26.28^{c}
Nadan	5.23^{c}	10.08^{b}	15.78^{b}	18.78^{b}	22.13^{b}	36.75^{b}
Muvattupuzha Local	4.68^{c}	9.53^{b}	13.55^{b}	18.30^{b}	21.00^{bc}	36.73^{b}

DAL – Days After Lowering

Table 11. Quantitative morphological characters of different betel vine cultivars of Malappuram district during 2013 – 14

Sl. No.	Morphological characters	*Puthukodi*	*Chelan*	*Karinadan*	*Nadan*	*Muvattupuzha Local*
1	Days to lateral branching	134.00^{ab}	80.75^{c}	151.25^{a}	85.00^{c}	127.50^{b}
2	Days between lateral branch emergence	18.23^{c}	9.35^{d}	26.35^{a}	18.03^{c}	21.78^{b}
3	Angle between orthotropic shoot and leaf petiole (degree)	49.85^{c} (narrow)	77.95^{a} (wide)	49.12^{c} (narrow)	59.92^{b} (narrow)	57.55^{b} (narrow)
4	Leaf length (cm)	17.70^{c} (long)	14.78^{d} (medium)	19.48^{a} (long)	19.23^{b} (long)	15.25^{d} (medium)
5	Leaf width (cm)	12.63^{c} (medium)	11.50^{d} (medium)	14.40^{a} (broad)	13.80^{b} (broad)	11.75^{d} (medium)
6	Leaf area (cm^2)	156.13^{d} (medium)	134.25^{e} (low)	196.23^{a} (high)	184.08^{b} (high)	164.25^{c} (medium)
7	Leaf weight (g)	4.78^{a}	2.82^{c}	4.85^{a}	4.53^{a}	3.95^{b}
8	Leaf weight per unit area (g/cm^2)	0.028^{a}	0.021^{d}	0.025^{b}	0.022^{c}	0.022^{c}
9	Leaf petiole length (cm)	2.93^{c} (short)	4.85^{a} (long)	2.75^{c} (short)	3.30^{b} (medium)	3.35^{b} (medium)
10	Leaf tip angle (degree)	35.20^{c} (narrow)	38.90^{b} (narrow)	51.62^{a} (wide)	42.62^{b} (medium)	40.95^{b} (medium)
11	Spike length (cm)	2.60^{b} (medium)	5.97^{a} (long)	2.52^{b} (medium)	2.40^{c} (medium)	2.68^{b} (medium)
12	Spike diameter (cm)	0.60^{a} (medium)	0.40^{d} (slender)	0.55^{b} (medium)	0.50^{c} (medium)	0.50^{c} (medium)
13	Spike peduncle length (cm)	3.77^{a} (medium)	3.07^{c} (short)	3.65^{a} (medium)	3.42^{b} (short)	3.25^{b} (short)

4.2.2.5 Days to lateral branching

Significant variation was observed in number of days for emergence of first lateral branch among cultivars. The maximum number of days (151.25) for emergence of lateral branch was recorded in *Karinadan* which was followed by *Puthukodi* (134.00 days) and *Muvattupuzha Local* (127.50 days). Male cultivar, *Chelan* took 80.75 days for emergence of first lateral branch which was statistically at par with *Nadan* (85days).

4.2.2.6 Days between Emergence of Lateral Branches

With respect to frequency of lateral branch emergence, significant variation was observed among cultivars. *Chelan* showed early lateral branch emergence and took significantly less number of days (9.35 days) for the emergence of two consecutive lateral branches which was followed by *Nadan* (18.03 days) and *Puthukodi* (18.23 days). *Karinadan* recorded more days (26.30) for the emergence of two consecutive lateral branches and it took more days for first lateral branching also.

4.2.2.8 Angle between Orthotropic Shoot and Leaf Petiole

The angle between orthotropic shoot and leaf petiole significantly varied from cultivar to cultivar. Except *Chelan,* all other cultivars showed narrow angle between orthotropic shoot and leaf petiole. Wide angle between orthotropic shoot and leaf petiole was obtained in *Chelan* (77.95^0) followed by narrow angle in *Nadan* (59.92^0). The lowest angle between orthotropic shoot and leaf petiole was observed in *Karinadan* (49.12^0) followed by *Puthukodi* (49.85^0). *Muvattupuzha Local* recorded 57.55^0 between orthotropic shoot and leaf petiole.

4.2.2.9 Leaf Length

Based on the data obtained on leaf length, leaves of *Karinadan, Nadan* and *Puthukodi* were grouped into long leaves whereas *Chelan* and *Muvattupuzha Local* showed medium long leaves.

The cultivars showed significant variation with respect to leaf length and the longest leaf (19.48 cm) was recorded in *Karinadan* followed by *Nadan* (19.23 cm) and *Puthukodi* (17.70 cm). Shortest leaf length was recorded in *Chelan* (14.78 cm). *Muvattupuzha Local* recorded a leaf length of 15.25 cm (Table 11).

4.2.2.10 Leaf Width

Significant variation was observed among cultivars with respect to leaf width. Broad leaves were recorded in *Karinadan* (14.40 cm) and *Nadan* (13.48 cm). The leaf width of all other cultivars was grouped in the class of medium.

4.2.2.11 Leaf Area

The cultivars showed significant variation with respect to leaf area and were grouped into cultivars having high leaf area (*Karinadan* and *Nadan*), medium leaf area (*Puthukodi, Muvattupuzha Local*) and low leaf area (*Chelan*). Significantly high leaf area (196.23 cm^2) was recorded in *Karinadan* followed by *Nadan* (184.08 cm^2) and *Muvattupuzha Local* (164.25 cm^2). Significantly lowest leaf area (134.25 cm^2) was noticed in *Chelan.*

4.2.2.12 Leaf Weight

Leaf weight varied significantly among cultivars. Significantly higher fresh leaf weight was observed in *Karinadan* (4.85 g), *Puthukodi* (4.78 g) and *Nadan* (4.53 g) whereas significantly lower fresh leaf weight was observed in Chelan (2.82 g). *Muvattupuzha Local* recorded leaf weight of 3.95 g.

4.2.2.13 Leaf Weight per Unit Area

Fresh leaf weight per unit area varied significantly among the cultivars. Significantly higher fresh leaf weight per unit area was observed in *Puthukodi* (0.028 g/cm^2) and lowest fresh leaf weight per unit area was recorded for *Chelan* (0.021 g/cm^2). *Muvattupuzha Local* and *Nadan* recorded 0.022 g/cm^2 as leaf weight per unit area.

4.2.2.14 Leaf Petiole Length

Long petiole was observed in *Chelan,* whereas petiole having medium length was recorded in *Karinadan* and *Puthukodi. Muvattupuzha Local* and *Nadan* recorded medium petiole length. Petiole length was significantly shortest in *Karinadan* (2.75 cm) and *Puthukodi* (2.93 cm). Longest petiole (4.85 cm) was recorded for *Chelan* followed by *Muvattupuzha Local* (3.35 cm) and *Nadan* (3.30 cm).

4.2.2.15 Leaf Tip Angle

Based on the observations on leaf tip angle, cultivars were grouped into classes having narrow leaf tip angle (*Puthukodi* and *Chelan*), medium leaf tip angle (*Nadan* and *Muvattupuzha Local*) and wide leaf tip angle (*Karinadan*). Highest leaf tip angle (51.62^0) was recorded in *Karinadan.* The leaf tip angles in *Nadan* (42.62^0), *Muvattupuzha Local* (40.95^0) and *Chelan* (38.90^0) were statistically on par. Minimum leaf tip angle was recorded in *Puthukodi* (35.20^0).

4.2.2.16 Floral Morphology

In the present study, male and female plants were identified at the stage of spike formation. Spikes appeared first in *Chelan* followed by other cultivars. *Chelan* was the only male cultivar identified in the study. All other cultivars *viz., Puthukodi, Karinadan, Nadan* and *Muvattupuzha Local* were identified as female. In all cultivars, axillary spikes opposite to the leaf were noticed. Individual florets were many in

number, sessile, naked and compactly arranged on the inflorescence axis. A bract subtended each floret, both in male and female spikes.

The matured male florets were yellow in color with the two black stamens. All other floret parts were modified into a bract and embedded on the rachis. Matured female spikes were identified by the presence of whitish, sessile stigmatic lobes. On ageing the stigmatic lobes became black. Number of stigmatic lobes in cultivars varied between six to nine.

4.2.2.17 Spike Length

Significant variation was observed with respect to spike length among different cultivars (Plate 6). Significantly long spikes were recorded in *Chelan* (5.97 cm). Spikes with medium length were produced by *Muvattupuzha Local* (2.68 cm), *Puthukodi* (2.60 cm), *Karinadan* (2.52 cm) and *Nadan* (2.40 cm). Among the cultivars, *Nadan* showed significantly low value for spike length.

4.2.2.18 Spike Diameter

The data showed that marked difference existed among the cultivars of betel vine for spike diameter. The female cultivar, *Puthukodi* recorded the significantly high diameter of 0.60 cm followed by *Karinadan* (0.55 cm), *Muvattupuzha Local* and *Nadan* (0.50 cm each). The male cultivar, *Chelan* had slender spikes with significantly low spike diameter of 0.40 cm.

4.2.2.19 Spike Peduncle Length

There was significant difference among cultivars for spike peduncle length. Peduncle having medium length was recorded in *Karinadan* (3.65 cm) and *Puthukodi* (3.77 cm). *Chelan*, *Muvattupuzha Local* and *Nadan* recorded short peduncles of 3.07, 3.25 and 3.42 cm respectively.

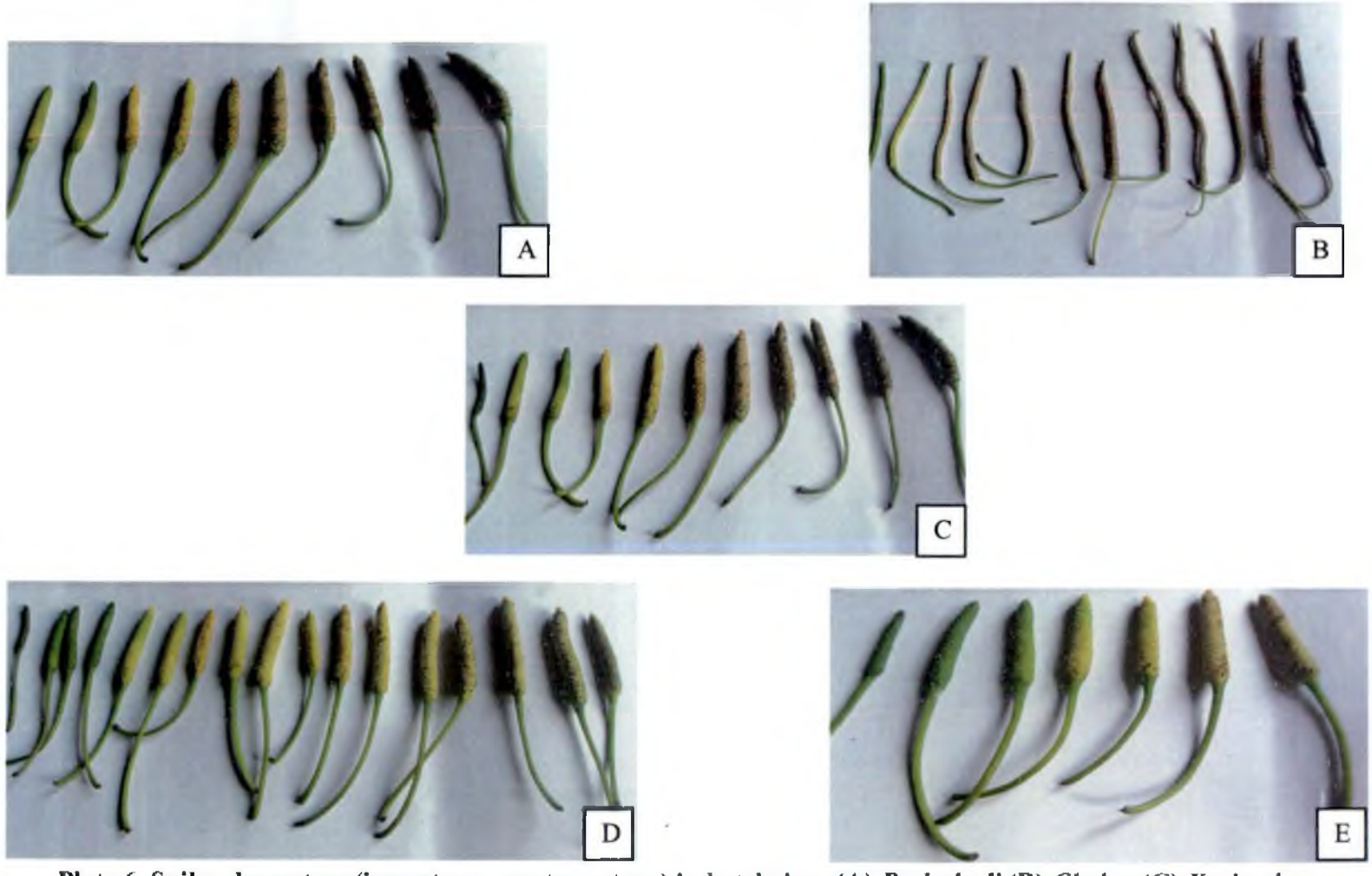


Plate 6. Spike characters (immature – mature stage) in betel vine: (A) *Puthukodi* (B) *Chelan* (C) *Karinadan* (D) *Nadan* (E) *Muvattupuzha Local*

4.3 BIOCHEMICAL CHARACTERS

4.3.1 Essential Oil – Yield and Components

Yield of essential oil in betel vine cultivars ranged from 0.45 to 0.57 per cent (Table 12) with a maximum content of essential oil in *Muvattupuzha Local* (0.57 per cent). *Karinadan*, *Puthukodi* and *Nadan* recorded 0.52, 0.50 and 0.47 per cent essential oil respectively. *Chelan* recorded the minimum essential oil content of 0.45 per cent.

Biochemical components of essential oil extracted from different cultivars were analyzed using Gas Chromatographic technique (GC). Most of the biochemical components of essential oil were isolated during the first 30 minutes of the gas chromatography. Eugenol showed a retention time of 13.54 minutes (Fig. 6), whereas 16.05 minutes was recorded as the retention time of methyl eugenol (Fig. 7). Chromatograms of isoeugenol (Fig. 8) and methyl isoeugenol (Fig. 9) had shown a retention time of 16.61 minutes and 18.78 minutes respectively.

Among the components studied, eugenol was the major component (11.02 – 20.80 per cent) of essential oil in all the cultivars under study followed by methyl isoeugenol with a range of 0.10 – 1.50 per cent. The highest percentage of eugenol (20.80) was seen in essential oil derived from *Chelan* cultivar. Methyl eugenol was found in *Chelan* and *Nadan* to the extent of 0.80 per cent. Except *Karinadan*, isoeugenol was present in all cultivars with a range of 0.80 – 1.00 per cent.

Number of peaks in essential oil, retention time with corresponding relative amount and its possible identity are presented in Table 13. The data showed the possible presence of hydroxychavicol in all the cultivars. Its possible presence in cultivars was to the tune of 39.50 (*Chelan*) to 44.60 per cent in *Nadan*. A total of 55 possible components with eight compounds as predominant (>2 %) were identified in

Table 12. Essential oil and biochemical components present in different betel vine cultivars of Malappuram district during 2013 - 14

Sl. No.	Betel vine cultivars	Yield of essential oil (v/w %)	Eugenol (%)	Methyl eugenol (%)	Isoeugenol (%)	Methyl isoeugenol (%)
1	*Puthukodi*	0.50	15.30	-	0.80	0.30
2	*Chelan*	0.45	20.80	0.80	1.00	0.20
3	*Karinadan*	0.52	11.60	-	-	0.10
4	*Nadan*	0.47	16.30	0.80	0.80	0.10
5	*Muvattupuzha Local*	0.57	11.00	-	0.80	1.50

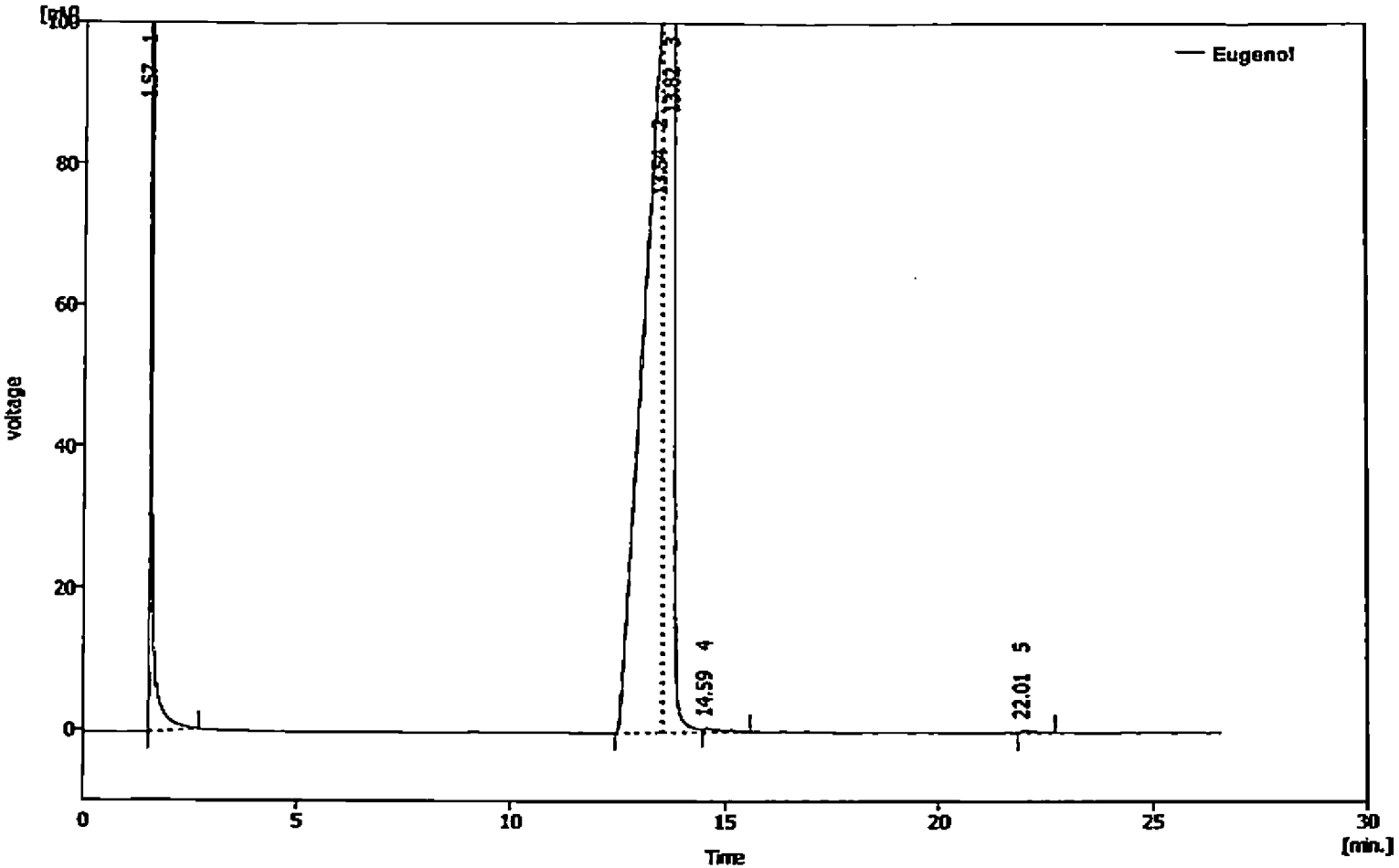


Fig. 6. Chromatogram of eugenol

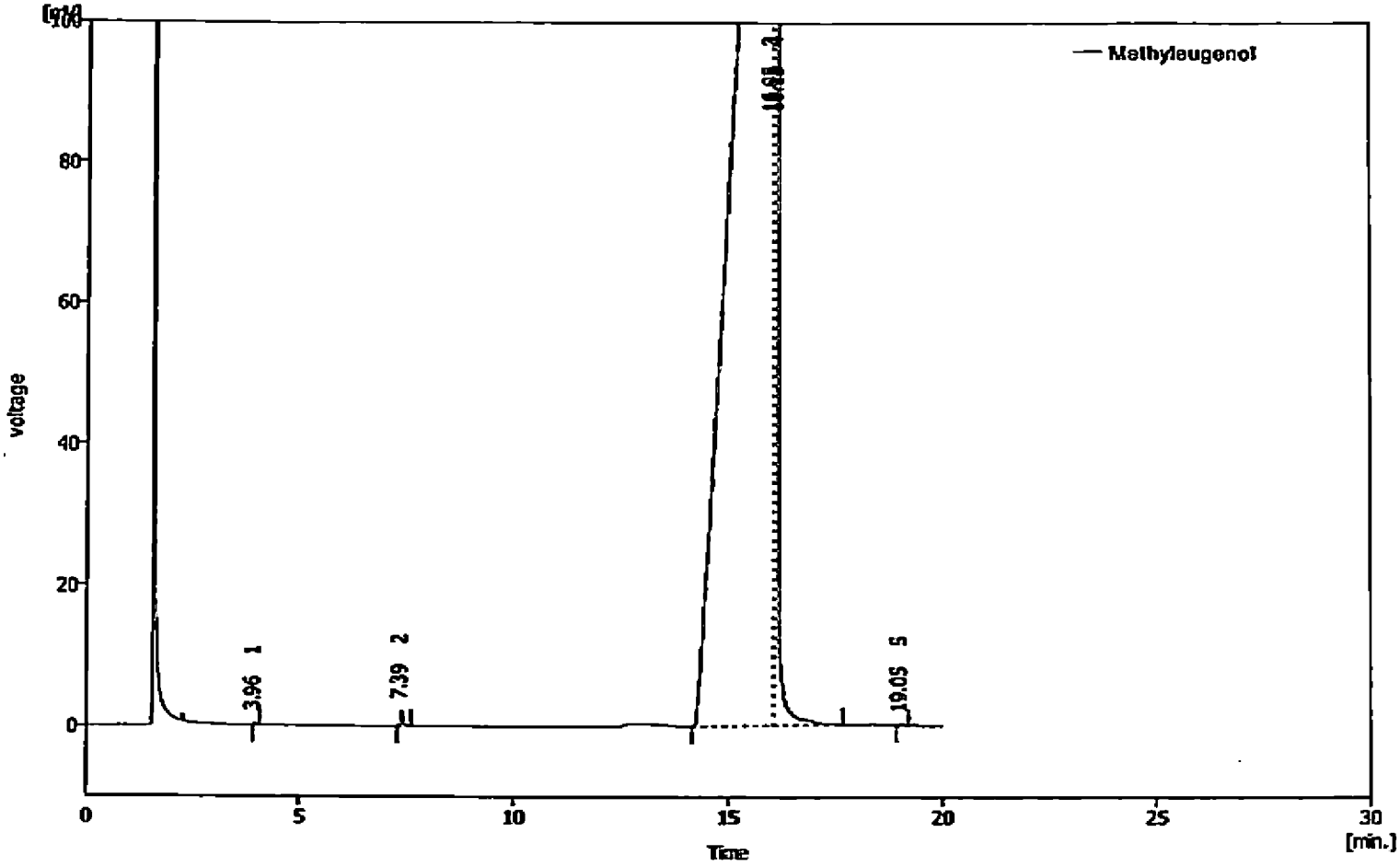


Fig. 7. Chromatogram of methyl eugenol

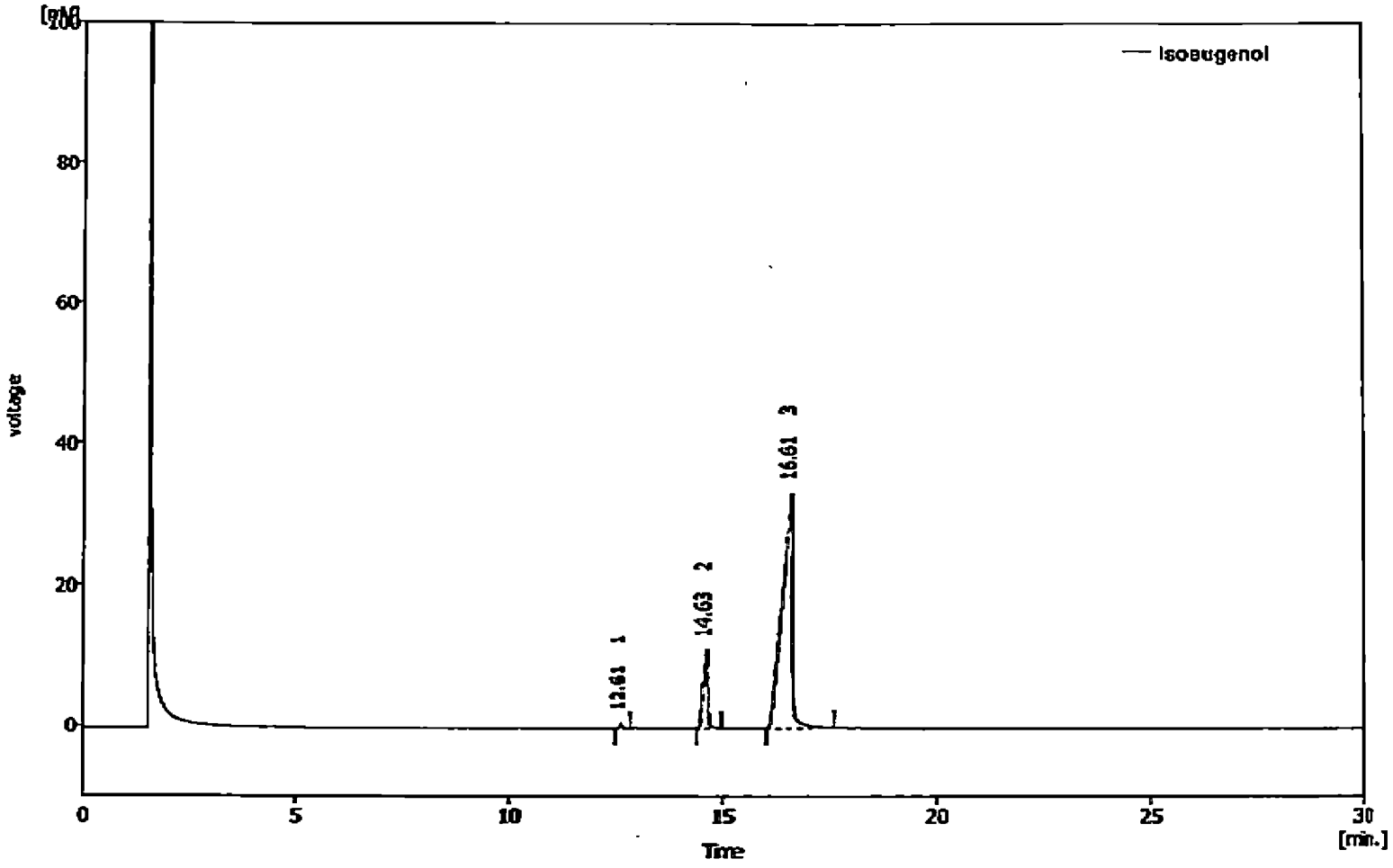


Fig. 8. Chromatgram of isoeugenol

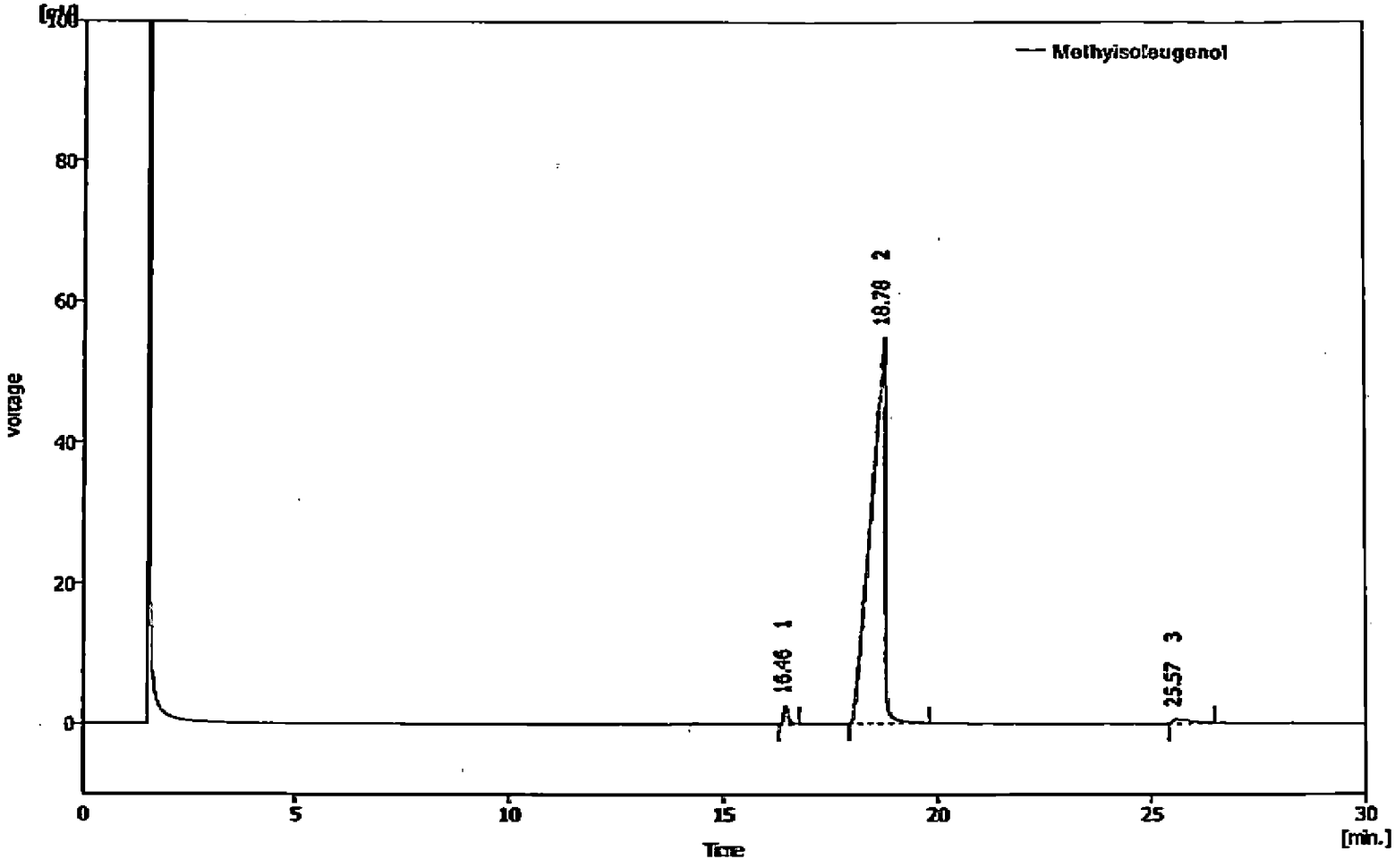


Fig. 9. Chromatogram of methyl isoeugenol

Table 13. Distribution of predominant chemical compounds (>2%) in the essential oil of different betel vine cultivars of Malappuram district during 2013 - 14

Betel vine cultivars	No. of peaks obtained	Retention time (minutes)	Relative amount (v/w %)	Possible identity
Puthukodi	55	11.05	45.30	Hydroxychavicol
		13.3	15.30	
		7.16	7.80	
		24.39	2.90	
		19.7	2.80	β caryophyllene
		4.47	2.70	
		3.34	2.50	
		4.06	2.10	
Chelan	56	11.49	39.50	Hydroxychavicol
		13.95	20.80	
		7.37	9.85	
		3.35	2.70	
		4.51	2.60	
		4.08	2.20	
Karinadan	39	11.60	44.50	Hydroxychavicol
		13.71	11.60	
		1.53	9.30	
		7.07	8.70	
		20.17	4.30	β caryophyllene
		3.85	2.80	
		24.55	2.40	5-(2-propenyl)-1,3-benzodioxole
		19.48	2.20	
Nadan	56	10.78	44.60	Hydroxychvicol
		13.07	16.30	
		4.45	3.20	
		4.04	2.40	
Muvattupuzha Local	55	11.34	41.10	Hydroxychavicol
		1.58	11.70	
		13.48	11.00	
		7.19	5.60	
		24.67	5.00	5-(2-propenyl)-1,3-benzodioxole
		19.99	4.10	β caryophyllene
		3.34	2.40	

the essential oil of *Puthukodi*. Hydroxychavicol and β caryophyllene were the few predominant possible compounds identified in essential oil of *Puthukodi* and accounted for 48.10 per cent of the total oil.

A total of 56 possible chemical components were identified in the oil from *Chelan*. In this cultivar, six predominant possible components, including hydroxychavicol accounted for 56.85 per cent of essential oil. Compared to other cultivars, quantity of hydroxychavicol was less in this cultivar.

Analysis of the relative composition of betel oil of *Karinadan*, showed 39 possible compounds with seven predominant components. Hydroxychavicol, β caryophyllene and 5-(2-propenyl)-1, 3-benzodioxole were some of the possible predominant components with a relative amount of 44.50, 4.30 and 2.40 per cent respectively.

Fifty six possible components were identified from the essential oil of *Nadan*. Hydroxychavicol with a relative amount of 44.60 was identified as the major predominant possible component in this cultivar.

GC analysis of essential oil of *Muvattupuzha Local* recorded a total of 55 possible components of which seven were predominant. Hydroxychavicol (41.10 per cent), 5-(2-propenyl)-1, 3-benzodioxole (5.00 per cent) and β caryophyllene (4.10 per cent) were the major possible components identified from this cultivar.

The table containing retention time, area and height of each volatile compound present in the essential oil of five betel vine cultivars and standards are given in Appendix I - IX.

4.3.2 Chlorophyll Content

The estimation of chlorophyll content in leaves included total chlorophyll, chlorophyll a and chlorophyll b.

4.3.1.1 Total Chlorophyll

Cultivars showed significant difference regarding total chlorophyll content in fresh leaves (Table 14). Among the cultivars, it ranged from 1.96 to 2.83 mg/g tissue. Maximum content (2.83 mg/g) of total chlorophyll was recorded by *Karinadan* followed by *Nadan* (2.55 mg/g) and *Puthukodi* (2.43 mg/g). Lowest total chlorophyll content (1.96 mg/g) was observed in *Chelan.* Total chlorophyll content in *Muvattupuzha Local* was 2.26 mg/g.

4.3.1.2 Chlorophyll a

There was a significant variation in chlorophyll a content among cultivars. It was found that *Karinadan* had the highest chlorophyll a content (1.73 mg/g) followed by *Puthukodi* (1.57 mg/g). *Chelan* had minimum content (1.29 mg/g) of chlorophyll a.

4.3.1.3 Chlorophyll b

Chlorophyll b content in leaves varied significantly among the cultivars and significantly high content of chlorophyll b (0.70 mg/g) was found in *Karinadan* and *Muvattupuzha Local*(0.62 mg/g) than *Chelan* (0.39 mg/g).

4.3.3 Total Soluble Protein

The cultivars had significant variation with respect to protein content in fresh leaves. The protein content was significantly high in *Karinadan* (4.05 mg/g) and *Nadan* (3.78 mg/g). The leaves of *Puthukodi* had a protein content of 3.23 mg/g and it was statistically on par with *Muvattupuzha Local* (3.10 mg/g). Lowest protein (1.23 mg/g) content was observed in *Chelan.*

4.3.4 Total Phenol Content

The data showed a significant variation among cultivars with respect to total phenol content. *Chelan* was having less total phenol content (2.21 g/100g) and *Karinadan* exhibited the highest total phenol content (3.36 g/100g) followed by *Puthukodi* (3.08 g/100 g) and *Nadan* (2.70 g/100 g). Leaves of *Muvattupuzha Local* had a phenol content of 2.48 g/100g.

4.3.5 Antioxidant Capacity

Statistically significant variation in the antioxidant capacity of betel leaves was recorded in all cultivars. Among the cultivars, *Karinadan* recorded significantly high antioxidant capacity (8.36 µg ascorbic acid/mg) in leaves followed by *Puthukodi* (7.5 µg ascorbic acid/mg) and *Nadan* (7.03 µg ascorbic acid/mg). *Muvattupuzha Local* recorded antioxidant capacity of 6.82 microgram ascorbic acid/mg. As in the case of total protein and phenol content, the antioxidant capacity was found low in *Chelan* (5.38 µg ascorbic acid/mg).

4.4 ORGANOLEPTIC PROPERTY – LEAF PUNGENCY

The study on pungency of betel vine cultivars revealed that *Muvattupuzha Local* was highly pungent, with a score of 4.12 while *Puthukodi, Karinadan* and *Nadan* were medium pungent cultivars (Table 15). *Chelan* was identified as less pungent cultivar with a mean score of 2.70.

4.5 GENETIC PARAMETERS

The extent of genetic variability with respect to different quantitative characters in betel vine cultivars of Malappuram district was estimated. The abstract of analysis of variance of these characters is given in Appendix X.

Table 14. Biochemical components of different betel vine cultivars of Malappuram district during 2013 - 14.

Sl. No.	Biochemical characters	Betel vine cultivars				
		Puthukodi	*Chelan*	*Karinadan*	*Nadan*	*Muvattupuzha Local*
1	Total chlorophyll (mg/g)	2.43^{b}	1.96^{d}	2.83^{a}	2.55^{b}	2.26^{c}
2	Chlorophyll a (mg/g)	1.57^{ab}	1.29^{b}	1.73^{a}	1.51^{ab}	1.45^{ab}
3	Chlorophyll b (mg/g)	0.50^{ab}	0.39^{b}	0.70^{a}	0.54^{ab}	0.62^{a}
4	Total protein (mg/g)	3.23^{b}	1.23^{c}	4.05^{a}	3.78^{a}	3.10^{b}
5	Total phenol content (g/100g)	3.08^{b}	2.21^{e}	3.36^{a}	2.70^{c}	2.48^{d}
6	Antioxidant capacity (µg ascorbic acid/mg)	7.50^{b}	5.38^{d}	8.36^{a}	7.03^{bc}	6.82^{c}

Table 15. Ranking of different betel vine cultivars of Malappuram district based on pungency

Betel vine cultivars	Mean score	Pungency class
Puthukodi	3.80	Medium
Chelan	2.70	Less
Karinadan	3.90	Medium
Nadan	3.90	Medium
Muvattupuzha Local	4.12	Highly

4.5.1 Genetic Variability

The variability parameters like range and mean for the above characters are presented in Table 16.

Results of analysis of variance revealed significant difference among all cultivars. Among the quantitative characters, plant height varied from 264.28 to 337.05 cm with an average of 298.28 ± 24.91cm. Total number of leaves and number of lateral branches ranged from 175.68 - 280.35 and 10.88 – 16.88 and with a mean of 205.58 ± 38.13 and 14.20 ± 1.95 respectively. Both the number of nodes per lateral branch and number of leaves per lateral branch varied from 26.20 to 43.95 with an average of 31.58 ± 6.46.

Days to lateral branching and days between emergence of two lateral branches varied from 80.75 – 151.25 and 9.35 – 21.28 days with an average of 115.7 ± 26.34 and 18.75 ± 5.52 days respectively. With respect to angle between orthotropic shoot and leaf petiole, the variability ranged between 49.12^0 and 77.95^0 with an average of 58.22 ± 10.44^0.

Length and width of leaf varied from 13.78 to 19.48 cm and 11.50 to 14.40 cm with mean of 16.29 ± 2.31 cm and 12.81 ± 1.12 cm respectively. In the case of leaf area, the range of variation was from 134.25 to 196.23 cm^2 with an average of 166.98 ± 21.62 cm^2. Leaf weight ranged from 2.83 – 4.85 g. For leaf weight per unit area, the range of variation was from 0.021 to 0.025 g/cm^2 with an average of 0.023 ± 0.003 g/cm^2. The range of variation for leaf petiole length and leaf tip angle was from 2.75 to 4.85 cm and 35.20 to 51.62^0, with an average of 3.43 ± 0.73 cm and 41.86 ± 5.40^0 respectively.

The range of variation for spike length and spike diameter was from 2.40 to 5.97 cm and 0.40 to 0.60 cm respectively. Spike peduncle length ranged between 3.25 and 4.77 cm with a mean of 3.44 ± 0.25 cm.

Table 16. Range and mean of different characters in betel vine cultivars of Malappuram district during 2013 - 14

Sl. No.	Characters	Range	Mean
	Morphological characters		
1	Plant height (cm) at 90 DAL	264.28 – 337.05	298.28 ± 24.91
2	Total number of leaves at 90 DAL	175.68 - 280.35	205.58 ± 38.13
3	Number of lateral branches at 90 DAL	10.88 – 16.88	14.20 ± 1.95
4	Number of nodes per lateral branch at 90 DAL	26.20 – 43.95	31.58 ± 6.46
5	Number of leaves per lateral branch at 90 DAL	26.20 – 43.95	31.58 ± 6.46
6	Days to lateral branching	80.75 – 151.25	115.7 ± 26.34
7	Days between emergence of two lateral branches	9.35 – 21.28	18.75 ± 5.52
8	Angle between orthotropic shoot and leaf petiole(^{0})	49.12 – 77.95	58.22 ± 10.44
9	Leaf length (cm)	13.78 – 19.48	16.29 ± 2.31
10	Leaf width (cm)	11.50 – 14.40	12.81 ± 1.12
11	Leaf area (cm^2)	134.25 – 196.23	166.98 ± 21.62
12	Leaf weight (g)	2.83 – 4.85	3.65 ± 0.25
13	Leaf weight per unit area (g/cm^2)	0.021 – 0.025	0.023 ± 0.003
14	Leaf petiole length (cm)	2.75 – 4.85	3.43 ± 0.73
15	Leaf tip angle	35.20 – 51.62	41.86 ± 5.40
16	Spike length (cm)	2.40 – 5.97	3.23 ± 1.37
17	Spike diameter (cm)	0.40 – 0.60	0.51 ± 0.06
18	Spike peduncle length (cm)	3.25 – 4.77	3.44 ± 0.25
	Biochemical characters		
19	Total chlorophyll (mg/g)	1.96 – 2.83	2.41 ± 0.28
20	Chlorophyll a (mg/g)	1.29 - 1.73	1.51 ± 0.12
21	Chlorophyll b (mg/g)	0.39 - 0.69	0.55 ± 0.08
22	Total protein (mg/ g)	1.22 - 4.05	3.05 ± 0.977
23	Total phenol (g/100 g)	2.20 - 3.36	2.76 ± 0.41
24	Antioxidant capacity (µg ascorbic acid/g)	5.38 - 8.36	7.03 ± 0 .96

Among biochemical characters, total chlorophyll content ranged from 1.96 - 2.83 mg/g with a mean value of 1.91 ± 0.28 mg/g. Chlorophyll a and chlorophyll b content varied from 1.29 - 1.73 and 0.39 - 0.69 mg/g with an average of 1.51 ± 0.12 and 0.55 ± 0.08 mg/g respectively. Total protein content varied from 1.22 - 4.05 mg/g, average being 3.05 ± 0.98 mg/g. With respect to total phenol content and antioxidant capacity, the variability ranged from 2.20 to 3.36 g/100 g and 5.38 to 8.36 µg ascorbic acid/mg with an average of 2.76 ± 0.41g/100 g and 7.03 ± 0.96 µg of ascorbic acid/mg respectively.

4.5.2 Phenotypic and Genotypic Coefficients of Variation

The genotypic and phenotypic coefficients of variation among yield and yield attributes are presented in Table 17.

Moderate estimates of GCV and PCV was observed for total number of leaves, number of lateral branches, angle between orthotropic shoot and leaf petiole, leaf length, leaf area, leaf tip angle and spike diameter. Low GCV and PCV was observed with respect to characters like spike peduncle length, leaf width and plant height. All other morphological characters showed high PCV and GCV.

Among the biochemical characters, total chlorophyll content, chlorophyll a content, total phenol content and antioxidant capacity showed moderate PCV whereas chlorophyll b content and total protein content showed high estimates of PCV. Low estimate of GCV was observed for chlorophyll a content while high estimate of GCV was observed for total protein content. Moderate GCV was observed with respect to all other biochemical characters.

4.5.3 Heritability, Genetic Advance and Genetic Gain

Genetic parameters like heritability, genetic advance and genetic gain were estimated for yield attributes of betel vine cultivars and are presented in Table 18.

Table 17. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) in different characters of betel vine cultivars during 2013 - 14

Sl. No.	Plant characters	Phenotypic coefficient of variation (PCV)	Genotypic coefficient of variation (GCV)
	Morphological characters		
1	Plant height	8.40	8.35
2	Total number of leaves	18.58	18.54
3	Number of lateral branches	14.40	13.75
4	Days to lateral branch emergence	29.00	22.76
5	Days between lateral branch emergence	31.00	29.48
6	Angle between orthotropic shoot and leaf petiole	18.22	17.94
7	Number of nodes per lateral branch	21.12	20.46
8	Number of leaves per lateral branch	21.124	20.46
9	Leaf length	14.30	14.22
10	Leaf width	9.02	8.76
11	Leaf area	13.02	12.95
12	Leaf weight	26.86	6.96
13	Petiole length	22.87	21.29
14	Leaf tip angle	13.72	12.91
15	Spike length	42.77	42.51
16	Spike diameter	14.13	12.70
17	Spike peduncle length	8.37	7.17
	Biochemical characters		
1	Total chlorophyll	16.15	14.64
2	Chlorophyll a	14.29	8.00
3	Chlorophyll b	29.76	14.99
4	Total protein	33.64	32.01
5	Total phenol	15.44	14.83
6	Antioxidant capacity	14.44	13.72

Table 18. Heritability, genetic advance and genetic gain of morphological characters of different betel vine cultivars of Malappuram district during 2013 - 14

Sl. No.	Plant characters	Heritability (H)	Genetic advance(GA)	Genetic gain
Morphological characters				
1	Plant height	98.86	5598.13	18.76
2	Total number of leaves	99.59	8600.39	41.83
3	Days to lateral branch emergence	61.62	4673.56	40.39
4	Days between lateral branch emergence	90.47	1188.59	63.39
5	Angle between orthotropic shoot and leaf petiole	96.93	2324.85	39.93
6	Number of lateral branches	91.22	421.70	29.69
7	Number of nodes per lateral branch	93.82	1414.51	44.79
8	Number of leaves per lateral branch	93.82	1414.51	44.79
9	Leaf length	98.82	520.58	31.95
10	Leaf width	94.45	246.71	19.25
11	leaf area	98.84	4859.23	29.10
12	Leaf weight	6.72	14.89	40.78
13	Petiole length	86.67	153.70	44.81
14	Leaf tip angle	88.57	1149.80	27.46
15	Spike length	98.79	308.51	95.51
16	Spike diameter	80.76	13.16	25.80
17	Spike peduncle length	73.42	47.75	13.90
Biochemical characters				
1	Total chlorophyll	82.14	57.27	29.98
2	Chlorophyll a	31.33	15.28	10.12
3	Chlorophyll b	25.37	9.38	17.06
4	Total protein	90.53	210.24	68.84
5	Total phenol	90.29	88.88	32.20
6	Antioxidant capacity	90.25	2069.30	29.46

For all morphological characters, heritability (in broad sense) estimates ranged between 61.62 per cent (days to lateral branch emergence) and 99.59 per cent (total number of leaves) and showed high heritability (> 60 per cent). Highest genetic advance of 8600.39 was observed in the case of total number of leaves.

Genetic gain among morphological characters varied from 13.90 for spike peduncle length to 95.51 for spike length. Genetic gain was high for total number of leaves (41.83 per cent), days to lateral branch emergence (40.39 per cent), days between lateral branch emergence (63.39 per cent), angle between orthotropic shoot and leaf petiole (39.93 per cent), number of lateral branches (29.69 per cent), number of nodes per lateral branch (44.79 per cent), number of leaves per lateral branch (44.79 per cent), leaf length (31.95 per cent), leaf area (29.10 per cent), petiole length (44.81 per cent), leaf tip angle (27.46 per cent), spike length (95.51 per cent) and spike diameter (25.80 per cent). Moderate genetic gain estimates were exhibited by plant height (18.76 per cent), leaf width (19.25 per cent) and spike peduncle length (13.90 per cent).

Among biochemical characters, heritability was high for most of the characters and it ranged from 25.37 to 90.53 per cent. Heritability was low for chlorophyll b (25.37 per cent) and moderate for chlorophyll a (31.33 per cent). Estimates of heritability were high for total protein (90.53 per cent), total phenol (90.29 per cent), antioxidant capacity (90.25 per cent) and total chlorophyll (82.14 per cent).

Genetic gain of biochemical characters ranged from 10.12 to 68.84 per cent. Chlorophyll a (10.12 per cent) and chlorophyll b (17.06 per cent) recorded moderate genetic gain. High genetic gain was observed for total protein (68.84 per cent), total phenol (32.20 per cent), total chlorophyll (29.98 per cent) and antioxidant capacity (29.46 per cent).

4.5.4 Correlation Coefficients between Total Number of Leaves and Yield Components in Betel vine Cultivars.

Correlation coefficients between total number of leaves and yield components in betel vine cultivars are presented in Table 19.

Genotypic correlation coefficient revealed highly significant positive correlation of total number of leaves with number of leaves per lateral branch (0.995), plant height (0.886) followed by number of lateral branches (0.805) while significantly high negative correlation was shown by days between lateral branch emergence (-0.908) and days to lateral branching (-0.802). Plant height had highly significant positive correlation with number of lateral branches (0.978) and number of leaves per lateral branch (0.904). Plant height recorded highly significant negative correlation with days to lateral branching (-1.014) and days between lateral branch emergence (-0.937). Days to lateral branching showed highly significant positive correlation with days between lateral branch emergence (0.885) whereas it recorded highly significant negative correlation with number of lateral branches (-0.964) and number of leaves per lateral branch (-0.846). Number of lateral branches was found to have highly significant positive correlation with plant height (0.978) and highly significant negative correlation with days between lateral branch emergence (-0.930). Number of leaves per lateral branch negatively correlated (-0.877) with days between lateral branch emergence significantly.

With regards to phenotypic correlation, total number of leaves showed significant positive correlation with number of leaves per lateral branch (0.968) followed by plant height (0.879) and number of lateral branches (0.779) while negative correlation was seen with days between lateral branch emergence (-0.864)

Table 19. Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients between total number of leaves and yield components in betel vine cultivars of Malappuram district during 2013 - 14

	X_1	X_2	X_3	X_4	X_5	X_6
X_1	1.000	0.886**	-0.802**	0.805**	0.995**	-0.908**
X_2	0.879**	1.000	-1.014	0.978**	0.904**	-0.937**
X_3	-0.658**	-0.842**	1.000	-0.964**	-0.846**	0.885**
X_4	0.779**	0.933**	-0.784**	1.000	0.831**	-0.930**
X_5	0.968**	0.877**	-0.612**	0.759**	1.000	-0.877**
X_6	-0.864**	-0.888**	0.706**	-0.894**	-0.802**	1.000

*Significant at 5% level, **Significant at 1% level

X_1 = Total number of leaves

X_2 = Plant height

X_3 = Days to lateral branching

X_4 = Number of lateral branches

X_5 = Number of leaves per lateral branch

X_6 = Days between lateral branch emergence

and days to lateral branching (-0.658). Plant height showed highly significant positive phenotypic correlation with number of lateral branches (0.933) and number of leaves per lateral branch (0.877) and negative correlation with days between lateral branch emergence (-0.888) and days to lateral branching (-0.842). Days to lateral branching had highly significant positive phenotypic correlation with days between lateral branch emergence (0.706) while had significant negative correlation with number of lateral branches (-0.784) and number of leaves per lateral branch (-0.612) Number of lateral branches had significant positive correlation with number of leaves per lateral branch (0.759) whereas had negative correlation with days between lateral branch emergence (-0.894). Number of leaves per lateral branch showed significant negative correlation with days between lateral branch emergences (-0.802).

4.5.5 Direct and indirect effects of plant growth parameters on total number of leaves

Direct and indirect effects of five plant growth parameters on total number of leaves are presented in Table 20.

It was observed that number of leaves per lateral branch showed very high direct positive effect (0.871) on total number of leaves. Days between lateral branch emergence had high negative direct effect (-0.361) on total number of leaves followed by plant height (-0.234) and days to lateral branching (-0.145), while number of lateral branches (-0.099) had low negative direct effect on total number of leaves.

Plant height exhibited high positive indirect effect on total number of leaves *via* number of leaves per lateral branch (0.764) and it had moderate positive indirect effect on leaf yield by days between lateral branch emergence (0.320), while it had high negative indirect effect *via* plant height (-0.234).

Table 20. Direct and indirect effects of plant growth parameters on total number of leaves in betel vine cultivars at Malappuram district during 2013 - 2014

	X_1	X_2	X_3	X_4	X_5
X_1	**-0.234**	0.122	-0.092	0.764	0.320
X_2	0.197	**-0.145**	0.077	-0.533	-0.255
X_3	-0.219	0.113	**-0.099**	0.661	0.322
X_4	-0.206	0.089	-0.075	**0.871**	0.289
X_5	0.208	-0.102	0.088	-0.698	**-0.361**

Residual value = 0.0331

X_1 = Plant height

X_2 = Days to lateral branching

X_3 = Number of lateral branches

X_4 = Number of leaves per lateral branch

X_5 = Days between lateral branch emergence

Days to lateral branching exhibited moderate positive indirect effect on leaf yield *via* plant height (0.197) followed by number of lateral branches (0.077) whereas negative indirect effect was exhibited through number of leaves per lateral branch (-0.533) and days between lateral branch emergence (-0.255).

Number of lateral branches exhibited moderate positive indirect effect on leaf yield *via* number of leaves per lateral branch (0.661) followed by days between lateral branch emergence (0.322) and days to lateral branching (0.113) while it had low negative indirect effect through plant height (-0.219).

Number of leaves per lateral branch was found to have moderate positive indirect effect with days between lateral branch emergence (0.308) and days to lateral branching (0.089), while plant height (-0.206) and number of lateral branches (-0.075) had significant negative indirect effects.

Days between lateral branch emergence had positive indirect effect with plant height (0.208) followed by number of lateral branches (0.088) and negative indirect effects through number of leaves per lateral branch (-0.698) followed by days between lateral branch emergence (-0.361).

4.5.6 Diversity Analysis

The proximity matrix arrived based on morphological and biochemical characters of five cultivars revealed that the least Euclidean distance (212.65) was between *Nadan* and *Muvattupuzha Local* whereas highest distance (2981.57) was between the *Chelan* and *Karinadan* (Table 21). The Euclidean distance between *Nadan* and *Chelan* was 1650.58.

Table 21. Proximity matrix based on morphological and biochemical characters in betel vine cultivars

Proximity matrix

Betel vine cultivars	Euclidean distance				
	Puthukodi	***Chelan***	***Karinadan***	***Nadan***	***Muvattupuzha Local***
Puthukodi	0.00	2118.79	863.66	470.37	677.72
Chelan	2118.79	0.00	2981.57	1650.58	1442.13
Karinadan	863.66	2981.57	0.00	1331.82	1540.63
Nadan	470.37	1650.58	1331.82	0.00	212.65
Muvattupuzha Local	677.72	1442.13	1540.63	212.65	0.00

Discussion

5. DISCUSSION

Characterization of varieties and genotypes of crops is important for protection of bio-wealth in the WTO era. The present work is an attempt to characterize betel vine cultivars of Malappuram district, Kerala based on morphological and biochemical characters. Betel vine is rated as second most popular daily consumption item in Asia (Pradhan *et al.*, 2013). Approximately 125 – 150 cultivars of betel vine are identified across India.

In Kerala, Malappuram district is famous for cultivation of betel vine. The cultivars growing in this area are generally known as *Tirur betel vine*. Leaves of *Tirur betel vine* are known for their characteristic pungency. The leaves have considerable market potential and fetch premium price in domestic and international markets. Betel oil is a great source of different biomolecules like hydroxychavicol, eugenol, β - caryophyllene *etc.* which reveals the potential of betel vine as a raw material for extraction of valuable biochemicals. However, *Tirur betel vine* farmers face price fluctuation for their product and get lower price during the period of surplus production of leaves. The income from betel vine cultivation could be enhanced and the price fluctuation could be minimized to some extent if leaves are used to extract essential oil and other neutraceutical ingredients. In spite of the market potential of leaves and therapeutic values of betel oil, information about morphological and biochemical characters of betel vine cultivars, especially those grown in Kerala, is scanty and no betel vine descriptor has been developed yet in India for varietal characterization and DUS testing.

In this background, it was essential to have in-depth studies to reveal the extent of variability among cultivars in the major betel vine growing tracts of Kerala. It would be useful for identification of suitable cultivars for specific purposes like commercial cultivation, manufacturing of drugs, food supplements, essential oil extraction and cultural and religious uses *etc.* The superior genotypes identified could

be utilized in crop improvement programmes based on specific breeding objectives. Information on morphological and biochemical characteristics would act as data base for the development of betel vine descriptor.

Characterization of betel vine cultivars was also a prerequisite for the protection of intellectual property rights with special emphasize on farmers' rights over these cultivars and their products. The data generated would reveal the uniqueness of *Tirur betel vine* which would accelerate initiatives of Kerala Agricultural University to register *Tirur betel vine* as a Geographical Indication. Finally, the documentation of genetic diversity and systems of cultivation would help to maintain the heritage and legacy of *Tirur Betel vine.*

5.1 SURVEY

The area of cultivation of *Tirur betel vine* in Malappuram district during 2012 - 13 was 183 ha (FIB, 2014). It was cultivated in seven Block Panchayaths *viz.*, Ponmundam, Parappanangadi, Valanchery, Malappuram, Vengara, Tirur and Kondotty. In these Block Panchayaths, farmers were highly depending on betel vine cultivation for meeting their livelihood. Variation was seen on different morphological characters of *Tirur betel vine* cultivars. Cultivars recorded from Tirur and nearby areas were *Puthukodi, Karinadan, Nadan* and *Chelan. Puthukodi* was cultivated in larger area compared to other cultivars and exported to Pakistan. Leaves of *Nadan* were mostly preferred in local markets of Kerala. *Karinadan* and *Chelan* cultivars were conserved by few farmers in Malappuram district. Farmers mainly adopted organic method of cultivation. In Malappuram area, there are two systems of cultivation *viz., Ottakodi* and *Koottakodi* (Plate 7). *Koottakodi* system was followed in pure crop system whereas *Ottakodi* system was practiced as an intercrop system of coconut and arecanut gardens. These two systems were entirely different from the Bareja system (Haider *et al.*, 2013) for raising betel vine in West Bengal, the leading state for betel cultivation in India.

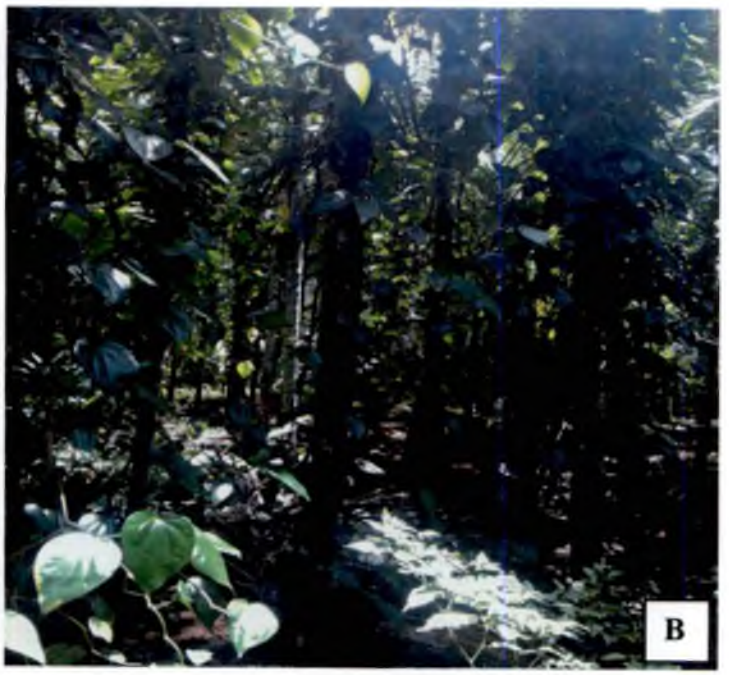


Plate 7. Systems of betel vine cultivation at Malappuram district:

(A) *Koottakodi* (B) *Ottakodi*

5.2 CHARACTERIZATION

Morphological and biochemical characters of betel vine cultivars of Malappuram district were recorded and compared with *Muvattupuzha Local.*

5.2.1 Morphological Characterization

Morphological characterization included both qualitative and quantitative characters. In general morphological characters of *Chelan* cultivar were different from that of other cultivars of the area and also from *Muvattupuzha Local.* The specific characteristics of each cultivar are presented in Table 22.

5.2.1.1 Qualitative Characters

5.2.1.1.1 Internodal Color

Orthotropic shoot and lateral branch expressed variability for internodal color. Green internode with purple color at nodal region was observed in *Nadan, Puthukodi,* and *Muvattupuzha Local. Chelan* showed intermodal color of light green with purple tinge whereas *Karinadan* showed uniform purple - green color for internode.

Internodal color of lateral branch varied before and after spike formation. Purple pigmentation was present in all cultivars before spike formation. Purple color with light green broken stripes was observed in lateral branches (without spike) of *Karinadan, Puthukodi, Nadan* and *Muvattupuzha Local* whereas *Chelan* showed light purple with light green broken stripes at internodal region. Purple pigmentation was absent in lateral branches with spikes. Green internode was noticed in *Karinadan, Nadan, Puthukodi* and *Muvattupuzha Local.* Light green internode was seen in *Chelan.*

Table 22. Morphological characters of betel vine cultivars of Malapuram district.

Sl. No.	Characters			*Puthukodi* (female)	*Chelan* (male)	*Karinadan* (female)	*Nadan* (female)	*Muvattupuzha Local* (female)
1	Internodal color	Orthotropic shoot		Green with purple color at nodal region	Light green with purple tinge	Uniform purple green color	Green with purple color at nodal region	Green with purple color at nodal region
		Lateral branch	With spike	Green	Light green	Green	Green	Green
			Without spike	Purple color with light green broken stripes	Light purple with light green broken stripes	Purple color with light green broken stripes	Purple color with light green broken stripes	Purple color with light green broken stripes
2	Lateral branch pattern			Hanging	Semi erect	Hanging	Mostly hanging, rarely horizontal	Mostly hanging, rarely horizontal
3	Shoot tip color			Dark purple color with broken green stripes	Light purple with light green broken stripes	Dark purple color with broken green stripes	Dark purple color with broken green stripes	Dark purple color with broken green stripes
4	Leaf color			Green,	Light green	Dark green	Green	Green
5	Leaf length (cm)			Long (17.70)	Medium (14.78)	Long (19.48)	Long (19.23)	Medium (15.25)
6	Leaf width (cm)			Medium (12.63)	Medium (11.50)	Broad (14.40)	Broad (13.80)	Medium (11.73)
7	Leaf area (cm^2)			Medium (156.13)	Low (134.25)	High (196.23)	High (184.08)	Medium (164.25)
8	Leaf petiole length (cm)			Short (2.93)	Long (4.85)	Short (2.75)	Medium (3.30)	Medium 3.35)
9	Leaf lamina shape			Mostly ovate elliptic, rarely ovate lanceolate	Ovate	Mostly ovate lanceolate, rarely ovate eliptic	Mostly ovate lanceolate, rarely cordate	Mosty ovate elliptic, rarely cordate
10	Leaf base shape			Cordate	Mostly round, rarely acute	Cordate	Mostly cordate, rarely round	Mostly cordate, rarely round
11	Leaf tip angle (^{0})			Narrow (35.20)	Narrow (38.90)	Wide (51.62)	Medium (42.62)	Medium (40.95)
12	Leaf apex shape			Aristulate	Aristulate	Accuminate	Accuminate	Apiculate
13	Spike length (cm)			Medium (2.60)	Long (5.97)	Medium (2.52)	Medium (2.40)	Medium (2.68)
14	Spike diameter (cm)			Medium (0.60)	Slender (0.40)	Medium (0.55)	Medium (0.50)	Medium (0.50)
15	Spike peduncle length (cm)			Medium (3.77)	Short (3.07)	Medium (3.65)	Short (3.42)	Short (3.25)

Pinkish coloration in the stem of the betel vine was reported by Chaveerach *et al.* (2006). Variation in the internodal color could be used as a morphological marker in cultivar identification.

5.2.1.1.2 Lateral Branch Pattern

Three types of lateral branch pattern namely hanging, horizontal and semierect were shown by the betel vine cultivars. Hanging lateral branches were mainly seen in betel vine cultivars whereas, semierect lateral branches were the unique character of *Chelan*. *Nadan* and *Muvattupuzha Local* rarely produced horizontal lateral branches.

5.2.1.1.3 Shoot Tip Color

All cultivars showed purple pigmentation at shoot tip. *Chelan* had light purple with light green broken stripes for shoot tip whereas all other cultivars showed dark purple colour with green broken stripes. Color of shoot tips of *Tirur betel vine* cultivars was same as that in *Muvattupuzha Local* (Plate 8). An experiment conducted by Sreedevi *et al.* (2005) on black pepper recorded light purple for young orthotropic shoot tip.

5.2.1.1.4 Leaf Margin

Two types of leaf margin (even and wavy) were found in betel vine cultivars. All cultivars, including check cultivar, *Muvattupuzha Local* showed even leaf margin except *Chelan* which produced wavy leaf margin. *Tirur betel vine* cultivars and *Muvattupuzha Local* showed same pattern in leaf margin. Similar findings were reported by Pariari and Imam (2012a).

5.2.1.1.5 Leaf Brittleness

Leaf brittleness varied among cultivars of betel vine. Highly brittle leaves were produced by *Karinadan* and low brittle leaves by *Chelan*. Medium brittle

Plate 8. Shoot tip color in betel vine: (A) *Puthukodi* (B) *Chelan* (C) *Karinadan* (D) *Nadan* (E) *Muvattupuzha Local*

leaves, which have more market preference were produced by *Puthukodi*, *Nadan* and *Muvattupuzha Local*. High brittleness of leaves in *Karinadan* and low brittleness of leaves in *Chelan* could have reduced their marketability.

5.2.1.1.6 Leaf Color

Light green leaves were the unique character of *Chelan* whereas dark green leaves were the character of *Karinadan*. The remaining three cultivars *viz.*, *Puthukodi*, *Nadan* and *Muvattupuzha Local* produced green leaves (Plate 9). Light green leaves and very dark green leaves generally have less consumer preference. The dorsal surface of leaf lamina of all cultivars showed slight anthocyanin pigmentation along main veins and at the point of insertion of petiole with leaf lamina.

5.2.1.1.7 Leaf Lamina Shape

Leaf lamina shape varied with different cultivars. Four types of leaf lamina namely ovate, cordate, ovate elliptic and ovate lanceolate were seen in leaves from the lateral branches of the cultivars. Mostly ovate elliptic lamina shape was observed in the leaves of *Puthukodi* and *Muvattupuzha Local*. *Karinadan* and *Nadan* mostly produced ovate lanceolate leaf lamina. *Chelan* was unique in the group to produce ovate leaf lamina. Chaveerach *et al.* (2006) observed ovate lamina for betel vine leaves whereas Pariari and Imam (2012a) observed cordate leaf lamina.

5.2.1.1.8 Leaf Base Shape

Generally three leaf base shapes were seen in betel vine cultivars. Cordate leaf base was most common except in *Chelan* (Plate 10). As in the case of other qualitative characters, in leaf base shape also, *Chelan* was different from other cultivars. Generally *Chelan* showed distinguishable round leaf base. Very rarely, *Chelan* produced leaves with acute leaf base. Other cultivars like *Puthukodi*, *Karinadan*, *Nadan* and *Muvattupuzha Local*, mostly produced cordate leaf base.

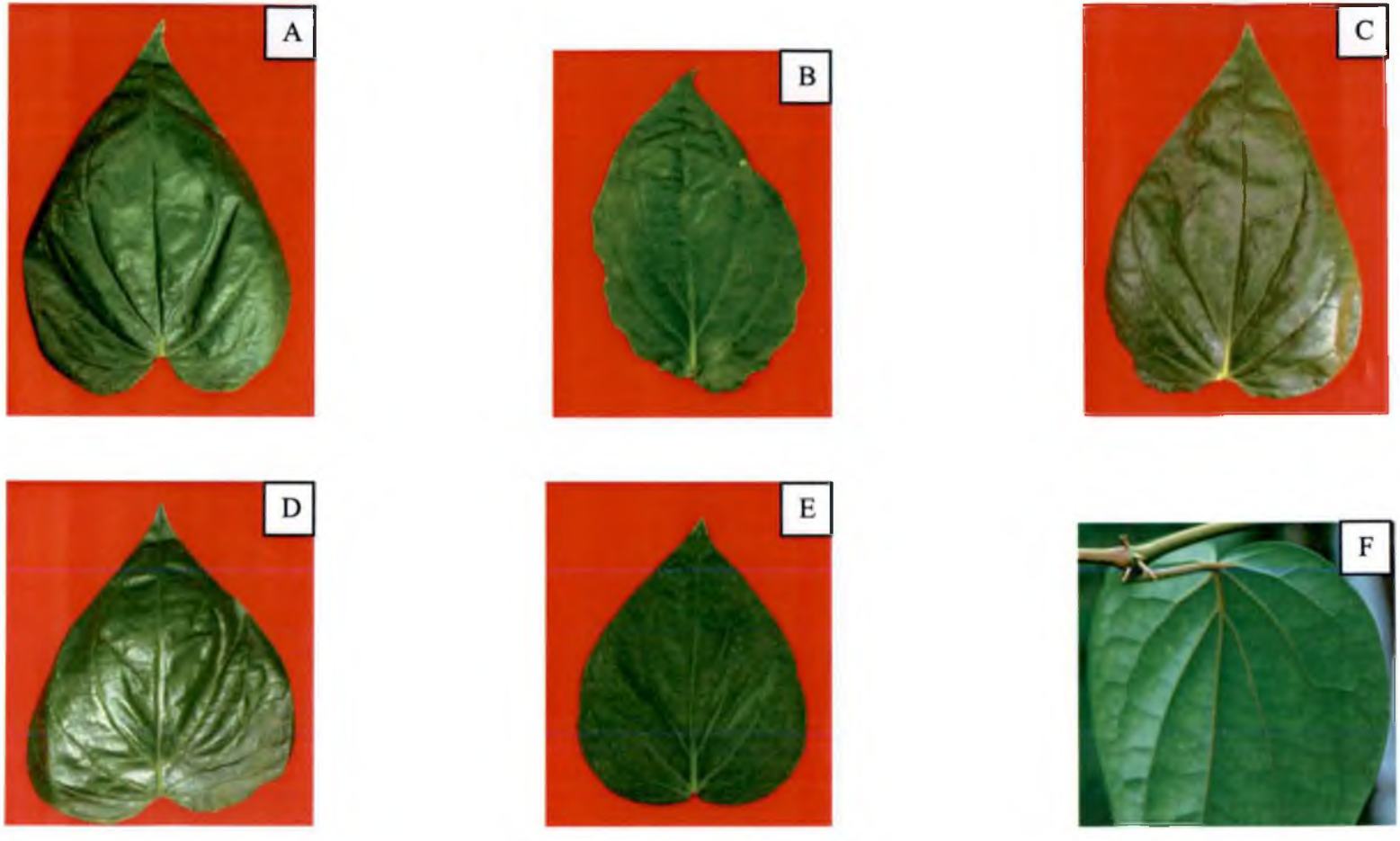


Plate 9. Leaf color in betel vine: (A) *Puthukodi* – Green (B) *Chelan* – Light green (C) *Karinadan* – Dark green (D) *Nadan* – Green (E) *Muvattupuzha Local* - Green (F) Anthocyanin pigmentation on dorsal surface

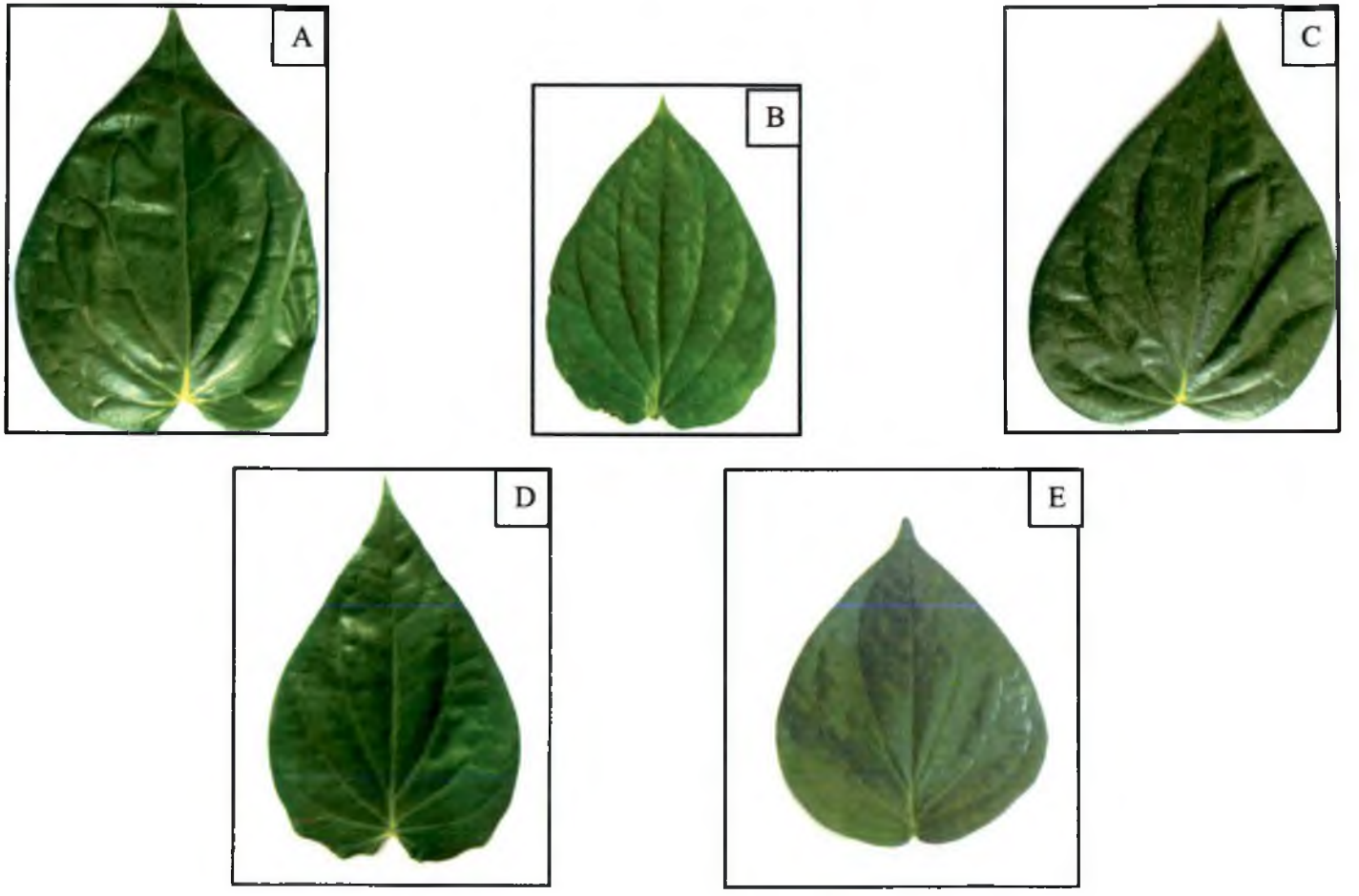


Plate 10. Leaf base shapes in betel vine: (A) *Puthukodi* – Cordate (B) *Chelan* - Round (C)*Karinadan* – Cordate (D) *Nadan* - Cordate (E) *Muvattupuzha Local* - Cordate

Nadan and *Muvattupuzha Local* rarely produced leaves with round leaf base. Chaveerach *et al.* (2006) found cordate base in *P. betle*. In black pepper, Sanchu (2000) reported cordate and round leaf bases.

5.2.1.1.9 Leaf Apex Shape

Leaf apex shape varied among cultivars. Three types of leaf apex namely accuminate, apiculate and aristulate were seen in leaves from the lateral branches of the cultivars (Plate 11). Leaf apex of *Nadan* and *Karinadan* was accuminate while aristulate leaf apex was observed for *Chelan* and *Puthukodi. Muvattupuzha Local* had apiculate leaf apex. However slight variations from these cited shapes of leaf apex were also observed. Repeated studies are required to confirm leaf apex shape in each cultivar.

From the above mentioned morphological characters it is clear that *Chelan* is a very distinct, easily distinguishable cultivar seen in Malappuram district with light green leaves, wavy leaf margin, ovate leaf lamina and round leaf base. The internodal color of orthotropic shoot was light green with purple tinge. The lateral branches were semierect. However *Puthukodi*, *Nadan* and *Muvattupuzha Local* were morphologically similar to some extent showing green internode with purple color at nodal region of orthotropic shoots, purple shoot tip, even leaf margin, medium leaf brittleness and green leaves. The shoot tip showed dark purple colour with green broken stripes in tender stem. The lateral branches were generally hanging.

5.2.1.2 Quantitative Characters

5.2.1.2.1 Plant Height

Chelan showed significantly high value for plant height at all stages of crop growth; might be due to higher growth rate. *Karinadan* recorded lower plant height at all stages (Fig. 10). At 90 DAL, all cultivars recorded a plant height of more than

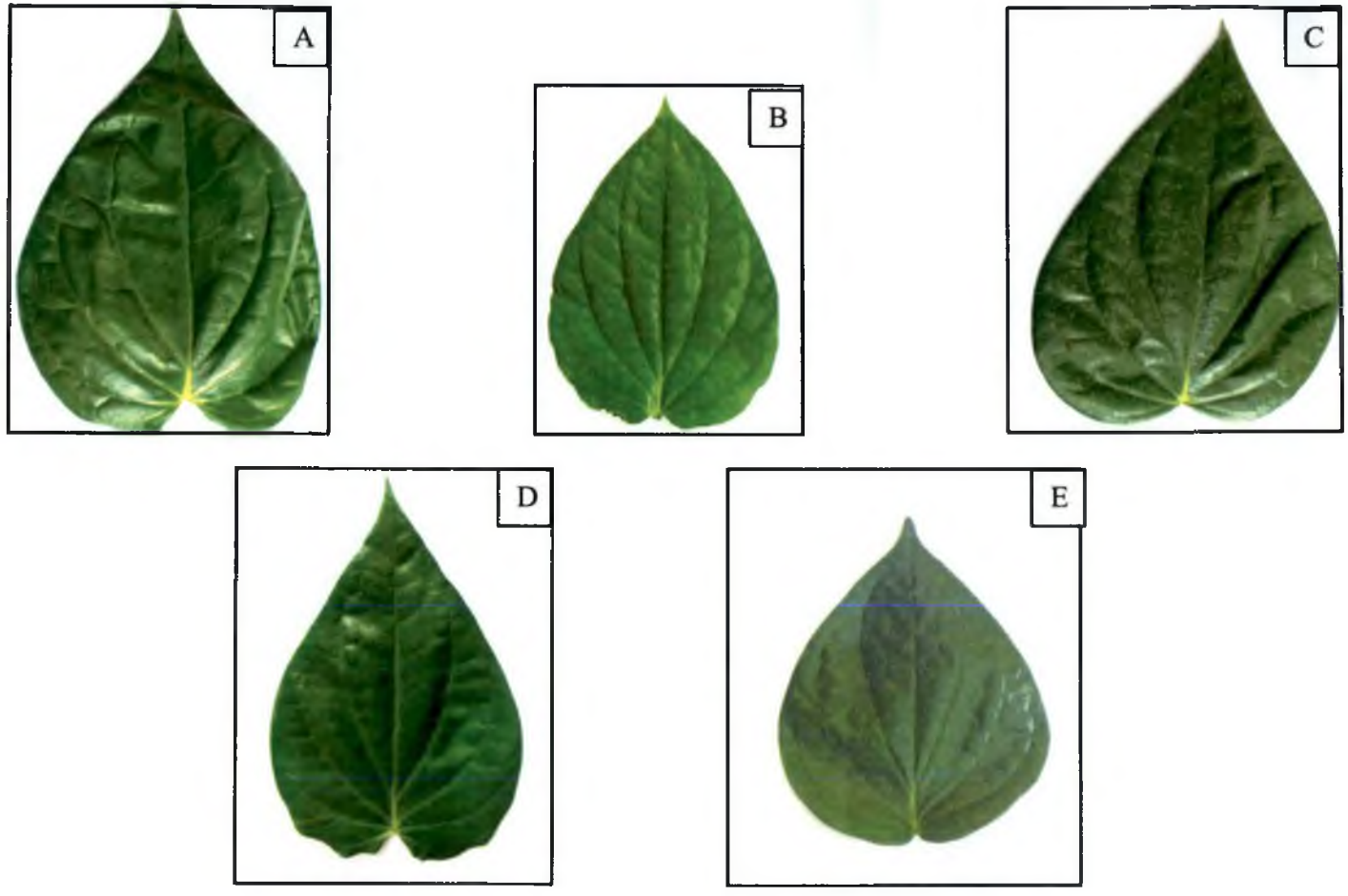


Plate 11. Leaf apex shapes in betel vine: (A) *Puthukodi* – Aristulate (B) *Chelan* – Aristulate (C) *Karinadan* - Accuminate (D) *Nadan* - Accuminate (E) *Muvattupuzha Local* - Apiculate

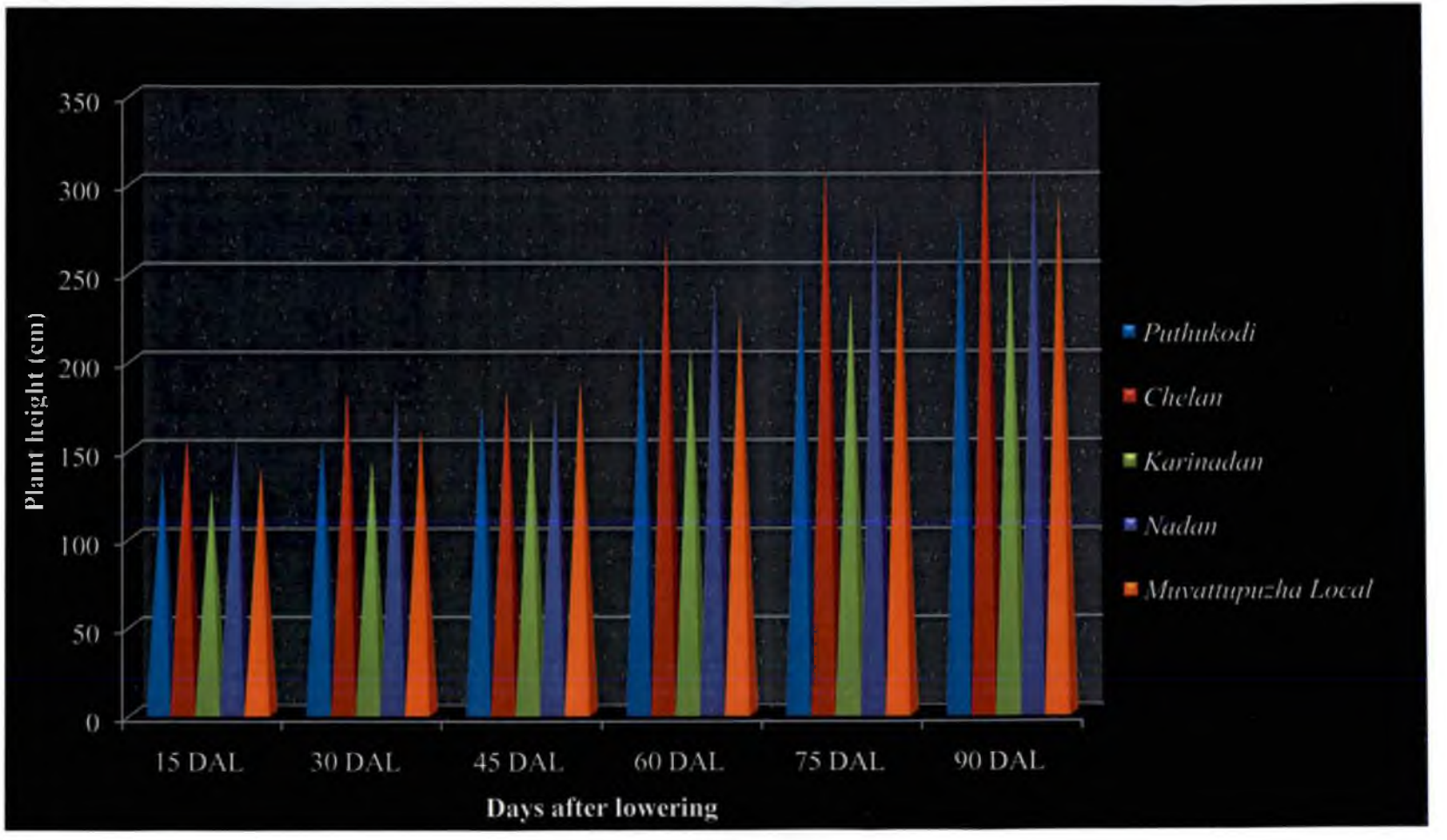


Fig. 10. Plant height at different growth stages of betel vine cultivars of Malappuram district during 2013 - 14

260 cms. A study conducted by Pariari and Imam (2012a) revealed a vine length of 145.37 cm.

5.2.1.2.2 Total Number of Leaves

Faster growth rate in *Chelan* as evidenced by highest plant height, led to highest (280.35 per vine) total number of leaves at 90 DAL. On the other hand *Karinadan*, might be due to slow growth rate, produced lowest number (175.68 leaves per vine) of leaves (Fig. 11). The work conducted by Pariari and Imam (2012a) showed higher annual leaf yield of 58.56 leaves per vine in *Simurali Deshi*. The number of leaves per vine at 90 DAL in *Tirur betel vine* cultivars ranged from 175.68 – 280.35. In the present study, method of trailing adopted in *Koottakodi* system would have resulted in higher leaf number per vine than the annual leaf yield (58.56 leaves per vine) in *Simurali Sanchi* recorded in '*bareja*' system by Pariari and Imam (2012a).

5.2.1.2.3 Number of Lateral Branches

As in the case of plant height and total number of leaves, number of lateral branches in *Chelan* recorded significantly high values (Fig. 12). This would be due to faster growth rate in this cultivar. *Karinadan* recorded less number of lateral branches as in other characters. *Chelan* recorded 16.88 lateral branches while *Karinadan* recorded 10.88 lateral branches at 90 DAL. *Muvattupuzha Local* recorded lower number of lateral branches than *Nadan* and more number than *Puthukodi*.

5.2.1.2.4 Number of Nodes per Lateral Branch

Longer vine with more number of lateral branches and more nodes in lateral branches is a desirable character in betel vine as it can produce more number of leaves. As in the case of other growth parameters, here also *Chelan* produced highest number of nodes per lateral branch (43.95) and *Karinadan* produced lowest number of nodes per lateral branch (26.28) at 90 DAL (Fig. 13). *Nadan* and *Muvattupuzha*

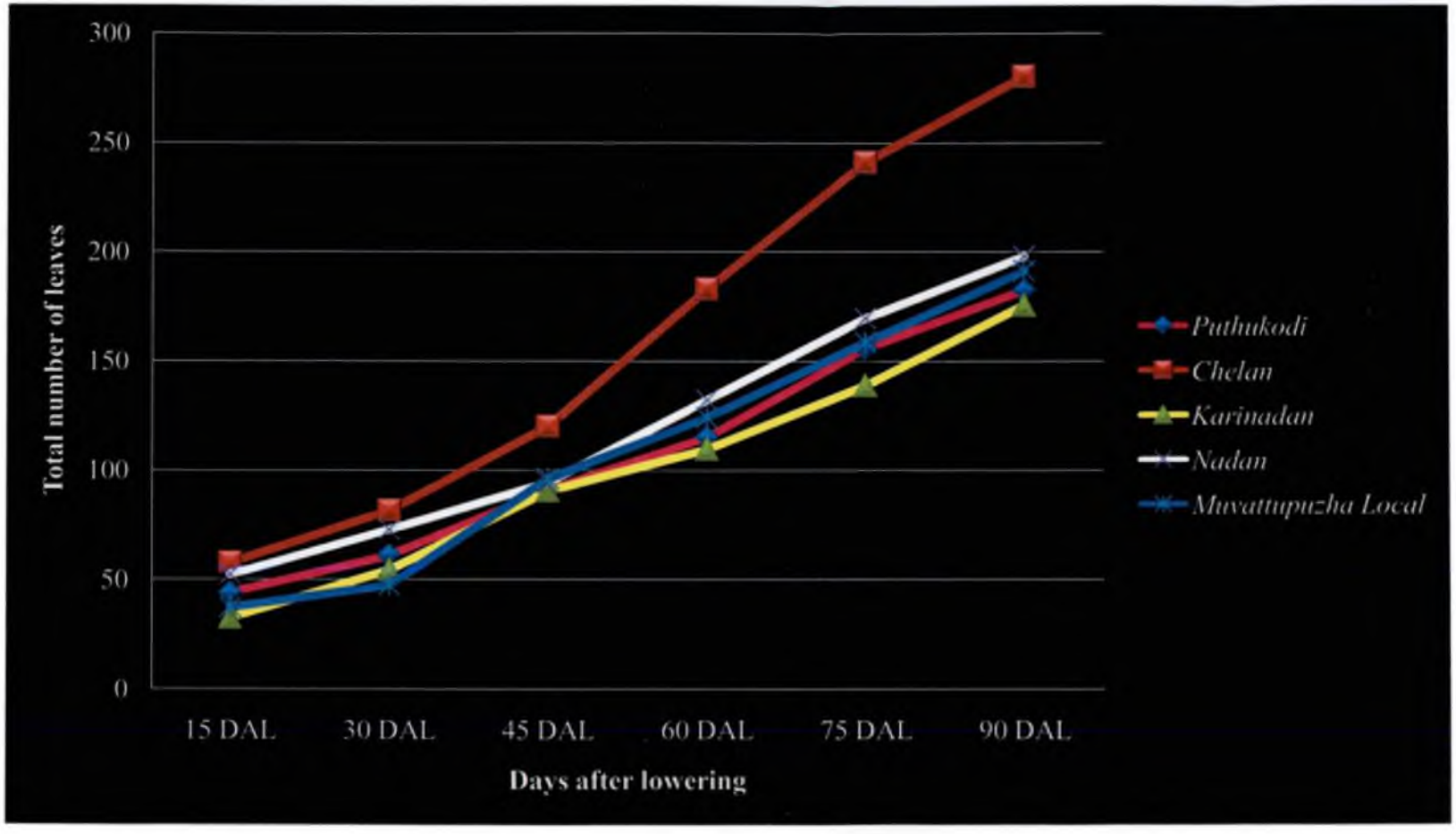


Fig.11. Total number of leaves at different growth stages in betel vine cultivars of Malappuram district during 2013 - 14

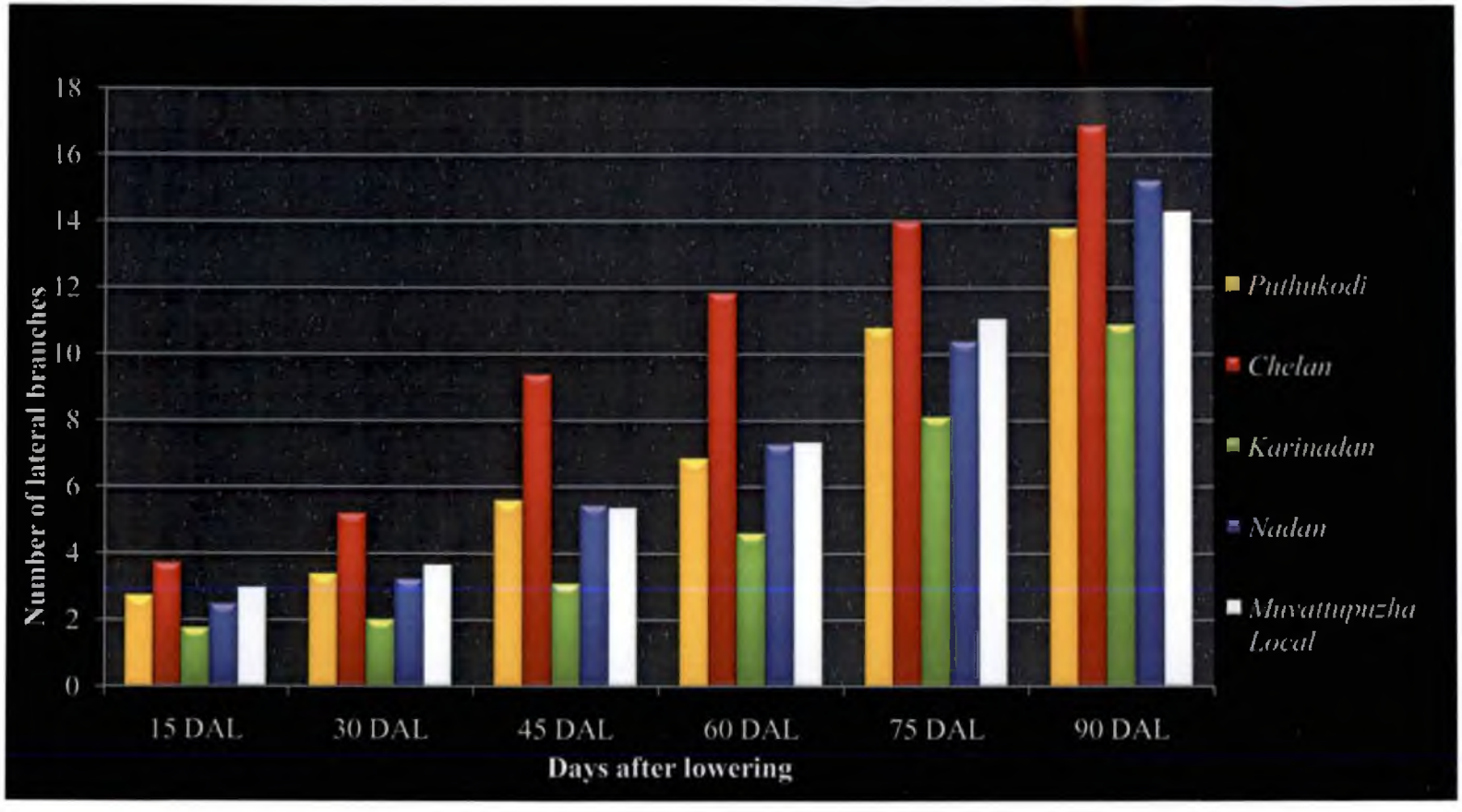


Fig. 12. Number of lateral branches at different growth stages of betel vine cultivars of Malappuram district during 2013 - 14

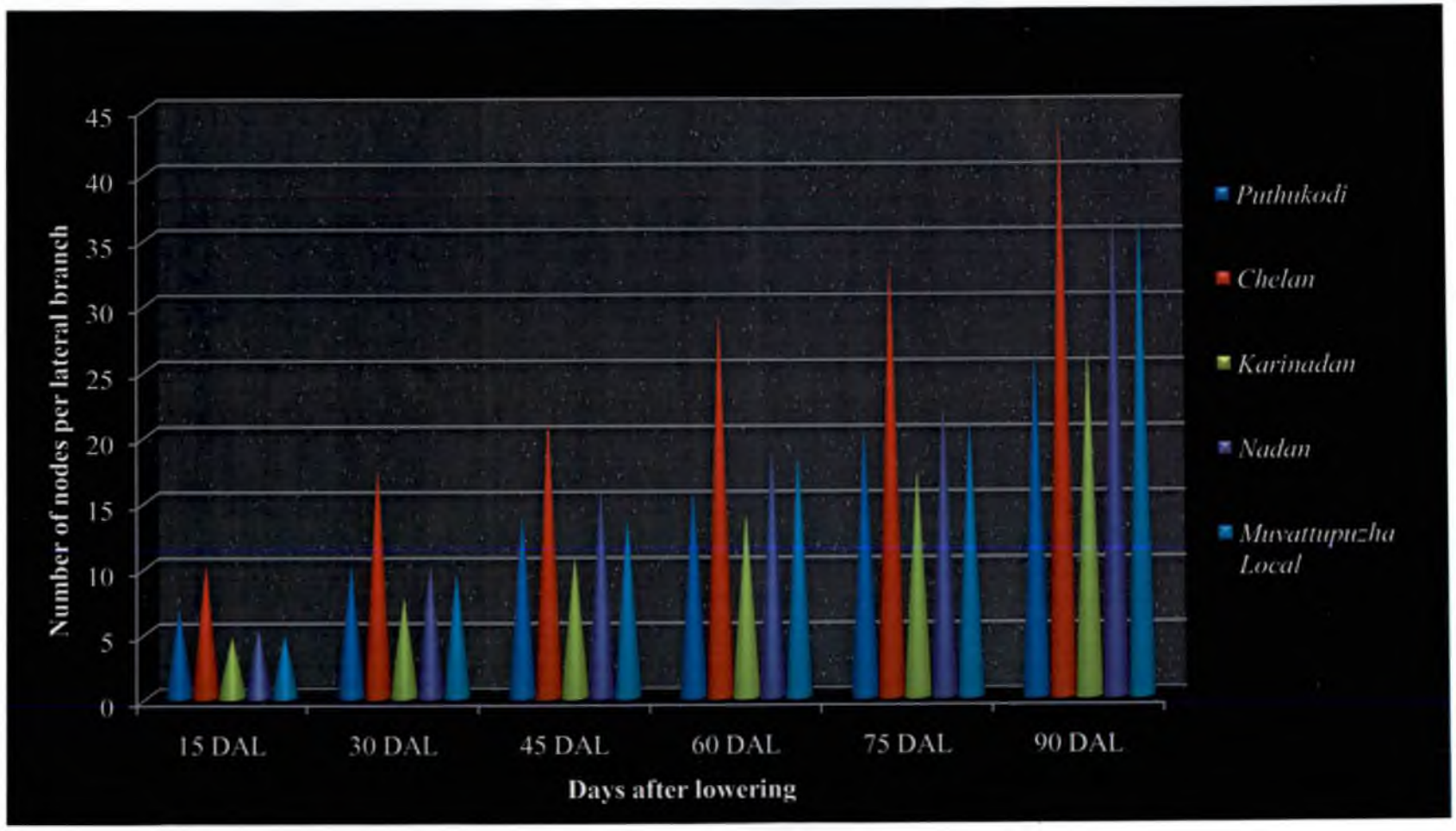


Fig. 13. Number of nodes per lateral branch at different growth stages of betel vine cultivars of Malappuram district during 2013 - 14

Local produced almost same number of nodes per lateral branch during the same period of crop growth. An experiment on black pepper revealed that number of nodes per lateral branch ranged from 21 – 44 (Sreedevi *et al.*, 2005).

5.2.1.2.5 Number of Leaves per Lateral Branch

The data on number of leaves per lateral branch and number of nodes per lateral branch were same because from each node only one leaf was produced. Number of leaves per lateral branch increased in tune to increase in days after vine lowering. *Chelan,* with faster growth rate, produced more number of leaves at all growth stages. *Karinadan* produced lesser number of leaves at all growth stages (Fig. 14).

Study on plant growth parameters, revealed that *Karinadan* had a slow growth rate indicated by less plant height, less number of total leaves, less number of lateral branches, less number of leaves per lateral branch and less number of nodes per lateral branch. On the other hand, *Chelan* had a higher growth rate indicated by higher plant height, more number of total leaves, more number of lateral branches, more number of leaves per lateral branch and more number of nodes per lateral branch.

5.2.1.2.6 Days to Lateral Branching

Leaves from lateral branches have more market value and are locally called as '*Kanni vettila*'. So, days to lateral branching is important with regard to production of marketable leaves. More days for the emergence of lateral branch would result in less leaf yield. Among the cultivars, *Karinadan* with slow rate of vine growth, took more number of days (151.25) for the commencement of lateral branching. This cultivar expressed less number of total leaves also. *Chelan* with more plant height at all stages, showed early emergence of first lateral branch. This cultivar had expressed higher number of leaves at all growth stages.

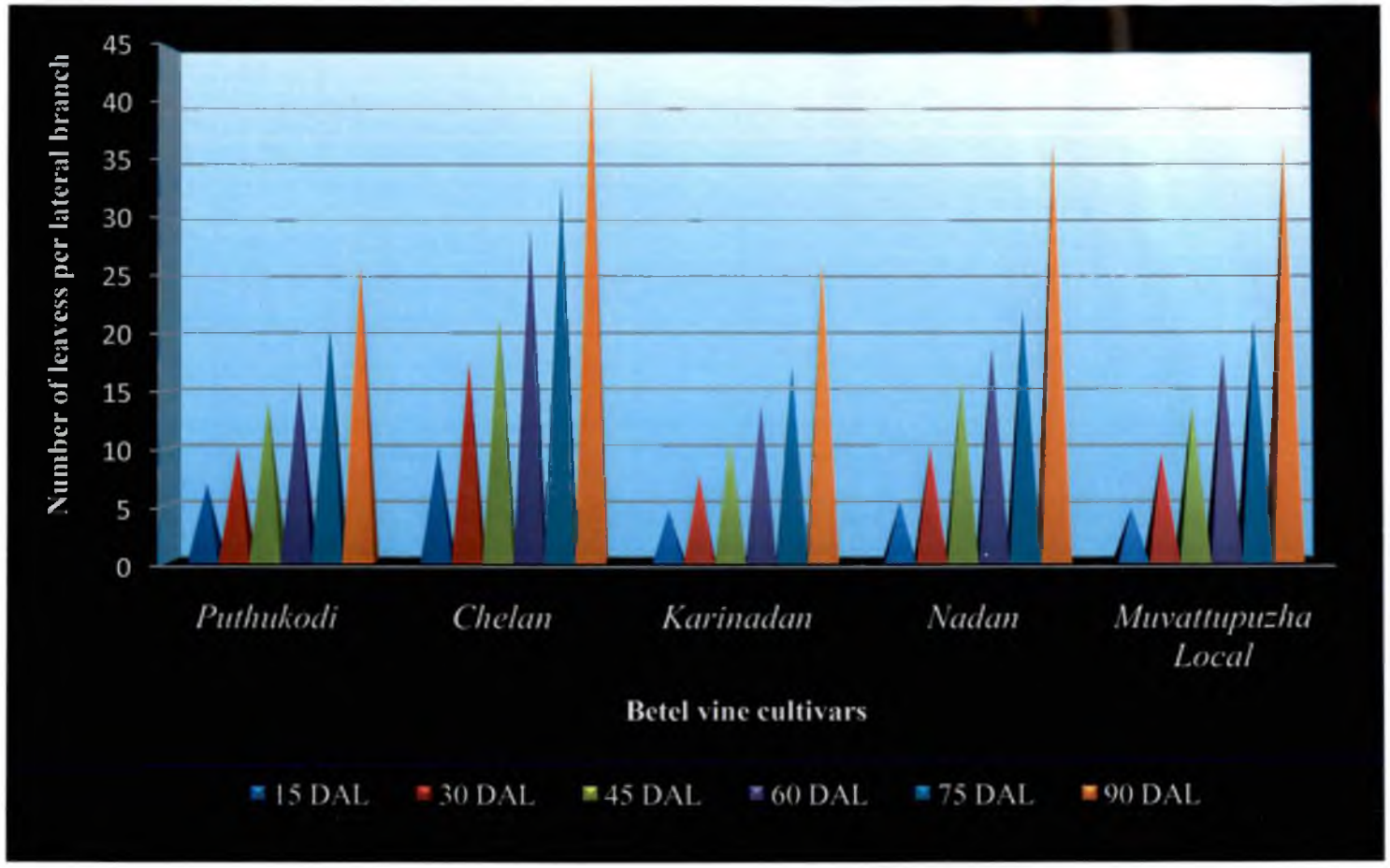


Fig. 14. Number of leaves per lateral branch at different growth stages of betel vine cultivars of Malappuram district during 2013 - 14

5.2.1.2.7 Days between Lateral Branch Emergence

Emergence of two consecutive lateral branches occurred very fastly in *Chelan* compared with other cultivars. *Chelan* with more plant height and less days to lateral branching recorded lesser days (9.35) between lateral branch emergence, indicating a faster growth rate in this cultivar. *Karinadan* with less plant height and more number of days for lateral branching took more days (26.35) between emergence of two lateral branches.

5.2.1.2.8 Angle between Leaf Petiole and Orthotropic Shoot

The angle between orthotropic shoot and leaf petiole is an important character in distinguishing different subtypes of *Piper* sp. Except *Chelan*, all other cultivars showed narrow angle between orthotropic shoot and leaf petiole. *Chelan* had wide angle between orthotropic shoot and leaf petiole. The wide angle in *Chelan* resulted in spreading nature of leaves. Krishnamurthy *et al.* (2010) suggested that in black pepper, the leaf angle should be more at the bottom compared to top for filtering more light to the bottom canopy.

5.2.1.2.9 Leaf Length

Leaf length varied significantly among cultivars with highest leaf length (19.48 cm) in *Karinadan*, the cultivar with slow growth rate, less lateral branches and less leaf production. On the other hand, lowest leaf length (14.78 cm) was observed in *Chelan*, the cultivar with more number of leaves and lateral branches. In general, *Karinadan*, *Nadan* and *Puthukodi* showed long leaves (Plate 12). Pariari and Imam (2012a) also observed variation in leaf length between 14.75 – 16.73 cm among six cultivars namely *Ghanagette*, *Simurali Sanchi*, *Simurali Jhal*, CARI -2, CARI -6 and *Sanchi*.

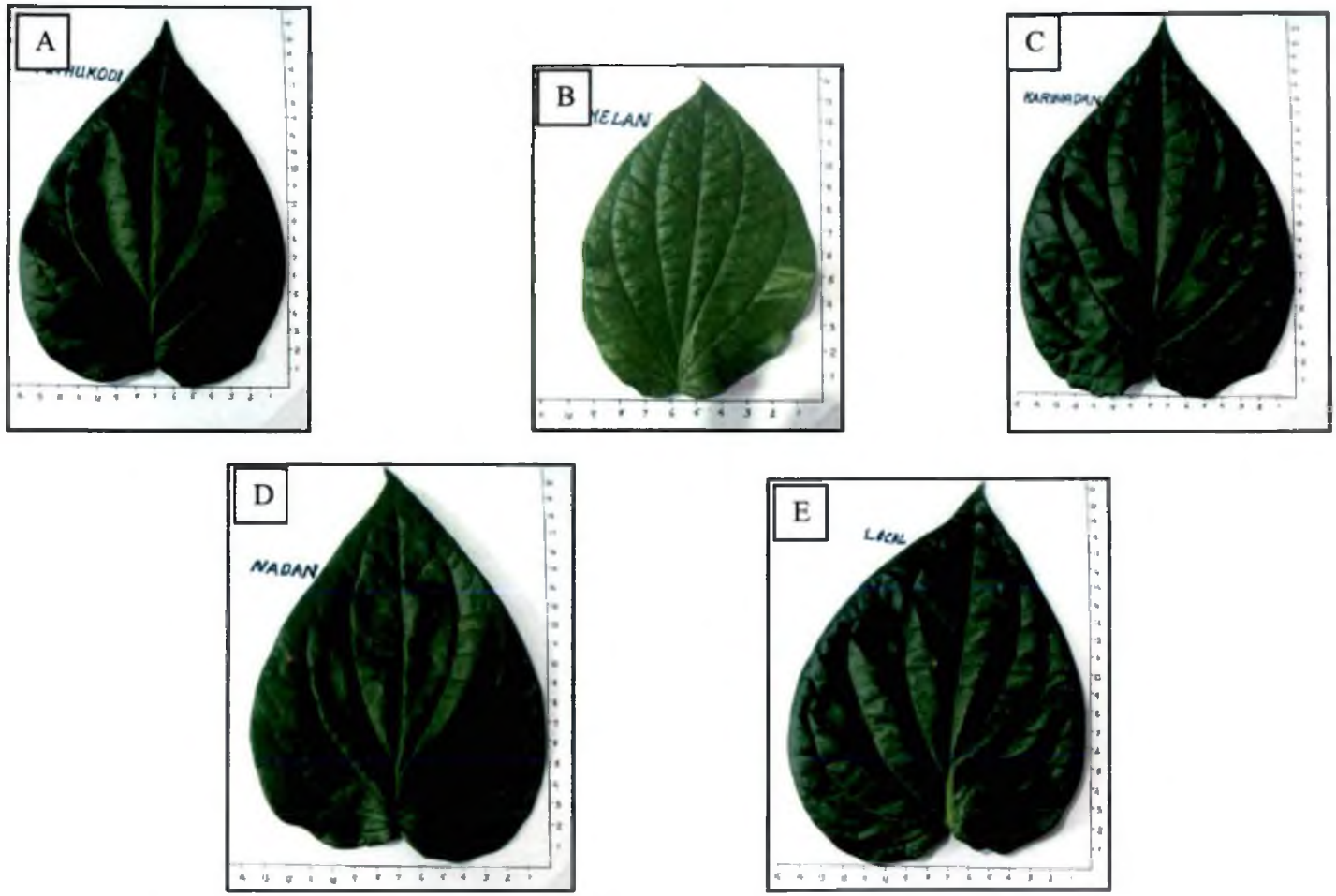


Plate12. Variation in leaf length and width in betel vine: (A) *Puthukodi* (B) *Chelan* (C) *Karinadan* (D) *Nadan* (E) *Muvattupuzha Local*

5.2.1.2.10 Leaf Width

The trend exhibited for leaf length was also seen in leaf width also. Leaf width was more in *Karinadan* (14.40 cm) and less (11.50 cm) in *Chelan*. *Puthukodi* showed a leaf width of 12.63 cm. Optimum leaf length and leaf width are always preferred by traders to reduce handling difficulties. Highest leaf length and leaf width of *Karinadan* could cause handling difficulties during harvesting, packing and transportation. Leaves with more leaf length and leaf width are less preferred by consumers also. In addition to this, less number of leaves and lateral branches could reduce potential of this cultivar for commercial cultivation.

5.2.1.2.11 Leaf Area

Among the cultivars, maximum leaf area was recorded in *Karinadan* (196.23 cm^2) and minimum in *Chelan* (134.25 cm^2). The bigger leaves as well as small leaves of these cultivars would have added to their less acceptance in the market. *Muvattupuzha Local* (164.25 cm^2) and *Puthukodi* (156.13) recorded medium leaf area. Pariari and Imam (2012b) reported leaf area from 114.17 to 129.00 cm^2 for betel vine cultivars grown with different sources of organic manures.

5.2.1.2.12 Leaf Weight

Leaf weight is an important criteria, deciding the market potential of betel leaf. *Karinadan* and *Puthukodi* produced leaves having maximum fresh weight while *Chelan* produced thin leaves with low weight. Pariari and Imam (2012b) reported a leaf weight of 3.64 g for *Simurali Sanchi* which was close to the fresh leaf weight of *Muvattupuzha Local* (3.95 g). The high fresh weight of leaves recorded in *Puthukodi*, would have been a reason for its high consumer acceptance.

5.2.1.2.13 Leaf Weight per Unit Area

Leaf fresh weight per unit area ranged from 0.022 - 0.028 g/cm^2 with maximum in *Puthukodi* and minimum in *Chelan*. Pariari and Imam (2012b), in their field experiment, observed maximum fresh weight of 328.83 g for 100 leaves. Leaf weight is considered as an important parameter in the betel vine markets (Sumanasena *et al*., 2005). High fresh leaf weight might be one of the reasons for the high export potential of *Puthukodi* and on the other hand, low fresh leaf weight and small leaf size are some of the probable reasons for less preference for leaves of *Chelan*. Leaves with low fresh weight could easily wither or dry, leading to low preference by consumers. Higher fresh leaf weight may indirectly lead to higher shelf life.

5.2.1.2.14 Leaf Petiole Length

In the present study, a range of 2.75 - 4.85 cm was recorded as leaf petiole length among the cultivars. Petiole length was significantly less in *Karinadan* and *Puthukodi*, significantly high in *Chelan*. Reddy (1996) reported 5.2 - 6.6 cm lengthy leaf petioles among the cultivars. Depetiolated betel leaves had higher shelf life than leaves with petioles irrespective of seasons (Pariari and Imam, 2012a). Farmers usually depetiolate the leaves before marketing which leads significant loss of biomass. This indicates that petiole length is an unfavourable character. So leaves with short petioles are always preferred. To harvest maximum light, varieties of black pepper with lower leaf petiole length at the top and increased petiole length at the bottom both in runner as well as lateral branch is ideal (Krishnamurthy *et al*., 2010).

5.2.1.2.15 Leaf Tip Angle

Narrow leaf tip angle (35.20^0) was seen in *Puthukodi* and *Chelan*. *Nadan* and *Muvattupuzha Local* showed medium leaf tip angle. Leaf tip angle in *Karinadan* was wide. Leaf tip angle determines the shape of leaf apex, and may act as morphological

character for cultivar identification. Detailed study on different cultivars to reveal the diversity in leaf tip angle is necessary.

From the above discussion on quantitative morphological characters it was evident that the cultivar *Chelan* showed more vegetative growth with lesser days to lateral branch initiation and lesser days between two consecutive lateral branch emergence. However, leaves of *Chelan* were distinct and small with less leaf length, less leaf width, less leaf area, less leaf weight per unit area and more petiole length reducing the market demand of the leaves. *Karinadan* showed less vegetative growth with more days to lateral branch emergence. The leaves were bigger in size with more leaf length, more leaf width, more leaf area and less petiole length.

5.2.1.2.16 Floral Morphology

During the study period cultivars showed profuse flowering. This was contradictory to the observation (CSIR, 1969) that flowering and fruit setting was rare in Indian climate. Initiation of spike was noticed from five months after planting. Observations on spike characters revealed that *Chelan* was male cultivar whereas all other cultivars under study were female. This is contradictory to report that usually male plants are cultivated throughout India to harvest green leaves (Lakshmi and Naidu, 2010).

Spikes appeared first in *Chelan* and followed by other cultivars. Spikes were axillary and opposite to leaf (Plate 13). Many individual, sessile florets were compactly arranged on the inflorescence axis. A bract subtended each floret, both in male and female spikes (Plate 14 and 15).

Immature female spikes were green in color. At the stage of anthesis, sessile stigmatic lobes became white clearly visible and white in color. Generally six to nine stigmatic lobes were seen in female cultivars. The stigmatic lobes became black

Plate 13. Flowering shoot tip of betel vine

Plate 14. Male spike of betel vine

Plate 15. Female spike of betel vine

coloured towards the end of spike maturation (Plate 16). Male spikes were yellow in color and the two black stamens of each floret protruded at the stage of anthesis (Plate 17).

5.2.1.2.17 Spike Length

Spike length varied significantly among cultivars with maximum spike length in *Chelan* (male cultivar) and minimum in *Nadan*. Extent of spike length varied from 2.40 to 5.97 cm. Spike was with medium length in all female cultivars. According to Chaveerach *et al.* (2006), 3.0 to 12.0 cm and 2.5 to 4.0 cm lengthy male and female spikes respectively were produced in betel vine.

5.2.1.2.18 Spike Diameter

Spike diameter showed significant variation in different betel vine cultivars. Normally, spikes of the *Chelan* (male cultivar) were slender compared with medium sized spikes in all female cultivars. In this study, male cultivar produced long slender spikes and female cultivars produced spikes with medium length and diameter.

5.2.1.2.19 Spike Peduncle Length

Generally spike peduncle length was lower in male cultivar when compared to female cultivars. Spike peduncle length was highest (3.77 cm) in *Puthukodi* (female cultivar) and lowest (3.07 cm) in *Chelan* (male cultivar). Chaveerach *et al.* (2006) reported that peduncle length of betel vine varied from 2 - 3 cm. Indirect relationship was seen in between spike peduncle length and spike length. *Chelan* produced longer spikes with shorter peduncles.

Plate 16. Female spike in different stages (A) Immature (B) At anthesis (C) Towards spike maturation

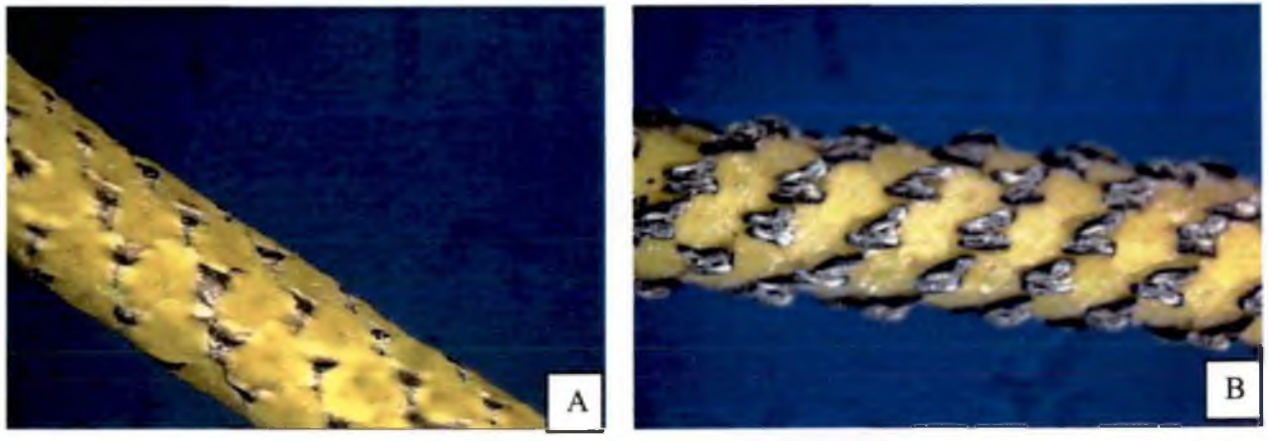


Plate 17. Male spike in different stages (A) Immature (B) Mature

5.2.2 Biochemical Characterization

5.2.2.1 Betel Leaf Oil

The flavor of betel leaf is due to the presence of essential oil. It is reported that essential oil contributed to the medicinal property of betel vine (Pradhan *et al.*, 2013). Betel vine was reported to have immunosuppressive activity and antimicrobial property (Banerjee, 2012). Essential oil content is an important character for identification of types for commercial cultivation with the objective of oil extraction, as betel oil fetches high price (186 dollar per 100 ml) in international markets (New Directions Aromatics, nd).

5.2.2.1.1 Betel Leaf Oil - Yield

Cultivars showed difference in yield of essential oil from leaves. Comparatively higher yield (0.57 per cent v/w) of essential oil was obtained from the check variety, *Muvattupuzha Local* and this could have contributed to the high pungency in this cultivar. The high content of essential oil makes *Muvattupuzha Local* cultivar more suited for oil extraction. *Chelan* recorded the minimum content of 0.45 per cent (v/w) of essential oil and probably this might be the reason for low pungency in this cultivar. Sugumaran *et al.* (2011) obtained an essential oil yield of 0.31 per cent in volume by weight basis in the cultivar *Vellaikodi*. Rani and Ramamurthy (2012) obtained 0.08 to 0.20 per cent (v/w) of essential oil from betel leaf. Guha (2006) found that *Mita*, *Sanchi* and *Bangla* varieties of betel vine had about 2, 1.70 and 0.80 per cent essential oil respectively. The variations in the essential oil content in different studies might be due to varietal difference, cultural practices followed, plant part used, lab conditions *etc.* More studies are required to confirm the content of essential oil in different cultivars.

5.2.2.1.2 Identification of Betel Leaf Oil Components

In the present study, essential oil from most of the cultivars (Fig. 15, 16, 17, 18, 19) showed the presence of 55 – 56 components. *Karinadan* showed the presence of less components (39) in its essential oil. Eugenol was the major component of essential oil in all cultivars under study and it ranged from 11.02 to 20.80 per cent in different cultivars with highest content of 20.80 per cent in *Chelan*. This is confirmation with the result of Guha (2003), who reported eugenol as the chief ingredient of essential oil in betel vine with a content of 29.50 per cent. Eugenol had antifungal and antioxidant properties (Pradhan *et al.*, 2013). Baliga *et al.* (2011) reported eugenol in betel vine as an excellent antimutagen. It could be used as a local anesthetic for tooth ache (Pradhan *et al.*, 2013). In the present study, identification of eugenol as the major component of essential oil confirmed the medicinal properties of this crop. Eugenol with its antioxidant property makes betel vine as a probable candidate in the treatment of dreaded diseases. Moreover eugenol is used in perfumeries, flavorings and medicine as a local antiseptic and anesthetic. Eugenol can be combined with zinc oxide to form a material known as zinc oxide eugenol which has restorative and prosthodontic applications in dentistry (Jadhav *et al.*, 2004). So *Chelan* with highest content of eugenol has more potential in manufacturing of drugs, perfumes and dentistry materials. Balasubramanyam and Rawat (1990) also suggested that eugenol contributed to the clove like aroma of certain cultivars like *Bangla* and *Sanchi*. So it could be assumed that the high content of eugenol could impart mild aroma to this cultivar.

Like eugenol, Methyl isoeugenol in trace amounts, was identified from all the cultivars. Methyl isoeugenol is a natural food flavor and is used for treating mood disorders (Fajemiroye *et al.*, 2011). *Muvattupuzha Local* had the highest content of methyl isoeugenol. Hence this cultivar has potential use in food flavours and medicines.

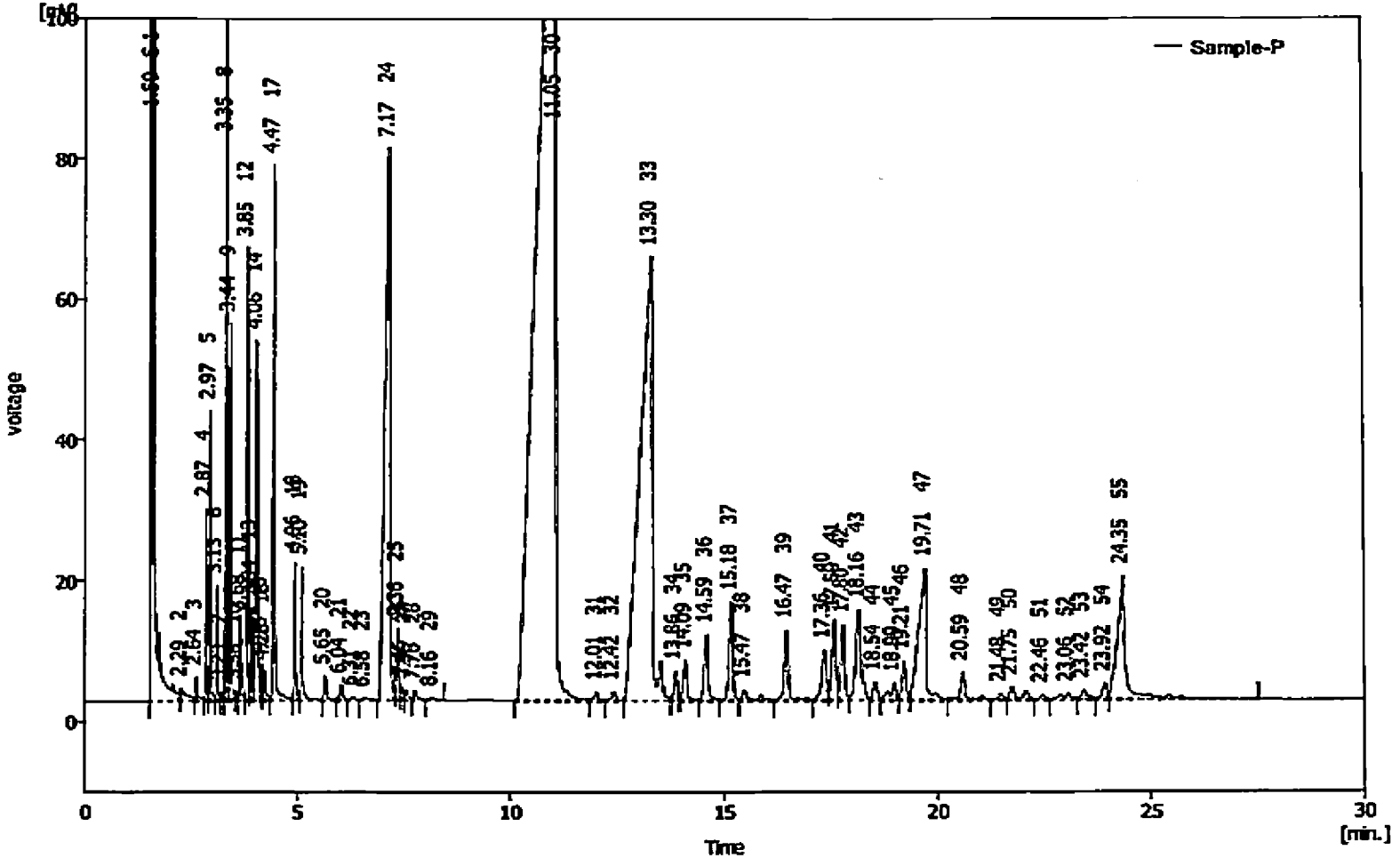


Fig. 15. Chromatogram of essential oil of *Puthukodi* cultivar of betel vine during 2013 - 14

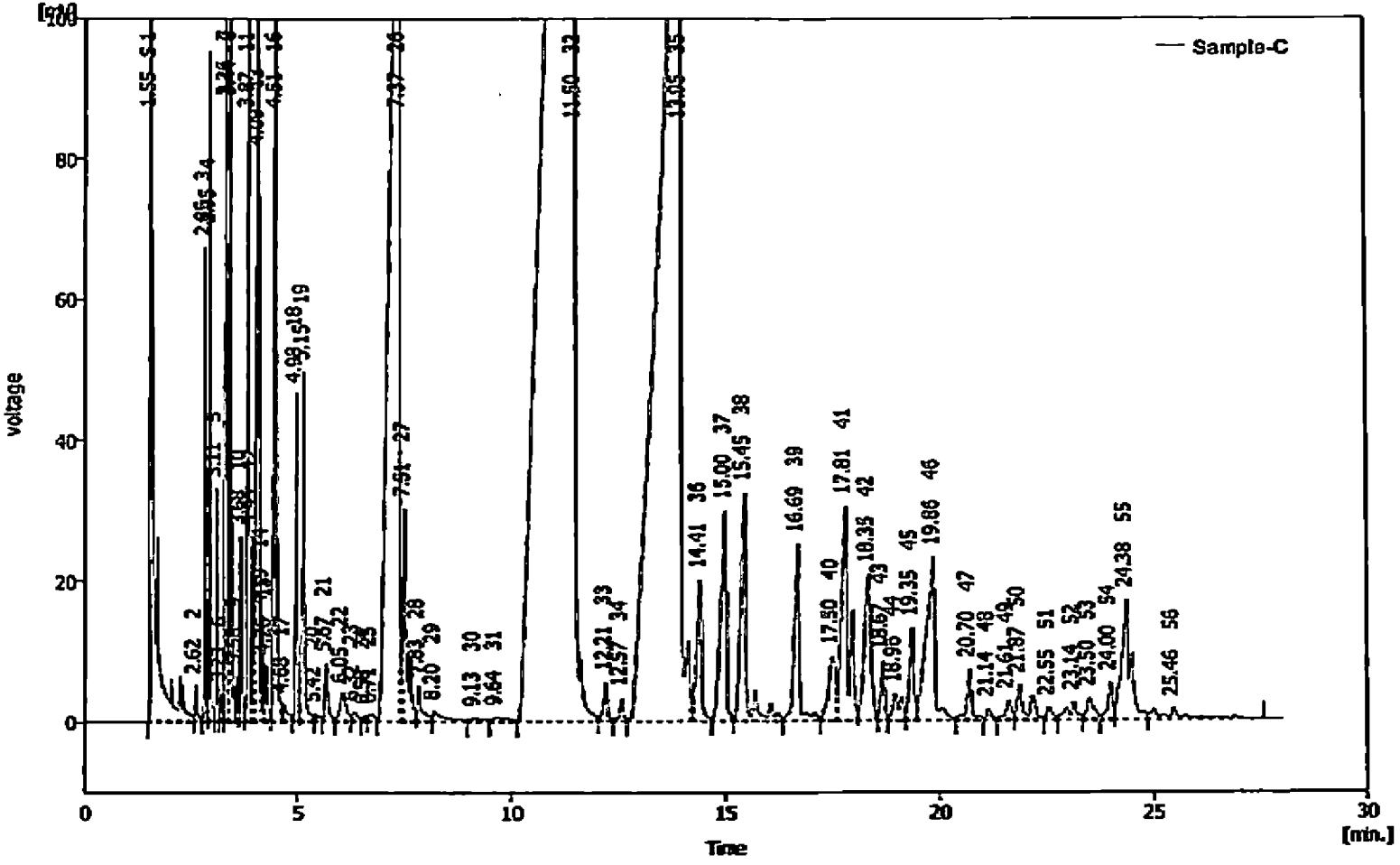


Fig. 16. Chromatogram of essential oil of *Chelan* cultivar of betel vine during 2013 - 14

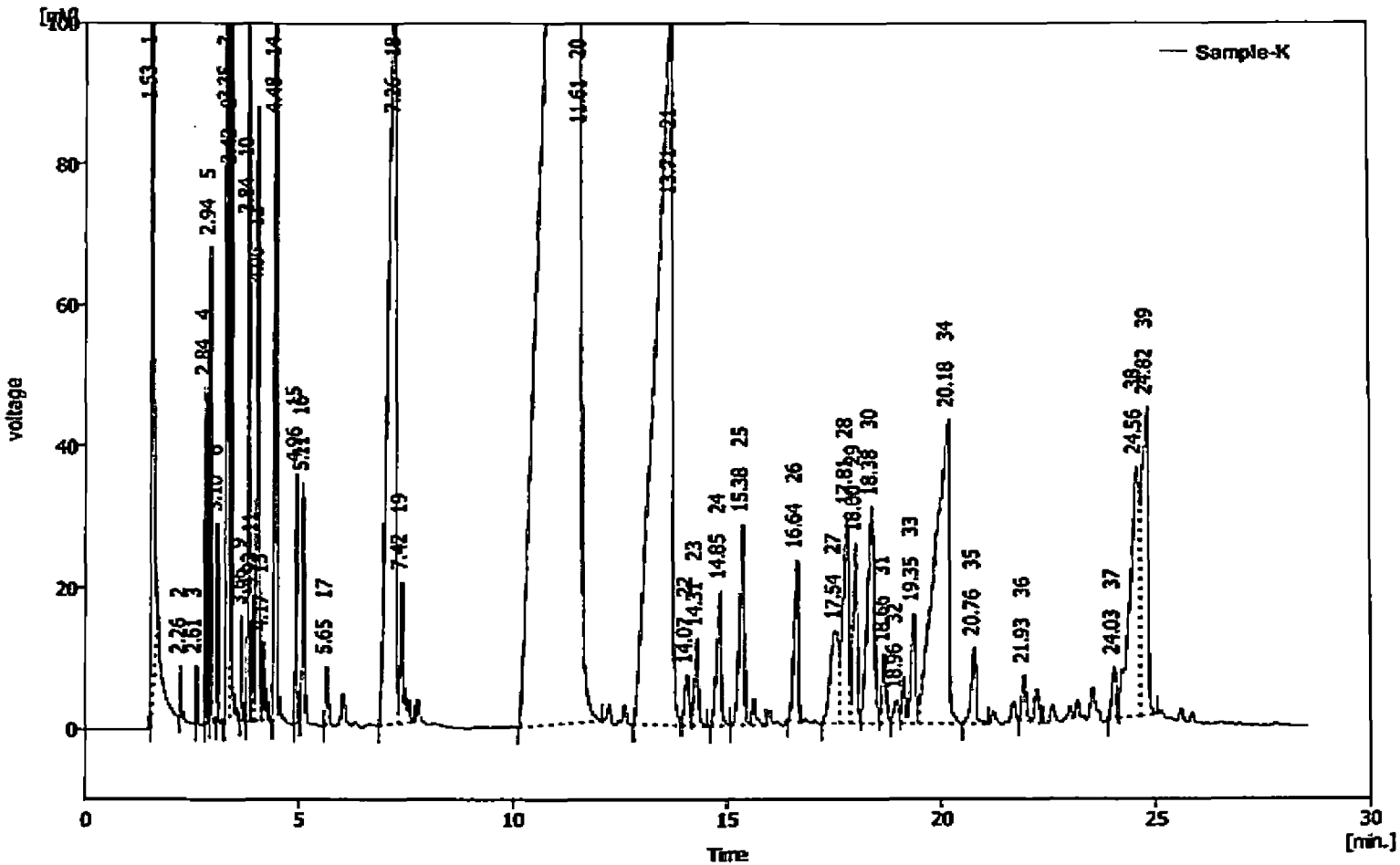


Fig. 17. Chromatogram of essential oil of *Karinadan* cultivar of betel vine during 2013 - 14

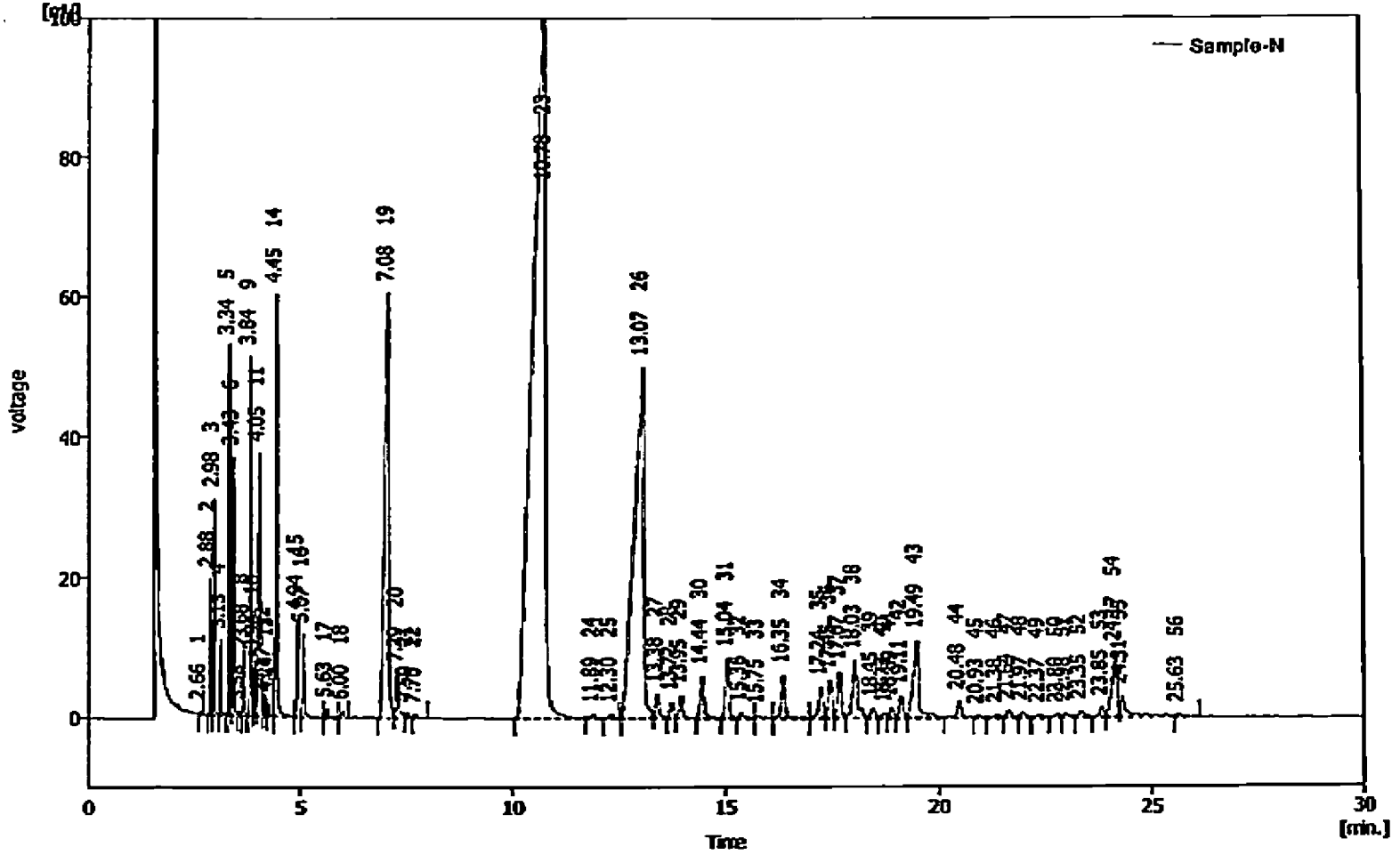


Fig. 18. Chromatogram of essential oil of *Nadan* cultivar of betel vine during 2013 - 14

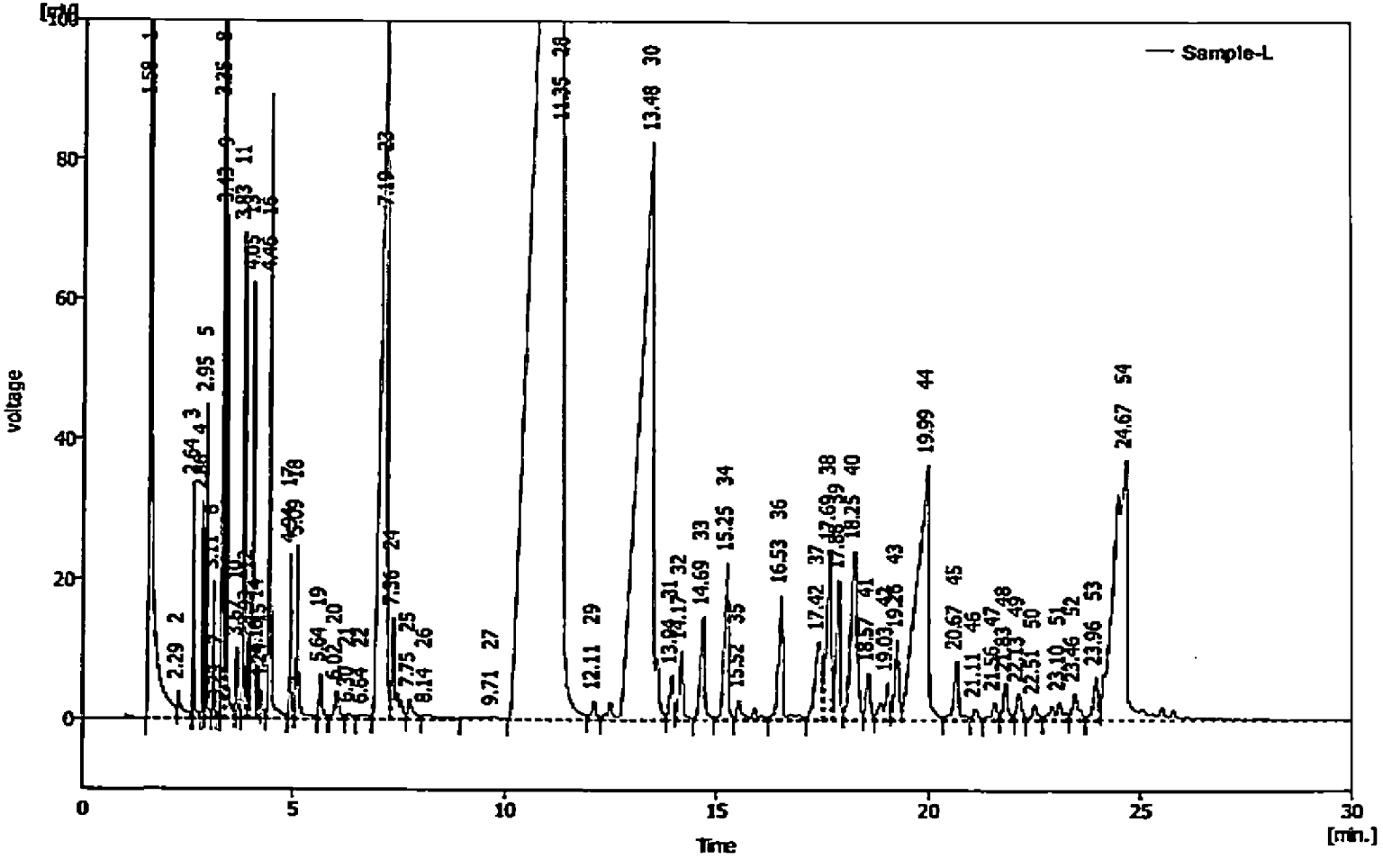


Fig. 19. Chromatogram of essential oil of *Muvattupuzha Local* cultivar of betel vine during 2013 - 14

Content of methyl eugenol was same in *Nadan* and *Chelan*. Methyl eugenol is used in aroma therapy and as massage oil (Government of Canada, 2010). It is also widely used as a fragrant ingredient in perfumes, toiletries and detergents. Methyl eugenol is also used as an insect attractant in combination with insecticides (NTP, 2000; HSDB, 2010). Hence *Nadan* and *Chelan* have more potential to use in perfume industry. Earlier the presence of high content of eugenol in *Chelan* had revealed its potential use in drugs, perfumes and dentistry materials.

Isoeugenol was also present in all cultivars except in *Karinadan* to the range of 0.80 – 1.00 per cent. Isoeugenol had been used in the manufacture of vanillin (Merck, 1996). As a flavoring agent, isoeugenol is added to nonalcoholic drinks, baked foods and chewing gums (National Toxicology Programme, 2010). *Chelan* recorded higher isoeugenol content, indicating its potential value in the production of flavoring agents.

Possible compounds identified from the chromatograms in comparison to known retention time were hydroxychavicol, β caryophyllene and 5-(2-propenyl)-1, 3- benzodioxole. Many studies have reported that hydroxychavicol is a major phenolic compound in the aqueous extract of betel leaves (Nalina and Rahim, 2007; Ali *et al.*, 2010; Pin *et al.*, 2010). Hydroxychavicol said to possess antibacterial (Ramji *et al.*, 2002; Sharma *et al.*, 2009), antioxidant and anticarcinogenic activities (Chang *et al.*, 2002b). The betel leaves were reported to possess anticancerous activity particularly against the tobacco carcinogens (Padma *et al.*, 1989; Chang *et al.*, 2002b; Wu *et al.*, 2004) due to the presence of hydroxychavicol (Amonkar *et al.*, 1989). Baliga *et al.* (2011) reported the use of hydroxychavicol as antimutagen. The possible presence of hydroxychavicol in all the cultivars indicated the medicinal properties of this crop. Betel leaves and betel juice if administered properly can contribute antibacterial and carcinogenic properties. The possible content of hydroxychavicol was more in *Puthukodi*.

Possible content of β caryophyllene in essential oil ranged from 2.80 - 4.30 per cent among the cultivars. This is an FDA approved food additive and contributes to the spiciness of black pepper (Ghelardini *et al.*, 2001).

The other possible component identified in the present study is 5-(2-propenyl)-1, 3- benzodioxole, commonly known as safrole. More in depth studies are needed to reveal the biochemical ingredients and medicinal properties of betel vine.

5.2.2.2 Chlorophyll Content

5.2.2.2.1 Total Chlorophyll

Significant variation in the total chlorophyll content was observed among the cultivars (Fig. 20). Higher chlorophyll content (2.83 mg/g) in *Karinadan* led to dark green color of leaves which is less preferred by customers. *Puthukodi* with a chlorophyll content of 2.43 mg/g had green leaves and has more market preference. *Chelan* with low total chlorophyll content (1.96 mg/g) had light green leaves, which lead to less market preference.

5.2.2.2.2 Chlorophyll a

Chlorophyll a content in leaves varied significantly in various cultivars with maximum content (1.73 mg/g) in *Karinadan* and minimum (1.29 mg/g) in *Chelan*. Approximately same content of chlorophyll was recorded in *Sanchi* by Pariari and Imam (2012b).

5.2.2.2.3 Chlorophyll b

Chlorophyll b was significantly low (0.39 mg/g) in *Chelan*. Pariari and Imam (2012b) reported almost same content of chlorophyll b. He suggested that source of organic manures had a significant effect on chlorophyll b content.

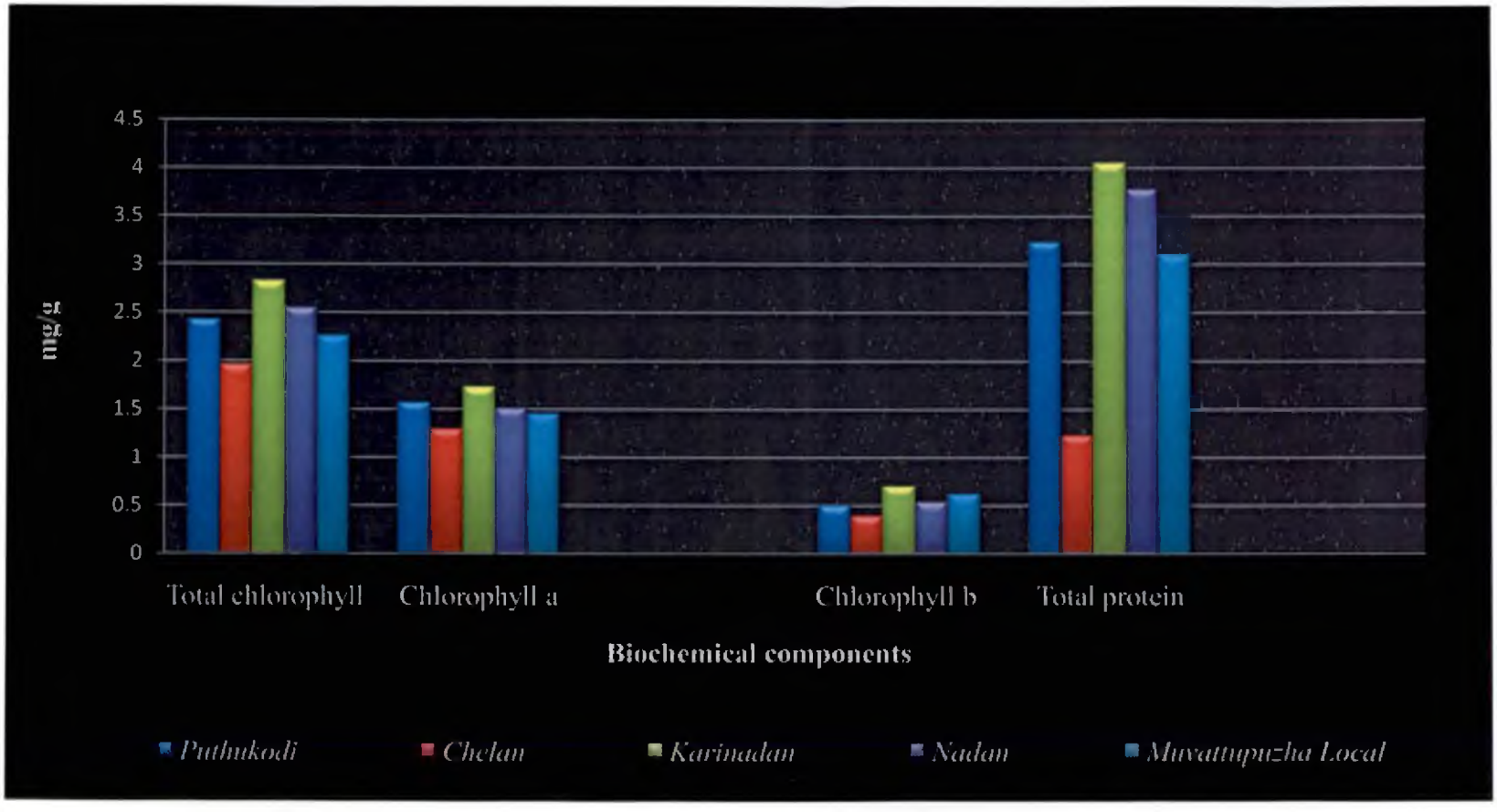


Fig. 20. Chlorophyll and total protein content in different betel vine cultivars of Malappuram district during 2013 - 14

Chlorophyll benefits the body in a number of distinct and unique ways. It is known to be a green element that helps to clean the body of harmful toxins. It is also an active agent that the body uses to fight infection. A regular and recommended intake of chlorophyll keeps the circulatory and digestive systems much healthier. To further assert its significance, chlorophyll is included in many of the natural nutritional supplements (Lewis, 2012). In general chlorophyll content is high in *Karinadan*. This emphasized the potential use of *Karinadan* for medicinal purpose.

5.2.2.3 Total Soluble Protein

Amount of protein present in different cultivars varied significantly with highest protein content in *Karinadan* (4.05 mg/g) and *Nadan* (3.78 mg/g). Since protein content is highly influenced by environmental conditions and soil nutritional conditions (Yoshida, 1981), further studies are needed to confirm the high protein content in the leaves of betel vine cultivars. In *Chilanthikarpooram red,* Chandini (1989) reported that nitrogen application also influenced the protein content of marketable leaves in betel vine.

5.2.2.4 Total Phenol

The phenolic compounds in plants are important due to their ability in UV protection, antimicrobial activity, antioxidant activity and insect resistance (Shirley, 2002).

All phenolic compounds are not antioxidants. However most of the phenolic compounds have antioxidant capacity. The phenolic compounds having antioxidant capacity are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron, chelating metal ions in aqueous solutions (Petti and Scully, 2009). High phenol content in *Karinadan* might have contributed to its high antioxidant capacity (Fig. 21) revealed in the present study.

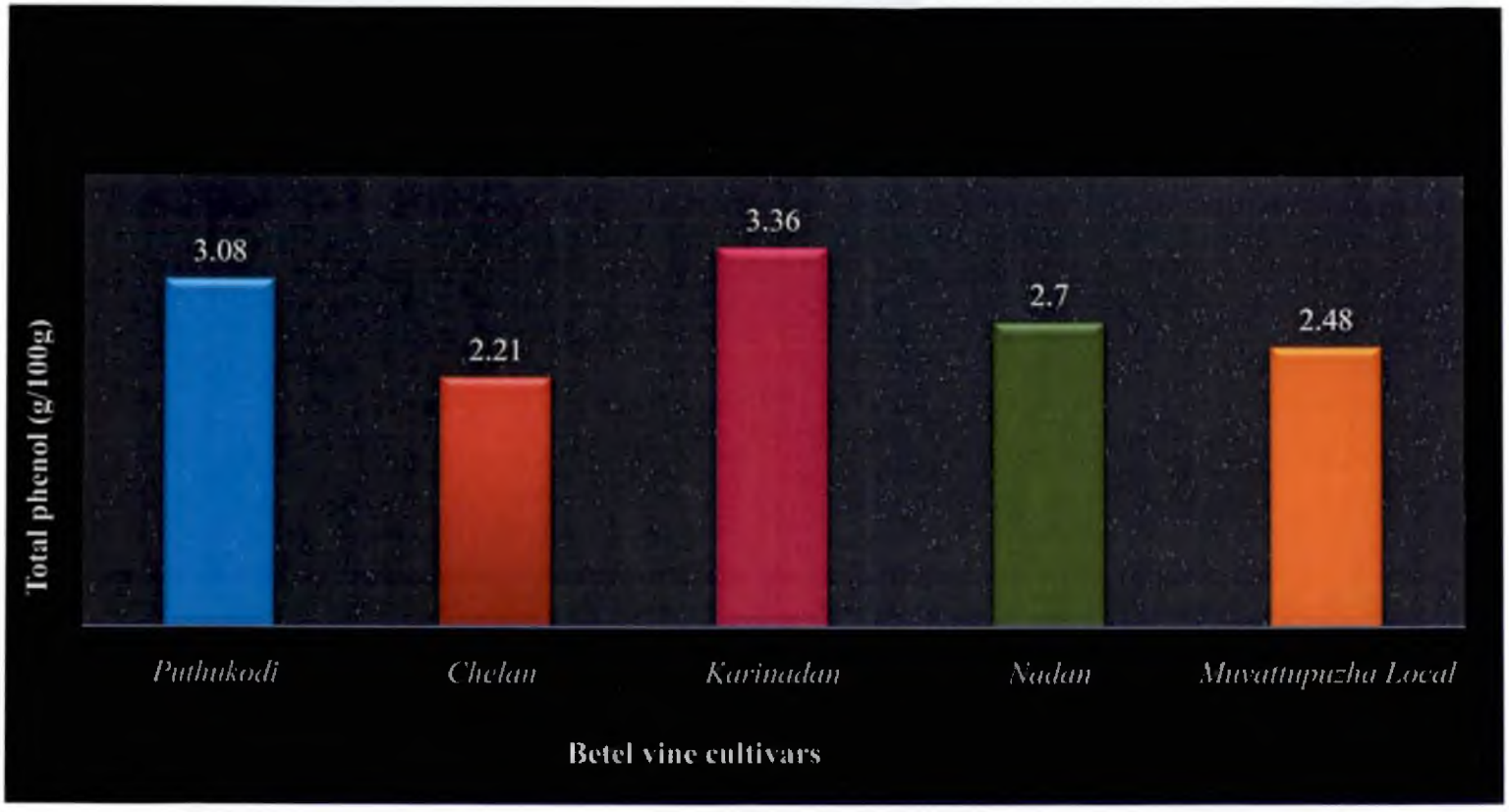


Fig. 21. Total phenol content in different betel vine cultivars of Malappuram district during 2013 - 14

5.2.2.5 Antioxidant Capacity

Antioxidant capacity is contributed by several molecules like vitamin B1, B2 and E, as well as several phenolic compounds (Sytar, 2014). Like all other biochemical characters under study, highest antioxidant capacity was recorded in *Karinadan* and lowest in *Chelan*. Generally plants that have significant therapeutic properties were found to be rich in phenolics and high antioxidant components (Sazwi *et al*., 2013). This relationship between high antioxidant activity and high phenol content was revealed in *Karinadan* (Fig. 22). *Muvattupuzha Local* was in between *Chelan* and *Karinadan*. More studies are needed in the antioxidant capacity of betel vine.

. 5.2.3 Orgnoleptic Character – Leaf Pungency

Pungency is the result of the interaction effect of chemical components present in the particular sample. So there will be correlation between the presence and quantity of chemical components and pungency of specific cultivar. High pungency was recorded in *Muvattupuzha Local* and this might be due to the high content of essential oil and phenol. Less pungency recorded in *Chelan* probably due to the presence of comparatively less amount of phenol as well as essential oil.

5.3 GENETIC PARAMETERS

The development of an effective plant breeding programme is depends upon the existence of genetic variability. An insight into the magnitude of variability present in the gene pool of a crop species is utmost importance for starting a judicious plant breeding programme.

The components of variation due to phenotype and genotype were studied in the present investigation.

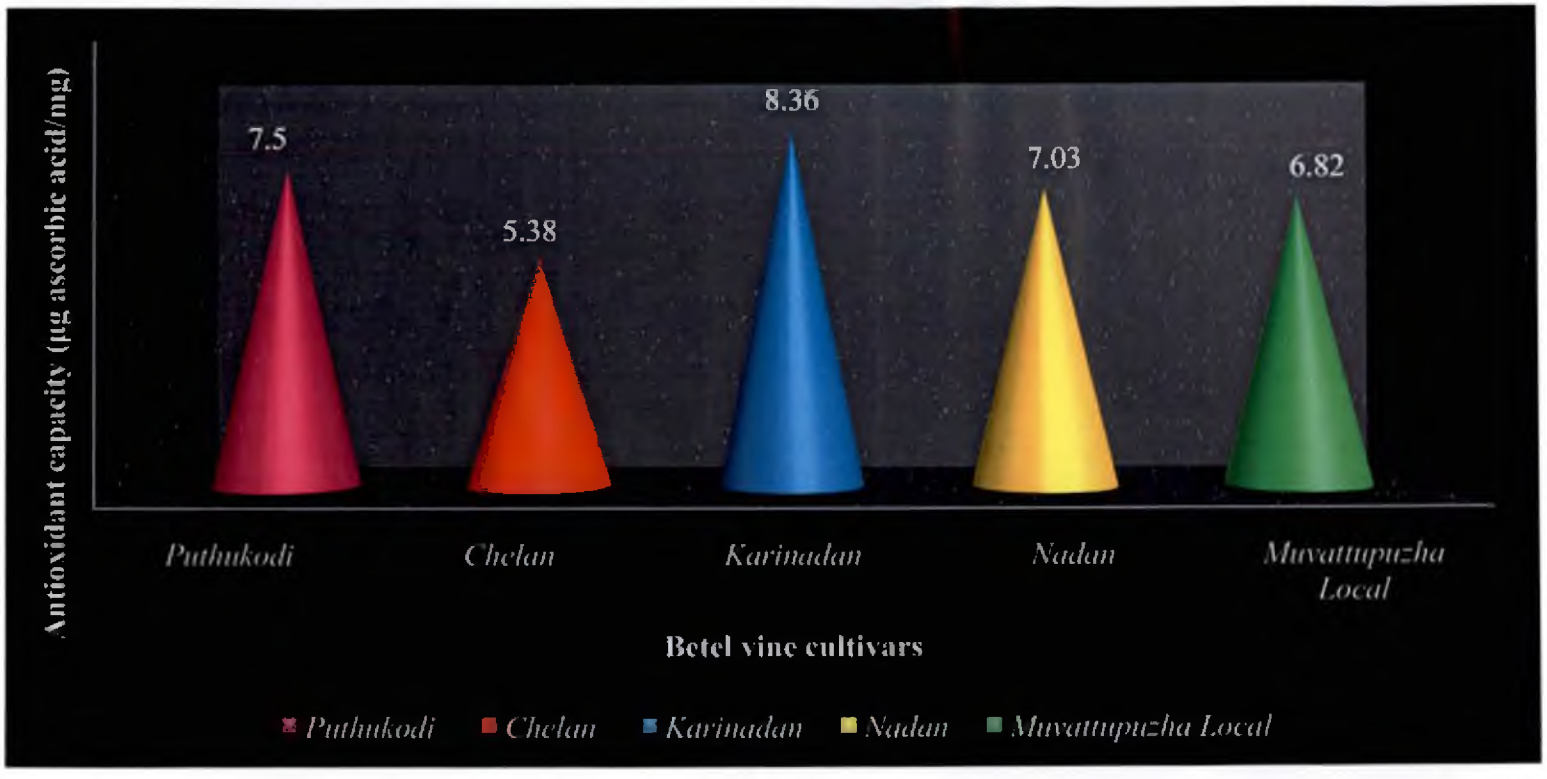


Fig. 22. Antioxidant capacity in different betel vine cultivars of Malappuram district during 2013 - 14

5.3.1 Range and Mean

Among cultivars, plant height recorded from 264.28 to 337.05 cm with 175.68 - 280.35 number of total leaves. Cultivars produced 10.88 – 16.88 number of lateral branches with in a period from 15 – 90 DAL. The number of nodes per lateral branch and number of leaves per lateral branch were recorded same during the period because from each node only one leaf was produced.

The first lateral branch emergence observed on 80.75 days after planting in *Chelan* whereas *Karinadan* produced first lateral branch on 151.25 days after planting. The frequency for lateral branch emergence was 9.35 days in *Chelan*. *Karinadan* recorded more days for two consecutive lateral branch emergence.

Length and width of leaf varied from 13.78 to 19.48 cm and 11.50 to 14.40 cm with mean of 16.29 ± 2.31 cm and 12.81 ± 1.12 cm respectively. In the case of leaf area, the range of variation was from 134.25 to 196.23 cm^2 with an average of 166.98 ± 21.62 cm^2. Leaf weight ranged from 2.83 – 4.85 g. For leaf weight per unit area, the range of variation was from 0.021 to 0.025 g/cm^2 with an average of 0.023 ± 0.003 g/cm^2. The range of variation for leaf petiole length and leaf tip angle was from 2.75 to 4.85 cm and 35.20 to 51.62^0, with an average of 3.43 ± 0.73 cm and 41.86 ± 5.40^0 respectively.

The range of variation for spike length and spike diameter was from 2.40 to 5.97 cm and 0.40 to 0.60 cm respectively. Spike peduncle length ranged between 3.25 and 4.77 cm with a mean of 3.44 ± 0.25 cm.

Among biochemical characters, total chlorophyll content ranged from 1.96 - 2.83 mg/g. Total protein content varied from 1.22 - 4.05 mg/g. With respect to total phenol content and antioxidant capacity, the variability ranged from 2.20 to 3.36 g/100 g and 5.38 to 8.36 µg ascorbic acid/mg respectively.

5.3.2 Phenotypic and Genotypic Coefficients of Variation

Both phenotypic and genotypic coefficient of variation, independent of the unit of measurement, is provided by the standard deviation expressed in percentage mean. So variability of characters expressed in various units can be compared through this estimation.

From the results (Fig. 23) it was observed that most of the characters were less affected by environmental factors, as the PCV for all the characters were close to GCV except characters like days to lateral branch emergence, leaf weight and chlorophyll a, b content. Low GCV and PCV were observed for characters like plant height, leaf width and spike peduncle length. Hence the scope of improvement through selection is less for these characters.

5.3.3 Heritability, Genetic Advance and Genetic Gain

In a general sense, heritability specified the proportion of the total variability that is due to genetic causes, or the ratio of genotypic variance to the total variance. It is a good index of the transmission of characters from parents to their offspring or it is the heritable portion of phenotypic variance (Nadarajan and Gunasekaran, 2005).

In the present study heritability estimates were high for all the characters except chlorophyll a, b content and leaf weight (Fig. 24). Similar reports were also made by Das *et al.* (1999) for sixteen morphological and chemical characters like leaf area, leaf length, leaf breadth, number of laterals/vine, vine length, diameter of internode, chlorophyll a and b content, and 100 leaf weight.

The heritability indicates only the effectiveness with which selection of genotype could be done based on the phenotypic performance, but failed to show the genetic progress (Johnson *et al.* 1955). High heritability, does not therefore,

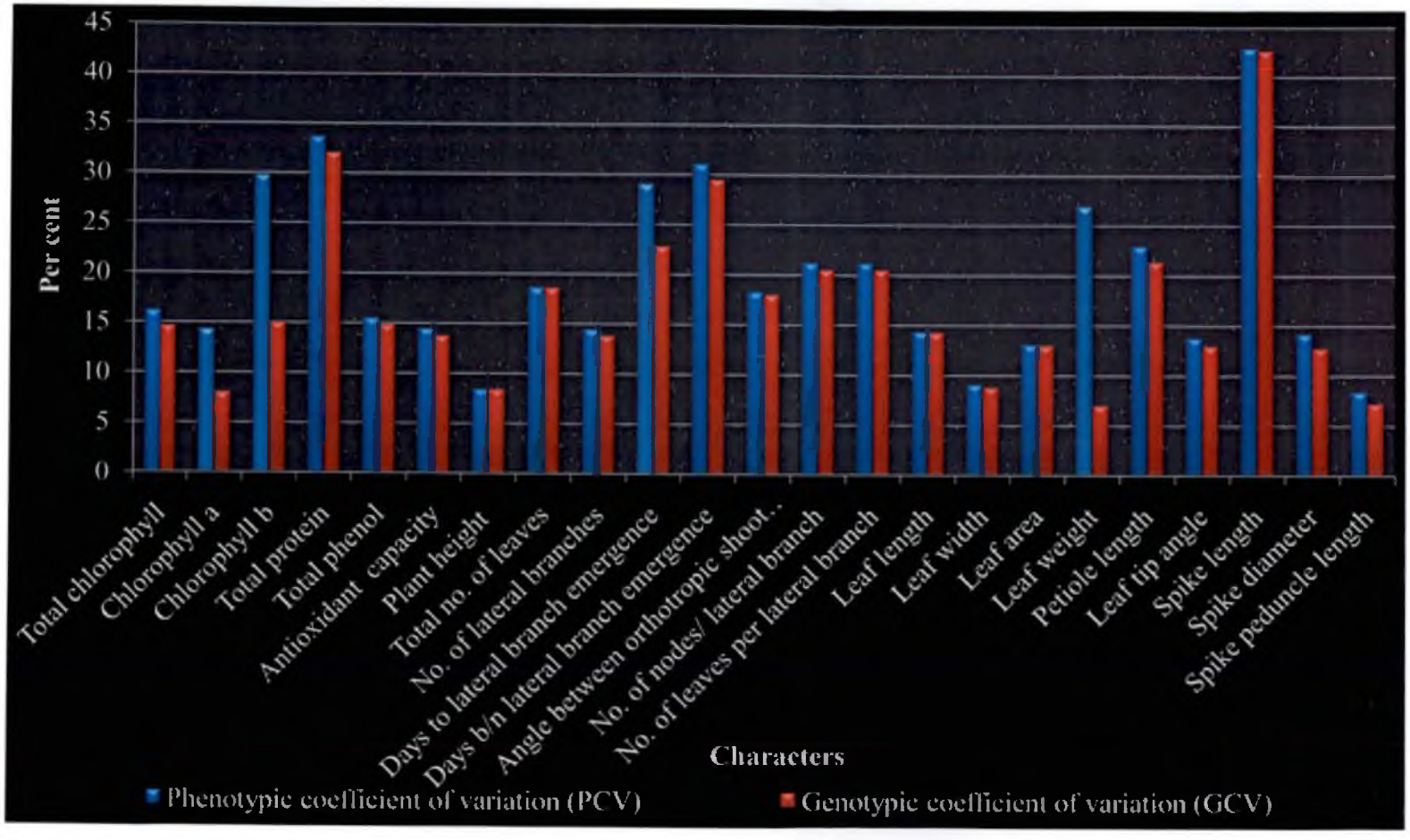


Fig. 23. Phenotypic and genotypic coefficient variation in different characters of betel vine cultivars of Malappuram district during 2013 - 14

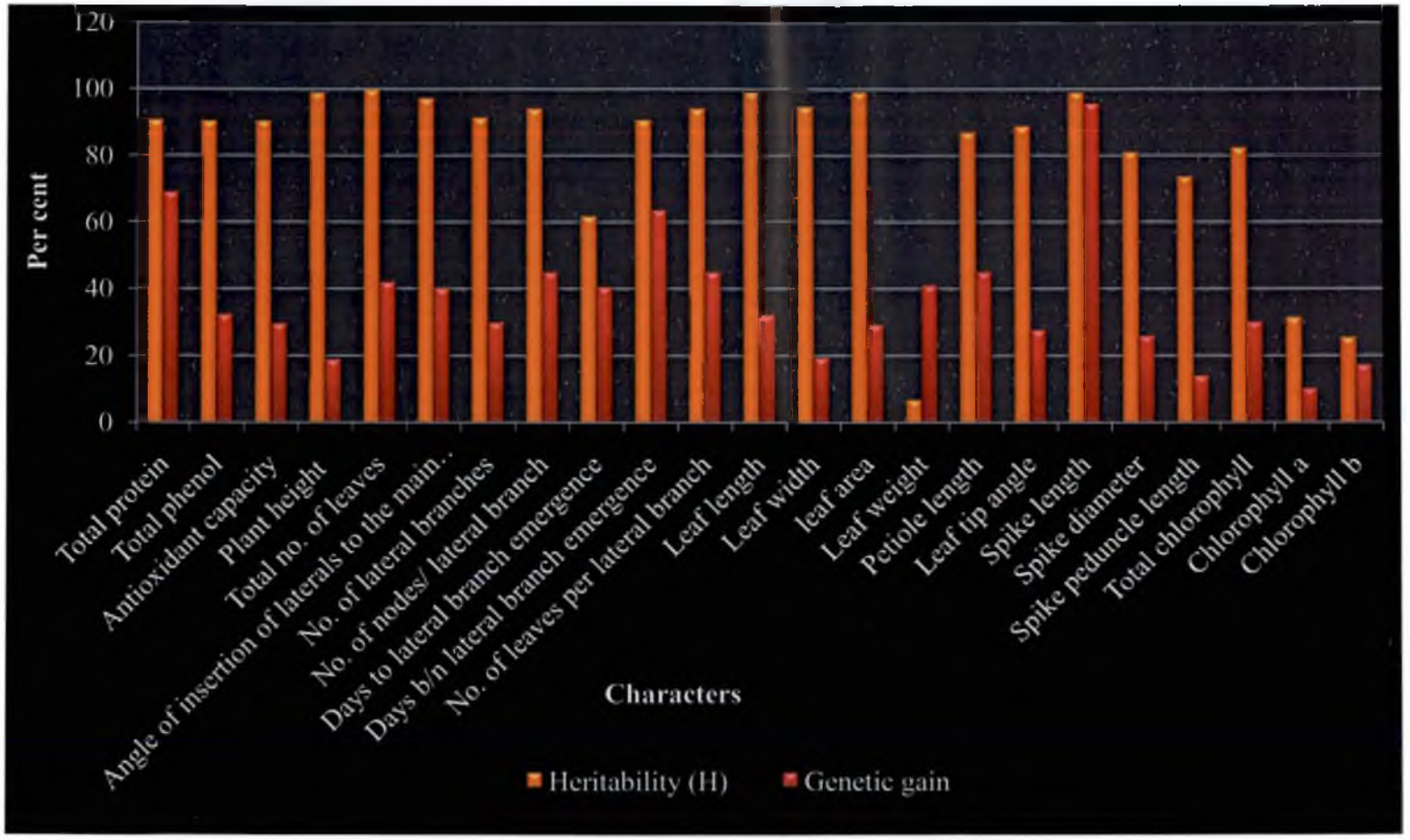


Fig. 24. Heritability and genetic gain of different morphological and biochemical characters of betel vine cultivars of Malappuram district during 2013 - 14

necessarily mean greater genetic gain. Genetic gain is calculated in order to ascertain its relative utility. The difference between the mean phenotypic value of the progeny of selected plants and the base or parental population is known as genetic gain. High heritability accompanied with high genetic gain indicates that most likely the heritability is due to additive gene effects and selection may be effective (Singh and Narayanan, 1993). When non additive gene effects govern heritability, the expected genetic advance would be low.

Among the characters studied most of the characters including total number of leaves, days to lateral branch emergence, days between lateral branch emergence, angle between orthotropic shoot and leaf petiole, number of lateral branch and number of nodes per lateral branch with high heritability also showed high genetic gain. It indicated that the heritability of these characters is governed by additive gene effects and selection must be effective. Selection based on these characters could lead to elite genotypes. However characters like plant height, leaf width and spike peduncle length had shown high heritability accompanied by moderate genetic gain. The reduction in genetic gain may be due to non additive gene action.

5.3.4 Correlation Coefficients between Total Number of Leaves and Plant Growth Parameters

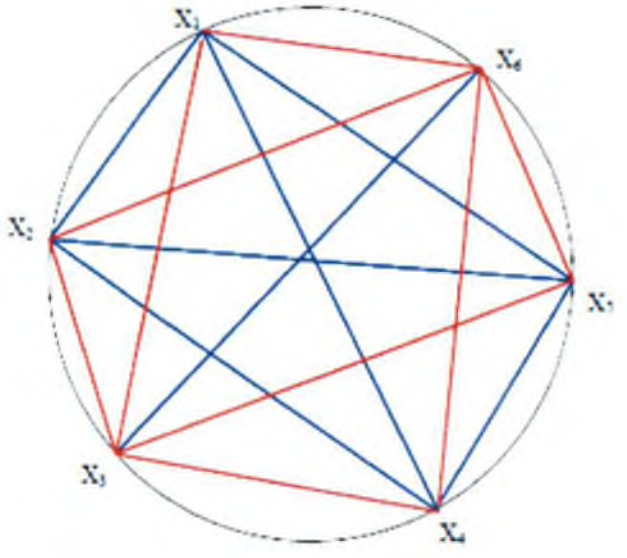
Study of the association of characters is a must to understand the genetics of the crop. Correlation study helps the plant breeder to understand genetic architecture of the crop as the correlation occur due to genetic reasons namely linkage or plieotropy. Since yield is a complex character, the practice of unilateral selection often results in retrograde or less optimum progress in isolating superior genotypes. From the knowledge of association of various characters with yield and among themselves, breeders can assess the complexity of the character and can practice selection based on appropriate selection criteria.

Among the correlation coefficients of five characters with total number of leaves, negative genotypic correlation was exhibited between days to lateral branch emergence and days between lateral branch emergence (Fig. 25). Days between lateral branch emergence negatively correlated with all the characters except days to lateral branch emergence. The highest significant positive genotypic correlation of total number of leaves was with number of leaves per lateral branch followed by plant height and number of lateral branches. This revealed that improvement of total number of leaves could be achieved by exercising selection simultaneously for increased number of leaves per lateral branch and plant height. Similar findings were reported in a study on character association, where number of leaves per vine had positive correlations with number of laterals per vine and vine length (Das *et al.*, 1999).

5.3.5 Direct and Indirect Effects of Plant Growth Parameters on Total Number of Leaves

Though the correlation studies were helpful in measuring the association between yield and yield components, they did not provide the exact picture of the direct and indirect causes of such association which could be obtained through path analysis (Wright, 1923). Path analysis is very useful to point out the important yield components which can be utilized for formulating selection parameters. Path coefficient analysis is a partial regression coefficient which splits the correlation coefficients into measures of direct and indirect contribution of various independent characters on a dependent character. The results also help in indirect selection for genetic improvement of yield.

Path coefficient analysis was performed using five quantitative characters (Fig. 27). Number of leaves per lateral branch showed highest positive direct effect on total number of leaves. This was mainly due to high positive significant



Positive correlation

Negative correlation

X_1 = Total number of leaves,

X_2 = Plant height,

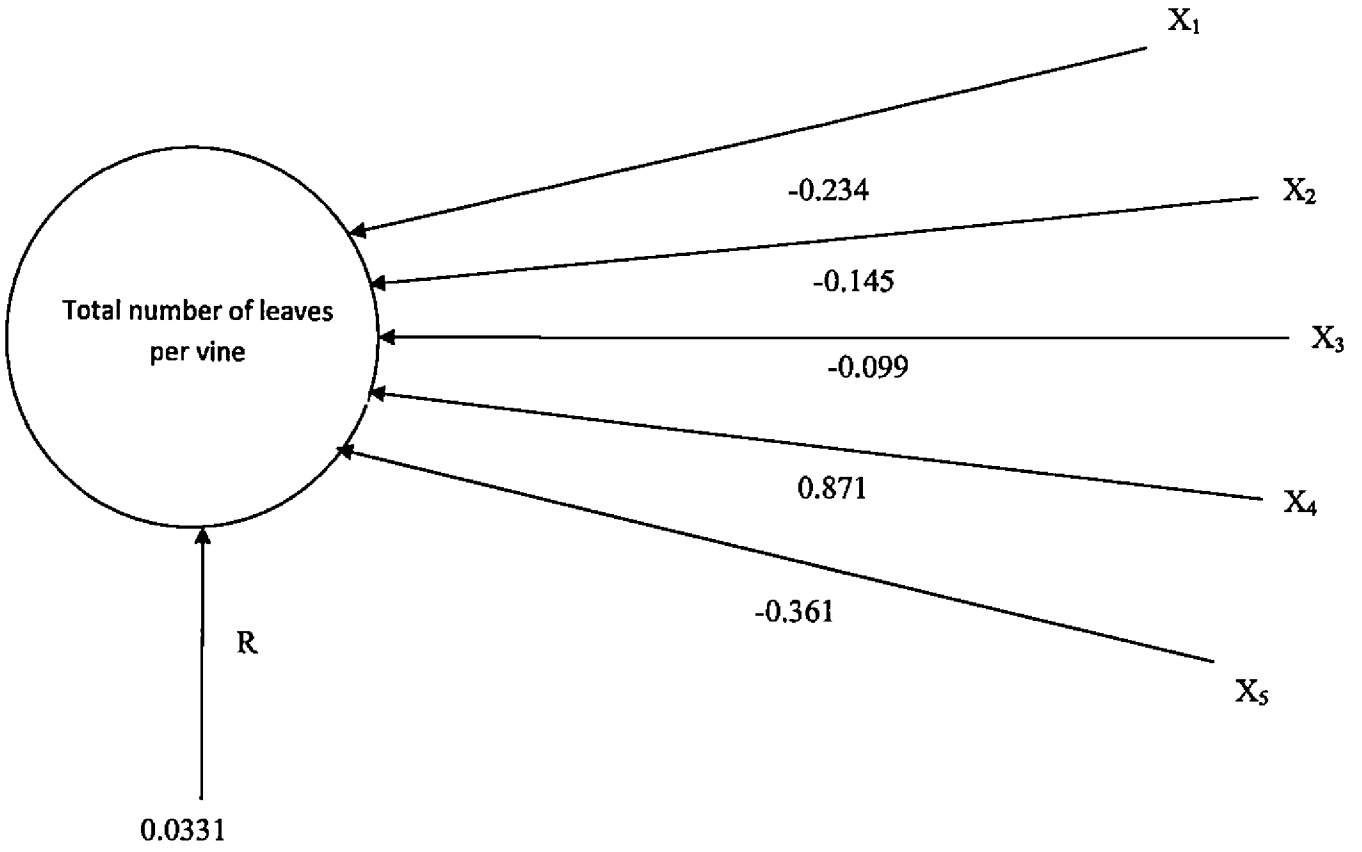
X_3 = Days to lateral branching,

X_4 = Number of lateral branches

X_5 = Number of leaves/ lateral branch,

X_6 = Days between lateral branch emergence

Fig. 25. Genotypic correlation between total number of leaves and plant growth parameters in betel vine cultivars of Malappuram district during 2013 - 14



X_1 = Plant height, X_2 = Days to lateral branching, X_3 = Number of lateral branches,

X_4 = Number of leaves per lateral branch, X_5 = Days between lateral branch emergence

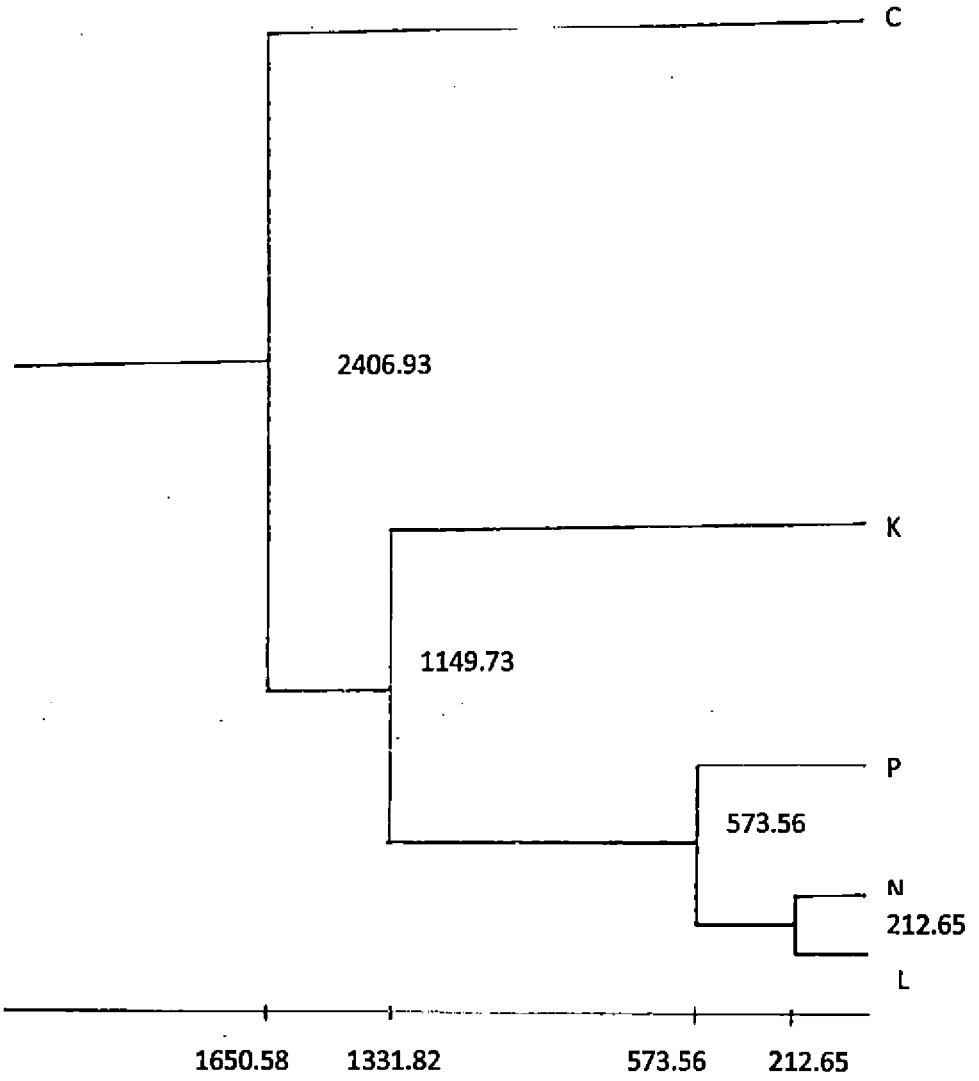
Fig. 26. Path diagram indicating direct effects of plant growth characters on total number of leaves of betel vine cultivars of Malappuram district during 2013 - 14

correlation between number of leaves per lateral branch and total number of leaves. The positive correlation of total number of leaves with number of leaves per lateral branch was expounded partly by its high positive direct effect and partly by its positive indirect effect through plant height. Selection based on number of leaves per lateral branch would be useful in increasing the leaf yield. High indirect effect of plant height based on number of leaves per lateral branch indicated that selection through plant height could lead to higher number of total number of leaves.

All other characters showed negative direct effect on total number of leaves. The residual effect obtained in path analysis was 0.0331. This indicated that 0.966 per cent variation in total number of leaves was contributed by five yield components namely plant height, days to lateral branching, number of lateral branches, number of leaves per lateral branch and days between lateral branch emergence.

5.3.6 Diversity Analysis

The results of diversity analysis are presented in a dendrogram (Fig. 27) which illustrates the relationship between five cultivars under study. The distance between two clusters or two cultivars is the measure of degree of diversification; greater the distance between two clusters, greater the divergence and *vice versa*. The dendrogram constructed, with Euclidean distance, based on morphological and biochemical characters revealed that *Chelan* cultivar was distinct from other cultivars. *Chelan* showed 2406.93 Euclidean distance from cluster containing *Karinadan, Puthukodi, Nadan* and *Muvattupuzha Local*. *Karinadan* formed distinct cluster with a distance of 1149.73 from the cluster containing *Puthukodi*, *Nadan* and *Muvattupuzha Local*. It was interesting to note that *Muvattupuzha Local* and *Nadan* collected from different geographical locations, having almost similar morphological and biochemical characters, proved their relatedness by being in a single cluster with lowest Euclidean distance of 212.65. The cultivars from different clusters are



C – *Chelan*; K – *Karinadan*; P – *Puthukodi*; N – *Nadan*; L – *Muvattupuha Local*

Fig. 27. Dendrogram of taxonomic relationship of betel vine cultivars of Malappuram district during 2013 - 14

generally opted for hybridization purpose and hence *Karinadan* and *Chelan* can be used for hybridization to combin their superior characters.

5.4 IDENTIFICATION OF SUPERIOR CULTIVARS FOR SPECIFIC PURPOSES

The acceptance of genotypes for commercial cultivation is based on yield and quality parameters. As leaves are the major economic product from betel vine, the growth parameters which influence total number of leaves have significant importance in the selection of ideal types. Along with growth parameters, leaf characters such as leaf length, leaf width, leaf area, leaf weight, leaf weight per unit area, leaf color, leaf brittleness, leaf lamina shape and leaf base shape also play a role in the selection of cultivars for commercial cultivation. Biochemical constituents like protein content, phenol content, antioxidant capacity and chlorophyll content together with leaf pungency also play an important role in the selection on cultivars for chewing and medicinal purpose.

In the present study, *Chelan* cultivar showed higher plant height, number of lateral branches and number of leaves per lateral branch and thus resulted in higher number of leaves per vine. However, leaf characters such as leaf length, leaf width, leaf area and leaf weight per unit area as well as biochemical characters like total protein content, chlorophyll content, total phenol content and antioxidant capacity were not promising for *Chelan*. Apart from this, the pungency was less for this cultivar and the leaves were small and light green in color. All the above characters make *Chelan* less suited for commercial cultivation to harvest marketable leaves for chewing purpose.. On the other hand, the high content of eugenol in this cultivar indicates its potential use in neutraceutical industry. Being a male cultivar with distinct morphological characters, *Chelan* can contribute to develop better genotypes through hybridization programmes.

The growth rate of *Karinadan* was slow compared to other cultivars of Malappuram area. It produced dark green larger leaves which are not much preferred

by the betel vine chewers. Optimum leaf size is a factor that adds to the market preference of betel leaves. Hence this cultivar is not ideal for the production of leaves for chewing purpose. On the other side, *Karinadan* is rich in biochemical constituents, like total protein, chlorophyll content, total phenol and high antioxidant capacity, making it more suited to use in indigenous medicine and drug manufacturing.

Puthukodi is the major cultivar of the Malappuram district. Growth parameters and leaf characters recorded in this cultivar are in between *Chelan* and *Karinadan.* Optimum leaf size of *Puthukodi* reduced handling damage during transportation to some extent. Green coloured leaves with medium brittleness and pungency are added qualities of this cultivar. Leaf weight per unit area is an important character with regard to export, as increased leaf weight will result in low withering during transportation and marketing. *Puthukodi* recorded maximum leaf weight per unit area which is an added advantage of this cultivar. This cultivar proved to be ideal for commercial cultivation for harvesting leaves for chewing purpose.

Muvattupuzha Local had green leaves with high pungency. With regard to growth parameters and leaf characters it is more related to *Nadan.* The high content of phenol and essential oil make its leaves more pungent and more suited to oil extraction.

Summary

6. SUMMARY

The present investigation on "Characterization of betel vine (*Piper betle* L.) types of Malappuram district" was carried out at the Department of Plant Breeding & Genetics, College of Horticulture, Thrissur and the farmer's field at Malappuram district, Kerala during 2012 - 2014 to study the diversity of betel vine types in Tirur and nearby areas of Malappuram district and to characterize the types based on morphological and biochemical features.

Puthukodi, Chelan, Karinadan and *Nadan* were the betel vine cultivars grown in Malappuram District. *Puthukodi, Chelan* and *Karinadan* along with *Nadan* (local check variety) and *Muvattupuzha Local* cultivar from Asamannoor, Ernakulum district were raised in farmer's field at Malappuram in a Randomized Complete Block Design with four replications, by following the cultural practices adopted by farmers. The morphological and biochemical characters were recorded at different stages of plant growth following standard procedures.

The salient findings are summarized below.

- Tirur, Malappuram, Valanchery, Vengara, Kondotty, Parappanangadi and Ponmundam were the major betel vine growing Block Panchayaths in Malappuram district with maximum area in Tirur Block Panchayath. The betel vine cultivars recorded from Tirur and nearby areas of Malappuram district were *Puthukodi, Chelan, Karinadan* and *Nadan*. *Puthukodi* and *Nadan* were the common cultivars whereas *Chelan* and *Karinadan* were the cultivars conserved by few farmers.

- In general, *Karinadan* and *Chelan* had shown distinct morphological and biochemical characters compared with other cultivars grown in Malappuram district. *Muvattupuzha Local* showed more similarity with *Nadan* with regard to morphological characters.

Karinadan had dark green leaves with even leaf margin, short petiole and high brittleness. The internodes of orthotropic shoots were uniform purple - green in colour where as shoot tip showed purple colouration. This cultivar produced hanging lateral branches. The plant growth parameters like plant height, total number of leaves, number of lateral branches and number of leaves per lateral branch were significantly low in this cultivar, indicating a slower growth rate. On the other hand the leaf characters like leaf length, leaf width, leaf area and leaf weight per unit area were comparatively high in this cultivar, making it less acceptable in the market. *Karinadan* was rich in biochemical constituents with significantly high content of total chlorophyll (2.83 mg/g), total protein (4.05 mg/g), total phenol (3.36 g/100g) and high antioxidant capacity (8.36 µg ascorbic acid/ mg). The leaves were medium pungent.

Chelan had light green leaves with wavy leaf margin, long petiole, ovate leaf lamina, low brittleness and low pungency. The cultivar had light green with purple tinge for the internodes whereas shoot tips were light purple. Lateral branches of *Chelan* were semierect in nature. The plant growth parameters like plant height, total number of leaves, number of lateral branches and number of leaves per lateral branch were significantly high in this cultivar at all growth stages, indicating a faster growth rate. On the other hand leaf characters including leaf length, leaf width, leaf area, leaf weight and leaf weight per unit area were comparatively low, leading to less market preference. *Chelan* recorded significantly low content of total chlorophyll (1.96 mg/g), total protein (1.23 mg/g), total phenol (2.21 g/100g) and low antioxidant capacity (5.38 µg ascorbic acid/mg). However high content (20.80 %) of eugenol was recorded in this cultivar.

Nadan, Puthukodi and Muvattupuzha Local had shown more similarity among themselves in morphological and biochemical characters. These cultivars had green leaves with even leaf margin and medium brittleness. Internodal region of orthotropic shoot of these cultivars were green with purple colour towards nodal region. *Puthukodi* recorded high fresh leaf weight per unit area and produced hanging lateral branches. *Nadan* and *Muvattupuzha Local* recorded comparatively similar trend in growth

parameters like plant height, total number of leaves, number of lateral branches and number of leaves per lateral branches. These cultivars mostly produced hanging lateral branches. Content of biochemical constituents in these cultivars was intermediate to *Karinadan* and *Chelan*. High yield of essential oil was obtained from *Muvattupuha Local,* which had high pungency. Medium pungency was recorded in *Nadan* and *Puthukodi.*

- Ovate elliptic (*Puthukodi* and *Muvattupuzha Local*) and ovate lanceolate (*Nadan* and *Karinadan*) were the mostly seen leaf lamina shapes in betel vine cultivars. Most of the cultivars produced leaves with cordate base. *Nadan* and *Karinadan* had acuminate leaf apex while aristulate leaf apex was observed for *Chelan* and *Puthukodi. Muvattupuzha Local* had apiculate leaf apex.

- Purple pigmentation was noticed at internodes of lateral branches of all betel vine cultivars under study before spike formation. However purple pigmentation was absent in lateral branches with spikes.

- Profuse flowering was observed in all cultivars under study. *Chelan* was identified as male cultivar and all others as female cultivars. Spikes from male and female cultivars differed significantly for morphological characters. *Chelan* (male) produced long slender spikes with short peduncle. Female cultivars produced comparatively short thick spikes having less peduncle length.

- Spikes were axillary and opposite to leaf. Sessile naked florets were compactly arranged on the inflorescence axis. A bract subtended each floret, both in male and female spikes. Female spike was very distinguishable at the stage of anthesis with the presence of 6 - 9 white coloured sessile stigmatic lobes on each floret. The stigmatic lobes became black towards the end of spike maturation. Male spikes were yellow in color and the two black stamens of each floret protruded at the stage of anthesis.

- Essential oil content was low (0.45 per cent) in *Chelan* whereas it was high (0.57 per cent) in *Muvattupuzha Local*. Eugenol was the major component of essential oil in all

cultivars. Possible compounds identified from the chromatograms of oil from different cultivars were hydroxychavicol, β caryophyllene and 5-(2-propenyl)-1, 3- benzodioxole.

- Most of the morphological and biochemical characters were less affected by environmental factors, as the PCV for the characters were close to GCV except characters like days to lateral branch emergence, leaf weight and content of chlorophyll a and chlorophyll b.

- Most of the characters except plant height, leaf width and spike peduncle length showed high heritability accompanied with high genetic gain, indicating additive gene effects. Selection based on these characters would lead to development of elite genotypes.

- Total number of leaves showed highest significant positive genotypic correlation with number of leaves per lateral branch followed by plant height and number of lateral branches. This revealed that improvement of total number of leaves could be achieved by exercising selection simultaneously for these characters.

- Number of leaves per lateral branch showed highest positive direct effect on total number of leaves. The positive correlation of total number of leaves with number of leaves per lateral branch was expounded partly by its high positive direct effect and partly by its positive indirect effect through plant height. Selection based on number of leaves per lateral branch will be useful in increasing the leaf yield.

- The dendrogram with Euclidean distance, based on morphological and biochemical characters, proved that *Chelan* was very distinct from other cultivars under study. *Karinadan* formed a separate cluster with a Euclidean distance of 1650.58 from *Muvattupuzha Local*. *Nadan* and *Muvattupuzha Local* proved their relatedness by being in a single cluster.

- *Puthukodi* was the most accepted and widely grown cultivar in Malappuarm district probably due to its medium growth rate and optimum leaf characters with medium pungent green leaves. *Chelan* and *Karinadan* were less preferred for the production of

leaves meant for chewing purpose. However *Karinadan,* rich in biochemical constituents, could be exploited in drug manufacturing and indigenous medicine.

Suggested future line of work

- Detailed morphological, biochemical and molecular characterization of betel vine cultivars grown in Kerala
- Development of betel vine descriptor
- Studies on floral morphology and biology
- Hybridization work for combining superior characters of *Karinadan* and *Chelan*
- Registration of *Tirur betel vine* as a Geographical Indication
- Studies on factors contributing to the high acceptance of *Puthukodi* among the farmers of Malappuram district
- Studies on antimicrobial, neutraceutical and medicinal properties of betel vine

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Appendices

Appendix I. GC result of standard - Eugenol

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.567	1084.717	761.932	17.1	76.3	0.02
2	13.540	3094.481	103.291	48.7	10.3	0.50
3	13.823	2147.555	132.601	33.8	13.3	0.29
4	14.587	15.107	0.581	0.2	0.1	0.38
5	22.007	6.264	0.287	0.1	0.0	0.37
	Total	6348.124	998.693	100.0	100.0	

Appendix II. GC result of standard - Isoeugenol

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	12.607	4.653	0.793	0.8	1.7	0.09
2	14.630	76.557	11.339	12.3	24.8	0.10
3	16.613	539.024	33.510	86.9	73.4	0.25
	Total	620.233	45.642	100.0	100.0	

Appendix III. GC result of standard - Methylisoeugenol

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	16.457	18.321	2.616	1.4	4.5	0.10
2	18.777	1270.757	54.994	97.1	94.3	0.37
3	25.570	19.134	0.724	1.5	1.2	0.44
	Total	1308.213	58.335	100.0	100.0	

Appendix IV. GC result of standard - Methyleugenol

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.960	0.573	0.290	0.0	0.1	0.03
2	7.393	7.872	2.137	0.1	0.5	0.06
3	16.050	10007.703	191.692	88.3	48.8	0.86
4	16.147	1310.234	198.240	11.6	50.5	0.10
5	19.047	1.317	0.237	0.0	0.1	0.09
	Total	11327.699	392.596	100.0	100.0	

Appendix V. Details of GC analysis of essential oil of *Chelan*

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
2	2.623	14.719	5.113	0.1	0.3	0.03
3	2.853	112.535	67.329	0.5	3.5	0.03
4	2.953	161.572	95.178	0.7	5.0	0.03
5	3.113	63.346	33.099	0.3	1.7	0.03
6	3.227	11.736	3.948	0.1	0.2	0.05
7	3.357	577.966	223.310	2.7	11.6	0.04
8	3.443	271.113	121.759	1.3	6.3	0.04
9	3.577	18.578	6.597	0.1	0.3	0.05
10	3.680	66.591	26.220	0.3	1.4	0.04
11	3.867	359.484	129.577	1.7	6.8	0.05
12	3.953	71.268	26.288	0.3	1.4	0.05
13	4.087	482.683	109.482	2.2	5.7	0.07
14	4.187	35.492	15.013	0.2	0.8	0.04
15	4.257	33.092	8.053	0.2	0.4	0.06
16	4.510	568.782	142.747	2.6	7.4	0.06
17	4.680	20.426	2.458	0.1	0.1	0.11
18	4.983	134.112	46.505	0.6	2.4	0.04
19	5.153	202.831	49.730	0.9	2.6	0.06
20	5.417	8.988	1.056	0.0	0.1	0.21
21	5.670	41.502	8.236	0.2	0.4	0.07
22	6.053	42.277	3.874	0.2	0.2	0.15
23	6.320	11.170	1.278	0.1	0.1	0.15
24	6.577	5.267	0.777	0.0	0.0	0.14
25	6.710	12.070	1.008	0.1	0.1	0.25
26	7.370	2113.570	150.187	9.8	7.8	0.23
27	7.507	184.425	30.232	0.9	1.6	0.06
28	7.827	37.414	5.007	0.2	0.3	0.07
29	8.200	26.083	1.440	0.1	0.1	0.20
30	9.133	10.506	0.443	0.0	0.0	0.49
31	9.643	14.490	0.559	0.1	0.0	0.46
32	11.497	8518.291	208.897	39.5	10.9	0.67

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
33	12.207	34.867	5.495	0.2	0.3	0.08
34	12.570	20.146	3.228	0.1	0.2	0.08
35	13.950	4485.642	120.510	20.8	6.3	0.60
36	14.410	164.703	20.048	0.8	1.0	0.12
37	15.000	269.385	29.802	1.2	1.6	0.14
38	15.453	320.199	32.450	1.5	1.7	0.13
39	16.690	211.474	25.120	1.0	1.3	0.12
40	17.503	108.957	9.099	0.5	0.5	0.20
41	17.813	358.363	30.524	1.7	1.6	0.14
42	18.347	228.934	20.785	1.1	1.1	0.16
43	18.667	52.753	8.428	0.2	0.4	0.10
44	18.957	49.513	3.746	0.2	0.2	0.12
45	19.353	97.799	13.135	0.5	0.7	0.11
46	19.863	336.824	23.184	1.6	1.2	0.23
47	20.697	59.724	7.214	0.3	0.4	0.10
48	21.137	12.481	1.571	0.1	0.1	0.11
49	21.610	27.959	2.754	0.1	0.1	0.14
50	21.867	72.922	5.032	0.3	0.3	0.12
51	22.553	17.983	1.819	0.1	0.1	0.13
52	23.140	40.442	2.503	0.2	0.1	0.11
53	23.500	36.925	3.063	0.2	0.2	0.17
54	23.997	43.334	5.269	0.2	0.3	0.12
55	24.380	246.265	17.011	1.1	0.9	0.14
56	25.463	56.064	1.659	0.3	0.1	0.10
	Total	21586.040	1918.850	100.0	100.0	

Appendix VI. Details of GC analysis of essential oil of *Karinadan*

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.533	2151.168	996.096	9.3	36.1	0.03
2	2.260	8.779	7.110	0.0	0.3	0.02
3	2.614	11.164	8.065	0.0	0.3	0.02
4	2.842	75.482	47.606	0.3	1.7	0.02
5	2.939	106.163	66.950	0.5	2.4	0.02
6	3.101	49.343	28.036	0.2	1.0	0.03
7	3.350	651.374	243.044	2.8	8.8	0.04
8	3.429	202.850	103.540	0.9	3.8	0.03
9	3.663	29.445	14.633	0.1	0.5	0.03
10	3.841	240.017	98.617	1.0	3.6	0.04
11	3.930	43.064	17.139	0.2	0.6	0.04
12	4.059	360.835	86.964	1.6	3.2	0.07
13	4.168	35.984	10.995	0.2	0.4	0.03
14	4.477	391.572	116.343	1.7	4.2	0.05
15	4.957	88.448	35.034	0.4	1.3	0.04
16	5.111	100.037	32.900	0.4	1.2	0.05
17	5.651	28.948	8.026	0.1	0.3	0.06
18	7.264	1345.594	117.290	5.8	4.3	0.19
19	7.416	102.864	20.021	0.4	0.7	0.06
20	11.607	10252.715	229.809	44.5	8.3	0.75
21	13.714	2675.699	104.227	11.6	3.8	0.40
22	14.069	56.241	7.150	0.2	0.3	0.12
23	14.306	78.128	12.338	0.3	0.4	0.10
24	14.845	138.980	19.014	0.6	0.7	0.11
25	15.377	234.161	28.321	1.0	1.0	0.11
26	16.636	150.592	22.812	0.7	0.8	0.11
27	17.541	180.446	13.021	0.8	0.5	0.23
28	17.805	268.858	29.326	1.2	1.1	0.15
29	18.003	170.091	25.520	0.7	0.9	0.11
30	18.382	348.121	30.658	1.5	1.1	0.17
31	18.665	62.383	9.901	0.3	0.4	0.09

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
32	18.957	26.973	3.190	0.1	0.1	0.14
33	19.353	148.608	15.437	0.6	0.6	0.11
34	20.178	994.024	43.109	4.3	1.6	0.37
35	20.763	85.735	10.827	0.4	0.4	0.11
36	21.931	76.769	6.403	0.3	0.2	0.10
37	24.032	49.460	7.295	0.2	0.3	0.11
38	24.556	556.853	35.309	2.4	1.3	0.24
39	24.823	463.537	43.709	2.0	1.6	0.20
	Total	23041.504	2755.788	100.0	100.0	

Appendix VII. Details of GC analysis of essential oil of *Puthukodi*

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W0 [min
2	2.290	18.112	1.829	0.2	0.2	
3	2.643	10.216	3.446	0.1	0.4	
4	2.870	42.179	27.464	0.5	2.8	
5	2.967	66.505	41.323	0.8	4.3	
6	3.127	28.942	16.456	0.4	1.7	
7	3.233	3.942	1.325	0.0	0.1	
8	3.347	204.207	106.916	2.5	11.0	
9	3.437	102.050	53.770	1.2	5.6	
10	3.580	4.474	1.472	0.1	0.2	
11	3.683	24.851	11.208	0.3	1.2	
12	3.850	140.721	64.357	1.7	6.7	
13	3.943	29.697	13.335	0.4	1.4	
14	4.060	176.795	51.291	2.1	5.3	
15	4.183	12.364	5.217	0.1	0.5	
16	4.250	14.349	4.219	0.2	0.4	
17	4.473	224.991	76.273	2.7	7.9	
18	4.963	49.851	19.643	0.6	2.0	
19	5.103	61.225	19.032	0.7	2.0	
20	5.653	13.778	3.531	0.2	0.4	
21	6.037	11.178	2.233	0.1	0.2	
22	6.310	3.278	0.451	0.0	0.0	
23	6.580	4.357	0.304	0.1	0.0	
24	7.167	642.360	78.408	7.8	8.1	
25	7.357	37.148	10.223	0.4	1.1	
26	7.457	7.063	1.817	0.1	0.2	
27	7.560	7.315	1.441	0.1	0.1	
28	7.757	8.573	1.398	0.1	0.1	
29	8.157	3.191	0.321	0.0	0.0	
30	11.053	3744.718	137.319	45.3	14.2	
31	12.010	8.382	1.245	0.1	0.1	
32	12.417	8.342	1.240	0.1	0.1	

	Relen. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
33	13.303	1266.688	63.011	15.3	6.5	0.32
34	13.860	24.137	4.174	0.3	0.4	0.09
35	14.087	35.107	5.728	0.4	0.6	0.08
36	14.593	58.337	9.407	0.7	1.0	0.09
37	15.177	85.161	14.051	1.0	1.5	0.09
38	15.473	18.323	1.494	0.2	0.2	0.09
39	16.467	68.002	9.951	0.8	1.0	0.10
40	17.357	59.527	7.192	0.7	0.7	0.13
41	17.590	78.831	11.509	1.0	1.2	0.11
42	17.800	64.242	10.785	0.8	1.1	0.09
43	18.160	114.708	12.946	1.4	1.3	0.12
44	18.540	18.533	2.550	0.2	0.3	0.11
45	18.990	25.375	2.432	0.3	0.3	0.10
46	19.210	37.051	5.513	0.4	0.6	0.10
47	19.707	235.230	18.734	2.8	1.9	0.19
48	20.590	35.049	3.971	0.4	0.4	0.10
49	21.483	7.670	0.862	0.1	0.1	0.12
50	21.747	25.227	1.872	0.3	0.2	0.11
51	22.457	6.378	0.685	0.1	0.1	0.13
52	23.060	15.053	0.900	0.2	0.1	0.11
53	23.423	14.331	1.426	0.2	0.1	0.12
54	23.920	17.099	2.378	0.2	0.2	0.11
55	24.347	243.579	17.595	2.9	1.8	0.15
	Total	8268.791	967.675	100.0	100.0	

Appendix VIII. Details of GC analysis of essential oil of *Nadan*

	Retan. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.660	0.834	0.524	0.0	0.1	0.03
2	2.880	28.493	19.619	0.6	2.9	0.02
3	2.977	47.097	30.975	1.0	4.6	0.02
4	3.133	18.368	10.825	0.4	1.6	0.03
5	3.340	90.393	52.762	1.9	7.8	0.03
6	3.433	66.463	36.981	1.4	5.5	0.03
7	3.580	1.598	0.689	0.0	0.1	0.04
8	3.683	18.060	9.390	0.4	1.4	0.03
9	3.843	103.783	51.399	2.1	7.6	0.03
10	3.937	16.553	7.882	0.3	1.2	0.03
11	4.047	115.655	37.647	2.4	5.6	0.04
12	4.170	7.284	3.182	0.2	0.5	0.04
13	4.240	5.588	1.839	0.1	0.3	0.04
14	4.450	156.153	60.343	3.2	9.0	0.04
15	4.943	33.062	13.747	0.7	2.0	0.04
16	5.073	33.987	11.951	0.7	1.8	0.04
17	5.627	4.196	1.315	0.1	0.2	0.05
18	6.003	3.854	0.903	0.1	0.1	0.06
19	7.077	397.480	60.786	8.2	9.0	0.11
20	7.287	27.540	6.363	0.6	0.9	0.06
21	7.503	4.005	0.781	0.1	0.1	0.08
22	7.703	3.180	0.547	0.1	0.1	0.07
23	10.780	2160.939	106.310	44.6	15.8	0.33
24	11.887	4.114	0.614	0.1	0.1	0.08
25	12.303	3.699	0.620	0.1	0.1	0.08
26	13.070	790.986	50.075	16.3	7.4	0.26
27	13.383	22.871	3.491	0.5	0.5	0.09
28	13.723	13.180	2.301	0.3	0.3	0.09
29	13.950	19.676	3.247	0.4	0.5	0.08
30	14.443	37.511	6.071	0.8	0.9	0.09
31	15.043	48.616	8.623	1.0	1.3	0.09

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
32	15.360	5.831	0.781	0.1	0.1	0.09
33	15.750	2.833	0.374	0.1	0.1	0.09
34	16.347	38.343	6.137	0.8	0.9	0.09
35	17.237	31.715	4.467	0.7	0.7	0.11
36	17.450	35.106	5.392	0.7	0.8	0.10
37	17.670	38.643	6.593	0.8	1.0	0.09
38	18.030	67.353	8.199	1.4	1.2	0.11
39	18.447	10.105	1.360	0.2	0.2	0.11
40	18.730	5.490	0.668	0.1	0.1	0.15
41	18.890	8.875	1.442	0.2	0.2	0.10
42	19.110	20.328	3.101	0.4	0.5	0.10
43	19.487	105.060	11.046	2.2	1.6	0.13
44	20.480	17.549	2.389	0.4	0.4	0.09
45	20.930	2.115	0.293	0.0	0.0	0.11
46	21.377	3.733	0.421	0.1	0.1	0.11
47	21.643	7.806	0.987	0.2	0.1	0.11
48	21.970	5.788	0.683	0.1	0.1	0.14
49	22.367	3.460	0.397	0.1	0.1	0.12
50	22.797	3.920	0.458	0.1	0.1	0.12
51	22.980	4.868	0.550	0.1	0.1	0.11
52	23.347	8.913	0.881	0.2	0.1	0.12
53	23.850	10.618	1.455	0.2	0.2	0.11
54	24.167	72.732	9.106	1.5	1.4	0.12
55	24.313	40.120	2.921	0.8	0.4	0.13
56	25.630	3.642	0.426	0.1	0.1	0.10
	Total	4840.162	672.333	100.0	100.0	

Appendix IX. Details of GC analyis of essential oil of *Muvaatupuzha Local*

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.580	2088.488	997.086	11.7	41.7	0.03
2	2.293	27.773	3.941	0.2	0.2	0.04
3	2.637	59.786	33.211	0.3	1.4	0.03
4	2.857	52.576	31.078	0.3	1.3	0.03
5	2.953	78.653	45.042	0.4	1.9	0.03
6	3.113	39.383	19.588	0.2	0.8	0.03
7	3.233	3.459	0.949	0.0	0.0	0.07
8	3.347	423.101	183.965	2.4	7.7	0.04
9	3.427	151.283	72.044	0.8	3.0	0.03
10	3.667	27.115	10.175	0.2	0.4	0.04
11	3.833	162.114	69.595	0.9	2.9	0.04
12	3.930	31.456	11.555	0.2	0.5	0.05
13	4.047	250.513	62.457	1.4	2.6	0.07
14	4.163	19.157	7.513	0.1	0.3	0.04
15	4.243	17.635	4.005	0.1	0.2	0.06
16	4.457	287.614	89.415	1.6	3.7	0.05
17	4.943	64.610	23.527	0.4	1.0	0.04
18	5.090	98.258	24.660	0.5	1.0	0.05
19	5.640	30.451	6.383	0.2	0.3	0.07
20	6.023	29.764	3.950	0.2	0.2	0.09
21	6.297	6.726	0.771	0.0	0.0	0.15
22	6.640	10.448	0.555	0.1	0.0	0.36
23	7.190	993.997	100.188	5.6	4.2	0.16
24	7.360	89.715	14.603	0.5	0.6	0.06
25	7.750	23.509	2.885	0.1	0.1	0.10
26	8.143	17.774	0.696	0.1	0.0	0.34
27	9.710	16.820	0.398	0.1	0.0	0.91
28	11.347	7351.369	195.085	41.1	8.2	0.62
29	12.107	20.639	2.603	0.1	0.1	0.09
30	13.483	1969.555	82.616	11.0	3.5	0.37
31	13.943	42.478	6.277	0.2	0.3	0.11

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
32	14.167	64.186	9.826	0.4	0.4	0.09
33	14.690	108.831	14.760	0.6	0.6	0.11
34	15.250	157.534	22.504	0.9	0.9	0.11
35	15.517	41.598	2.892	0.2	0.1	0.09
36	16.527	139.833	17.799	0.8	0.7	0.10
37	17.420	133.349	11.214	0.7	0.5	0.21
38	17.687	200.532	24.082	1.1	1.0	0.14
39	17.883	127.536	20.001	0.7	0.8	0.10
40	18.253	260.843	24.209	1.5	1.0	0.16
41	18.573	47.043	6.703	0.3	0.3	0.11
42	19.030	56.806	5.344	0.3	0.2	0.10
43	19.260	85.413	11.479	0.5	0.5	0.11
44	19.990	726.953	36.466	4.1	1.5	0.33
45	20.667	73.259	8.454	0.4	0.4	0.11
46	21.113	14.164	1.665	0.1	0.1	0.12
47	21.563	24.027	2.507	0.1	0.1	0.12
48	21.827	42.912	5.314	0.2	0.2	0.11
49	22.133	31.707	3.858	0.2	0.2	0.13
50	22.507	21.519	2.151	0.1	0.1	0.13
51	23.100	44.203	2.594	0.2	0.1	0.11
52	23.457	41.108	3.846	0.2	0.2	0.13
53	23.960	49.195	6.183	0.3	0.3	0.12
54	24.673	888.974	37.280	5.0	1.6	0.34
55	35.310	4.760	0.636	0.0	0.0	0.09
	Total	17872.502	2388.584	100.0	100.0	

Appendix X. Mean sum of squares of morphological and biochemical characters

Sl. No.	Characters	Replication mean sum of square	Treatment mean sum of square
	Morphological characters		
1.	Plant height	0.131	435.07
2.	Total number of leaves	1.28	1240.93
3.	Number of lateral branches	2.80	52.94
4.	Number of nodes per lateral branch	3.02	76.95
5.	Leaf length	0.11	420.57
6.	Leaf width	1.78	86.63
7.	Petiole length	0.11	33.48
8.	Leaf weight	1.05	1.36
9.	Leaf area	4.37	427.66
10.	Leaf weight per unit area	4.91	69.97
11.	Days between lateral branch emergence	0.45	48.50
12.	Leaf tip angle	3.77	149.88
13.	Angle between stem and petiole	3.49	549.28
14.	Days to lateral branch emergence	402.86	3901.92
15.	Spike length	0.023	9.45
16.	Spike diameter	0.001	0.022
17.	Spike peduncle length	0.022	0.326
	Biochemical characters		
18.	Chlorophyll a	0.105	0.032
19.	Chlorophyll b	0.054	0.020
20.	Total chlorophyll	0.408	0.107
21.	Protein	4.88	0.10
22.	Phenol	0.852	0.014
23.	Antioxidant capacity	4744.15	100.23

CHARACTERIZATION OF BETEL VINE (*Piper betle* L.) TYPES OF MALAPPURAM DISTRICT

by

PREETHY T.T.
(2012-11-118)

ABSTRACT OF THE THESIS

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Kerala Agricultural University

Department of Plant Breeding and Genetics
COLLEGE OF HORTICULTURE
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ABSTRACT

The present investigation on "Characterization of betel vine (*Piper betle* L.) types of Malappuram district" was carried out at the Department of Plant Breeding and Genetics, College of Horticulture, KAU, Vellanikkara and farmer's field in Malappuram district during 2013 – 2014 aiming to study the diversity of betel vine types in Tirur and nearby areas of Malappuram district and to characterize the types based on morphological and biochemical features.

Puthukodi, Chelan, Karinadan and *Nadan* were the betel vine cultivars recorded from Malappuram District. *Puthukodi* and *Nadan* were the most common cultivars whereas *Chelan* and *Karinadan* were the cultivars conserved by few farmers.

Morphological characterization revealed distinctness of *Karinadan* and *Chelan* from other cultivars. *Karinadan* had dark green leaves with even leaf margin, short petiole, mostly ovate lanceolate leaf lamina, high brittleness and medium pungency. Leaf characters including leaf length, leaf width, leaf area and leaf weight per unit area were comparatively high in this cultivar. Orthotropic shoots of this cultivar showed uniform purple – green color. It produced hanging lateral branches. The plant growth parameters like plant height, total number of leaves, number of lateral branches and number of leaves per lateral branch were significantly low in this cultivar.

On the other hand, *Chelan* had light green leaves with wavy leaf margin, long petiole, ovate leaf lamina and round leaf base. Leaf characters including leaf length, leaf width, leaf area and leaf weight per unit area and brittleness were significantly low in this cultivar leading to low market preference. In this cultivar, internodes of

orthotropic shoots showed light green colour with purple tinge. Lateral branches of *Chelan* were semierect in nature. The plant growth parameters like plant height, total number of leaves, number of lateral branches and number of leaves per lateral branch were significantly high in this cultivar, resulting in higher number of leaves per plant.

Nadan, Puthukodi and Muvattupuzha Local cultivars had green leaves with even margin and medium brittleness. *Puthukodi* recorded maximum leaf weight per unit area and optimum leaf parameters, making it as the most preferred cultivar in Malappuram district.

Profuse flowering was observed in all cultivars during the study period. *Chelan* was identified as male cultivar and all others were female cultivars. Spikes produced on *Chelan* were long, slender with short peduncle. Female cultivars produced medium lengthy spikes having medium diameter.

Spikes were axillary and opposite to leaf. Sessile naked florets were compactly arranged on the inflorescence axis. A bract subtended each floret, both in male and female spikes. Female spike was very distinguishable with the presence of 6 - 9 white coloured sessile stigmatic lobes on each floret. Male spikes were yellow in color and at the stage of anthesis, two black stamens protruded from each floret protruded.

Essential oil content was maximum in *Muvattupuha Local,* a cultivar with high pungency and it was low in *Chelan.* GC studies revealed that, eugenol was the major component of essential oil in all cultivars with high content (20.80 per cent) in *Chelan.* Possible compounds identified from the chromatograms of oil from different cultivars were hydroxychavicol, β caryophyllene and 5-(2-propenyl)-1, 3-benzodioxole.

Karinadan was rich in biochemical constituents whereas *Chelan* was significantly low in biochemical constituents. *Puthukodi* with optimum leaf characters recorded medium pungency.

Studies on genetic parameters revealed that most of the characters were less affected by environmental factors. Many of the characters under study showed high heritability with high genetic gain, indicating additive gene effects and selection must be effective. Correlation studies showed that the highest significant positive genotypic correlation of total number of leaves was with number of leaves per lateral branch followed by plant height and number of lateral branches. Number of leaves per lateral branch showed highest positive direct effect and all other characters showed negative direct effect on total number of leaves.

The dendrogram based on Euclidean distance proved that *Chelan* was very distinct from other cultivars under study. *Karinadan* also formed a separate cluster with a Euclidean distance of 1650.58 from *Muvattupuzha Local*. *Nadan* and *Muvattupuzha Local* proved their relatedness by being in a single cluster.