EVALUATION OF ECOTYPES OF LONG PEPPER

(*Piper longum* L.)

By

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THESIS

Submitted in partial fulfillment of the requirementfor the degree of

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DECLARATION

I, hereby declare that this thesis entitled **"Evaluation of ecotypes of long pepper (*Piper longum* L.)"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me 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 **"Evaluation of ecotypes of long pepper (*Piper longum* L.)"** is a bonafide record of research work done independently by **Mrs. Riya Joseph** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associate ship to her.

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I INTRODUCTION

Piper longum L. (Long pepper or Pippali) is an important medicinal plant belonging to the family Piperaceae. Vedas and all most all Samhitas have described this plant in detail. In Charakasamhita, Pippali has been mentioned for 264 times. Susruta also referred this plant with at most importance. Long pepper was well known in ancient India and was mainly used as source of pungency and for flavoring various beverages and dishes. Ravindran *et al.* (2005) reported that it was from India long pepper reached the rest of Asia and Mediterranean region through "spice route" and it was used as spice in this entire region.

Long pepper of commerce is the dried mature spikes of female plant. The dried roots and lower stems of male plant constitute Pipalamool, which also has medicinal properties. Iyer (1983) reported that long pepper is used mainly in Ayurvedic system of medicine, for about 320 classical preparations and in many herbal formulations. The medicinal uses of *Piper longum* in Ayurvedic, Unani and Sidha preparations have been described by several workers (Kirtikar and Basu, 1935; Suseelappan, 1991; Joseph and Skaria, 2001; Sasikumar, 2004). This forms an important component in Ayurvedic preparation such as Trikadu (dry ginger- black pepper-long pepper) and Panchakolam. It is considered as rejuvenating and revitalizing drug in Ayurveda and mature spikes of female plant, thick stems, roots and leaves are extensively used in treatment of bronchial diseases, dyspepsia, worms, amoebiasis etc.

Indian long pepper is mostly derived from the wild plants, the main sources of supply being Assam, West Bengal and Uttar Pradesh including Uttaranchal. Long pepper is reported to be cultivated at lower elevations in the Annamalai hills in Tamil Nadu and parts of Meghalaya, particularly in Cheerapunji area. It is propagated by cuttings, requires optimum shade and heavy organic manuring for proper growth and productivity. Due to its peculiar climatic requirement, long pepper is not recommended as a sole crop in Kerala, but as intercrop in irrigated coconut plantations (Viswanathan, 1995).

There is relatively high demand for this spice cum medicinal plant in domestic market in India. A survey carried out by State Government in 1987 revealed that annual requirement of Pippali in Kerala is 313 t and the demand for this crop is increasing day by day. Consequently, a large quantity of long pepper is imported to India from Malaysia and Singapore. Over the years industrial demand of long pepper is continuously increasing which is largely met by collection from its natural habitat, endangering the genetic variability and natural resource of this species. Hence it is essential to meet this demand by production through cultivation.

Kerala state is blessed with wide variability in long pepper. Identification of ecotypes with superior therapeutic and agronomic traits is essential for profitable cultivation of long pepper as under shrub in tropical plantations. Hence this investigation has been taken up with an objective to evaluate the various ecotypes of *Piper longum* available at Department of Plantation Crops and Spices for their variability in yield, morphological and biochemical characters so as to identify superior types with respect to yield and quality.

Review of literature

II. REVIEW OF LITERATURE

Piper longum, a member of the Piperaceae family, is the genuine source of Indian long pepper. Long pepper is cultivated all over India mostly on hotter parts. It is cultivated on a large scale in lime stone soil and in heavy rainfall areas where relative humidity is high. The major production areas have their own special adapted clones, such as suali clone (Assam and North East) and Asli clone (Bengal and Assam). Being a semi domesticated crop, collection of diverse germplasm within and outside the country and selection appears to be the first and foremost method of developing the high yielding varieties which suit to different agro climatic conditions and cropping systems

2.1 Origin and Distribution

Theophrastus had recognized two types of pepper, namely black pepper, *Piper nigrum* and long pepper, *Piper longum*, both of which were used by Greeks and Romans. Long pepper is indigenous to south Asian countries such as India, Srilanka, Bhutan and Nepal. It also occurs in Malaysia, Indonesia and Philippines. Purseglove *et al.* (1981) assume that *Piper longum* may be the first pepper to reach the Mediterranean and was more highly regarded by the Greek and Romans than *Piper nigrum*. In India it is distributed widely in low altitude of evergreen forests, occurring in sub Himalayan hills, Assam, Khasi regions, Eastern ghats and Western ghats, and in low elevation of forest lands and lower hills of West Bengal. Long pepper is cultivated in the tribal belt of Andhra Pradesh, parts of Orissa, Northwestern Bengal, Assam and in North Eastern states.

Piper longum has been located by Rao (1914) in the evergreen forests of Travancore, Arienkavu, Ponmudi and at elevations of 2000-3000 feet. Kirtikar and Basu (1935) reported the distribution of *Piper longum* in hotter provinces of India, Ceylon and Malay Islands. Krishnamurthy (1969) described the occurrence of *Piper longum* in the hotter parts of India, from central Himalayas to Assam, Khasi and Mikir hills, lower hills of Bengal and evergreen forests of Western Ghats from Konkan to Travancore and has been recorded from Nicober Islands. The distribution of *Piper longum* in Calicut was recorded by Manilal and Sivarajan (1982). The cultivation of *Piper longum* in Kerala was reported by Ravindran *et al.* (2005)

2.2 Taxonomy

Hooker (1890) divided the order *Piperaceae* in to two tribes namely *Saurureae* and *Pipereae.* The tribe *Pipereae* is further divided in to genus *Piper* and *Peperomia*. The genus Piper is further divided in to six selections namely *Muldera, Cubeba, Chavica, Pseudochavica, Eupiper and Heckeria*. *Piper longum* comes under the selection *Chavica.* The selection *Chavica* includes fifteen species other than *Piper longum*. They are *Piper peepuloides* or *Piper officinarum, Piper chaba* or *Piper retrofactum, Piper sylvaticum, Piper petiolatum, Piper betle, Piper miniatum, Piper boehmeriaefolium, Piper pothiformae, Piper anisotis, Piper aurantiacum, Piper hapinum, Piper bachystachyum, Piper thomsoni, Piper rostratum* and *Piper penangense.* Purseglove *et al.* (1981) listed out the economic species of *Piper* other than *Piper longum,* as *Piper nigrum* which is used as a major spice; *Piper betle L.* (Betel pepper) whose leaves are chewed as masticatory; *Piper methysticum Fors.t*, whose roots provide the national beverage of Polynesians etc. . Species like *Piper cubeba*, *Piper retrofractum* or *Piper chaba* (Java long pepper), *Piper clusii, Piper guineense, Piper saigonense* and *Piper longifolium* are used as spice.

2. 3 Habit and external morphology

Long pepper plant is a slender, creeping shrub, spreading on the ground and rooting at the nodes. It grows and creeps over small shrubs, rocks etc but does not climb on trees as in the case of black pepper or other wild peppers. The plant produces distinct dimorphic branches; those main branches creeping on the ground have cordate leaves with long petioles (plagiotropes); and those axillary branches that grow erect produces leaves that are sessile or with short petiole. The former shoots are vegetative and grow by the activity of terminal bud, while the short axillary branches are the fruiting branches, produce fruits (spikes) opposite to leaves, and growth is sympodial. Leaves are simple, alternate and variable in size and shape. Long pepper plant is dioecious, male and female plants are separate.

2.3.1 Stem Characters

Purseglove *et al.* (1981) reported that branches in black pepper are dimorphic in nature; the orthotropic vegetative climbing branches give the framework of the plant. These are stout branches with 5-12 cm long internodes; at each swollen node there is a leaf and an axillary bud, which can grow out to give a plagiotropic fruiting branch and short adventitious roots, which adhere firmly to the climbing support. Lateral fruiting branches have no roots. Both types of stems branch, but only orthotropic shoots will produce further climbing shoots and can be used for propagation. Ravindran and Balachandran (2005) reported that long pepper produce distinct dimorphic branches, those main branches creeping on the ground called orthotrope and those axillary branches that grow erect called plagiotrope. The former shoots are vegetative and grow by the activity of the terminal bud, while the short axillary branches are fruiting branches, produces spikes opposite to the leaves and the growth is sympodial. Kumar (1998) reported that stems of long pepper are numerous, ascending, cylindrical or globose. Stems are swollen and irregularly knotty with each piece quarter inch long, irregularly thick, hard and of a brownish color. Branchlets are erect, prostrate or creeping, soft and grooved when dry. The whole stem is finely pubescent

Sujatha and Namboodiri (1995) reported that, in black pepper stem characters like thickness of nodes of orthotrope, thickness of internodes of orthotrope and angle of insertion of plagiotrope influenced the yield positively. Earlier during 1979 Chandy and Pilli also observed that the drooping, horizontal or erect nature of plagiotropes varied between plant types in black pepper and this stem characters determined the photosynthetic efficiency and yield. It was reported by Ibrahim *et al.* (1986) that internodal length in black pepper varies more than any other morphological characters.

Manuel (1994) conducted a comparative evaluation of selected types of *Piper longum* in coconut plantation and reported significant variability in stem characters like the length of longest stem, number of stems per hill, number of vegetative branches per stem and these characters influenced the dry spike yield in *Piper longum*. Internodal length of main stem, number of spike bearing branches per stem and angle of insertion of spike bearing branches significantly varied in different accessions in *Piper longum*. Jaleel (2006) studied eight accessions of the *Piper longum* viz. Assam, Kanjur, Maharashtra, NL-84-68, Viswam, Pattambi, Nilambur, Odakkali and reported that length of spike bearing branch and internodal length of different accessions differ significantly. At the same time, angle of insertion of reproductive branches of different accessions did not differ significantly

2.3.2 Leaf Characters

According to Purseglove *et al.* (1981) *Piper nigrum* leaves on both climbing and fruiting branches are alternate and simple with petiole 2-5 cm long, which is grooved above. Lamina is ovate, entire, coriaceous with the base oblique, obtuse or rounded and tip acuminate. Leaves are dark green and shiny above, pale and gland dotted beneath. The size varies greatly between cultivars and may be 8-20 cm or more long and 4-12cm or more broad. There are usually 5-7 main veins.

Chatterjee and Pakrashi (1997) reported that leaves of *Piper longum* types are 5-9 cm long, 3-5 cm wide, subacute, entire, glabrous, cordate with broad rounded lobes at base. Kumar (1998) observed that leaves in long pepper are numerous, simple, stipulate and petiolate or sessile according to their position on the plant. Leaf blade varies in shape in the same plant. The upper leaves are generally sessile, damplexicaul or stem creeping, ovate or ovate oblong, acute and most often unequally sided or unequally cordate at base. It was also reported that leaves are 6.5-9 cm long and 3-5 cm wide. Lower leaves are broadly ovate, pale dull beneath; cordate at base. Petioles of lower leaves are 0.5-7.5cm long and stout but that of the upper ones is very short and absent. Stipules are about 1-3cm, membraneous, lanceolate, obtuse and falling soon. CSIR (1998) reported that leaves of *Piper longum* are 5-9cm long, 3-5cm wide, ovate, cordate with broad rounded lobes at base, subacute, entire, and glabrous. Ravindran and Balachandran (2005) found that orthotropes of *Piper longum* have cordate leaves with long petioles and plagiotropes produce leaves that are sessile or with short petiole. Leaves are simple alternate and variable in size and shape

Manuel (1994) studied four different accessions of long pepper and reported significant variability in leaf characters like leaf length, leaf width, and length of leaf, petiole and number of leaves per hill and found that these leaf characters do not influence the dry spike yield. Jaleel (2006) worked with eight accessions of *Piper longum* and reported that length of leaves on vegetative branch and length of leaves produced on reproductive branch differ significantly. The mean length of leaf lamina on vegetative branches was maximum for accession namely NL-84-68 (11.25cm) and minimum for the variety Viswam (6.37cm). On the reproductive branch, the longest leaves were observed in the male accession, Nilambur (8.97cm) and the accession Pattambi recorded the shortest lamina length (4.49cm) followed by Viswam (5.14cm). It was also reported that area of leaves on vegetative branches and the leaves on reproductive branches showed significant difference between accessions. Petiole length of different accessions also showed significant variability. Shape of leaf lamina for all the eight accessions for the vegetative branch was chordate, where as it was lanceolate for the leaves of reproductive branches. All accessions had dark green color for leaves of vegetative branch except Viswam, which had medium green colour and in the leaves of reproductive branches all accessions except Odakkali (Medium green) recorded dark green color.

2.3.3 Inflorescence, Flowers & Spikes

Purseglove *et al.* (1981) reported in black pepper, the pendant spikes are borne opposite to the leaves on plagiotropic branches and is 3-5 cm long, bearing 50-150 minute flowers borne in the axils of ovate fleshy bract. The flowers may be unisexual, with monoecious or dioecious or hermaphrodite but most of the cultivated types are bisexual. This may be a condition that probably originated from the wild ones as a result of continuous selection and vegetative propagation through ages. He also reported that the cultivars exhibit great variability in the percentage of bisexual or productive flowers on their spikes. Higher the percentage of bisexual flowers, greater will be the productivity and most of the high yielding and popular cultivars produce as much as 70-98 percentage bisexual flowers. Purseglove *et al.* (1981) added that under intense shade conditions the bisexual types produce more female flowers and less hermaphrodite flowers.

Kumar (1998) reported that *Piper longum* produce spikate inflorescence, whose flowers are sessile, usually unbranched, elongated, simple, and indeterminate. Flowers are without perianth, very densely packed and male and female parts are on separate plants. Spikes are 5cm long, cylindrical, solitary, pedunculate and upright. Flowers are unisexual, minute and sessile. Male spikes are large, narrow, and slender, bracts narrow, 1-3 inch long, peltate, stamens two in number. Female spikes are 1.3-2.5cm long and 4-5mm in diameter, bracts circulate, flat and peltate, stigma, 3-4 very short and persisting. Flowering season is July-August. CSIR (1998) reported that spikes are cylindrical pedunculate, male larger and slender, female 1.3-2.5cm long and 4-5mm diameter.

Ravindran and Balachandran (2005) found that long pepper plant is dioecious, male and female plants are separate. Flowers are arranged on a short cylindrical spike, male spikes are much longer than the female spikes. Female spikes are short, stout, flowers fused laterally. Female flower consists of one ovary only that arises from axil of the bract.

Kumar (1998) reported that fruits in *Piper longum* are short, consists of multitude of minute buccate fruits closely packed among a common axis, whole forming a spike, one and half inch length and quarter inch thickness. Fruits are ovoid, crowned with a stigma and arranged with small peltate bracts beneath each. When ripe, fruits are grayish green or nearly blackish and particularly sunk in fleshy axils. Fruiting season is November to March. Seeds are globose, testa thin, with in the hardened periphery. Ravindran and Balachandran (2005) reported that fruits of *Piper longum* are small, closely packed. Fruiting is apomictic and fruits are produced without pollination. Hence male plants are not required for fruit production. He also reported that fruits of *Piper chaba* are larger, more conical and not cylindrical as in *Piper longum*, become orange red on ripening and are more pungent with less flavour. Spikes of *Piper peepuloids* look similar to *Piper longum*. *Piper mollesua* spikes are globose with minute fruits that are not laterally fused.

2.4 Chemical constituents

Kumar (1998) reported that two alkaloids, Piperlongumine (piplartine) and piperlonguminine isolated from roots and stem bark of *Piper longum* and characterised as N-(3,4,5- trimethoxy cinnamoy) – piperidin- 2 one- 5-ene and isobutyl amine of piperic acid respectively. Atal and Ojha (1966) reported the presence of sesamine, dihydrosterol and a new sterol, piplasterol in the fruits of *Piper longum*. According to Tabuneng *et al.* (1983) chemical constituents of *Piper longum* include amides (greater than 12 in numbers) and lactams (greater than10 in numbers). Of the amides, piperine is most abundant and is present in fruits to the extent of 3-4 percentages, while piperlonguminine is the next major amide. Other noted amides in fruits include pipernonaline, dehydropipernonaline, longamide etc. Roots contain amides like piperlongumine and piperlonguminine and stem bark contain amide called piplartin. *Desai et al.* (1989) reported that lactams were obtained from the roots, and these are alkaloids belonging to the group aristolactam (e.g., Piperolactam B, Aristolactam AII, Cepharanone B) and 4, 5 – dioxo apoorphines (e.g., Cepharadione A, Cepharadione B, Piperadione). CSIR (1998) reported that compounds obtained from this plant are ligans (sesamin, pluviatilol, methyl pluviatilol, asarinin) and sterols (dihydro stigma sterol). It was also reported that the fruit of *Piper longum* had shown the presence of alkaloids piperine (4-5 %), piplartine, two new liquid alkaloids, one of which designated as alkaloid A, which is closely related to pellitrorine. It was quoted by Rastogi and Mehotra (1993) that three alkaloids piperolactam A, piperolactam B and piperodione isolated from roots and characterized as 10-amino – 4 hydroxy -2,3- dimethoxy – phenanthrene -1-carboxylic acid lactam; 10- amino 4 hydroxy -2 , 3- dimethoxy phenanthrene -1 – carboxylic acid lactam and 2- hydroxy -1-methoxy-6- methyl – 4H- dibenzo quinoline – 4,5 (6H)-dione respectively.

Ravindran and Balachandran (2005) reported that dried long pepper spikes contain 9.5 percentage moisture, 12.2 percentage protein, 39.5 percentage starch, 5.8 percentage fiber, and 5.9 percentage ash, 4.2 percentage ash insoluble in acid, 1.5 percentage volatile oil, 6.6 percentage fixed oil and 0.5-4.5 percentage piperine. Spikes contain constituents like sylvatin, sesamin, diaeuolemin, piperine, pipalartine, asparinin, pulviatilol, fragerin (E), pipericide, guineenside, longamide, piplasterol and dihydro – piperonaline. Roots contain chemical constituents like piperine, piperlongumine, piperlonguminine, piplasterol, triacontane, cepharanone B, aristolactum AII, piperolactum A, piperolactum B, cepharadione A, noncepharadione B, sesamin, 22,23-dihydro stigmasterol, methyl 3,4,5-timethoxycimamate and piperadione etc. It was also reported that leaves contain compounds like Henriacontane, B-sitosterol, hernia contane -16-one and triacontanol. Manuel (1994) studied the total alkaloid content of five types of *Piper longum* and recorded maximum percentage of alkaloid for Panniyur (2.91

%) followed by Cheematippali (2.87 %), Pattambi (2.85 %) and Kaanjur (2.85 %). The minimum alkaloidal content of 2.80 % was recorded in Mala.

Richest and Flegem (1966) identified a new sesquiterpene hydrocarbon containing tetra-substituted double bond from essential oil. Chopra *et al.* (1999) reported that *Piper longum* contains essential oil consisting of long chain hydrocarbon, mono and sesquiterpenes, caryophyllene being the main product. CSIR (1998) reported that fruits of *Piper longum* on steam distillation furnish an essential oil yield of 0.7 percentage, in which caryophyllene is the major constituent. Ravindran and Balachandran (2005) reported that long pepper on steam distillation yield 0.7-1.5 percentage of light green viscous essential oil with spicy odour, having the following characteristics vise density 0.8484, specific gravity 1.4769, optical rotation +40.1degree, acid value 7.2, saponification value 8.9. Main constituents are n-hexadecane, n-heptadecane, n-octadecane, n-eisocane, n-heneicosane, thujene, terpinolene, zingiberene, dihydro carveol, phenyl ethyl alcohol,p-methoxy acetophenone, p-cymene.

2.5 Biological properties

Atal and Ojha (1966) reported that Alkaloid A in *Piper longum* showed significant antitubercular activity against *Mycobacterium tuberculosis* H-37 Rv strain and also it inhibited the growth of the bacillus in 20 microgram /ml concentrations. Koul and Kapil (1993) evaluated Long pepper and piperine for their hepatoprotective formulations and find that these have significant protection against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity reducing *in vitro* and *in vivo* lipid peroxidation. Ravindran and Balachandran (2005) reported that alcohol extracts of dry fruits and aqueous extracts of leaves had shown activity against *Micrococcus pyogenes* var *aureus* and *Escheritia coli*. Ravindran and Balachandran (2005) reported that the alkaloid fraction had shown significant *in vitro* antitubercular activity against *Mycobacterium tuberculosis H37 Rv* strain. Dihydro piperonaline isolated from dried fruits had significantly inhibited KCl- induced contraction of the rabbit isolated coronary artery. Long pepper increased phenobarbitone-sleeping time, which was suggested to be due to the enhancement of barbiturate uptake. Methanolic extract of *Piper longum* shows nematological activity on the second stage larvae of *Toxocara* even at 0.1-0.2 mg/ml.

2.6 Pharmacognosy

Ravindran and Balachandran (2005) reported that even though *Piper longum* is the genuine source of Indian long pepper, in raw drug market, the spikes of certain other species are also traded as long pepper such as *Piper peepuloids, Piper chaba (Piper officinarum, Piper retrofactum)* and *Piper mullsuca*. *Piper chaba* is the source of raw drug 'Chavya' and is commonly known as Java long pepper (Java tippali). Chavya is used as separate raw drug in classical medicinal formulations. The fruits of this species are larger, more conical and not cylindrical as in *Piper longum*, become orange red on ripening (not black as in case of *Piper longum*) and are more pungent with fewer flavors. *Piper peepuloids* looks similar to *Piper longum*; while *Piper mullesua* spikes are globose with minute fruits that are not laterally fused and is usually known as 'Golmirch' in north Indian markets. The entire above are often traded mixed as Indian long pepper. Based on size and taste, certain other varieties are also recognized in trade such as suali pipali (comes mainly from Manipur and adjacent regions of North East), Asli pipali (comes mainly from Uttaranchal, Assam and North Bengal), Desi, pippali (comes mainly from Andhra Pradesh and Orissa), Golpippali (mainly from Uttaranchal).

2.7 Uses

According to Iyer (1983) Long pepper (fruit, root or both) gets in to more than 320 classical Ayurvedic formulations. Long pepper is also a component of various modern herbal formulations meant for digestion, dyspepsia, asthma, bronchitis, chest congestion etc.It is also reported that Pippali cures cough,dyspnoea, ascites, leprosy, diabetes, piles, colic indigestion, anaemia, thirst and dispels cardiac and splenic disorder, fever, loss of appetite and worm troubles. CSIR (1998) reported the medicinal uses of Pippali as for diseases of respiratory tract, cough, and bronchitis, asthma etc. It is used as counter-irritant and analgesic when applied locally for muscular pains and inflammation.

2.8 Characters to be considered for comparative evaluation

2.8.1 Vegetative characters

Sujatha and Namboodiri (1995) studied the influence of vegetative characters on yield of black pepper (*Piper nigrum*) observed that characters like thickness of node of orthotrope, thickness of internode of orthotrope and angle of insertion of plageotrope influenced the yield positively. In this study data on 580 genotypes of black pepper were utilized to estimate correlation between yield and 20 quantitative characters. The thickness of internodes also showed high intercorrelation with these reproductive characters. Thickness of main stem of pepper vine also influenced the yield. Chandy and Pilli (1979) reported that the thickness of orthotrope increases as the vine matures. According to George and Mercy (1978), a pepper vine starts yielding around fifth year of planting and yield increases and stabilizes as the vine matures.

Sujatha and Namboodiri (1995) also reported the significant positive correlation of angle of insertion of plageotrope with yield. It may be due to the fact that as the angle increases the plageotrope will be more or less horizontal and this will enable the vine to hold most of leaves on these fruiting branches directly against sunlight, thus increasing photosynthetic efficiency and yield in black pepper. Chandy and Pilli (1979) observed that the drooping, horizontal or erect nature of plagiotropes determined the photosynthetic efficiency and yield. In this study it was also reported that length of leaf, length of internodes of plagiotrope, thickness of node of plagiotrope showed non significant and positive correlation with yield, while length of petiole, breadth of leaf, area of leaf and thickness of internode of plagiotrope showed negative and non significant correlation with yield.

Ibrahim *et al.* (1985) reported that in black pepper internodal length varied with the varieties more than any other morphological character. The internodal length in pepper is of economic importance, as shorter internodes tend to increase total number of spikes. The variety Panniyur- 1 showed most variability for internodal length.

Manuel (1994) reported that the length of longest stem, number of stems per hill, the number of vegetative branches per stem exhibited significant positive correlation with dry spike yield in *Piper longum*. The number of spike bearing branches and angle of insertion of spike bearing branch in *Piper longum* was significantly influenced the dry spike yield during 7 and 8 month after planting. Vegetative characters like length of petiole, area of leaf lamina, internodal length of spike bearing branches, etc. did not influence the yield of *Piper longum*.

Manuel (1994) conducted a variability study in five accessions of *Piper longum* and reported the significant variability in vegetative characters like length of longest stem, number of vegetative branches per stem, internodal length of longest stem, length of leaf, width of leaf, length of petiole, number of leaves per hill, spread of plant, number of spice bearing branches per stem and angle of insertion of spice bearing branches. Manuel (1994) concluded that selection for improvement of dry spike yield in *Piper longum* will be efficient, if it is based on vegetative characters like angle of insertion of spike bearing branch, number of stems per hill, number of spikes per spike bearing branches and number of spike bearing branches per stem.

2.8.2 Yield contributing characters

Sujatha and Namboodiri (1995) conducted a study on heterosis in black pepper (*Piper nigrum L.*) and reported that the reproductive characters viz. number of spikes per vine, number of developed berries per spike and length of spike influenced the dry berry yield per vine. Sujatha and Namboodiri (1995) reported that in black pepper reproductive characters viz. green spike yield per vine, green berry yield per vine, number of spikes per vine and number of under developed berries per spike are positively and significantly correlated with yield. Ibrahim *et al*. (1985) also reported the positive and significant relation ship between yield and spike number, spike length and number of under developed berries per spike.

Manuel (1994) conducted a comparative evaluation of selected types of *Piper longum* in coconut plantations and reported that the five types of *Piper longum* did not differ significantly for the productive characters namely length of spike, diameter of spike, length of peduncle, days from planting to emergence of spike, days from emergence to maturity of spike and ratio of weight of green spike to weight of dry spike. At the same time the *Piper longum* types differ significantly for the productive characters namely number of spikes per spike bearing branch, yield of green spike in Kg/ha and yield of dry spike in Kg/ha.

Manuel (1994) evaluated *Piper longum* type's viz. Cheematippali, Panniyur, Mala, Pattambi, Kaanjur and reported that the length of spike did not differ significantly for the five types of *Piper longum* at the first and second harvest. The length of spike varied from 4.00 cm (Pattambi) to 4.33 cm (Cheematippali). Diameter of the spike also did not vary significantly for the five types and it ranges between 2.22cm (Pattambi) and 2.54 cm (Mala). Number of spike bearing branches differed significantly between the different accessions and was recorded in the range of 1.33cm for Mala, which was minimum value and 3.93cm, which was maximum value for Cheematippali. All the five types of Piper longum took all most same time from planting to emergence of spike and it varied like 163.7 days for Mala, which was the minimum and 180.56 days, which was the maximum duration. Manuel (1994) also reported that all the *Piper longum* types took almost same period for the maturity of spike. The five types differed significantly in the yield of green spikes and recorded as 2621.94Kg/ha (Cheematippali), 2499.44Kg/ha (Kaanjur), 2233.06 Kg/ha (Pattambi), 1813.33 Kg/ha (Panniyur) and 1530.55Kg/ha (Mala).In case of dry spike yield it was reported the maximum yield for Cheematippali (580.83Kg/ha) and minimum yield for Mala (201.95Kg/ha).

Jaleel (2006) reported the significant variability of spike length while evaluating the eight accessions of *Piper longum* viz. Assam, Kanjur, Maharashtra, NL-84-68, Viswam, Pattambi and two male accessions Nilambur and Odakkali and recorded that male accessions viz. Nilambur and Odakkali produce longest spikes with mean length of 7.55cm and 7.31cm respectively. At the same time for female accessions the longest spike length recorded for NL-84-64 (4.23cm) and shortest spike length for the KAU variety Viswam (2.4cm). Spike diameter also varied between the different accessions and NL-84-68 recorded the maximum diameter 3.59cm and the male accessions Nilambur and Odakkali recorded minimum spike diameter 1.31cm and 1.4cm respectively. The female accession Viswam that recorded a spike diameter of 1.53 cm, which was very near to the male accessions. The accession NL-84-68 had the boldest spike in terms of spike length and spike diameter. Jaleel (2006) also reported a maximum of four spikes per spike bearing branch for Assam followed by Viswam and male accession Odakkali 93.6 spikes. NL-84-68 recorded the minimum number of spikes per spike bearing branch (2.6). For the spike initiation, the male accessions Nilambur took the minimum days (132 days) and the female accession NL-84-68 took the maximum number of days (178 days). The later recorded the maximum number of days for the maturity of spike (69.6). The male accessions took 56 days for the maturity recorded the minimum. Jaleel (2006) recorded variation in fresh weight of spikes for all the eight accessions NL-84-68 recorded the maximum mean spike weight (1.639g) followed by Maharashtra (1.58g). The minimum fresh weight was for Viswam, 0.36g per spike.

2.8.3 Characters of yield and their correlation with path analysis

Sujatha and Naboodiri (1995) studied the characters of yield and their correlation in black pepper (*Piper nigrum* L.) and found that the dry berry yield per vine and the three reproductive characters viz. number of spikes per vine, number of developed berries per spike and length of spike which were having highly significant correlation with dry berry yield per vine. Sujatha and Namboodiri (1995) studied 580 genotypes of black pepper and estimated the correlation between yield and 20 quantitative characters. The results revealed that the reproductive characters viz. green spike yield per vine, green berry yield per vine, number of spikes per vine and number of under developed berries per spike as well as vegetative characters like thickness of node and internode of orthotrope and angle of insertion of plagiotrope are positively and significantly correlated with yield. The intercorrelations among these characters were also positive and significant. Path analysis revealed positive direct effect on yield by the characters green berry yield, spike number, spike length and angle of insertion of plagiotrope. The highest correlation was exhibited by green berry yield per vine, spike number. Sujatha and Namboodiri (1995) also reported that green spike yield per vine is also highly correlated with green berry yield per vine, spike number, spike length, number of developed berries per spike, thickness of node and internode of orhotrope and angle of insertion of plagiotrope. Positive and highly significant correlation was reported between pairs of characters viz. spike length and number of developed berries per spike, spike length and number of under developed berries per spike. This is because, as the length of spike increases, the total number of berries also increases. Ibrahim *et al.* (1985) also reported the positive and significant relationship between yield and spike number, spike length and number of developed berries per spike.

Sujatha and Namboodiri (1995) reported the positive and significant correlation with yield and vegetative characters like thickness of node of orthotrope, thickness of internode of orthotrope and angle of insertion of plagiotrope in black pepper. The intercorrelation between thickness of node and green spike yield per vine, spike number and spike length were also positive and highly significant. The thickness of internode also showed high intercorrelation with these reproductive characters. Path analysis revealed that, the direct effect of above two characters is negative and small but the high and positive indirect effects through their influence on green berry yield per vine are the reason for high correlation with yield.

Sujatha and Namboodiri (1995) reported the Intercorrelation of angle of insertion of plagiotrope with green spike yield per vine; green berry yield per vine and thickness of internode of orthotrope was significant. In path analysis the direct effect was found positive though small and it had a positive indirect effect through green berry yield per vine. Among the rest of the vegetative characters studied, length of leaf, length of internode of plagiotrope and thickness of node of plagiotrope showed no significant and positive correlation with yield, while the length of petiole, breadth of leaf, area of leaf and thickness of internode of plagiotrope showed no significant and negative correlation with yield.

Manuel (1994) conducted correlation studies between the vegetative and reproductive characters and spike yield in *Piper longum*, and it was found that vegetative characters like length of longest stem and number of stems per hill was significantly correlated to dry spike yield. The number of vegetative branches per stem exhibited significant correlation with dry spike yield at 5, 6, 7 month after planting. During 7th and 8th month the correlation between numbers of spike bearing branches per stem to dry spike yield was significant. The angle of insertion of spike bearing branch had found to be significantly correlated to dry spike yield. The number of spikes per spike bearing branch showed significant correlation with dry spike yield at 5, 6, 7 month after planting. Manuel (1994) reported highest positive correlation of green spike yield with dry spike yield followed by angle of insertion of spike bearing branch and number of stems per hill. The characters such as number of spikes per spike bearing branches per stem, length of longest stem, number of vegetative branches per stem and number of leaves per hill showed significant positive correlation with dry spike yield. Path analysis revealed that angle of insertion of spike bearing branch, number of vegetative branches per stem, number of spikes per spike bearing branch, length of longest stem, number of leaves per hill, number of stems per hill showed positive direct effect towards yield. Out of this, maximum contribution to dry spike yield is through angle of insertion of spike bearing branch.

2.9 Phytochemical Methods

2.9.1 Alkaloids

Daniel (1991) reported the presence of piperine on the TLC plate , when methanol extract of finely powdered black pepper was run in solvent system Toluene-ethyl acetate (70;30).The developed chromatograms were seen in UV360 nm and the major spot was fluorescing blue with Rf value 0.4 and reported the same as Piperine. Two more spots were observed below piperine which is dipiperine and piperettine. All these alkaloids turned reddish brown on spraying with concentrated sulphuric acid and subsequent heating. The extract of cubeb (*Piper cubeba L)* exhibited a bright violet spot (Rf about 0.45) in the toluene-ethyl acetate (70:30) solvent system, on spraying with concentrated sulphuric acid. It was observed that the compound is cubebin, which is a ligan. Piperine was not seen in Cubeb. It was also reported that the alkaloids present in genus Piper are of the Piperidine type. Sankar (1998) conducted *in vivo* and *in vitro* screening of *Sida spp*. for ephedrine content and found separation in the solvent system hexane-ethyl acetate (3:1) with concentrated sulphuric acid as spray reagent. It was also found the separation of the compound in butanol-glacial acetic acid-water (4:1:1) system with ninhydrin reagent as spray solution. Manjusha (2001) studied the biochemical variation in *Adathoda* types and observed the pink and orange spots of alkaloids under Chloroform: Methanol (9:1) solvent system on spraying with dragendorff reagent.

2.9.2 Phenols

The phenolics present in spices are simple in structure mostly containing a single aromatic ring, which contribute taste, colour and flavour to a number of spices. Daniel (1991) reported that simple phenols are present in vanilla (vanillaldehyde), winter green (methyl salicilate) and ginger (gingerols), while phenolic amines are pungent principles of red pepper (capsaicins), phenyl propanoids are present in tonco seeds (coumarin) and cubeb (ligan) where as phenyle propenes are seen in cloves (eugenol), nutmeg (myristicin) and fennel (anethole).

The distribution pattern of different phenolic acids in the Tribe Amhersticae (Caesalpinioideae) was studied by Nageshwar *et al.* (1987). Phenolic compounds in various olive varieties were determined by HPLC analysis by Esti *et al.* (1998). Dimethyl oleuropecin was found in two of the eight varieties studied. Manjusha (2001) performed qualitative tests for secondary metabolites (phenols) present in *Adathoda zeylanica* and *Adathoda beddomei* using thin layer chromatography and found separation for compound in Hexane –ethyl acetate (3:1) solvent system with Folins ciocalteus phenol as spraying reagent. The spots turned blue in colour.

2.9.3 Flavanoids

Flavanoides are mainly water-soluble compounds structurally derived from the parent substance flavone. As they are phenolic, they change colour when treated with base or ammonia. These compounds contain conjugated aromatic system and these show intense absorption bands in the UV and visible regions of the spectrum. Rangaswami and Seshadri (1971) identified luteolin as flavone in *Adhathoda vasica*. Total flavanoid content of ten species of *Artemissia subgenus Dracunculus* of Kazhakstan flora were compared by Alyukina and Ryakhovskaya (1980). The content of ariel parts was found to vary from 36 to 57 mg per gram (of dry matter) in eight sp while in the other two; this was only 18.0 and 5.7 mg per gram respectively.

Erythroxylum rufum and *Erythroxylum ulei* were examined by Bohm *et al.* (1981) and different pattern of flavanoids were seen in different collection of *E .ulei* where as four specimens of *E rufum* were identical. There was also difference in pattern between the species. The flavanoides of two geographically distinct variety of *Passiflora foetidae* had been reported by Ulubelin *et al* (1982) and found difference in composition of flavanoids between *Passiflora foetide var hibiscifolia* and *Passiflora foetide var hispida.* Chemotaxonomical study of South Indian taxa of *Piper* was carried out by Ravindran and Babu (1994). Fourteen taxa were analyzed for their flavanoids and based on the presence or absence of these compounds, percentage similarity indices were calculated. A chemical dichotomy was evident between the two sub genera Pipali (having erect spikes) and Muricha (having pendant spikes), there by supporting the validity of the sectional classification.

2.9.4 Terpenoids

Holden and Mablbery (1992) studied chemotaxonomy in *Euphorbia sp.* and qualitative and quantitative differences for components of terpenoid profiles were employed to distinguish between accessions of *Euphorbia sp.* Triterpenoid profiles were diagonised for a species and were similar for each of the accessions in the species collected from distant areas of Europe. Meragelmun *et al.* (1996) analyzed terpenoid constituents of *Viguiera sp.* and two varieties collected from different geographic regions of Argentina were shown to have very close chemical constituents.

Materials and Methods

III. MATERIALS AND METHODS

The investigations on "Evaluation of ecotypes of long pepper (*Piper longum* L.)" were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during September 2006 to September 2007. The experiment was conducted to evaluate the various ecotypes of *Piper longum* for their variability in yield, morphological and biochemical characters so as to identify superior types with respect to yield and quality. The experimental field is located at an altitude of 22.5m above MSL, between 10º 32^1 latitude and 76º 16 m longitude. The experimental site has a sandy loam soil which is acidic in reaction (pH 5.3). The area lies in tropical monsoon climatic region, with more than 80 per cent of the rain fall getting distributed through south west and north east monsoon shower. Data on temperature, rain fall, relative humidity, number of rainy days and sun shine hours during the entire cropping period were collected from meteorological observatory of College of Horticulture, Vellanikkara (Appendix-II). The details regarding experimental materials and methodology adopted for the study are presented in this chapter

Studies were undertaken under the following two major heads

3.1 Genetic cataloguing of long pepper

3.2 Evaluation of ecotypes of long pepper

3.1 Genetic cataloguing of long pepper

Twenty accessions (Table1) were genetically catalogued based on the descriptor developed for *Piper nigrum* by IPGRI (Table 2).

3.2Evaluation of ecotypes of long pepper

3.2.1 Plant material

Germplasm collections of long pepper (15 types) maintained as part of the project on "Germplasm collection and evaluation of medicinal plants" ongoing at Department of Plantation Crops and Spices and four accessions collected from different locations were utilized for the study. The released variety, Viswam was included as check. Details of *Piper longum* accessions selected for study are given in Table 1.

Table.1. Details of *Piper longum* selected for study

SI No	Accession no	Description
1	PL1	Viswam
2	PL2	IC 85299
3	PL3	IC 85301
4	PL4	IC 85247
5	PL5	IC 85278
6	PL6	IC 85270
7	PL8	Mananthody I
8	PL9	Mananthody II
9	PL10	IC 85277
10	PL11	IC85285
11	PL12	IC 85298
12	PL13	IC 266468
13	PL15	Yercaud
14	PL16	Marotichal I
15	PL17	Marotichal II
16	PL18	Kutanellur
17	PL19	Ollur
18	PL20	Mala
19	PL21	Kottakkal
20	PL22	Thiruvananthapuram

Plate – 1 – Field Photo

Table .2. Genetic cataloguing of long pepper (*Piper longum* L.)

1. Vegetative characteristics

1 Growth habit	-	Climbing / trailing /Erect
2. Branching type	-	Dimorphic / Polymorphic/ others
3. Runner shoots production	-	Few / Many
4. Pubescence on stem	-	Absent / present
5. Lateral branch habit	-	Erect/ Horizontal/ Hanging
6. Leaf lamina Shape	-	Ovate/ Ovate eliptic / Ovate lanceolate / Eliptic lanceolate / Cordate
7. Leaf base shape	-	Round/ Cordate / Acute / Oblique
8. Leaf margin	-	Even / wavy
9. Types of veining	-	Acrodromous / Camphylodromous/ Eucamptodromous
10. Leaf texture	-	Glabrous Coriaceous/ Glabrousmembraneous/ Glabrous sarcous/ Downy membraneous / Downy along the veins
11. Leaf Sales	-	Absent/ Present

2. Inflorescence and fruit characteristics

1 Spike orientation	-	Erect/ Prostrate
2. Spike Shape	-	Filiform / cylindrical / Globular/ Conical

3. Immature spike color	-	Green / Greenish yellow/ Light yellow/ Light Purple/ others.
4. Color change while fruit ripening	-	Green to black / Green to yellow, orange and then to red
5. Spike fragrance	-	Not fragrant / Fragrant
6. Flower arrangement on spike	-	Free / fused laterally
7. Spike texture	-	Glabrous / Hirtellous
8. Bract type	-	Sessile oblong and adnate to the rachis/ Cupular with decurrent base/ Fleshy, Connate, transformed in to a cup/ Deeply cupular with decurrent base/ Others.
9. Flower nature	-	Sessile/ shortly stipitate/ pedicillate
10. Fruit shape	-	Round / ovate / oblong / others
11. Fruit taste	-	Bitter / Pungent/ spicy.

3.2.2 Experiment Method

Two nodded cuttings of *Piper longum* accessions were raised in the nursery and two rooted cuttings were planted per hill in the field as intercrop in coconut garden in Randomized Block Design with two replications. The plot size adopted was 3mx1.8m and planting was made at a spacing of 60 cm x 60 cm.

Gap filling was carried out occasionally after planting in the main field. Weeding and earthing up were done at regular intervals. Irrigation was carried out weekly once during summer months. Farmyard manure was applied in three split doses, one at time of planting and consequent ones at three-month intervals. Staking was done two months after planting to provide support to the plant.

3.2.3 Observations

Observations on vegetative and productive characters were recorded from five hills randomly chosen from each plot for all the 20 types, leaving a border row on all sides. A total of five hills were selected at random from each plot and tagged for recording observations

3.2.3.1 Stem characters

a. Number of stems per hill

The number of stems per hill from each of five hills per plot was counted at monthly intervals and the mean of stems per hill was arrived.

b. Number of vegetative branches per stem

The number of vegetative branches per stem was recorded at monthly interval for two stems chosen at random from each of five hills per plot and mean arrived based on ten observations per plot.

c. Number of spike bearing branches per stem

Number of spike bearing branches per stem was recorded for ten stems chosen at random from five hills per plot and mean arrived at monthly intervals

d. Angle of insertion of spike bearing branch

The angle subtended by the spike-bearing branch with the main stem was measured in degrees with the help of protractor. Observations were recorded for ten-spike bearing branches chosen at random from five hills per plot and mean arrived at.

e. Internodal length of spike bearing branch

The length of two internodes of spike bearing branches selected at random from each of the five hills per plot was measured in cm using a meter scale and mean arrived at based on ten observations per plot at monthly intervals

f. Length of longest stem

The length of longest stem was measured in cm from ground level to the tip of the stem using meter scale and was recorded from five hills per plot and mean arrived at, monthly intervals

3.2.3.2 Leaf character

a. Leaf shape

Shape of leaf lamina was recorded by visual observation. The shape of leaf lamina was compared with the different types of leaf shapes suggested in *Piper nigrum* by IPGRI (1995).

b. Leaf area

Leaf area was determined using leaf area meter. The observations were recorded for each of twenty leaves chosen at random from five hills per pot and mean arrived.

c. Leaf color

Leaf color was assessed by visual observation.

d. Petiole length

The length of petiole was measured in cm using a meter scale for each of ten leaves chosen at random from five hills per plot and mean arrived at monthly intervals.

e. Number of leaves per hill

The number of leaves from each of the five hills per plot was counted and mean arrived at monthly intervals.

3.2.3.3 Spike characters

a. Number of spikes per spike bearing branch

The number of spikes was recorded for ten spike bearing branches chosen at random from five hills per plot and mean arrived, at monthly intervals

b. Length of spike

Length of spike was measured in cm immediately after harvest with the help of meter scale, for fifteen spikes chosen at random from five hills per plot and mean arrived at.

c. Girth of the spike

The diameter of the spike at maximum width was measured in cm with the help of a long strip of paper calibrated in mm and cm and wound over the spike. Observations were recorded for fifteen spikes chosen at random from five hills per plot after harvest.

d. Nature of spike apex

Nature of spike apex was assessed through visual observation

e. Days from planting to emergence of spike

The number of days required from planting to emergence of spike was recorded for fifteen spikes per plot and mean arrived at based on fifteen observations per plot.

f. Days from emergence to maturity of spike

The fruits after emergence when attain a bright cream color were tagged with paper tags bearing the date of tagging. When the fruits reach maturity, i.e. when the fruits attain a blackish green color with shining surface they were harvested and number of days required for maturity of spike was recorded. The observations were taken for fifteen spikes chosen at random from five hills per plot and mean arrived.

g. Green spike yield per plant

Yield of green spike was recorded from each of the five hills per plot and average green spike yield per hill was found out.

h. Dry spike yield per plant

The green spikes were oven dried for a period of four days at 60^0C and the dry spike yield from each of five hills were recorded and average dry spike yield per hill was found out.

i. Driage

Fifteen mature green spikes were selected at random from each plot and weighed. The same way fifteen spikes were then oven dried and again weighed and the ratio of weight of dry spike to the weight of green spike was worked out for each of the spike and multiplied by hundred, mean arrived at, based on fifteen observations per plot.

3.2.3.4 Biochemical characters

3.2.3.4.1 Estimation of essential oil

Clevenger apparatus was used for essential oil estimation. 20 g of ground dry pippali was taken in round-bottomed flask; added 200ml distilled water and distilled. The volatile oil being lighter than water condensed and collected on top of the Clevenger trap. The percentage of essential oil in the sample was worked out on a volume by weight basis.

3.2.3.4.2 Estimation of piperine

The piperine content in the dried spikes was estimated spectroscopically by the method of Soubhagya *et al.* (1990). Freshly powdered dry pippali (0.1g) was taken in a volumetric flask and extracted with 100 ml of acetone. The flask was kept in room temperature and shaken well for 2hrs. Then 0.25 ml of clear solution from the flask was taken in a cuvette and made up to 5ml with (4.75ml) acetone. The solution was shaken well and the absorbance of the solution read at 337nm with acetone as blank.

Preparation of the standard curve

0.1, 0.2, 0.3, 0.4 and 1ppm of pure piperine solution was prepared and their absorbance values were found out. The values were plotted on a graph and from the graph the concentrations corresponding to the absorbance of the sample were found out and piperine content in percentage were worked out.

3.2.3.4.3 Qualitative tests for secondary metabolites

Twelve pippali accessions were subjected to qualitative analysis of secondary metabolites like phenols, flavanoids, terpenoids and alkaloids. Thin layer chromatography (TLC) method was adopted for this purpose.

A. Preparation of crude extract

Five gram of finely powdered sample was extracted with 20 ml of methanol and concentrated to five ml. The concentrate was used for spotting in TLC plate.

B. TLC plate

Ready made silica gel G plates of 0.25 mm thickness were used for spotting. The plates were activated in chromatography oven. These activated plates were used for spotting samples.

C. TLC plate spotting

Silica gel plate of 0.25mm thickness was used. Sample was applied at a distance of 2cm. The TLC plates were placed in CAMAG TLC glass tank for elution. Rf value was recorded for each fraction as follows

Rf value = Distance moved by spots from the origin /Distance moved by solvent front from origin

D. Running solvent system

Various running solvent systems were tried for phenols, flavanoids and terpenoids and the following system, which gave better elution of spots, were selected. For alkaloids the solvent system suggested by Daniel (1995) was followed and for phenols, fllavanoids and terpenoids, solvent system reported by Manjusha (2001) in *Adathoda* was followed.

Compound	**Running solvent system**	**Ratio**
Phenol	Hexane: Ethyl acetate	3:1
Flavanoid	Hexane: Ethyl acetate	3:1
	Hexane: Ethyl acetate	1:2
	Hexane: Ethyl acetate	1:3
Alkaloid	Toluene: Ethyl acetate	93:7
Terpenoid	Hexane: Ethyl acetate	9:1

The solvent system was poured in the tank and the lid was placed tightly. To saturate with vapors of the volatile components of solvent system, filter paper sheets were placed adjacent to the inside walls of the tank. After 30 minutes the spotted plates were placed in the tank such that the spots were above the surface of solvent system. In about 45 to 60 minutes elution of the spots achieved 2/3 rd length of the plate then it was removed and placed under an exhaust flow of air to evaporate the solvents from the silica gel coat on the plate.

E. Spray reagents

Phenol	Folins ciocalteu's phenol reagent
Alkaloid	Concentrated sulphuric acid
Terpenoid	50% sulphuric acid

3.2.3.4.3.1 Phenol

Solvent system	Hexane: Ethyl acetate (3:1)
Spray reagent:	Folins ciocalteu's phenol reagent

Uniform spraying was done with fine droplets of spray reagent in an exhaust chamber. The sprayed plates were kept at 110^0C in a chromatographic oven to develop spots.

3.2.3.4.3.2 Flavanoids

Three solvent systems viz. Hexane: Ethyl acetate (3:1), Hexane: Ethyl acetate (1:2), Hexane: Ethyl acetate (1:3) was tried for the separation of flavanoids. Maximum separation was obtained in Hexane: Ethyl acetate (3:1) system. Plates were viewed under CAMAG UV betrachter (256 nm) and yellowish green fluorescent spots indicated the presence of flavanoids.

3.2.3.4.3.3 Terpenoid

Solvent:	-	Hexane: Ethyl acetate (9:1)
Spray reagent:	-	50% sulphuric acid

Plates were uniformly sprayed with fine droplets of 50% sulphuric acid. The sprayed plates were kept at 110^0c in a chromatographic oven to develop spots.

3.2.3.4.3.4 Alkaloid

Solvent - Toluene: Ethyl acetate (93:7)

Spray reagent - Concentrated sulphuric acid

After elution, the plates were uniformly sprayed with fine droplets of concentrated sulphuric acid. The sprayed plates were kept at 110^0c in a chromatographic oven to develop spots.

Photographs were taken on the same day before spreading the plate on cooling

3.2.3.5 Statistical analysis

Data on different characters were subjected to statistical analysis, using MSTATC package. The analysis of variance technique suggested by Fisher (1954) was employed for the estimation of various genetic parameters like analysis of variance, Correlation coefficients and path coefficient analysis for estimation of direct and indirect effects.

Results

IV. RESULTS

The results obtained from the present investigation are presented under the following heads

4.1 Genetic cataloguing in long pepper

4.2 Evaluation of variability in long pepper

4.1 Genetic cataloguing in long pepper

Based on descriptor mentioned in Table 2 twenty accessions of long pepper were genetically catalogued for vegetative, flowering and spike characters (Table 3).

The accessions included in the study showed erect and trailing growth habit with runner shoot production vary from few to many. Leaf lamina shape was found to be cordate and ovate lanceolate.

Spike shape was found to be cylindrical and filiform and immature spike color was found to be yellow and green. During fruit maturity spike colour changed from green to yellow. Accessions were male or female.

4.2 Variability

4.2.1 Stem characters

a. Number of stems per hill

The number of stems per hill of twenty *Piper longum* accessions from four months after planting to eight month after planting at an interval of one month is presented in Table 4. The twenty accessions differed significantly at all stages except at fifth month after planting.

The accessions PL 5 (2.50) and PL 22 (7.40) attained highest positions at four and six months after planting. At maximum vegetative growth period (seven month after planting) the accession PL 20 (11.00) showed maximum number of stems per hill which was statistically on par with PL 22 (10.60), PL 4 (8.79) and the check variety PL 1 had 7.30 stems per hill.

Table.3.Morphological characters of long pepper.

SL. No.	ACC. No	Growth habit	Branching type	Runner shoot production	Pubescence on stem	Lateral branch habit	Leaf lamina shape	Leaf base shape	Leaf margin	Types of veining	Leaf texture	Leaf scale
1	PL1	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
2	PL2	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
3	PL3	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
4	PL4	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
5	PL5	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
6	PL6	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
7	PL8	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Ovate lanccolate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
8	PL9	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
9	PL10	Erect and Trailing	Dimorphic	Many	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
10	PL11	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
11	PL12	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
12	PL13	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
13	PL15	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
14	PL16	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
15	PL17	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
16	PL18	Erect and Trailing	Dimorphic	Many	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
17	PL19	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
18	PL20	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
19	PL21	Erect and Trailing	Dimorphic	Few	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent
20	PL22	Erect and Trailing	Dimorphic	Many	Finely pubescent	Erect	Cordate	Cordate	Wavy	Acrodromous	Glabrous Coriaceous	Absent

Sl. No.	ACC. No.	Spike Orientation	Spike Shape	Immature Spike colour	Colour change while fruit ripening	Spike fragrance	Flower arrangement on spike	Bract type	Flower nature	Fruit shape	Fruit taste
1	PL1	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
2	PL2	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
3	PL3	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
4	PL4	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
5	PL5	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
6	PL6	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
7	PL8	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
8	PL9	Erect	Filiform	green	Green to yellow	Fragrant	laterally	Peltate orbicular	sessile	-	-
9	PL10	Erect	Cylindrical	yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
10	PL11	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
11	PL12	Erect	Filiform	green	Green to yellow	Fragrant	laterally	Peltate orbicular	sessile	-	-
12	PL13	-	-	-	-	-	-	-	-	-	-
13	PL14	Erect	Cylindrical	yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
14	PL15	Erect	Cylindrical	yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
15	PL16	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
16	PL17	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
17	PL18	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
18	PL20	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
19	PL21	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy
20	PL22	Erect	Cylindrical	Yellow	Green to black	Fragrant	laterally	Peltate orbicular	sessile	ovate	Spicy

Table. 4. Variability in number of stems of *Piper longum* accessions

Accession no	No of stems per hill				
	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP
PL 1 (Viswam)	2.17^{abc}	3.20^{a}	5.10^{abcd}	7.30^{cdef}	8.60^{bc}
PL 2	2.10^{abc}	4.00^{a}	6.60^{ab}	6.30^{defg}	10.90^{ab}
PL 3	2.00^{abcd}	3.30^{a}	5.50^{abc}	8.50^{bcd}	7.90^{bcd}
PL 4	2.00^{a}	2.90^{a}	5.10^{abcd}	8.79^{abc}	5.20^{cdefg}
PL 5	2.50^{a}	3.10^{a}	5.40^{abc}	8.00^{cde}	7.60^{bcd}
PL 6	1.60^{bcd}	2.80^{a}	3.50^{bcde}	4.90^{ghij}	5.90^{cdefg}
PL 8	2.00^{abcd}	3.50^{a}	5.40^{abc}	7.80^{cde}	10.90^{ab}
PL 9	1.80^{abcd}	3.10^{a}	3.90^{bcde}	5.40^{fghij}	5.60^{cdefg}
PL 10	1.50^{bcd}	1.92^{a}	2.77^{cde}	3.60^{jk}	3.60^{fg}
PL 11	1.30^{de}	2.63^{a}	3.20^{cde}	5.20^{fghij}	2.90^{g}
PL 12	1.60^{bcde}	2.50a	3.50^{bcde}	3.80^{ijk}	4.60^{defg}
PL 13	1.50^{bcde}	2.00^{a}	3.50^{bcde}	5.70^{efghij}	5.80^{cdefg}
PL15	1.00^{e}	1.70^{a}	2.10^{de}	1.85^{k}	3.10^{g}
PL 16	1.80^{abcde}	2.20^{a}	2.90^{cde}	4.20^{hij}	4.10^{efg}
PL 17	1.40^{cde}	1.60^{a}	1.40^{e}	4.70ghij	6.80^{cdef}
PL 18	2.20^{ab}	3.50^{a}	5.70^{abc}	6.00^{efghi}	8.30^{bc}
PL 19	$1.60b^{cde}$	2.00^{a}	1.80^{e}	4.60^{ghij}	3.50^{fd}
PL 20	2.00^{abcd}	3.40^{a}	7.20^{a}	11.00^{a}	12.00^{a}
PL 21	2.00^{abcd}	2.30^{a}	5.40^{abc}	6.90^{cdefg}	7.20^{cde}
PL 22	1.90^{abcd}	1.90^{a}	7.40^{a}	10.60^{ab}	10.70^{ab}

MAP- Months after planting

b. Number of vegetative branches per stem

The number of vegetative branches per stem of twenty accessions of *Piper longum* differed significantly at all stages except at six and eight months after planting (Table 5). At four months after planting PL 3 (4.15) recorded maximum number of vegetative branches per stem which was statistically on par with PL 8 (4.00). At five months after planting PL 8 (4.00) recorded maximum number of vegetative branches per stem. At maximum vegetative growth of plant Viswam the check variety (2.35) recorded maximum number vegetative branches per stem.

c. Number of spike bearing branches per stem

The twenty accessions differed significantly for number of spike bearing branches per stem at five, seven and eight months after planting (Table 6). Viswam the check variety (1.50) recorded maximum number of spike bearing branches per stem at five months after planting. During the maximum vegetative growth of plant, at seven months after planting PL 3 (3.45) recorded maximum number of spike bearing branches per stem which was statistically on par with PL 20 (3.20), PL 8 (3.15) and PL 2 (3.15).

d. Angle of insertion of spike bearing branch

The angle of insertion of spike bearing branches ranges between 47^0 (Viswam) to $73^{\ 0}$ (PL 21). Highest angle of insertion of spike bearing branch was given by PL 21 ($73^{\ 0}$) (Table 9).

e. Internodal length of spike bearing branch

There was significant difference between accessions at four, five, six, seven and eight months after planting. At maximum vegetative growth of plant PL 9 (5.05 cm) recorded maximum internodal length of spike bearing branch which was statistically on par with PL 21 (5.00 cm) (Table 7).

Table. 5. Variability in number of vegetative branches of *Piper longum* accessions

Accession no	No of vegetative branches per stem				
	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP
PL 1 (Viswam)	2.55^{b}	2.55^{bc}	3.17^{a}	2.35^{a}	1.20^{a}
PL 2	2.00^{bc}	2.00^{bcd}	1.45^{a}	1.00^{bcde}	0.80^{a}
PL 3	4.15^{a}	2.60^{bc}	2.57^{a}	0.90^{bcdef}	1.05^{a}
PL 4	2.05^{bc}	2.05^{bcd}	1.67^{a}	1.20^{bc}	0.55^{a}
PL 5	2.20^{bc}	2.20^{bcd}	1.70^{a}	0.85^{bcdefg}	0.55^{a}
PL 6	1.70^{bc}	1.65^{bcd}	2.05^{a}	1.00^{bcde}	0.60^{a}
PL 8	4.00^{a}	4.00^{a}	2.55^{a}	1.00^{bcde}	0.15^{a}
PL 9	2.30^{bc}	2.30^{bcd}	1.65^{a}	1.25^{b}	0.30^{a}
PL 10	1.15^{bc}	1.10^{d}	1.17^{a}	0.65^{defg}	0.75^{a}
PL 11	2.55^{b}	1.65^{bcd}	1.32^{a}	0.55efg	0.80^{a}
PL 12	1.05^{c}	1.05^{d}	1.24^{a}	1.20^{bc}	1.05^{a}
PL 13	1.05^{c}	0.90^{d}	1.01^{a}	1.10bcd	0.35^{a}
PL15	1.00^{c}	1.80^{bcd}	0.55^{a}	0.70^{defg}	0.40^{a}
PL 16	1.50^{bc}	1.03^{d}	1.25^{a}	0.50^{fg}	1.10^{a}
PL 17	1.60^{bc}	0.90^{d}	0.80^{a}	1.05^{bcd}	0.70^{a}
PL 18	1.85^{bc}	1.80^{bcd}	1.12^{a}	0.75^{cdefg}	0.75^{a}
PL 19	1.00^{c}	1.15^{cd}	0.05^{a}	0.40^{g}	0.55^{a}
PL 20	1.45^{bc}	1.25^{bcd}	0.87^{a}	0.95^{bcdef}	0.70^{a}
PL 21	2.10^{bc}	2.10^{bcd}	1.45^{a}	0.90^{bcdef}	1.15^{a}
PL 22	1.45^{bc}	1.60^{bcd}	1.14^{a}	0.85^{bcdefg}	1.70^{a}

MAP- Months after planting

Table. 6. Variability in number of spike bearing branches of *Piper longum* accessions

Accession no	No of spike bearing branches per stem				
	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP
PL 1(Viswam)	0.70^{a}	1.50^{a}	0.20^{a}	0.95^{efgh}	4.80^{a}
PL 2	0.75^{a}	0.75^{abc}	1.55^{a}	3.15^{ab}	3.65^{abc}
PL 3	0.45^{a}	0.40^{c}	0.95^{a}	3.45^{a}	3.00^{abcd}
PL 4	0.81^{a}	0.25^{c}	0.65^{a}	0.25^{h}	1.75^{bcd}
PL 5	0.50^{a}	0.60^{abc}	1.30^{a}	2.75^{abc}	4.55^{ab}
PL 6	1.20^{a}	0.90^{abc}	1.80^{a}	1.70^{cdef}	2.40^{abcd}
PL 8	0.45^{a}	1.40^{ab}	2.50^{a}	3.15^{ab}	4.90^{a}
PL 9	0.35a	0.85^{abc}	1.55^{a}	1.90^{cde}	0.60^{d}
PL 10	Nil	Nil	0.15^{a}	0.40^{gh}	1.20^{cd}
PL 11	0.25^{a}	Nil	0.50^{a}	1.10defgh	1.90^{bcd}
PL 12	0.55^{a}	0.50^{bc}	0.60^{a}	2.10^{bcd}	1.35^{cd}
PL 13	Nil	0.20^{c}	0.35^{a}	Nil	1.20^{cd}
PL15	Nil	Nil	Nil	0.25h	1.47^{cd}
PL 16	0.10^{a}	Nil	0.20^{a}	Nil	0.95^{cd}
PL 17	0.10^{a}	0.50^{bc}	0.90^{a}	1.40^{defg}	2.85^{abcd}
PL 18	Nil	0.60^{abc}	1.90^{a}	0.64^{fgh}	2.95^{abcd}
PL 19	Nil	0.10^{c}	0.30^{a}	0.40^{gh}	1.00^{cd}
PL 20	0.25^{a}	0.20^{c}	1.60^{a}	3.20^{ab}	4.80^{a}
PL 21	0.25^{a}	0.20^{c}	0.95^{a}	2.60^{abc}	0.75^{cd}
PL 22	0.20^{a}	0.05^{c}	Nil	Nil	0.30^{d}

MAP- Months after planting

f. Length of longest stem

Stem length differed significantly in all months except at seven months after planting. PL13 at four (28.50 cm), five (45.80 cm) and six (52.90 cm) months after planting recorded maximum stem length. At maximum vegetative growth period there was no significant different between accessions (Table 8).

Variability in stem characters at maximum vegetative growth stage is given in Table 9

4.2.2 Leaf characters

a. Leaf shape

All the accessions recorded cordate leaf shape except for PL 8 (ovate lanceolate)

b. Leaf Area

The leaf area of twenty accessions of *Piper longum* was recorded and is presented in Table 12. The leaf area of twenty accessions differed significantly. The maximum leaf area was recorded in PL 13 (66.13 cm^2). The accessions PL 17 and PL 12 recorded relatively higher leaf area of 60.15 cm^2 and 54.31 cm^2 respectively. The minimum leaf area was observed in the accessions PL 19 (24.30cm^2).

c. Leaf colour

All the accessions recorded dark green colour for leaves except for the accessions PL 20 which was light green in colour (Table 12).

d. Petiole length

The twenty accessions differed significantly with respect to petiole length in all the months. The maximum petiole length at fourth month was recorded by PL 13 (4.47 cm), at fifth month by PL 17 (4.34 cm), and at sixth month by PL 19 (5.80 cm). At maximum vegetative growth stage, PL 13 (6.66 cm) recorded maximum petiole length (Table 10)

Table. 7. Variability in internodal length of spike bearing branch of *Piper longum* accessions

Accession no	Internodal length of spike bearing branch (cm)				
	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP
PL 1 (Viswam)	4.72^{a}	2.90^{cde}	4.76^{a}	4.20^{bcde}	5.89^{a}
PL 2	3.16^{de}	2.80^{cde}	3.54^{ab}	4.63^{abcd}	3.87^{cde}
PL 3	3.85^{bc}	3.52^{abc}	4.17^{ab}	4.01^{cde}	5.04^{b}
PL 4	2.87^{e}	3.70^{abc}	4.77^{a}	4.16^{cde}	3.83^{cde}
PL 5	2.76^{e}	4.05^{ab}	4.16^{ab}	4.19^{bcde}	4.29^{bcd}
PL 6	3.47^{cd}	3.58^{abc}	4.18^{ab}	3.57^{ef}	4.80^{b}
PL 8	2.83^{e}	2.47^{de}	3.26^{b}	4.50^{abcd}	3.81^{cde}
PL 9	4.14^{b}	4.36^{a}	4.75^{a}	5.05^{a}	3.84^{cde}
PL 10	Nil	Nil	4.48^{ab}	3.64^{ef}	3.36^{e}
PL 11	Nil	Nil	3.07^{b}	3.18f	3.50^{e}
PL 12	3.55^{cd}	3.28^{bcd}	3.87^{ab}	3.85^{def}	4.95^{b}
PL 13	Nil	Nil	4.25^{ab}	4.35^{abcde}	4.50^{bc}
PL15	Nil	Nil	Nil	Nil	3.84^{cde}
PL 16	Nil	Nil	Nil	Nil	3.62^{cde}
PL 17	Nil	4.48^{a}	4.50^{ab}	4.33^{abcde}	5.90^{a}
PL 18	Nil	4.23^{ab}	4.46^{ab}	4.24^{abcde}	4.76^{b}
PL 19	Nil	4.13^{ab}	4.39^{ab}	4.81^{abc}	4.95^{b}
PL 20	Nil	2.26^{e}	3.81^{ab}	4.34^{abcde}	3.61^{de}
PL 21	3.77^{bc}	3.01^{cde}	3.26^{b}	5.00^{ab}	3.91^{cde}
PL 22	Nil	Nil	Nil	Nil	Nil

MAP- Months after planting

Table. 8. Variability in stem length of *Piper longum* accessions

Accession no	Length of longest stem (cm)				
	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP
PL 1 (Viswam)	16^{de}	20.60^{fg}	31.30^{ef}	49.50^{a}	76.50^{defg}
PL 2	24^{ab}	33.50^{d}	32.30^{de}	41.30^{a}	58.40^{fg}
PL 3	16^{de}	25.40^{ef}	30.80^{ef}	73.70^{a}	102.50^{bcde}
PL 4	14^{e}	26.10^{e}	32.60^{de}	40.40^{a}	55.50^{fg}
PL 5	19^{cd}	33.20^{d}	42.30^{c}	55.90^{a}	61.90^{fg}
PL 6	23^{bc}	38.90^{bc}	47.50^{b}	66.90^{a}	118.40^{bc}
PL 8	16.50^{de}	27^{e}	31.80^{ef}	44^{a}	47.00^{g}
PL 9	13^{e}	23.20^{ef}	28.80^{fg}	35.80^{a}	46.70^{g}
PL 10	14^{e}	23.40^{ef}	27.00^{gh}	38.20^{a}	56.40^{fg}
PL 11	12.50^{e}	16.50^{g}	25.50^{h}	43.10a	65.50^{efg}
PL 12	27^{ab}	39.80^{b}	46.60^{b}	72.50^{a}	105.70^{bcd}
PL 13	28.50^{a}	45.80^{a}	52.90^{a}	71.10^{a}	51.10^{fg}
PL15	5^{f}	10.70^{a}	14.50^{i}	24.70^{a}	50.90^{fg}
PL 16	4^{f}	8.80^{h}	11.80^{i}	31.50^{a}	134.00^{b}
PL 17	17^{de}	34.30^{cd}	35.10^{d}	46.70^{a}	88.70^{cdef}
PL 18	13^{e}	22.70^{ef}	24.10^{h}	36.30^{a}	58.80^{fg}
PL 19	15^{de}	36.30^{bcd}	43.60^{c}	59.40^{a}	170.50^{a}
PL 20	17.50^{de}	27.60^{e}	32.50^{de}	47.00^{a}	56.50^{fg}
PL 21	14.50^{de}	25.90^{e}	33.10^{de}	46.20^{a}	51.50^{fg}
PL 22	15.50^{de}	37.60^{bcd}	42.10^{c}	57.00^{a}	126.90^{bc}

MAP- Months after planting

Table. 9. Variability of stem characters at maximum vegetative growth phase.

Accession no	Number of stems per hill	Number of vegetative branches per stem.	Number of spike bearing branches per stem	Internodal length of spike bearing branch (cm)	Length of longest stem. (cm)	Angle of insertion of spike bearing branch (degrees)
PL1(Viswam)	7.30 ^{cdef}	2.35 ^{a}	0.95^{efgh}	4.20^{bcde}	49.50 ^{a}	47 ^{d}
PL2	6.30 ^{defg}	1.00 ^{bcde}	3.15^{ab}	4.63 ^{abcd}	41.30 ^{a}	59 ^{abcd}
PL3	8.50 ^{bcd}	0.90 ^{bcdef}	3.45^{a}	4.01 ^{cde}	73.70 ^{a}	66 ^{abc}
PL4	5.50 ^{bed}	1.20 ^{bc}	0.25^{h}	4.16^{cde}	40.40 ^{a}	54 ^{abcd}
PL5	8.90 ^{cde}	0.85 ^{bcdefg}	2.75^{abc}	4.19^{bcde}	55.90^{a}	63^{abcd}
PL6	8.00 ^{cde}	1.00^{bcde}	1.70^{cdef}	3.57^{ef}	66.90^{a}	56^{abcd}
PL8	4.90 ^{yhij}	1.00^{bcde}	3.15^{ab}	4.50 ^{ef}	44^{a}	63^{abcd}
PL9	7.80 ^{cde}	1.25^{b}	1.90^{cde}	5.05 ^{a}	35.80^{a}	60 ^{abcd}
PL10	5.40 ^{fghij}	0.65 ^{defg}	0.40^{gh}	3.64 ^{ef}	38.20^{a}	68 ^{ab}
PL11	3.60^{jk}	0.55 ^{efg}	1.10d^{efgh}	3.18 ^{f}	43.10^{a}	49 ^{cd}
PL12	5.20^{fghij}	1.20 ^{bc}	2.10^{bcd}	3.85 ^{def}	72.50 ^{a}	52 ^{cd}
PL13	3.80 ^{ijk}	1.10 ^{bcd}	Nil	4.35 ^{abcde}	71.10 ^{a}	Non flowering
PL15	5.70 ^{efghij}	0.70 ^{defg}	0.25^{h}	Nil	24.70 ^{a}	62 ^{abcd}
PL16	1.85 ^{k}	0.50 ^{jg}	Nil	Nil	31.50 ^{a}	61 ^{abcd}
PL17	4.20 ^{hij}	1.05 ^{bcd}	1.40^{defg}	4.33 ^{abcde}	46.70 ^{a}	62 ^{abcd}
PL18	4.70 g^{hij}	0.75^{cdefg}	0.64^{fgh}	4.24 ^{abcde}	36.30 ^{a}	72 ^{ab}
PL19	6.00 ^{efghi}	0.40^{g}	0.40^{gh}	4.81 ^{abc}	59.40 ^{a}	52 ^{bcd}
PL20	11.00^{a}	0.95^{bcdef}	3.20^{ab}	4.34 ^{abcde}	47.00 ^{a}	58 ^{bcd}
PL21	6.90^{cdelg}	0.90^{bcdef}	2.60^{abc}	5.00^{ab}	46.20 ^{a}	73 ^{a}
PL22	10.60^{ab}	0.85^{bcdefg}	Nil	Nil	57.00 ^{a}	64 ^{abcd}

e. Number of leaves per hill

Number of leaves varied significantly between the accessions at all months. Viswam the check variety recorded maximum number of leaves at four (36), five (69), six (140), seven (201) and eight (294) months after planting (Table 11).

Variability in leaf characters at maximum vegetative growth stage is given in Table 12.

4.2.3 Yield contributing characters

Out of twenty accessions studied only twelve accessions viz. PL 1(Viswam), PL 2, PL 3, PL 5, PL 8, PL 10, PL 11, PL 15, PL 18, PL 19, PL 20 and PL 21 were flowered, set fruits and mature spikes were harvested. The accessions PL 9 and PL 12 appeared to be male. PL 6, PL 13, PL 16, PL 18 and PL 22 were sparse flowering and mature spikes could not be harvest during first crop period. The productive characters of the 12 accessions are narrated below.

a. Number of spikes per spike bearing branch

The twenty accessions differed significantly at all stages except at six and eight months after planting (Table 13). The maximum number of spikes per spike bearing branch at four month after planting was recorded in PL 6 (1.21) and at five months after planting was recorded in PL 1 (1.58). At maximum vegetative growth phase of plant, the maximum number of spikes per spike bearing branch was observed in PL 3 (2.18) which was statistically on par with PL 5 (1.76), PL 20 (1.73), PL 2 (1.73) and PL 21 (1.63) Viswam the check variety recorded the spike number of 1.46.

b. Length of spike

The spike length of twelve accessions of *Piper longum* is presented in Table 14. The maximum length of spike was observed in PL8 (2.66cm) that was statistically on par with check variety Viswam (2.44cm) and others not differed significantly.

c. Girth of spike

The spike girth of twelve accessions of *Piper longum* is presented in Table 14. The twelve *Piper longum* accessions did not differed significantly with respect to girth of spike.

Table. 10. Variability in Petiole length of *Piper longum* accessions

Accession no	Petiole length (cm)				
	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP
PL 1 (Viswam)	3.41^{bcdef}	3.79^{abcde}	3.11^{defg}	3.65^{ef}	4.44^{cd}
PL 2	2.62^{gh}	3.25^{abcdef}	2.92^{efg}	3.59^{ef}	3.19^{e}
PL 3	2.59^{h}	3.01^{cdefg}	3.12^{defg}	2.85^{gh}	3.89^{cde}
PL 4	2.83^{efgh}	3.26^{abcdef}	4.13^{cde}	4.14^{d}	4.05^{cde}
PL 5	2.76^{fgh}	2.86^{defgh}	2.81^{fg}	3.36^{f}	3.00^{e}
PL 6	3.58^{bcde}	3.49^{abcdef}	3.59^{cdefg}	3.43^{ef}	3.63^{de}
PL 8	3.03^{defgh}	3.35^{abcdef}	4.08^{cde}	4.33^{d}	3.92^{cde}
PL 9	3.65^{bcd}	3.92^{abcd}	4.24^{cd}	3.79^{e}	4.10^{cde}
PL 10	3.44^{bcd}	2.28^{h}	2.73^{fg}	3.01^{g}	3.00^{e}
PL 11	2.54^{h}	2.98^{cdefg}	3.16^{defg}	$^{3.54}$ef	3.14^{e}
PL 12	3.37^{bcdef}	3.34^{abcdef}	3.98^{cdef}	4.16^{d}	5.89^{ab}
PL 13	4.47^{a}	4.05^{abc}	4.74^{abc}	6.66^{a}	5.01^{bc}
PL15	4.44^{a}	2.59^{fgh}	4.53^{bc}	4.26^{d}	4.00^{cde}
PL 16	3.11^{cdefg}	3.58^{abcdef}	2.66^{g}	2.43^{i}	3.89^{cde}
PL 17	3.05^{defgh}	4.34^{a}	4.70^{abc}	4.80^{c}	3.75^{de}
PL 18	2.45^{h}	3.20^{bcdefg}	3.21^{defg}	2.67^{ghi}	3.76^{de}
PL 19	3.85^{abc}	3.82^{abcde}	5.80^{a}	5.39^{b}	6.06^{ab}
PL 20	2.44^{h}	2.75^{efgh}	2.68^{g}	2.63^{hi}	3.64^{de}
PL 21	2.77^{fgh}	2.46^{gh}	3.13^{defg}	3.67^{ef}	3.80^{de}
PL 22	4.06^{ab}	4.21^{ab}	5.45^{ab}	5.08^{bc}	6.63^{a}

MAP- Months after planting

Table. 11. Variability in number of leaves of *Piper longum* accessions

Accession no	Total number of leaves				
	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP
PL 1 (Viswam)	36^{a}	69^{a}	140^{a}	201^{a}	294a
PL 2	13^{ef}	26^{def}	44^{cdef}	73^{bcd}	116^{c}
PL 3	14^{def}	25^{def}	42^{cde}	70^{bcde}	73cdef
PL 4	11^{efgh}	20^{efgh}	30^{cdef}	48^{cdef}	50^{cdef}
PL 5	25^{bc}	36^{cd}	58^{bcd}	102^{b}	87^{cdef}
PL 6	24^{bc}	30^{cde}	47^{cde}	71^{bcde}	54^{cdef}
PL 8	24^{bc}	50^{b}	86^{b}	158^{a}	231^{b}
PL 9	17^{de}	21^{efg}	36^{cdef}	54^{bcdef}	30^{f}
PL 10	5^{ij}	8^{hi}	11^{f}	20^{ef}	113^{cd}
PL 11	9^{fghij}	15^{fghi}	33^{cdef}	51^{bcdef}	92^{cdef}
PL 12	13^{efgh}	24^{ef}	40^{cdef}	71^{bcde}	40^{ef}
PL 13	12^{efgh}	12^{i}	20^{ef}	31^{def}	44^{ef}
PL15	5^{j}	6^{fghi}	9^{f}	11^{f}	26^{f}
PL 16	6^{hij}	10^{efg}	14^{ef}	20^{ef}	44^{def}
PL 17	11^{ghij}	17^{ghi}	24^{def}	39^{cdef}	56^{cdef}
PL 18	11^{ghij}	21^{efg}	37^{cdef}	56^{bcdef}	107^{cde}
PL 19	7^{efghij}	10^{defg}	14^{ef}	20^{ef}	38^{ef}
PL 20	20^{cd}	38^{c}	66^{bc}	104^{b}	195^{b}
PL 21	30^{b}	38^{c}	59^{bcd}	86^{bc}	41^{ef}
PL 22	12^{efgh}	21^{efg}	41^{cdef}	62^{bcdef}	84^{cdef}

MAP- Months after planting

Table. 12. Variability of leaf characters at maximum vegetative growth phase

Accession no	Leaf Shape	Leaf area (cm^2)	Leaf colour	Petiole length (cm)	Number of leaves per hill
PL1	Cordate	42.17^{efg}	Dark Green	3.65^{ef}	201^{a}
PL2	Cordate	32.76^{ijk}	Dark Green	3.59^{ef}	73^{bed}
PL3	Cordate	35.87^{hi}	Dark Green	2.85^{gh}	70^{bcde}
PL4	Cordate	34.88^{i}	Dark Green	4.14^{d}	48^{cdef}
PL5	Cordate	39.97^{g}	Dark Green	3.36^{f}	102^{b}
PL6	Cordate	44.69^{def}	Dark Green	3.43^{ef}	71^{bcde}
PL8	Ovate lanceolate	41.52^{fg}	Dark Green	4.33^{d}	158^{a}
PL9	Cordate	45.11^{de}	Dark Green	3.79^{e}	54^{bcdef}
PL10	Cordate	38.89^{gh}	Dark Green	3.01^{g}	20^{ef}
PL11	Cordate	32.93^{ij}	Dark Green	3.54^{ef}	51^{bcdef}
PL12	Cordate	54.31^{c}	Dark Green	4.16^{d}	71^{bcdef}
PL13	Cordate	66.13^{a}	Dark Green	6.66^{a}	31^{def}
PL15	Cordate	47.07^{d}	Dark Green	4.26^{d}	11^{f}
PL 16	Cordate	29.28^{k}	Dark Green	2.43^{i}	20^{ef}
PL 17	Cordate	60.15^{b}	Dark Green	4.80^{c}	39^{edef}
PL 18	Cordate	34.50^{ij}	Dark Green	2.67^{ghi}	56^{bcdef}
PL 19	Cordate	24.30^{l}	Dark Green	5.39^{b}	20^{ef}
PL 20	Cordate	30.86^{jik}	Light Green	2.63^{hi}	104^{b}
PL 21	Cordate	29.17^{jk}	Dark Green	3.67^{ef}	86^{bc}
PL 22	Cordate	32.72^{ijk}	Dark Green	5.08^{bc}	62^{bcdef}

d. Fresh weight of spike

The fresh spike weight of twelve types of *Piper longum* differed significantly. The maximum fresh weight of spike was recorded in PL 8 (0.91g). The accession PL 21 recorded fresh weight of spike (0.67g), which was statistically on par with PL 15 (0.62g), PL18 (0.56g), PL 3 (0.51g), PL 5 (0.51g), PL 2 (0.50g).The minimum fresh weight of spike was observed in Viswam (0.37g). (Table 14)

e. Dry weight of Spike

The dry weight of twelve accessions of *Piper longum* was presented in table 14. The twelve *Piper longum* accessions did not differed significantly.

f. Driage

The driage of twelve accessions of *Piper longum* vary significantly. The maximum driage was observed in PL2 (19.1). The check variety Viswam recorded a driage of 18.91%, which was statistically on par with PL5 (17.64%), PL19 (17.02%), PL11 (16.66%), PL20 (16.66%), PL21 (16.4%) and PL18 (16.07). (Table 14)

g. Nature of spike apex

In all twelve accessions of *Piper longum*, blunt spike apex was observed.

h. Days from planting to emergence of spike

The number of days taken by all the twenty accessions of *Piper longum* for spike emergence is presented in Table 15. The twenty types did not differ significantly with respect to this character.

i. Days from emergence to maturity of spikes

The number of days taken for maturity of spike of the twelve flowered accessions of *Piper longum* from which spikes were harvested is presented in Table 15. The twelve accessions differed significantly with respect to this character.

The maximum number of days for spike maturity was observed for PL10 (97 days) followed by PL 5 (92 days). The minimum days for spike maturity were observed in the check variety Viswam (66days).

Table. 13. Variability in number of spikes per spike bearing branch of *Piper longum* accessions

Accession no	Number of spikes per spike bearing branch				
	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP
PL 1 (Viswam)	1.16^{a}	1.58^{a}	0.88^{a}	1.46^{bcd}	2.09^{a}
PL 2	1.13^{ab}	1.12^{bcde}	1.55^{a}	1.73^{abc}	2.17^{a}
PL 3	1.00^{abc}	1.02^{bcde}	1.40^{a}	2.18^{a}	2.50^{a}
PL 4	1.13^{ab}	1.09^{bcde}	0.97^{a}	1.17^{cdef}	1.49^{a}
PL 5	1.06^{abc}	1.15bcd	1.50^{a}	1.76^{ab}	1.97^{a}
PL 6	1.21^{a}	1.08^{bcde}	1.36^{a}	1.14^{def}	1.62^{a}
PL 8	1.16^{a}	1.39^{ab}	1.42^{a}	1.45^{bcd}	2.02^{a}
PL 9	1.01^{abc}	1.36^{ab}	1.57^{a}	1.57^{bcd}	1.17^{a}
PL 10	0.71^{c}	0.77^{de}	0.85^{a}	1.18^{bcde}	1.44^{a}
PL 11	0.87^{abc}	0.71^{e}	0.90^{a}	0.71f	1.54^{a}
PL 12	1.05^{abc}	0.90^{cde}	1.16^{a}	1.53^{bcd}	1.40^{a}
PL 13	0.71^{c}	0.89^{cde}	0.95^{a}	0.71^{f}	1.56^{a}
PL15	0.71^{c}	0.71^{e}	0.71^{a}	0.71^{f}	1.89^{a}
PL 16	0.84^{abc}	0.71^{e}	0.88^{a}	0.88^{ef}	1.58^{a}
PL 17	0.74^{bc}	0.71^{e}	0.95^{a}	1.28^{bcde}	1.30^{a}
PL 18	0.71^{c}	1.30^{abc}	1.25^{a}	1.52^{bcd}	2.17^{a}
PL 19	0.71^{c}	0.74^{de}	0.71^{a}	0.80^{ef}	0.80^{a}
PL 20	1.02^{abc}	0.77^{de}	1.39^{a}	1.73^{abc}	2.48^{a}
PL 21	0.91^{abc}	0.80^{de}	1.23^{a}	1.63^{abcd}	1.70^{a}
PL 22	0.83^{abc}	0.90^{cde}	0.71^{a}	0.71^{f}	1.23^{a}

MAP- Months after planting

Table. 14. Variability in spike characters - I

Accession no	Spike length (cm)	Spike girth (cm)	Fresh weight of single spike (g)	Dry weight of single spike (g)	Driage (%)
PL 1 (Viswam)	2.44^{ab}	1.53^{a}	0.37^{d}	0.07^{a}	18.91^{ab}
PL 2	2.17^{cd}	2.63^{a}	0.50^{bcd}	0.10^{a}	19.1^{a}
PL 3	2.10^{cd}	2.06^{a}	0.51^{bcd}	0.08^{a}	15.68^{cd}
PL 5	2.01^{d}	2.54^{a}	0.51^{bcd}	0.09^{a}	17.64^{bc}
PL 8	2.66^{a}	2.49^{a}	0.91^{a}	0.13^{a}	14.28^{d}
PL 10	2.06^{cd}	1.96^{a}	0.44^{cd}	0.07^{a}	15.90^{cd}
PL11	1.97^{d}	2.05^{a}	0.48^{bcd}	0.08^{a}	16.66^{bc}
PL15	2.03^{cd}	2.01^{a}	0.62^{bc}	0.07^{a}	11.29^{ef}
PL18	2.18^{cd}	2.10^{a}	0.56^{bcd}	0.09^{a}	16.07^{bc}
PL19	2.05^{cd}	2.00^{a}	0.47^{bcd}	0.08^{a}	17.02^{bc}
PL20	2.26^{bc}	2.07^{a}	0.48^{bcd}	0.08^{a}	16.66^{bc}
PL21	2.10^{cd}	2.07^{a}	0.67^{b}	0.11^{a}	16.4^{bc}

Table. 15. Variability in spike characters - II

Accession no	No. of days from planting to emergence of spikes	No. days from emergence to maturity of spikes
PL 1 (Viswam)	103[a]	66[e]
PL 2	101[a]	89[c]
PL 3	130[a]	85[d]
PL 4	74[a]	-
PL 5	92[a]	92[b]
PL 6	75[a]	-
PL 8	151[a]	86[cd]
PL 9	73[a]	-
PL 10	88a	97a
PL 11	91[a]	85[d]
PL 12	85[a]	-
PL 13	Absolutely non flowering	Absolutely non flowering
PL15	87[a]	88[c]
PL 16	85[a]	-
PL 17	108[a]	-
PL 18	146[a]	89[c]
PL 19	90[a]	87[cd]
PL 20	107[a]	87[cd]
PL 21	102[a]	88[c]

Table. 16. Variability in spike yield of *Piper longum* accessions

Accession no	Fresh yield of spike per plant (g)	Dry yield of spikes per plant (g)
PL 1 (Viswam)	15.59^{bcd}	2.94^{ab}
PL 2	27.10^{a}	2.87^{ab}
PL 3	14.00^{cde}	2.01^{bc}
PL 5	23.00^{abc}	2.27^{bc}
PL 8	15.74^{bcd}	2.07^{bc}
PL 10	4.32^{ef}	0.62^{c}
PL11	6.20^{def}	1.04^{bc}
PL15	9.91^{def}	1.36^{bc}
PL18	13.44^{cde}	1.76^{bc}
PL19	3.43^{f}	0.43^{c}
PL20	15.50^{bcd}	2.25^{bc}
PL21	24.00^{ab}	3.09^{a}

j. Fresh spike yield per plant

Fresh spike yield of twelve accessions of *Piper longum* are presented in Table 16. The maximum fresh yield of spikes per plant during first harvest was recorded in PL 2 (27.10g), which was statistically on par with PL 21 (24.00g) and PL 5 (23.00 g). Viswam the check variety yielded 15.59 g of fresh spike, which was statistically at par with PL 8 (15.74g) and PL 20 (15.50g).

l. Dry spike yield per plant

The dry yield of spikes of twelve accessions of *Piper longum* is presented in Table 16. Twelve accessions of *Piper longum* differ significantly with respect to dry spike yield per plant. The maximum dry yield of spikes was observed in PL 21(3.09 g) followed by check variety Viswam (2.94 g). The minimum dry spike yield was observed in PL 19 (0.43), which was statistically at par with PL10 (0.62 g).

Analysis of variance is presented in Table 17

4.2.4 Biochemical characters

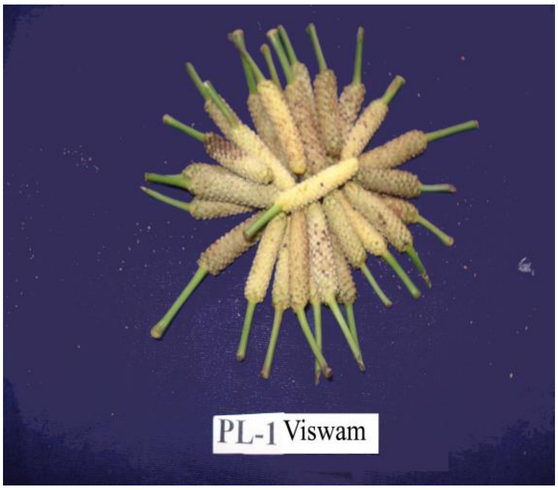
4.2.4.1 Essential Oil

The essential oil content of different accessions of *Piper longum* is given in Table 18. Viswam recorded maximum oil content (1.50%). The accession PL5, PL18 and PL21 recorded an oil content of 1.00%. The minimum oil content was recorded by PL2, PL3, PL8, and PL20.

4.2.4.2 Piperine content

The piperine content of twelve accessions of *Piper longum* is presented in Table 18. The maximum piperine content was recorded in PL11 (3.08. %). Viswam the check variety recorded a piperine content of 1.23% which was statistically on par with PL18 (1.78%), PL2 (1.72%), PL5 (1.68%), PL8 (1.62%), PL3 (1.50%), PL20 (1.50%), PL21 (1.24%). The minimum piperine content was recorded in PL10 (0.92%).

Plate- 2. ***Piper longum*** accessions

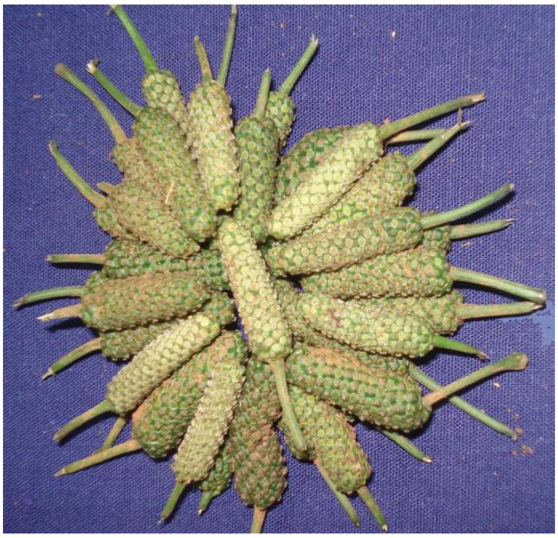


PL 1(Viswam)

PL 2

PL 5

Plate -3. ***Piper longum*** accessions

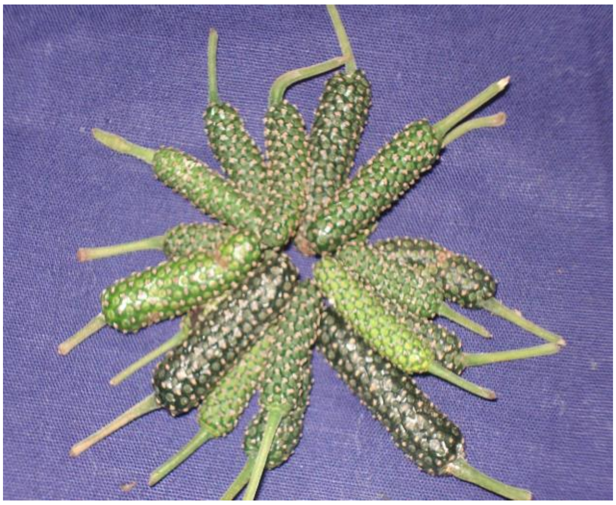
PL 8


PL 9


PL 12

Plate - 4. ***Piper longum*** accessions

PL 20



PL 21

Plate – 5. *Piper longum* accessions

Plate – 6. Variability of spikes in ***Piper longum***

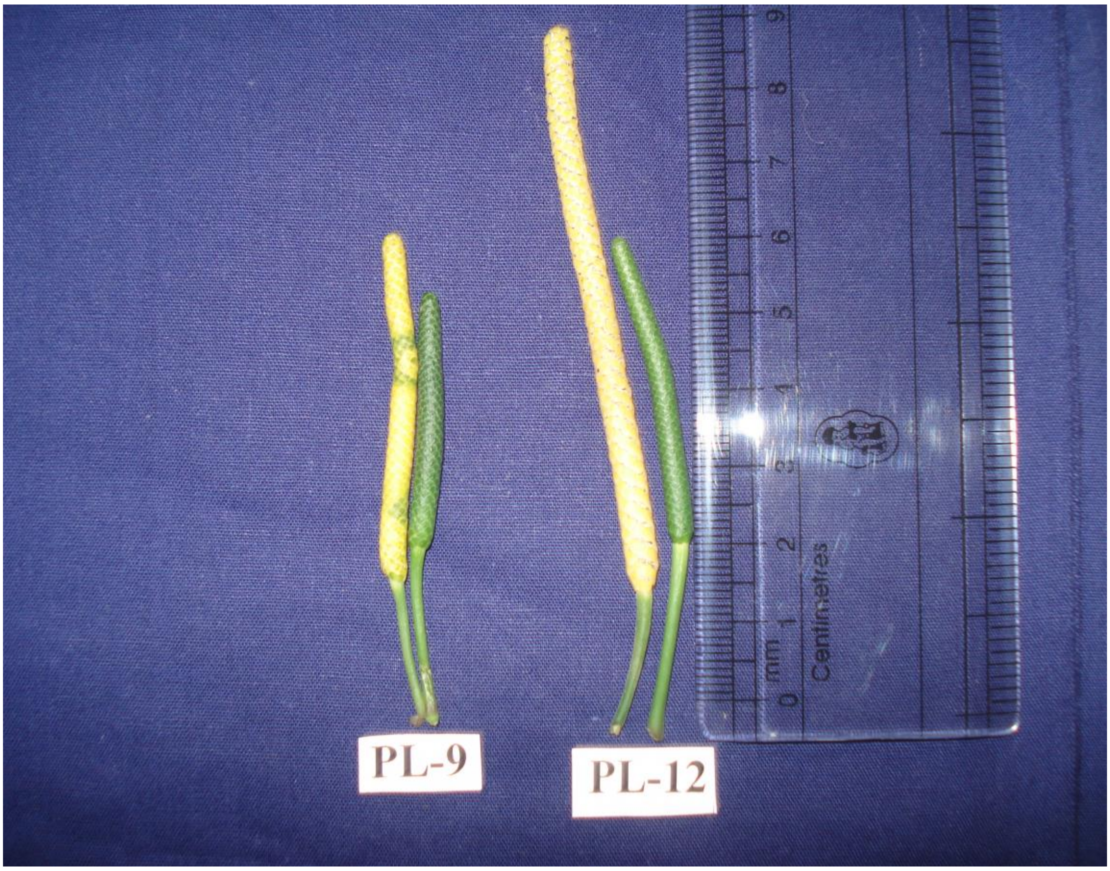


Plate -7. Variability of leaves in ***Piper longum***

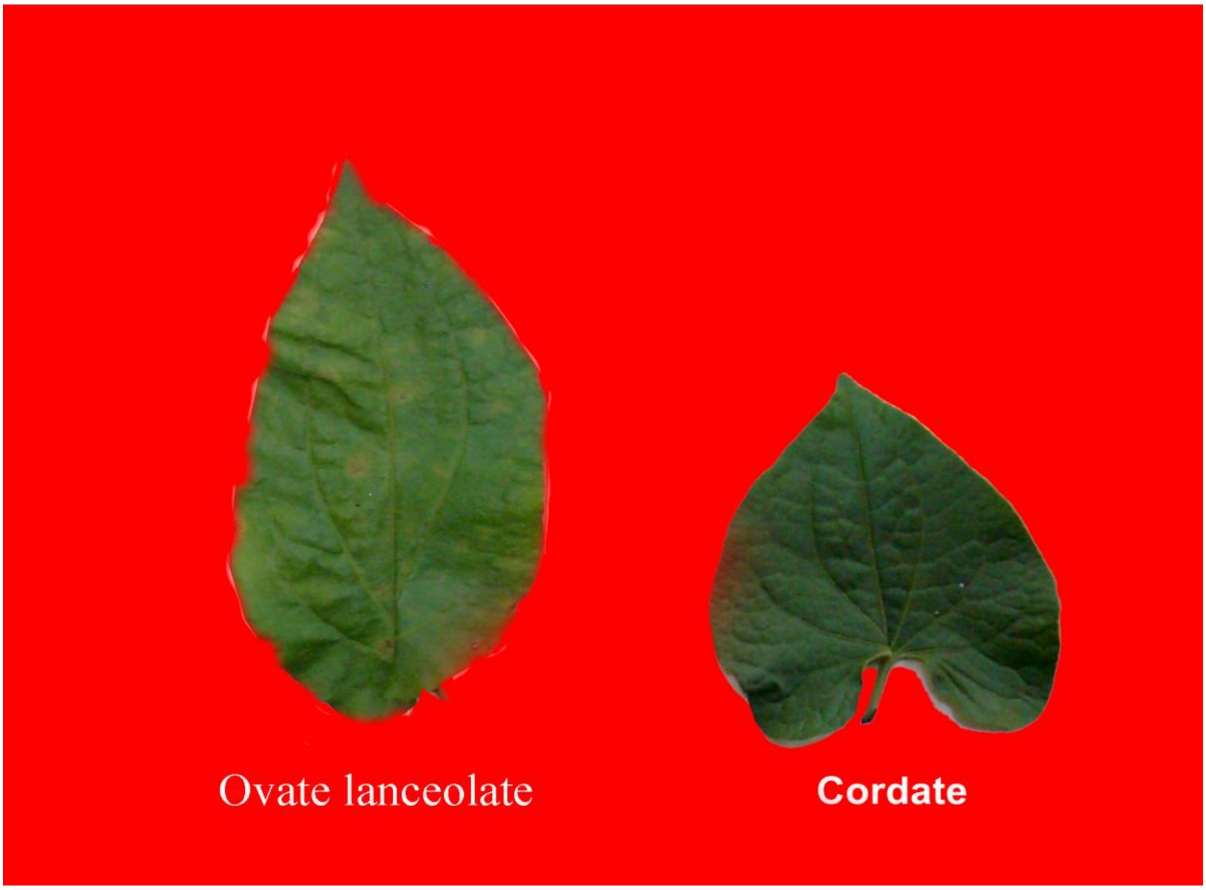


Table .17.Analysis of variance for various characters

Character	Degrees of Freedom	Mean sum of squares	F value
Number of stem per hill			
Four month after planting	19	0.257	2.5407
Five month after planting	19	0.937	1.2543
Six month after planting	19	6.169	3.3865
Seven month after planting	19	11.188	11.4723
Eight month after planting	19	15.927	7.6962
Number of vegetative branches per stem			
Four month after planting	19	1.573	4.5213
Five month after planting	19	1.136	3.2752
Six month after planting	19	1.4102	0.2304
Seven month after planting	19	0.326	8.7376
Eight month after planting	19	2.0269	0.0662
Number of spike bearing branches per stem			
Four month after planting	19	0.220	2.1121
Five month after planting	19	0.403	2.6314
Six month after planting	19	1.053	1.9296
Seven month after planting	19	3.049	12.8987
Eight month after planting	19	4.699	3.3408
Petiole length			
Four month after planting	19	0.803	7.8784
Five month after planting	19	0.677	3.2322
Six month after planting	19	1.783	6.5842
Seven month after planting	19	2.157	85.5073
Eight month after planting	19	2.050	8.1104
Internodal length of spike bearing branch			
Four month after planting	19	6.869	148.1732
Five month after planting	19	6.059	33.1231
Six month after planting	19	5.007	13.7632
Seven month after planting	19	5.232	44.1101
Eight month after planting	19	2.994	29.4109

Length of longest stem			
Four month after planting	19	75.705	16.9025
Five month after planting	19	186.918	36.4854
Six month after planting	19	217.499	112.2955
Seven month after planting	19	394.759	1.7850
Eight month after planting	19	2513.343	9.0927
Total number of leaves per hill			
Four month after planting	19	6.804	22.0342
Five month after planting	19	473.620	18.9460
Six month after planting	19	1825.802	8.3505
Seven month after planting	19	4424.876	9.2224
Eight month after planting	19	10335.114	12.8766
Angle of insertion of spike bearing branch	19	460.134	7.8588
Days from planting to emergence of spike	19	2222.532	2.0560
Days from emergence to maturity of spikes	11	104.850	62.23692
Fresh yield of spikes per plant	11	116.770	7.1407
Dry yield of spikes per plant	11	2.706	3.2618
Fresh weight of spikes	11	0.040	57.1566
Dry weight of spikes	11	0.001	2.7224
Driage	11	3.261	5.1513
Spike length	11	0.081	8.4654
Spike girth	11	0.179	1.9103
Piperine content	11	0.559	4.4966
Essential oil content	11	0.496	0.0000

4.2.4.3 Phenols

Phenol spot pattern of flowered accessions of *Piper longum* are given in Table 19. In *Piper longum* spikes a total of ten spots were resolved with Rf value ranging from 0.03 to 0.97 with a maximum of seven spots in PL18 and PL 15. All other types had the two spots with Rf value 0.85 and 0.97 except in PL1. PL8 lack the compound with Rf value 0.85. The accession PL 20 had compound with Rf value 0.03. The accession PL21 alone possess compound with Rf value 0.06. PL10 gave compound with Rf value 0.04. The accessions PL3, PL11, PL15, PL18 and PL20 expressed the compound of Rf value 0.05 and it was absent in all other accessions. PL8 possess the minimum of three spots with Rf values 0.97, 0.72 and 0.37.

4.2.4.4 Alkaloids

Alkloid spot pattern of flowered accessions of *Piper longum* are given in Table 20. In *Piper longum* spikes a total of ten spots were resolved with Rf value ranging from 0.05 to 0.52 with a maximum of eight spots in PL1, (Viswam) and a minimum of single spot in PL8. All the accessions had the compound with Rf value 0.05. All the accessions had an elution pattern lacking the compound with Rf value 0.5, but PL1 (Viswam), PL2, PL5 possess that particular compound. In a similar way all the accessions lack the compound with Rf value 0.45 but PL1 (Viswam), PL10 and PL11 which possess the same. All the accessions lack the compound with Rf value 0.33 but it could be identified in accessions PL1 (Viswam), PL5 and PL21. The compound with Rf value 0.05 was common for all the accessions.

4.2.4.5 Terpenes

Chromatogram of terpene pattern of eleven flowered accessions of *Piper longum* is given in Table 21. In *Piper longum* spikes a total of eight spots were resolved with Rf value ranging from 0.14 to 0.93, with a maximum of seven spots in PL2 and a minimum of two spots in PL21. The two compounds with Rf values 0.14 and 0.76 were absent in all the accessions except in PL2 and Viswam. The compond with Rf value 0.22 is absent in PL2, PL3 and PL21, but that particular compound was present in all other accessions. The compound with Rf value 0.37 was present in PL1, PL2, PL3, PL5, PL8 and PL10, but the particular compound was absent in other samples.

Table. 18. Variability in biochemical characters of *Piper longum* accessions

Accession no.	Piperine content (%)	Oil content (%)
PL 1 (Viswam)	1.23^{bc}	1.50^{a}
PL 2	1.72^{bc}	0.50^{c}
PL 3	1.50^{bc}	0.50c
PL 5	1.68^{bc}	1.00^{b}
PL 8	1.62^{bc}	0.50^{c}
PL 10	0.92^{c}	-
PL11	3.08^{a}	-
PL15	1.84^{b}	-
PL18	1.78^{bc}	1.00^{b}
PL19	1.88^{b}	-
PL20	1.50^{bc}	0.50^{c}
PL21	1.24^{bc}	1.00^{b}

Table. 19. Phenol spot pattern of different accessions of *Piper longum*

Sample	Rf values									
	.03	.04	.05	.06	.07	.24	.37	.72	.85	.97
PL1	-	-	-	-	+	+	+	+	-	-
PL2	-	-	-	-	+	+	+	+	+	+
PL3	-	-	+	-	-	+	-	+	+	+
PL5	-	-	-	-	-	+	+	+	+	+
PL8	-	-	-	-	-	-	+	+	-	+
PL10	-	+	-	-	-	+	+	+	+	+
PL11	-	-	+	-	-	+	+	+	+	+
PL15	-	-	+	-	+	+	+	+	+	+
PL18	-	-	+	-	+	+	+	+	+	+
PL20	+	-	+	-	-	+	+	+	+	+
PL21	-	-	-	+	-	+	+	+	+	+

Table. 20. Alkaloid spot pattern of different accessions of *Piper longum*

Sample	Rf values									
	.05	.09	.17	.22	.25	.28	.33	.45	.5	.52
PL1	+	-	+	+	+	+	+	+	+	-
PL2	+	-	-	+	-	+	-	-	+	+
PL3	+	+	-	-	+	+	-	-	-	-
PL5	+	-	+	+	-	-	+	-	+	+
PL8	+	-	-	-	-	-	-	-	-	-
PL10	+	-	-	-	+	-	-	+	-	+
PL11	+	-	+	+	-	-	-	+	-	+
PL15	+	+	-	+	-	-	-	-	-	-
PL18	+	-	-	+	+	-	-	-	-	+
PL20	+	+	-	-	-	+	-	-	-	+
PL21	+	+	-	-	-	-	+	-	-	-

Table. 21. Terpenoid spot pattern of different accessions of *Piper longum*

Sample	Rf values							
	.14	.16	.22	.29	.37	.76	.90	.93
PL1	+	-	+	+	+	+	-	-
PL2	+	+	-	+	+	+	+	+
PL3	-	-	-	+	+	-	-	+
PL5	-	-	+	+	+	-	-	+
PL8	-	-	+	+	+	-	-	+
PL10	-	-	+	+	+	-	-	+
PL11	-	-	+	+	-	-	-	+
PL15	-	-	+	+	-	-	-	+
PL18	-	-	+	+	-	-	-	+
PL20	-	-	+	+	-	-	-	+
PL21	-	-	-	+	-	-	-	+

Table. 22. Flavanoid spot pattern of different accessions of *Piper longum*

Sample	Rf Values								
	.05	.14	.21	.26	.35	.44	.54	.66	.80
PL1	+	+	+	+	+	-	+	+	+
PL2	+	+	-	+	+	-	-	+	+
PL3	+	+	-	+	-	+	-	-	-
PL5	+	-	+	+	+	-	+	+	+
PL8	+	-	-	+	+	-	-	-	+
PL10	+	+	-	+	+	+	-	-	+
PL11	+	+	-	+	+	-	+	-	+
PL15	-	+	-	+	+	+	-	-	+
PL18	+	+	-	+	+	+	-	-	+
PL20	+	+	-	+	-	+	-	-	+
PL21	+	+	-	+	+	-	-	-	+

Plate-8a. Chromatogram - Alkaloid

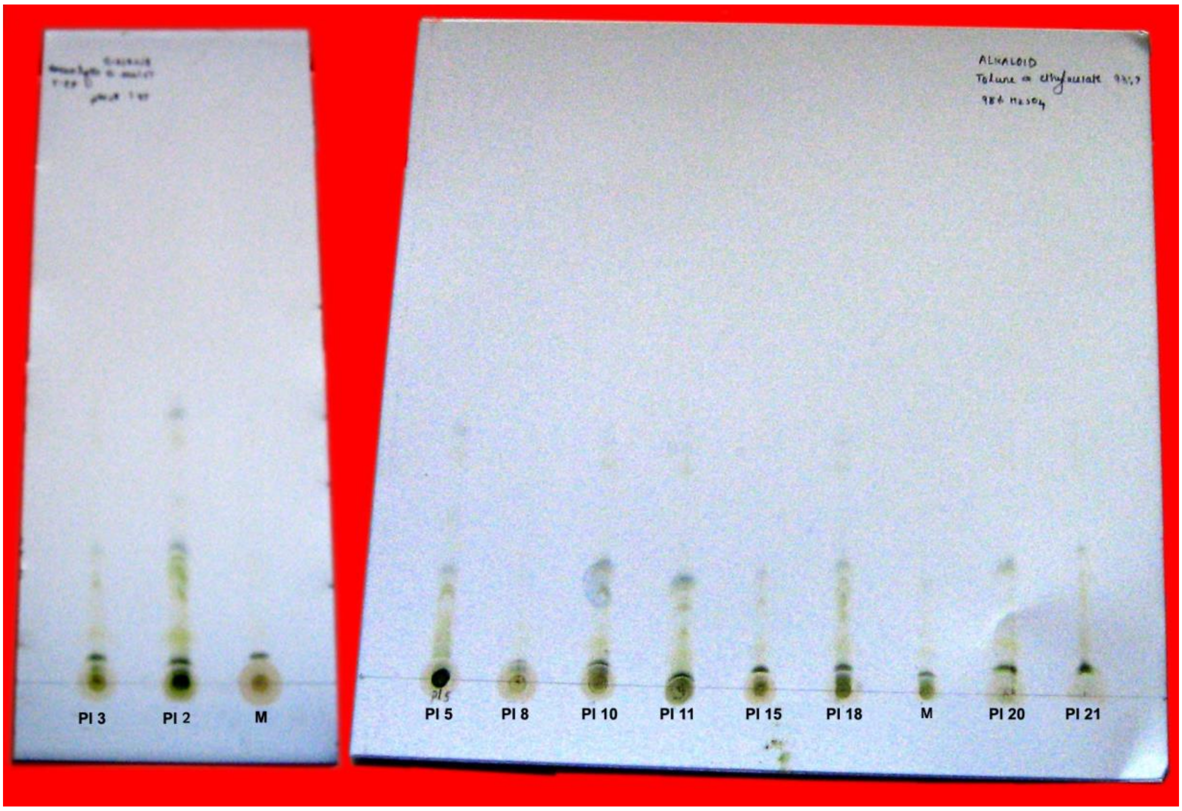


Plate 8b – Chromatogram - Flavanoid

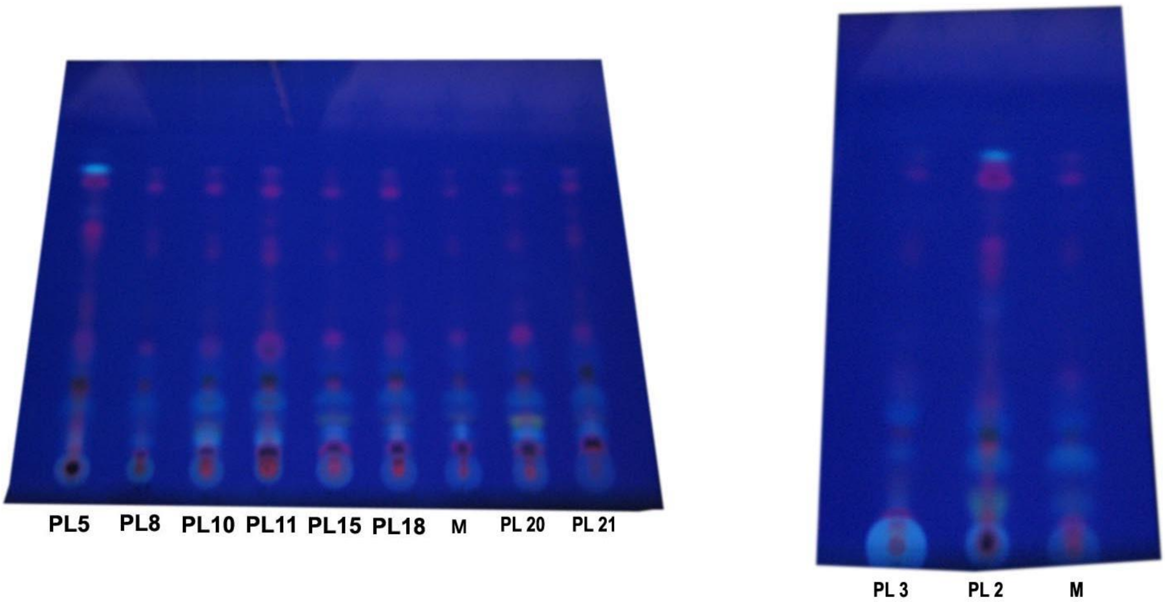


Plate 9a - Chromatogram -Terpenes

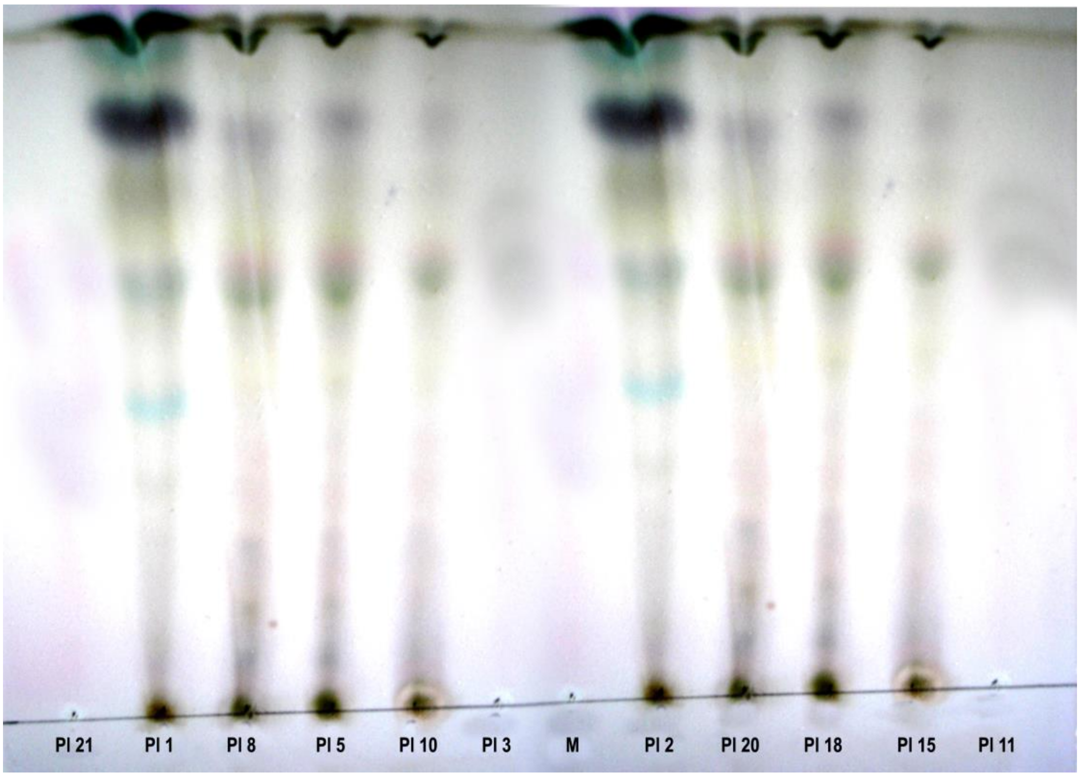
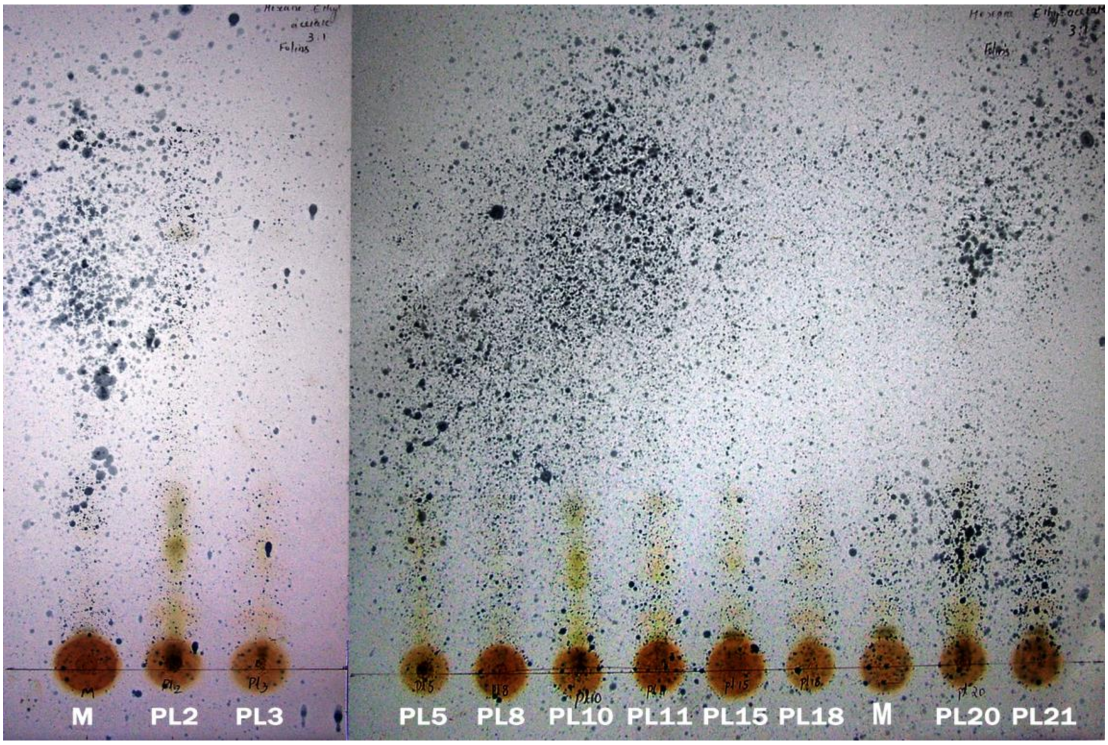


Plate 9b – Chromatogram- Phenols



4.2.4.6 Flavanoid

Chromatograms of flavanoid pattern of eleven flowered accessions of Piper longum are given in Table 22. In *Piper longum* spikes a total of nine spots were resolved with Rf values ranging from 0.05 to 0.80, with a maximum of eight spots in PL1 (Viswam) and a minimum of four spots in PL8 and PL 3. All the accessions had the compound with Rf value 0.05, except PL15. All the accessions possess the compound with Rf value 0.14 in the elution pattern except for the accessions PL5 and PL8. All the accessions possess the compound with Rf value 0.80 except PL3. All the accessions lack the spot of Rf value 0.66 but the accessions PL1, PL2 and PL5 possess the particular spot.

All the eleven accession possess the spots of Rf value 0.26 and 0.35 in their elution pattern except in PL3 and PL20 the spot with Rf value 0.35 is absent. At the same time the elution pattern of all the accessions lack the spot of Rf value 0.21 except in PL1 (Viswam) and PL5 which was present.

4.2.5 Correlation

The phenotypic correlations between different pairs of characters were estimated and presented in Table 23. Data revealed that dry spike yield was significantly and positively correlated with the vegetative branches per stem (0.441), spike bearing branches per stem (0.489), total number of leaves per stem (0.733), number of spikes per spike bearing branch (0.534), spike length (0.793), spike girth (0.721), fresh weight of spike (0.692), dry weight of spike (0.844) and fresh yield of spike (0.880)

Significant positive correlation was observed between number of stems per hill and total number of leaves (0.515), number of vegetative branches and total number of leaves (0.710). But number of vegetative branches was negatively correlated with angle of insertion (-0.250), spike length (-0.050), spike girth (-0.201) and fresh weight of spike (-0.186). Number of stems per hill was negatively correlated with petiole length (-0.089) and leaf area (-0.338).

Significant positive correlation was observed between numbers of spike bearing branch and inter nodal length of spike bearing branch (0.515), leaf number (0.514), number of spikes per spike bearing branch (0.581), spike girth (0.463), fresh weight of spike (0.444), dry weight of spike (0.460) and fresh yield of spike

(0.649). But number of spike bearing branches per stem negatively correlated with Petiole length (-0.361) and leaf area (-0.100).

With regard to petiole length its correlation with leaf area (0.506) was significant and positive. At the same time its correlation with angle of insertion of spike bearing branch was negative and significant (-0.657)

Significant positive correlation was found between internodal length of spike bearing branch and number of spike bearing branches per stem (0.515) and number of spikes per spike bearing branch (0.567)

No significant correlation was found between length of longest stem and any other character

With regard to leaf number there was significant correlation with number of stem (0.516), number of vegetative branches per stem (0.711), number of spike bearing branches per stem (0.515), number of spikes per spikes bearing branch (0.495), dry weight of spike (0.514) and fresh yield of spike (0.521)

Significant negative correlation was found between angle of insertion of spike bearing branch and petiole length (-0.657) and leaf area (-0.551).

With respective leaf area there was significant positive correlation with petiole length (0.506). Its correlation with angle of insertion of spike bearing branch (-0.551), spike girth (-0.465) and dry weight (-0.440) was negative and significant

Number of spikes per spike bearing branch was positively and significantly correlated with number of spike bearing branches per stem (0.836), internodal length of spike bearing branch (0.567), total leaves per plant (0.449) and fresh yield of spike (0.610). Its correlation with petiole length (-0.523) was negative and significant.

Spike length was positively and significantly correlated with spike girth (0.555), fresh weight of spike (0.947), dry weight of spike (0.978) and fresh yield of spike (0.798). Its correlation with leaf area was negative and significant (-0.449).

Spike girth was positively and significantly correlated with number of spike bearing branch (0.463), spike length (0.555), fresh weight of spike (0.947), dry weight of spike (0.950) and fresh yield of spike (0.831). Its correlation with leaf area was negative and significant (-0.465).

With regard to fresh weight of spike there was significant and positive correlation with number of spike bearing branches per stem (0.445), spike length (0.969), spike girth (0.941), dry weight of spike (0.952) and fresh yield of spike (0.776)

Dry weight of spike was positively and significantly correlated with number of spike bearing branches per stem (0.461), total number of leaves per hill (0.514), spike length (0.947), spike girth (0.950), fresh weight of spike (0.952) and fresh yield of spikes per plant (0.848). It was negatively correlated with leaf area (-0.440).

Fresh yield of spike was positively and significantly correlated with number of spike bearing branches per stem (0.650), total number of leaves per hill (0.524), number of spikes per spike bearing branch (0.610), spike length (0.790), spike girth (0.831), fresh weight of spike (0.776) and dry weight of spike (0.848).

4.2.6 Path analysis

Path coefficient analysis was done with nine characters in order to study the association of these characters with dry spike yield by isolating the direct as well as indirect effect of the causative factors on the dry spike yield and presented in Table 24.

The path analysis revealed that the fresh spike yield per plant exerted the maximum positive direct effect (0.7057) followed by length of spike (0.3749), number of vegetative branches per stem (0.2854), total number of leaves per hill (0.1000), dry weight of spike (0.0632) and the girth of spike (0.0222). Negative direct effect was observed in case of number of spike bearing branches per stem (-0.0813), number of spikes per spike bearing branches per stem (-0.0478) and fresh weight of spike (-0.2529).

The indirect effect of fresh yield per plant through the length of spike was positive and high (0.2865), where as the indirect effect through all other characters though low and increased the total effect to 0.8771. There is low and negative indirect effect on fresh yield per plant through the number of spike bearing branches per stem (-0.0526), number of spikes per spike bearing branch (-0.0286) and fresh weight of spike (-0.1913).

The number of vegetative branches per stem had a positive direct effect (0.2854) where as the indirect effect through all other characters were low i.e., total number of leaves per hill (0.0649), fresh weight of spike (0.0438), dry weight of spike (0.0012) there by elevating the total effect to 0.4358. The number of vegetative branches per stem had low negative indirect effect through number of spike bearing branches per stem (-0.0126), number of spikes per spike bearing branches per stem (-0.0137), length of spike (-0.0228) and girth of spike (-0.0039).

Even though total number of leaves had low positive direct effect (0.1000) its indirect effect through fresh yield of spikes per plant was high and positive (0.4565). The indirect effect of total number of leaves through number of vegetative branches per stem was (0.1854) and through the length of spike (0.1430) was positive but low. The indirect effect through the girth of spike (0.0064) and dry weight of spikes (0.0275) were low. The total number of leaves had low negative effect through the number of spike bearing branches per stem (-0.0394), the number of spikes per spike bearing branch (-0.0205) and fresh weight of spike (-0.1096).

The length of spike had a positive and direct effect (0.3749) and its indirect effect through fresh yield of spikes per plant were high and positive (0.5391). The indirect effect of length of spikes through total number of leaves (0.0381), girth of spikes (0.0213), and dry weight of spikes (0.0617) was low. There was low indirect effect on length of spikes through number of vegetative branches per stem (-0.0174), the number of spike bearing branches per stem (-0.0322), number of spikes per spike bearing branches (-0.0163) and fresh weight of spike (-0.2395).

Even though direct effect of girth of spike was positive and low (0.0222), its indirect effect through fresh yield of spikes per plant (0.5746) and the length of spike (0.3602) was positive and high. The indirect effect of the girth of spike through total number of leaves per hill (0.0288), dry weight of spike (0.0593) was low. The indirect effect of this character through number of vegetative branches per stem (-0.0499), number of spike bearing branches per stem (-0.0370), number of spikes per spike bearing branches (-0.0175), and fresh weight of spike (-0.2378) were negative and low.

Even though the dry weight of a spike (0.0632) had low positive effect, its indirect effects through fresh yield of spikes per plant (0.5721) and the length of spike (0.3661) were high and positive which elevate the total effect to 0.7869. The indirect effect of dry weight of spike through number of vegetative branches per stem (0.0055), total number of leaves per hill (0.0487) and the girth of spike (0.0208) were positive but low. There were low negative indirect effect through the number of spike bearing branches per stem (-0.0354), the number of spikes per spike bearing branch (-0.0163), and fresh weight of spike (-0.2379).

The fresh yield of spikes per plant exerted highest positive direct effect (0.7057). Even though indirect effect on fresh yield of spikes per plant through the length of spike was positive and high (0.2865), the indirect effect of fresh yield of spikes per plant through all other characters were low i.e. number of vegetative branches per stem (0.0378), total number of leaves per hill (0.0503), girth of spike (0.0181) and dry weight of spike (0.0512). The characters like number of spike bearing branches per stem (-0.0526), number of spikes per spike bearing branch (-0.0286) fresh weight of spike (-0.1913) exerted a low negative effect on fresh spike yield per plant.

The direct effect of number of spike bearing branches per stem was negative and low (-0.0813) but its indirect effect through fresh spike yield was high and positive (0.4565). Its indirect effect through the number of vegetative branches per stem (0.0443), total number of leaves per hill (0.0485), girth of spike (0.0101) and the dry weight of spike (0.0275) are positive but low. Its indirect effect through the number of spikes per spike bearing branch (-0.0366) and fresh weight of spike (-0.1096) were negative.

The direct effect of number of spikes per spike bearing branch was negative and low (-0.0478), its indirect effect through fresh spike yield per plant (0.4217) and length of spike (0.1276) were positive and high. Its indirect effect through number of vegetative branches per stem (0.0820), total number of leaves per hill (0.0429), girth of spike (0.0081) and dry weight of spike (0.0215) was positive but low which elevate the total effect to positive and high 0.5167. Its indirect effect through the number of spike bearing branches per stem (-0.0622) and fresh weight of spikes (-0.0772) was negative and low. The nine characters alone and in combination contributed nearly, 91.97 percentage of the variability in dry spike yield. The residual effect (0.0803) obtained was very low.

Table 23. Correlation Coefficients between yield and its components.

Character	No. of Stems per hill	No. of vegetative branch / stem	No. of Spike bearing branches	Petiole length	Internodal length	Length of longest stem	Leaf Number	Angle of insertion	Leaf area	No. of spike/ spike bearing branch	Spike length	Spike girth	Fresh weight of spike	Dry weight of spike	Fresh yield of spike	Dry yield of spike
No. of Stems per hill		0.247	0.368	-0.089	0.170	0.225	**0..515***	0.082	-0.338	0.385	0.129	0.112	0.089	0.170	0..313	0.316
No. of vegetative branch / stem	0.247		0.133	0.081	0..310	0.170	**0.710****	-0.250	0..366	0..317	-0.050	-0.201	-0.186	0.017	0.107	**0.440***
No. of Spike bearing branches / stem	0.368	0.133		-0.361	**0..515***	0..236	**0..514***	0..310	-0.100	**0..581***	0.405	**0.463***	**0.444***	**0.460***	**0.649****	**0.489***
Petiole length	-0.088	0.081	-0..361		0.049	0..352	0.175	**-0.657****	**0..506***	**-0.522***	-0..344	0.343	-0..272	-0..306	-0..366	-0.349
Internodal length	0.170	0.310	**0..515***	0.049		0..272	0..344	-0.123	0.101	**0.567***	0.251	0.247	0.205	0..309	0..316	0.151
Length of longest stem	0.180	0.171	0..237	0..352	0..272		0.157	-0.417	0..301	0.175	-0.224	-0.222	-0.263	-0.319	-0.142	-0.118
Leaf number	**0.516***	**0.711****	**0..515***	-0.175	0..344	0.157		0.065	-0.048	**0.495***	0.415	0.300	0..348	**0..514***	**0..521***	**0.733****
Angle Insertion	0.083	-0.250	0.311	**-0.657****	-0.123	-0.417	0.044		**-0.551***	0.404	0..322	0.351	0..376	0..321	0..333	0..217
Leaf area	-0.338	0.367	-0.100	**0.506***	0.101	0..301	-0.048	0..216		-0.106	**-0.449***	**-0.465***	-0.405	**-0.440***	-0.360	-0.295
No. of spike/ spike bearing branch	0.385	0.317	**0.836****	**-0.523***	**0..567***	0.175	**0.449***	**-0..551***	-0.106		0..353	0.370	0..313	0..391	**0.610****	**0.534***
Spike length	0..129	-0.050	0.405	-0..345	0..251	-0.224	0.415	0..322	**-0.449***	0.166		**0.555***	**0..947****	**0..978****	**0.798****	**0.793****
Spike girth	0..112	-0.201	**0.463***	-0344	0..247	-0.222	0..300	0..351	**-0.465***	0.370	**0..555***		0.**947****	**0..950****	**0.831****	**0.721****
Fresh weight of spike	0.089	-0.186	**0.445?***	-0.273	0..205	0..263	0..348	0..376	-0.405	0..313	**0..969****	**0.941****		**0..952****	**0.776****	**0.692****
Dry weight of spike	0.170	0.017	**0.461***	-0.307	0..309	-0.204	**0..514***	0..321	**-0.440***	0..391	0**..947****	**0.950****	**0..952****		**0.848****	**0.844****
Fresh yield of spike	0.313	0.108	**0.650****	-0..366	0..316	-0.142	**0..524***	0..333	-0..360	**0.610***	**0.790****	**0.831****	**0.776****	**0.848****		**0.880****
Dry yield of spike	0.316	**0.441***	**0.489***	-0..350	0..289	-0.118	**0.733****	0.217	-0.295	**0..534***	**0.793****	**0.721****	**0.692****	**0.844****	**0.880****	

* Significant at $p < 0.05$ ** Significant at $p < 0.01$

Table.24. Path coefficient analysis of spike yield and component characters

	Vegetative branches per stem	Spike bearing branches per stem	Total number of leaves	Spikes per spike bearing branch	Spike length	Spike girth	Fresh weight of spike	Dry weight of spike	Fresh yield of spike	Correlation with yield
Vegetative branches per stem	0.2854	-0.0126	0.0649	-0.0137	-0.0228	-0.0039	0.0438	0.0012	0.4356	0.4358
Spike bearing branches per stem	0.0443	-0.0813	0.0485	-0.0366	0.1484	0.0101	-0.1096	0.0275	0.4565	0.5077
Total number of leaves	0.1854	-0.0394	0.1000	-0.0205	0.1430	0.0064	-0.1096	0.0275	0.4565	0.6771
Spikes per spike bearing branch	0.0820	-0.0622	0.0429	-0.0478	0.1276	0.0081	-0.0772	0.0215	0.4217	0.5167
Spike length	-0.0174	-0.0322	0.0381	-0.0163	0.3749	0.0213	-0.2395	0.0617	0.5391	0.7299
Spike girth	-0.0499	-0.0370	0.0288	-0.0175	0.3602	0.0222	-0.2378	0.0593	0.5746	0.7029
Fresh weight of spike	-0.0494	-0.0352	0.0328	-0.0146	0.3551	0.0209	-0.2529	0.0594	0.5339	0.6499
Dry weight of spike	0.0055	-0.0354	0.0487	-0.0163	0.3661	0.0208	-0.2379	0.0632	0.5721	0.7869
Fresh yield of spike	0.0378	-0.0526	0.0503	-0.0286	0.2865	0.0181	-0.1913	0.0512	0.7057	0.8771

Discussion

V. DISCUSSION

The results of evaluation of twenty accessions of *Piper longum* based on eleven vegetative characters, eight yield contributing characters, piperine content, oil content of dried spikes and other qualitative aspects are briefly discussed in this chapter.

Piper longum the Indian long pepper plant is a slender, creeping shrub, spreading on the ground and rooting at the nodes. It grows and creeps over small shrubs, rocks etc but does not climb on trees as in the case of black pepper or other wild pepper. The plant produces distinct dimorphic branches, those main branches creeping on the ground have cordate leaves with long petioles, and axillary branches grow erect producing leaves that are sessile or with short petioles. The vegetative shoots grow by the activity of terminal bud, and the short axillary branches (fruiting branches) produces spikes opposite to leaves, with sympodial growth. Leaves are simple alternate and variable in size and shape.

Long pepper plant is dioecious, male and female plants are separate. Flowers are arranged on short cylindrical spike. The male spikes are much longer than female spikes. Female spikes are short, stout, flowers fused laterally. Each flower consists of one ovary only that arises from the axil of a bract. Fruits are small and closely packed. Fruiting is apomictic and fruits are produced without pollination. Hence male plants are not required for fruit production.

Kerala state is blessed with wide variability in long pepper. But very little attention has been paid for the improvement of this crop. There is only one released variety of this medicinal plant which is Viswam from Kerala Agricultural University. In present study twenty accessions of *Piper longum were* evaluated for their variability in yield, morphological and biochemical characters for identifying superior type.

5.1 Genetic cataloguing in *Piper longum*

Twenty *Piper longum* accessions collected from different locations were catalogued for morphological characters using IBPGR descriptor list for *Piper nigrum*. Wide rage of variation was not observed in morphological characters of different accessions of *Piper longum*. Runner shoot production ranged from few two many. Leaf lamina of different accessions expressed cordate and ovate lanceolate shape (Plate 7). Immature spike colour ranged from light yellow to green. Variation in colour change from immature to mature spike (green to dark green/ black) was also observed. Wide variability in spike shape, size, colour were also observed (Plate 6).

5.2 Variability

The success of any crop improvement program depends up on the precise information available on the genetic variability of the crop.

In the present study significant differences among the accessions for the characters such as number of stems per hill (Fig.1), number of vegetative branches per stem (Fig.2), number of spike bearing branches per stem (Fig.3), angle of insertion of spike bearing branch, internodal length of spike bearing branch (Fig.4), leaf area, petiole length (Fig.6), number of leaves per hill (Fig.7), number of spikes per spike bearing branch (Fig.9), spike length (Fig.8), days from emergence to maturity of spikes, fresh weight of spike(Fig.11), fresh yield of spikes per plant, dry yield of spikes per plant (Fig.10) and driage (Fig.12) were noticed . The existence of considerable variation indicated enough scope for crop improvement. Variability in many of the economic characters in this crop had been observed by workers like Manuel (1994) and Jaleel (2006).

Manuel (1994) conducted a comparative evaluation of four types of *Piper longum* in coconut plantation and reported significant variability in characters like number of stems per hill, number of vegetative branches per stem, number of spike bearing branches per stem, angle of insertion of spike bearing branches per stem, leaf length, leaf petiole length, and number of leaves per hill.

Jaleel (2006) reported significant variability of spike length, spike girth, period for spike maturity and fresh weight of spike.

Present study also indicates that there is significant variability among accessions with respect to biochemical characters such as essential oil content (fig.13) and piperine content (fig.14). Jaleel (2006) and Manuel (1994) reported variability of oil content and piperine content in different accessions of *Piper longum*.

The accessions differed qualitatively with respect to the presence of biochemical constituent like phenols, flavanoids, alkaloids and terpenes (Plate 8, Plate 9). Several workers have reported the variability of bio chemical constituents in medicinal plants. Variability of saponins in *Gymnema sylvestre* was reported by Nair (2005), plumbagin in *Plumbago spp* by Menon (1999). Manjusha (2001) reported the variability of phenols, flavanoids and alkaloids in different accessions of *Adathoda*.

5.3 Correlation

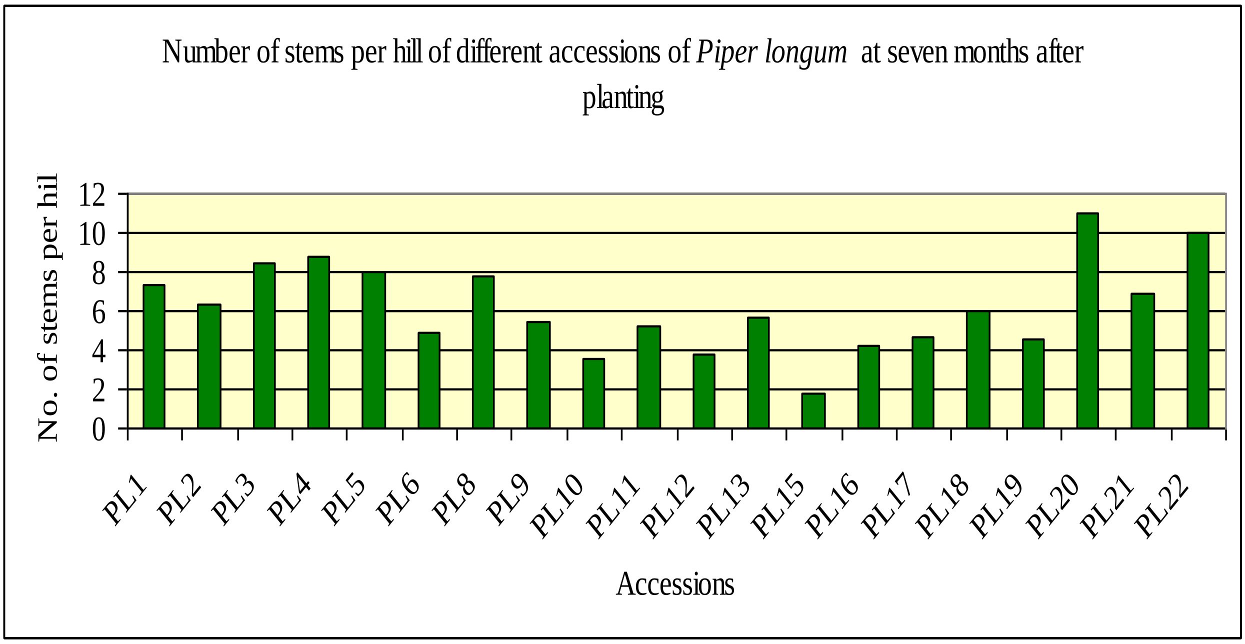
Yield is a complex character contributed by many related components. Hence knowledge of the relationship of yield and its component character is essential for the simultaneous improvement of yield components and in turn for the yield increase to be effective.

In the present study the dry spike yield was found to be significantly and positively correlated with characters like number of vegetative branches per stem, number of spike bearing branches per stem, total number of leaves per hill, number of spikes per spike bearing branches, spike length, spike girth, fresh weight of spike, dry weight of spike and fresh yield of spikes. Correlation of above characters with yield in many crop plants has been reported by many workers.

Manuel (1994) conducted correlation studies in *Piper longum* and found that characters like angle of insertion of spike bearing branch, number of vegetative branches per stem, number of leaves per hill, number of spike bearing branches per stem and number of spikes per spike bearing branches showed positive and significant correlation with dry spike yield and also reported that out of these characters maximum contribution to dry spike yield was exhibited by angle of insertion of spike bearing branch. Present study indicate that angle of insertion of spike bearing branch was not a significant yield contributing character, may be due to the presence of sparse flowering accessions included in the study. In this study positive correlation of number of stems per hill, angle of insertion of spike bearing branch, internodal length of spike bearing branch with dry spike yield was also observed. However these correlations were found to be insignificant indicating the independent nature of these characters in relation to yield

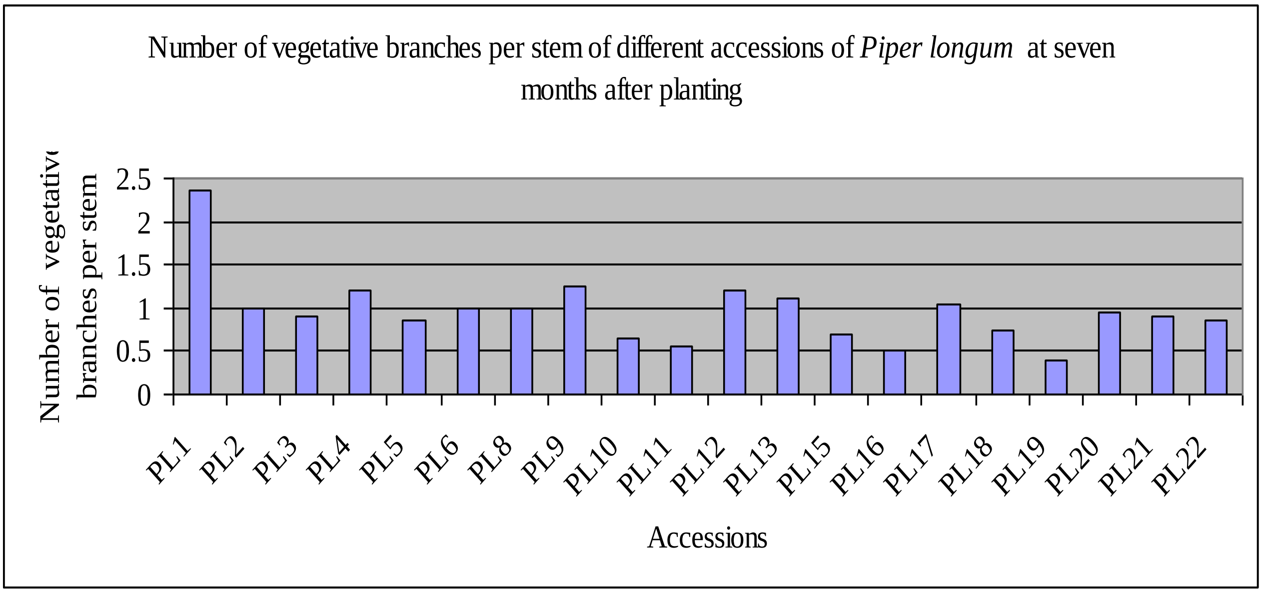
Dry spike yield was found to be negatively correlated with stem length, leaf area and petiole length. But these correlations were found to be insignificant.

Similar studies in chilly was conducted by Philipos (1986), Mehrotra *et al* (1977), Rao and Chonkar (1983), Bavaji and Murthy (1982), Joshi and Singh



Number of stems per hill of different accessions of *Piper longum* at seven months after planting
No. of stems per hil
12
10
8
6
4
2
0
PL1
PL2
PL3
PL4
PL5
PL6
PL8
PL9
PL10
PL11
PL12
PL13
PL15
PL16
PL17
PL18
PL19
PL20
PL21
PL22
Accessions

Fig. 1



Number of vegetative branches per stem of different accessions of *Piper longum* at seven months after planting
Number of vegetativ
branches per stem
2.5
2
1.5
1
0.5
0
PL1
PL2
PL3
PL4
PL5
PL6
PL8
PL9
PL10
PL11
PL12
PL13
PL15
PL16
PL17
PL18
PL19
PL20
PL21
PL22
Accessions

Fig. 2

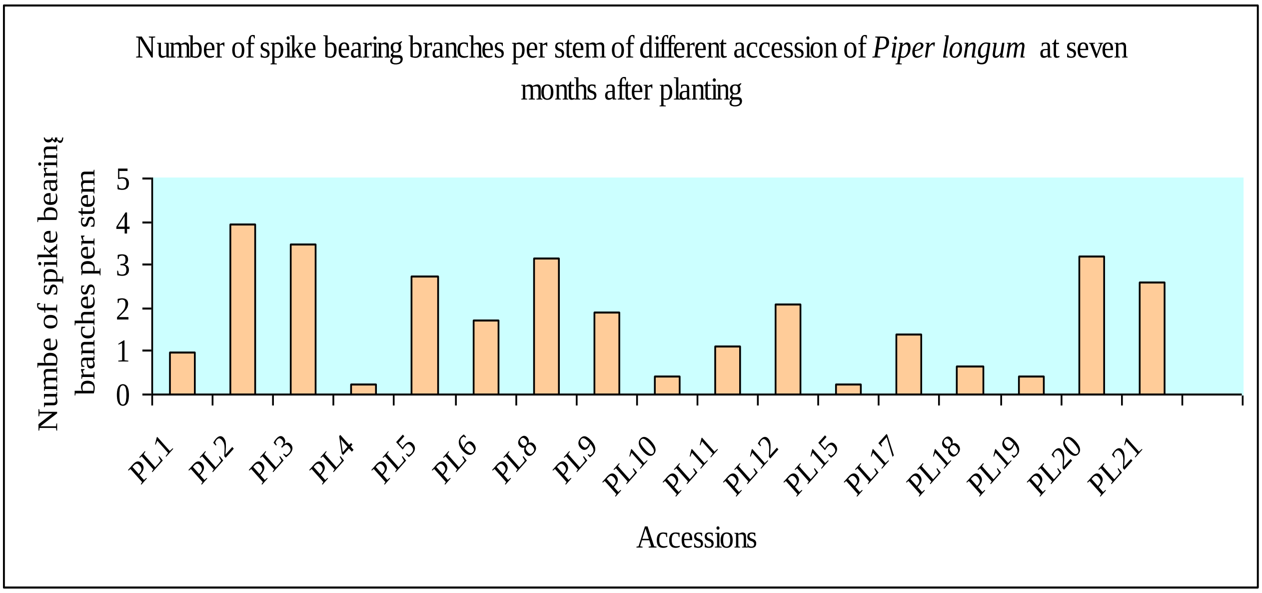

Number of spike bearing branches per stem of different accession of *Piper longum* at seven months after planting
Numbe of spike bearing branches per stem
0
1
2
3
4
5
PL1
PL2
PL3
PL4
PL5
PL6
PL8
PL9
PL10
PL11
PL12
PL15
PL17
PL18
PL19
PL20
PL21
Accessions

Fig. 3

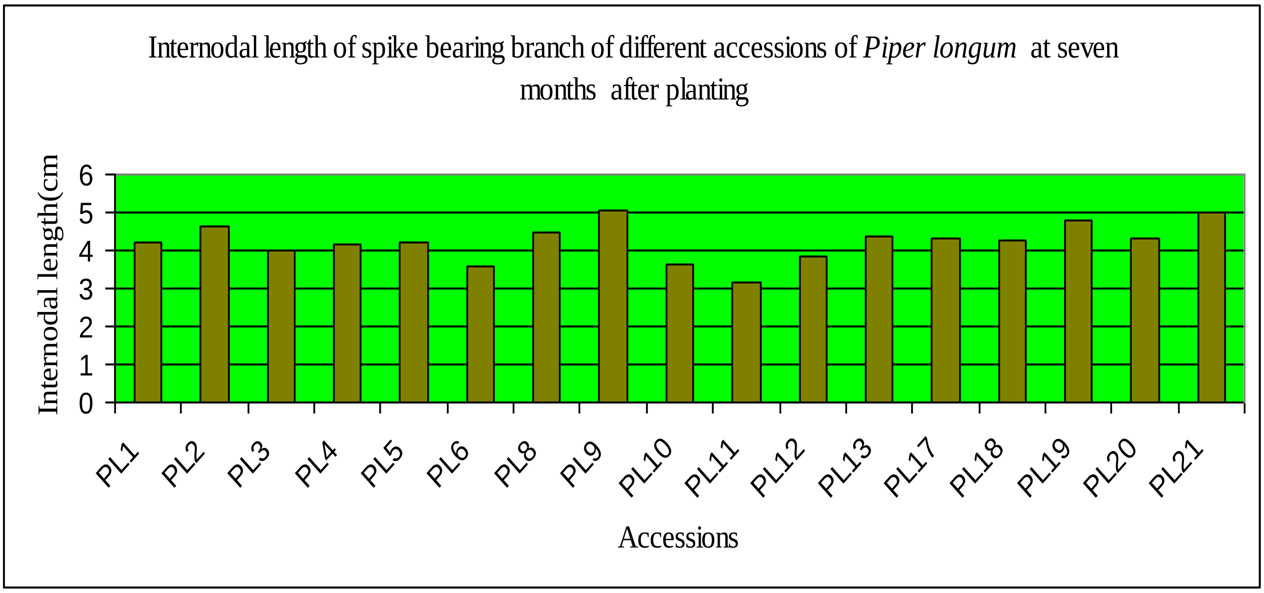

Internodal length of spike bearing branch of different accessions of *Piper longum* at seven months after planting
Internodal length(cm
0
1
2
3
4
5
6
PL1
PL2
PL3
PL4
PL5
PL6
PL8
PL9
PL10
PL11
PL12
PL13
PL17
PL18
PL19
PL20
PL21
Accessions

Fig. 4

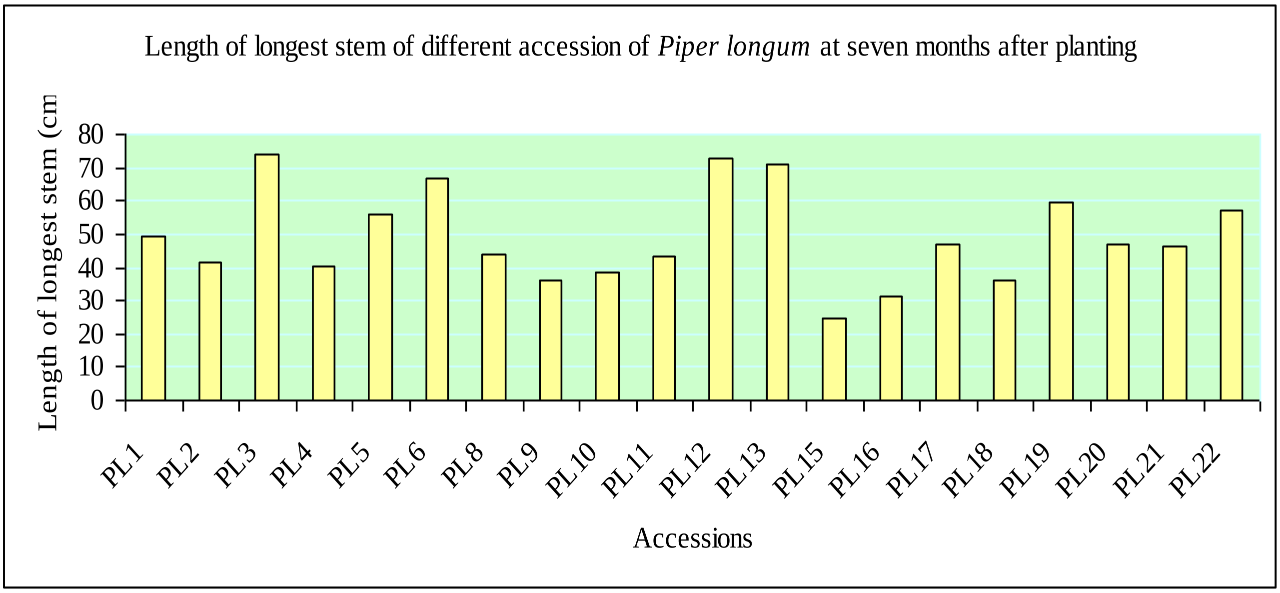

Length of longest stem of different accession of *Piper longum* at seven months after planting
Length of longest stem (cm
0
10
20
30
40
50
60
70
80
PL1
PL2
PL3
PL4
PL5
PL6
PL8
PL9
PL10
PL11
PL12
PL13
PL15
PL16
PL17
PL18
PL19
PL20
PL21
PL22
Accessions

Fig. 5

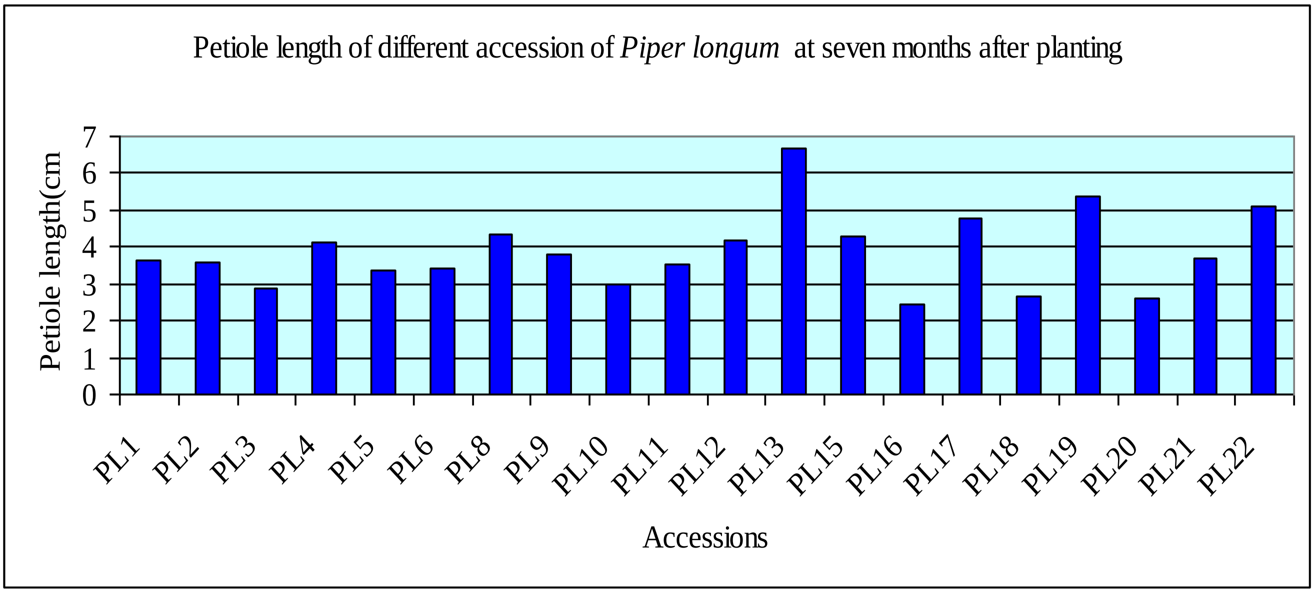

Petiole length of different accession of *Piper longum* at seven months after planting
Petiole length(cm
0
1
2
3
4
5
6
7
PL1
PL2
PL3
PL4
PL5
PL6
PL8
PL9
PL10
PL11
PL12
PL13
PL15
PL16
PL17
PL18
PL19
PL20
PL21
PL22
Accessions

Fig. 6

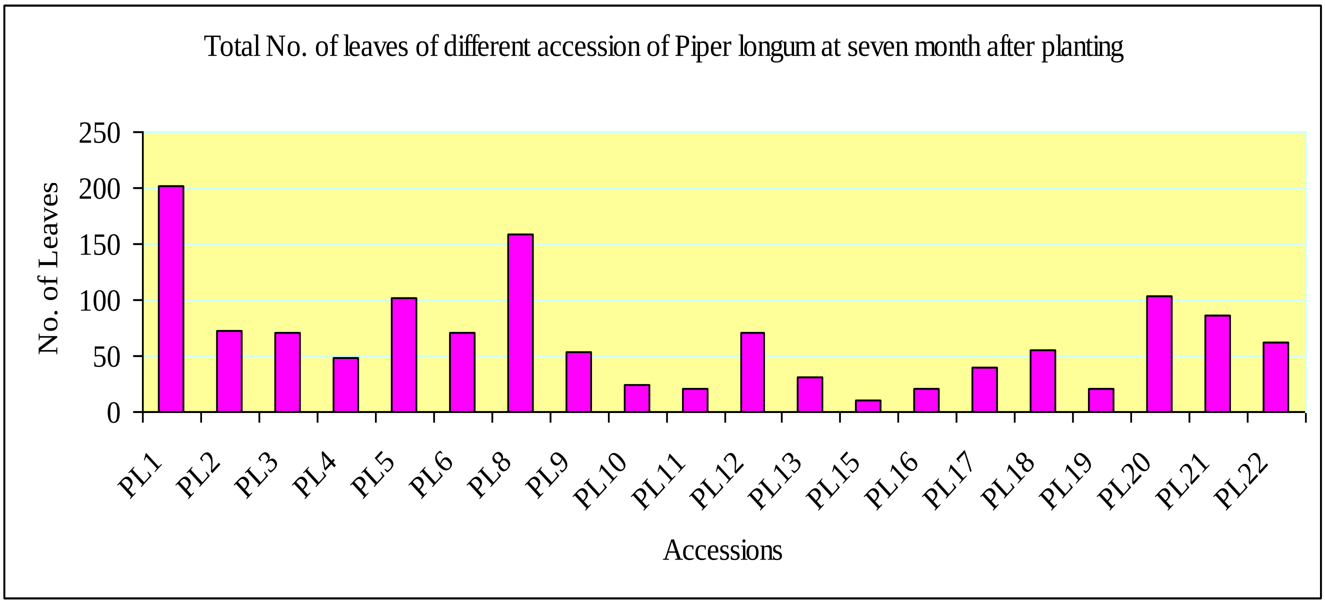

Total No. of leaves of different accession of Piper longum at seven month after planting
No. of Leaves
250
200
150
100
50
0
PL1
PL2
PL3
PL4
PL5
PL6
PL8
PL9
PL10
PL11
PL12
PL13
PL15
PL16
PL17
PL18
PL19
PL20
PL21
PL22
Accessions

Fig. 7

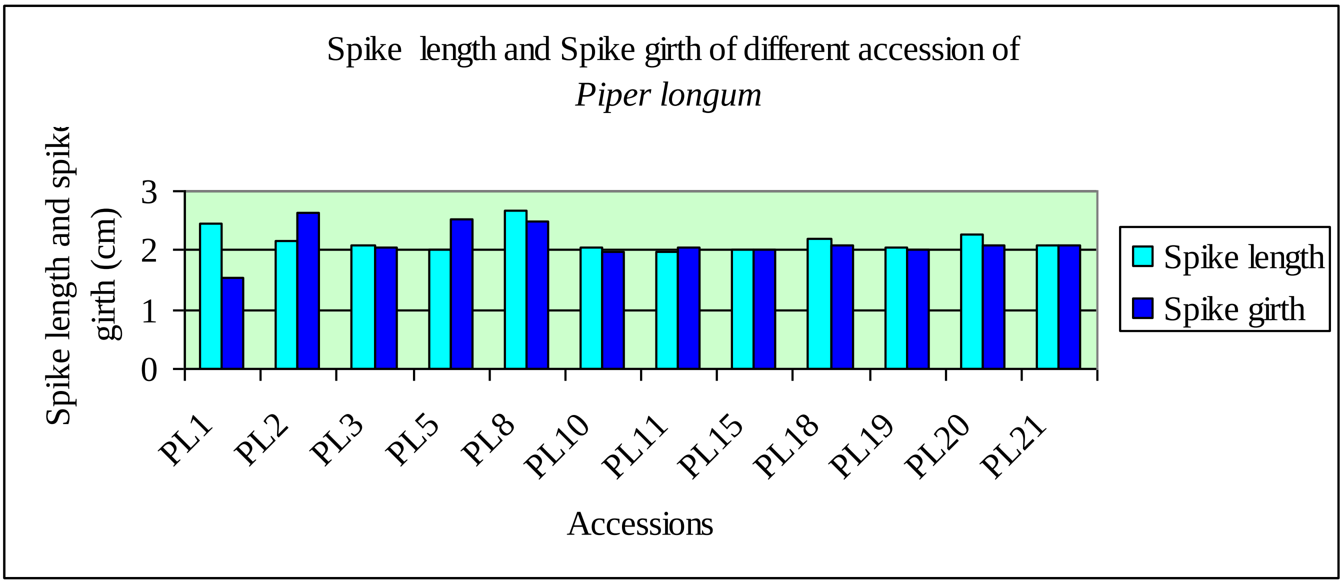
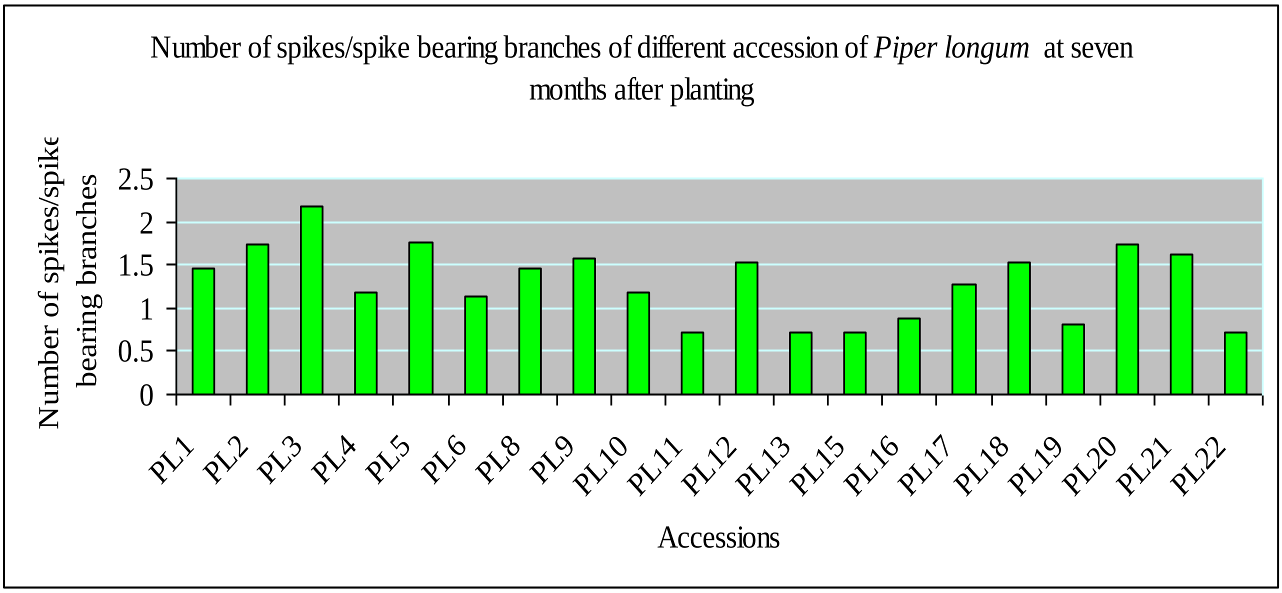
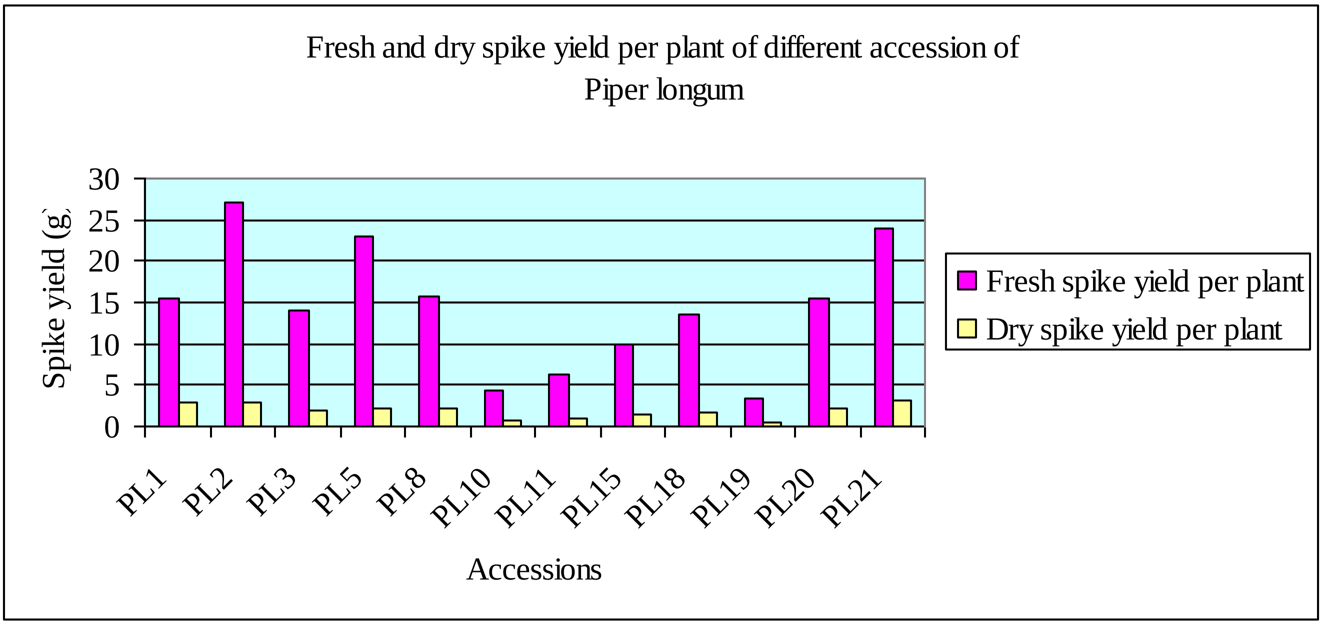

Spike length and Spike girth of different accession of
Piper longum
Spike length and spike girth (cm)
3
2
1
0
PL1
PL2
PL3
PL5
PL8
PL10
PL11
PL15
PL18
PL19
PL20
PL21
Accessions
Spike length
Spike girth

Fig. 8



Number of spikes/spike bearing branches of different accession of *Piper longum* at seven months after planting
Number of spikes/spike bearing branches
2.5
2
1.5
1
0.5
0
PL1
PL2
PL3
PL4
PL5
PL6
PL8
PL9
PL10
PL11
PL12
PL13
PL15
PL16
PL17
PL18
PL19
PL20
PL21
PL22
Accessions

Fig. 9



Fresh and dry spike yield per plant of different accession of Piper longum
Spike yield (g)
30
25
20
15
10
5
0
PL1
PL2
PL3
PL5
PL8
PL10
PL11
PL15
PL18
PL19
PL20
PL21
Accessions
Fresh spike yield per plant
Dry spike yield per plant

Fig. 10

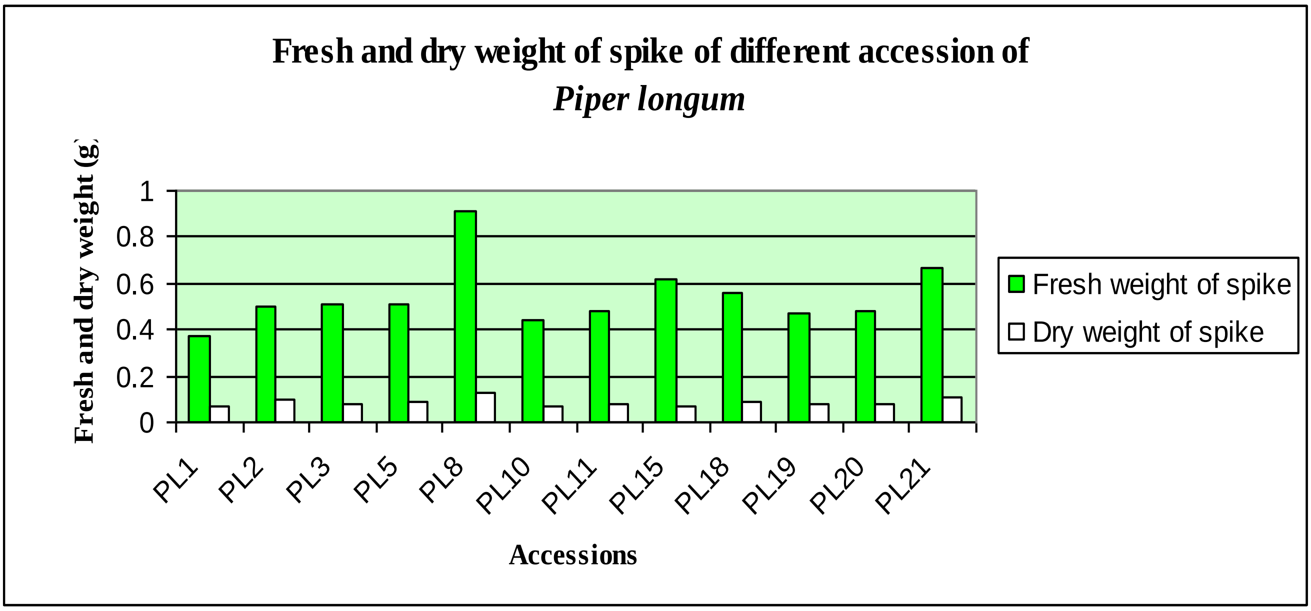

Fresh and dry weight of spike of different accession of *Piper longum*
Fresh and dry weight (g)
1
0.8
0.6
0.4
0.2
0
PL1
PL2
PL3
PL5
PL8
PL10
PL11
PL15
PL18
PL19
PL20
PL21
Accessions
Fresh weight of spike
Dry weight of spike

Fig. 11

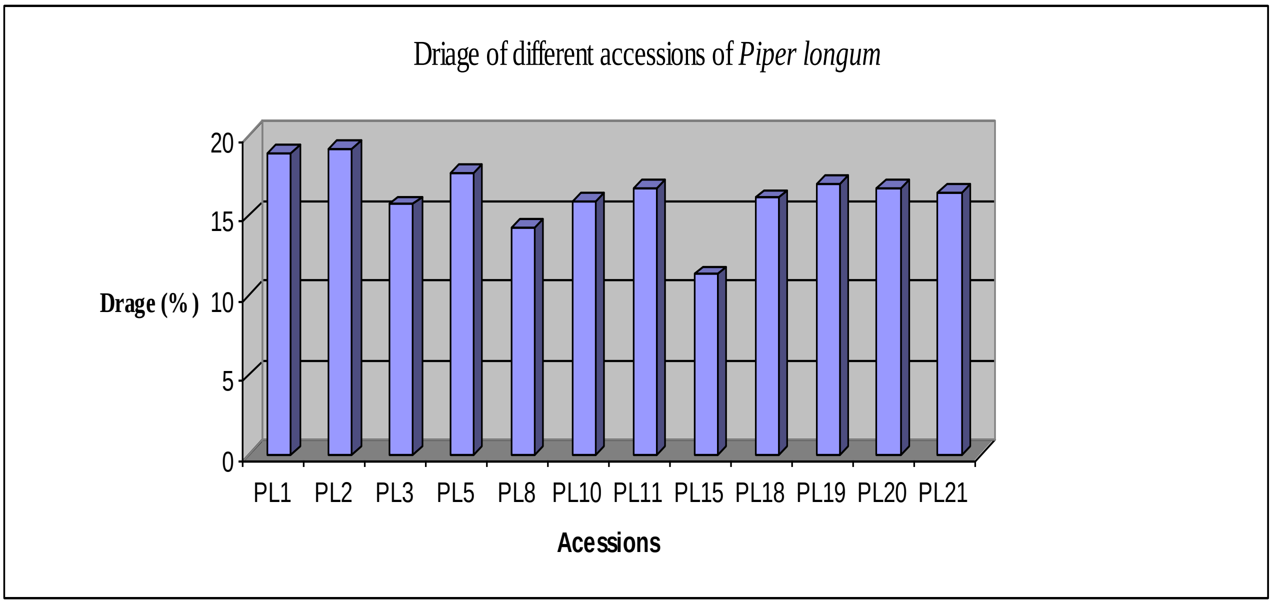

Driage of different accessions of *Piper longum*
Drage (%)
20
15
10
5
0
PL1
PL2
PL3
PL5
PL8
PL10
PL11
PL15
PL18
PL19
PL20
PL21
Acessions

Fig. 12

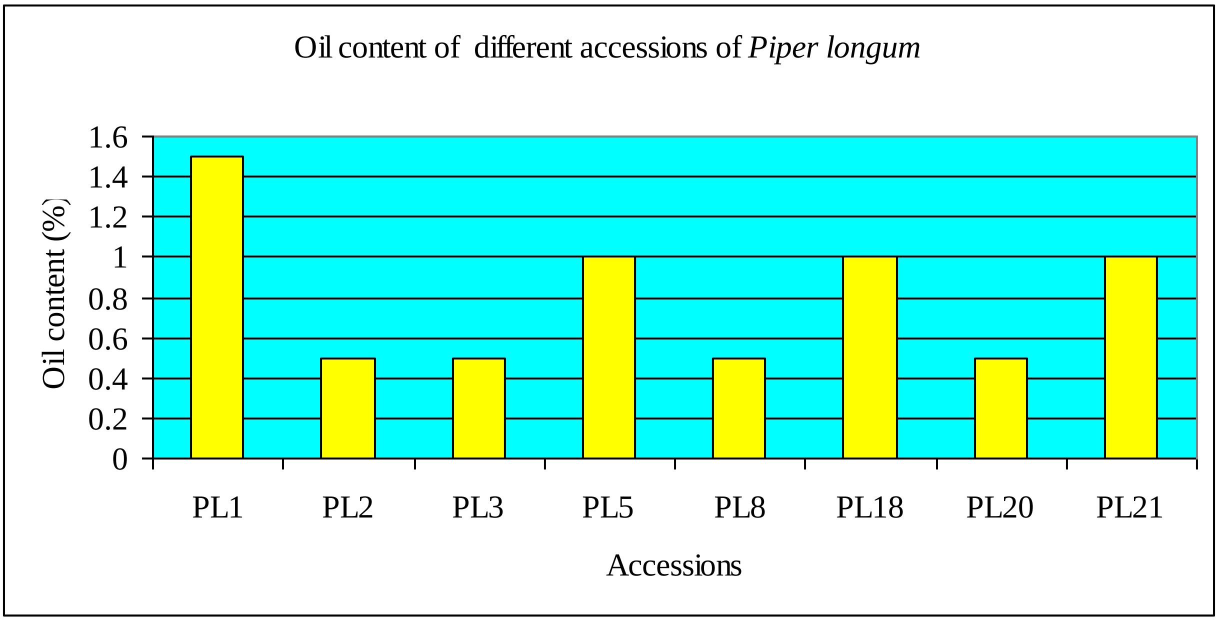

Oil content of different accessions of *Piper longum*
Oil content (%)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
PL1
PL2
PL3
PL5
PL8
PL18
PL20
PL21
Accessions

Fig.13

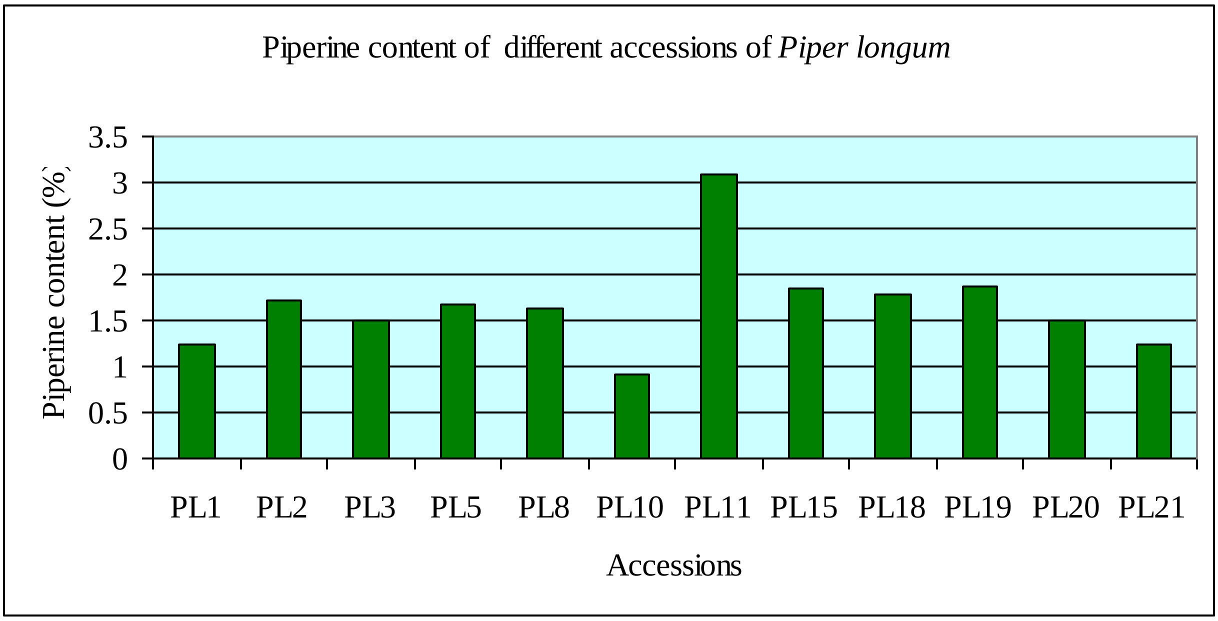

Piperine content of different accessions of *Piper longum*
Piperine content (%)
3.5
3
2.5
2
1.5
1
0.5
0
PL1
PL2
PL3
PL5
PL8
PL10
PL11
PL15
PL18
PL19
PL20
PL21
Accessions

Fig.14

(1983) and Nair *et al* (1984) and found that plant height, number of branches per plant and number of leaves showed positive correlation with yield characters like number and weight of fruits per plant and number and weight of fruits per plot. Pillai (1980), Muthiah and Sivasubramanian (1981), Philip (1987) and Abraham (1988) reported that the number of branches per plant was positively correlated with grain yield of black gram.

In this study it was found that the number of spike bearing branches per stem was positively and significantly inter correlated with total number of leaves per hill, the number of spikes per spike bearing branches per stem, spike girth, fresh weight of spike, dry weight of spike and fresh yield of spike. The total number of leaves per hill was positively and significantly correlated with the number of spikes per spike bearing branch, length of spike, dry weight of spike and fresh yield of spike. It was also found that the number of spikes per spike bearing branch was correlated with fresh spike yield per plant indicating that fresh spike yield per plant can be increased by selecting plant type with more number of spikes per spike bearing branch. Present study also indicates that length of spike was positively and significantly correlated with girth of spike, fresh weight of spike, dry weight of spike and fresh yield of spike.

Ibrahim et *al* (1985) reported that in *Piper nigrum*, the characters green spike yield per vine, green berry yield per vine, number of spikes per vine and number of under developed berries per spike, as well as vegetative characters like thickness of node and internodes of orthotrope and angle of insertion were positively and significantly correlated with yield. The inter correlation among these characters was also reported to be positive and significant.

Thamburaj (1973) found that in ridge gourd the fruit weight and number of seeds per fruit had a significant positive effect on yield per plant. Mehra and Peter (1980), Raju (1980), Shrama *et al* (1981), Rao and Chonkar (1983) and Nair *et al* (1984) reported that in chilly, there is significant association of character, individual fruit length with fruit yield per plot. Radhika (1984) reported the genotypic correlations of fresh weight of mature pods per plant with dry pod yield.

54. Path analysis

Correlation studies are helpful in measuring the association between yield and yield components. But they do not provide a clear picture of the direct and indirect causes of such associations. However these can be obtained through path analysis suggested by Dewey and Lu (1959). Path coefficient analysis provides effective measures of untangling direct and indirect course of association and permits a critical examination of specific cause acting to produce a given correlation and measures of relative importance of each factor.

On partitioning of the correlation in to direct and indirect effects, it was observed that fresh spike yield per plant, spike length, vegetative branches per stem, total number of leaves per hill, dry weight of spike and spike girth had direct positive effect respectively. The characters spike number of spike bearing branches per stem, number of spikes per spike bearing branch exert a negative direct effect on dry spike yield. It revealed a true relationship between the characters and yield and hence direct selection for these traits would be rewarding for yield improvement.

The direct effect of fresh spike yield per plant, spike length, vegetative branches per stem, total number of leaves per hill, dry weight of spike and spike girth was positive and their correlation coefficients were positive and significant hence direct selection via these characters should be considered. This is in agreement with the report of Manuel (1994) and Sujatha and Namboodiri (1995).

The number of spike bearing branches per stem and number of spikes per spike bearing branch exhibited negative direct effect on dry spike yield, while correlation coefficient was positive and significant. At the same time their indirect effect through fresh spike yield per plant and spike length was high and positive. This emphasizes the need for selection of spike bearing branch per stem and number of spikes per spike bearing branches through fresh spike yield per plant and spike length.

The indirect effect of all the yield contributing characters through fresh spike yield on dry spike yield was considerably large. Similarly the indirect effect of all characters through total number of leaves per hill was positive. Hence this emphasizes the importance of selection of types based on these characters, fresh spike yield per plant and total number of leaves per hill. This is in agreement with the report of Sujatha and Namboodiri (1995), that fresh spike yield per vine in black pepper had high positive direct effect on dry spike yield per vine.

5.6 Selection

The selection process involve all the yield components namely number of vegetative branches per stem, number of spike bearing branches per stem, total number of leaves per hill, number of spikes per spike bearing branch, spike length spike girth, fresh weight of spike, dry weight of spike and fresh yield of spike. Selection based on above characters revealed that the accessions PL 21, PL 2, PL 5, PL 20, PL 8 and PL 3 were high yielding along with the check variety Viswam.

Considering the quality aspects viz oil yield, piperine content and presence of biochemical constituents, the check variety Viswam and accessions PL 2, PL5 and PL21 were found to be promising. Even though PL8 and PL 20 were high yielding, the quality of spike was comparatively low as measured by the biochemical constituents.

Considering yield and quality aspects, accessions PL 2, PL 5 and PL 21were found to be promising compared to the released variety Viswam

Future line of work

The present study gives only a preliminary indication of the yield capacity and quality of the long pepper accessions evaluated. Being a perennial crop with a longevity of 3-4 years, its stability in yield and quality over seasons have to be studied before arriving at definite conclusions. Performance of the accessions at different shade intensity is also worth investigating.

Summary

SUMMARY

The present investigation entitled "Evaluation of ecotypes of long pepper (*Piper longum* L.) was carried out at the Department Plantation Crops & Spices at Collage of Horticulture, Vellanikara during the period 2005-2007. The salient features could be summarized as follows.

1. Twenty accession of *Piper longum* where genetically catalogued based on descriptor listed for *Piper nigrum*. Wide variations were not observed. Runner shoot production varied from few to many. Leaf lamina shape was found to be cordate and ovate lanceolate. Spike shape was found to be filiform and cylindrical. Immature spike colour was found to be yellow and green. Coulor change of spike while fruit ripening was varied between green to black and green to yellow.
2. Vegetative character, number of stems per hill vary significantly between the twenty accessions studied but the particular character was not contributed to the dry spike yield significantly
3. Number of vegetative branches per stem varies significantly between the accessions and the vegetative character significantly contributed to the dry spike yield .The maximum vegetative branches per stem at seven month after planting was observed in Viswam and the minimum vegetative branches per stem was observed in PL 19.
4. Number of spike bearing branch per stem varies significantly between the accessions and these character significantly contribute to the dry spike yield. Even though the direct effect of this character on dry spike yield was negative but its indirect effect through fresh spike yield was significant and highly positive. The maximum number of spike bearing branches per stem was resulted in PL 3 at seven month after planting.
5. Vegetative characters like petiole length, internodal length of spike bearing branch and length of longest stem varies between different accessions but these characters do not contributed to the dry spike yield.

6. Vegetative character total number of leaves per hill vary between the different accessions and these character correlated with dry spike yield significantly. The highest position with respect to this character was observed in Viswam, which was statistically at par with PL8.
7. Yield contributing character like Angle of insertion of spike and days from emergence to maturity of spike varies between the different accessions but this character did not contributed to dry spike yield.
8. All the flowered twelve accessions took same duration for first few spikes to emerge.
9. Yield contributing characters like length of spike vary between the twelve flowered accession and these characters contributed to the dry spike yield. The maximum length of the spike was resulted in PL 8, which was statistically at par with Viswam. The minimum spike length was observed in PL 11 followed by PL5.
10. Yield contributing character girth of spike did not vary significantly between the accession, but this character contributed to dry spike yield.
11. Dry weight of spike did not vary significantly between the fairly flowered twelve accessions. This character contributed to dry spike yield positively.
12. Fresh weight of green spike varies significantly between twelve fairly flowered accessions and this character contributed to dry spike yield positively. The maximum fresh weight of spike was observed in PL 8.
13. Maximum positive correlation to dry spike yield was exhibited by the character fresh spike yield and this character varies between fairly flowered accessions. The maximum fresh spike yield was resulted in PL 2, which was statistically at par with PL 21 and PL5.
14. Maximum dry spike yield was observed in PL 2, which was statistically at par with PL 21, and Viswam, the check variety. The minimum dry spike yield was observed in PL 10.
15. Yield contributing character like number of spikes per spike bearing branch vary significantly between the twenty accessions at seven month after planting. Even though the direct effect of this particular

character on dry spike yield was negative its indirect effect though fresh spike yield on dry spike yield was positive and highly significant.

16. Maximum number of spikes per spike bearing branch was observed in PL 3. The minimum spike number was observed in PL 11, PL 13, PL 15 and PL 22.
17. Leaf area of twenty accessions varies significantly. But this particular character did not contributed to dry spike yield. The maximum leaf area was observed in PL 13 and minimum leaf area was observed in PL 16 and PL 21.
18. All accession exhibited leaf colour of dark green except PL20, which was with light green coloured leaves.
19. Leaves on vegetative branch of all most all accession appeared in cordate shape. PL 8 was ovate laceolate in shape
20. Three vegetative characters wise number of vegetative branches per stem, number of spike bearing branches per stem and total number of leaves per hill was found to be correlated with dry spike yield. Out of these characters the total number of leaves per hill exhibited maximum correlation.
21. Yield contributing character like the number of spike per spike bearing branches, the length of spike, the girth of spike, fresh weight of spike, dry weight of spike and fresh yield of spike, found to be correlated with dry spike yield significantly and positively. The maximum correlation to dry spike yield was exhibited by fresh yield of spikes per plant.
22. The piperine content in spikes of twelve fairly flowered accessions varies significantly and the maximum piperine content was resulted in PL 11. The minimum piperine content was observed in PL 10.
23. The maximum essential oil content was resulted in Viswam followed by PL 5 and PL 21.
24. There was biochemical variation within the flowered twelve accessions with respect to the qualitative factors like phenol, alkaloids, terpenes, and flavaniod. Viswam and PL 5 were qualitatively superior. Even though the dry spike yield of the accessions PL 2, PL 20, PL 21, were

higher, qualitatively these accessions were medium. PL 8 was highly inferior with respect to qualitative character.

25. Viswam PL 5, PL 2, PL 20, PL 21, PL 8 and PL 3 were found to be promising types and further evaluation could be carried out to confirm their performance.

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Appendices

Appendix I

Laboratory equipments used for study

Silica Gel (G) TLC Plate

CAMAG TLC glass tank

Centrifuge

Spectrophotometer

Hot air Oven

Clevenger Apparatus

Area Meter

Electronic Weighing balance

UV betractor

Appendix II

Mean monthly parameters for crop growth

2006-2007

Month	Air temperature (^{0}C)		Total R.F (mm)	Rainy days	Total evaporation (mm)	Mean sunshine hrs.
	Max	Min				
September 2006	29.60	23.00	522.20	17.00	82.40	116.40
October	31.00	23.00	323.70	11.00	95.00	147.00
November	31.70	23.70	79.50	5.00	3.70	6.50
December	31.50	23.60	0.00	0.00	6.40	7.80
January 2007	32.50	22.00	0.00	0.00	196.00	268.50
February	34.00	22.20	0.00	0.00	1.00	275.50
March	36.00	24.40	0.00	0.00	173.3	254.40
April	35.00	25.00	61.00	4.00	188.50	230.00
May	32.80	24.60	240.50	10.00	163.00	6.60
June	30.00	23.50	826.50	23.00	4.20	105.50
July	28.40	22.60	1131.9	28.00	88.00	22.10
August	29.00	22.80	549.70	19.00	69.00	100.50
September	29.40	22.90	769.90	23.00	83.80	75.10

Appendix III

Lay out plan

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R1

PL 1	PL 18	PL 13	PL 10	PL 12	PL 9	PL 6	PL 5	PL 2	PL 17	PL 8	PL 22	PL 4	PL 11	PL 3	PL 21	PL 15	PL 16	PL 19	PL 20

PL 21	PL 22	PL 17	PL 1	PL 16	PL 19	PL 20	PL 12	PL 10	PL 8	PL 11	PL 15	PL 4	PL 6	PL 13	PL 9	PL 5	PL 2	PL 18	PL 3

R2

Abstract

EVALUATION OF ECOTYPES OF LONG PEPPER

(*Piper longum* L.)

By
RIYA JOSEPH

ABSTRACT OF THE THESIS

submitted in partial fulfillment of the requirementfor the degree of

MASTER OF SCIENCE IN HORTICULTURE

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ABSTRACT

Investigations on "Evaluation of ecotypes of long pepper (Piper *longum L.*) was undertaken at the Department of Plantation Crops and Spices at College of Horticulture, Vellanikkara during the period 2005-2007.The major objective of the study was to evaluate various ecotypes of *Piper longum* available at the Department of Plantation Crops and Spices for their variability in yield, morphological and biochemical characters so as to identify the superior types. Twenty accessions genetically catalogued and studied for their variability in vegetative, reproductive and biochemical characters and found that all the accessions varied with respect to these characters.

The vegetative, reproductive and biochemical characters were compared with the characters of the released variety; Viswam and the accessions, which performed on par with Viswam, were identified. Correlations of the various vegetative and reproductive characters were worked out with the dry spike yield and significant positive correlations were observed for nine characters like number of vegetative branches per stem, number of spike bearing branches per stem, total number of leaves per hill, number of spikes per spike bearing branch, length of spike, girth of spike, fresh weight of spike, dry weight of spike and fresh yield per plant Based on the above studies a few accessions which were statistically on par with the check variety Viswam could be identified. This preliminary evaluation gives only an indication and evaluation of the accessions has to be 3-4 years for obtaining confirmatory result.