HABITAT ANALYSIS AND DOMESTICATION STUDIES ON 'ORILATHAMARA' (*Nervilia aragoana* Gaud.)

By

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Habitat analysis and domestication studies on 'Orilathamara' (*Nervilia aragoana* Gaud.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Introduction

1. INTRODUCTION

'Orilathamara' (*Nervilia aragoana* Gaud.) is a less known, under utilized medicinal plant from the Orchidaceae family. The drug is used in the traditional systems of medicine for preparing various formulations, most of its requirement being met from wild sources. Known as "*Padmacarini*" in Sanskrit; "*Stalapadma*" and "*Sthalakamal*" in Hindi; "*Orilathamara*", "*Kalthamara*" and "*Nilathamara*" in Malayalam and "*Orilathamarai*" in Tamil, the plant is a terrestrial orchid with a single lotus like leaf. The underground fleshy tubers which are white in colour are the officinal part.

The drug is reported to be bitter, acrid, cooling, galactagogue, diuretic and tonic and is useful in uropathy, lithiasis, colic, agalactia, mental instability, epileptic fits, haemoptysis, diarrhoea, asthma, cough, vomiting and vitiated conditions of *pitha*. In Malaya, the decoction of leaves is used as a protective medicine after child birth. 'Orilathamara' (*Nervilia aragoana* Gaud.) is an inevitable constituent drug in many Ayurvedic preparations. Major Ayurvedic formulations containing 'Orilathamara' include *Matsyakshyadi kashaya, Vasthyamayanthaka ghritha, Sathavaryadi ghritha* and *Mahapaishachika ghritha.*

Botanical sources of this drug are highly controversial and Kerala physicians consider three species of *Nervilia* and *Habenaria diphylla* of Orchidaceae family as the source plants. The genus *Nervilia* is reported to be present in the forests of Western Ghats, in Kerala, Tamil Nadu and Maharashtra. In Kerala, *Nervilia aragoana* Gaud. has been located in Peechi, Parambikulam, Thenmala and Thekkady forests and in some sacred groves of Malabar.

At present, the entire requirement of the drug is met from wild sources. Because of over exploitation, the natural population is getting depleted day by day. Various workers have placed the genus *Nervilia* in the rare/endangered category.

The genus *Nervilia* has not been domesticated from the cultivation point of view. Under domestication outside the normal habitat or ecological range, many of the medicinal plants tend to behave differently. An understanding of the biological and ecological background of the species in their normal habitat is hence essential to understand their conservation biology as well as to predict their behavior under artificial cultivation.

Present investigation is an attempt to collect and describe the botanical sources of the drug and to study the response to domestication of the species. Outcome of the study is expected to elucidate some valuable information regarding the botanical identity of 'Orilathamara'. A positive response to domestication will not only help in the *in situ* conservation of this rare medicinal orchid, but also will facilitate its commercial cultivation, thereby ensuring the availability of genuine drug in sufficient quantity to the user industry.

These are the circumstances that led to the present study entitled "Habitat analysis and domestication studies on 'Orilathamara' (*Nervilia aragoana* Gaud.)". The study was taken up at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2009 – 2011 with the following objectives:

1. To collect the source plants of the drug 'Orilathamara' from wild.
2. To carry out detailed natural habitat analysis of *Nervilia aragoana* Gaud.
3. To understand the response of the species to domestication.

Review of Literature

2. REVIEW OF LITERATURE

"Orilathamara" (*Nervilia aragoana* Gaud.) is a less known under utilized medicinal plant from Orchidaceae family. It is used in the traditional systems of medicine for preparing various formulations, most of its requirements being met from wild sources. At present, the drug is not available in sufficient quantities for the user industry and it is categorized as rare or endangered. Researches conducted on "Orilathamara" is scarce. In this chapter, the available information on the genus *Nervilia* are presented along with the literature on other medicinal orchids and related medicinal species.

2.1 MEDICINAL ORCHIDS

Orchids are perennial herbaceous plants distributed throughout the world. They have a significant place as medicinal plants too. However, this fact is not popularly known. Medicinal orchids are those orchids which have medicinal value and are used in various systems of medicine. *Jeevakom* of Kerala, *Ondele thavare* of Karnataka, *Salaam Panja* of the Himalayas are only a few examples of this unique sub group called medicinal orchids. In fact there are an estimated 110 species of medicinal orchids in use from over 25,000 known species of the family (Udayan, 1999).

The early Greeks and Romans looked to the orchids more for their medicinal than for their aesthetic qualities. Orchids were used in Indian systems of medicine and in traditional Chinese medicine. They were mainly used as aphrodisiacs in ancient civilizations.

Medicinal orchids, like other orchids exhibit a variety of life forms. Majority are small herbaceous plants that continue their life cycle for many years. Several of these plants are epiphytic in nature, growing on other plants and trees,

while some others are terrestrial, growing on damp and moist ground. Interestingly, quite a few are saprophytes growing on dead and decaying organic matter, whereas the rest are climbers, seen clinging onto other support trees. The characteristic feature of epiphytic orchids is the presence of aerial roots. Even though orchids vary a lot in their vegetative forms, all the species can be grouped by their floral characteristics which are unique to the family and aid in their identification.

Medicinal orchids, like their fellow members of the family grow well in tropical climate though there are examples of a few of them growing under extreme conditions. Udayan (1999) observed that a humid climate, temperature around 27^0C and rainfall of 150-250 cm are ideal growing conditions for wild orchids.

2.1.1. History

The term 'orchid' was coined by Theophrastus as the anatomy of the plant resembles testicles. This may account for the use of orchids as aphrodisiacs in ancient civilizations. It is chiefly as a source of *salep* that orchids were therapeutically famous and it is still retained in the Pharmacopoeias of Austria, Belgium, Germany, Holland, Japan, Norway, Portugal, Russia and Switzerland. In India too it was considered as a royal food. *Salep* is the name given to the tubers or tuberous roots of various wild orchids, collected in the flowering season, immersed in boiling water for a short time and dried. The tubers are collected from wild plants growing in Asia Minor, South West Asia, France and Germany. From ancient times, *salep* has been considered to possess great invigorating virtues and has been extensively prescribed both in Europe and the East for recruiting the exhausted vitality of aged and enervated persons. Though its medieval and oriental reputation as an aphrodisiac has been irretrievably lost in the west, *salep* is still considered highly nutritive and as most useful article of diet for those who suffer from chronic diarrhoea (Britto and Senthilkumar, 2004).

2.1.2 Important Genera of Medicinal Orchids

The medicinal orchids of the world belong to 38 distinct genera. According to Caius (1936), they include *Acampe* (Indo- Malaya, Africa); *Aceras* (Mediterranean); *Anacamptis* (Europe, North Africa); *Cymbidium* (Tropical Asia, Africa, Australia); *Cypripedium* (Mexico); *Dendrobium* (Tropical Asia, Africa, Japan, Australia); *Epidendrum* (Tropical America); *Eulophia* (Africa); *Habenaria* (Whole world); *Microstylis* (Cosmopolitan, chiefly Tropical); *Orchis* (Mediterranean, Temperate Europe, Asia, North America, North Africa); *Rhynchostylis* (Indo-Malaya); *Vanda* (Asia, Australia) and *Zeuxine* (Tropical Asia, Africa).

Medicinal orchids of India belong to 15 distinct genera. They include *Acampe, Cymbidium, Dendrobium, Eulophia nuda, Habenaria, Hataeria, Luisia, Oberonia, Orchis, Rhynchostylis, Saccolabium, Vanda, Vanilla, Zeuxine* etc.

2.1.3. Geographical Distribution

In terms of geographical distribution, medicinal orchids are found from the humid tropics to the frozen areas of Alaska, including the snow covered areas of the Himalayas and the sandy deserts of Australia and Africa.

2.1.3.1. Medicinal orchids in the Himalayas

The distribution of orchids in the Himalayan region is of great interest especially the rich orchid belt of the Eastern Himalaya, particularly of the Sikkim - Darjeeling region. Pandey *et al*. (2003) reported that in the Western Himalaya, except in the lower temperate, subtropical belts of Eastern Kumaon, epiphytic orchids are hardly of any significance and further west, terrestrial orchids are common in the forest undergrowth of temperate zone.

Medicinal orchids of Kumaon and Garhwal Himalaya include *Dactylorhiza hatagirea, Eulophia nuda, Cymbidium aloifolium, Saccolabium chrysanthum, Malaxis acuminata, Habenaria commelinifolia, Pholidota articulata, Plathera susanne, Pogonia gammiena, Dendrobium fimbriatum, Herminium monophyllum, Zeuxine strateumatica* etc.

2.1.3.2. Medicinal orchids of Eastern Ghats

Eastern Ghats of Tamil Nadu is a potential site of orchids having luxuriant and gregarious medicinal orchid vegetation in different areas. Medicinal orchids seen in this area include *Acampe praemossa, Anoectochilies elatus, Bulbophyllum fischeri, Calanthe triplicata, Cymbidium aloifolium, Eulophia epidendraea, Geodorum densiflorum, Seidenfia rheedii, Pholidota pallida, Rhynchostylis retusa, Satyrium nepalense, Vanda tessellata, Dendrobium nanum, Nervilia aragoana* etc (Xavier and Senthilkumar, 2007).

2.1.3.3. Medicinal orchids of Western Ghats

Medicinal orchids of Western Ghats include *Seidenfia rheedii, Nervilia aragoana, Vanda tessellata, Vanilla walkeria, Dendrobium nanum, Cymbidium aloifolium* etc. Some valuable medicinal orchids have also been reported from Kerala forests. Unnikrishnan (1993) recorded the presence of *Seidenfia rheedii* in the sacred grooves of Malabar and Miniraj and Nybe (1999) recorded its occurrence in the Peechi, Silent Valley and Parambikulam forests. Four species of *Nervilia* have been reported from Kerala forests by Sasidharan (2006). They are *Nervilia aragoana, Nervilia crociformis, Nervilia infundibulifolia* and *Nervilia plicata*. Joseph and Mukkattu (2007) have reported two species of *Nervilia* viz., *Nervilia aragoana* and *Nervilia plicata* from the forests of Kerala. The former was found growing in the forested area as well as non forested areas of the state while the latter was a typical species of deep forests.

2.1.4. Orchids in Indian Systems of Medicine

In India, orchids are employed for a variety of medicinal uses in traditional systems of medicine like Ayurveda, Siddha and Unani.

2.1.4.1 Orchids in Ayurveda

'*Ashtavarga*' listed by Bhavamisra is a group of eight tubers or condensed stems obtained from Orchidaceae and Liliaecae families (Dey, 1998). These are well known for their nutritive and tonic properties. Ashtavarga include *Riddhi, Vriddhi, Kakoli, Kshirakakoli, Meda, Mahameda, Jeevaka and Rishabaka*; out of which *Riddhi Vriddhi, Jeevaka and Rishabaka* are medicinal orchids. They are reported to be cooling, spermatopoetic and nourishing as reported by Singh (2006). They also promote lactation and conception. Singh and Duggal (2009) have published the orchid representation in *Ashtavarga* drugs and it is presented in table.1.

Table 1.Orchid representation in *Ashtavarga*

Ayurvedic name	**Botanical name**	**Family**	**Part used**
Jeevaka	*Seidenfia rheedii*	Orchidaceae	bulb
Rishabhaka	*Seidenfia rheedii*	Orchidaceae	pseudo bulb
Riddhi	*Habenaria edgeworthii*	Orchidaceae	root
Vriddhi	*Habenaria latilabris*	Orchidaceae	root

Kerala physicians generally consider *ashtavarga* drugs as '*abhavadravya*' (unobtainable). They are either deleted or substituted with other permitted drugs. These are considered as rare drugs which have not been satisfactorily identified. Some of the ayurvedic preparations containing *ashtavarga* are *Dhanwantharam*

kashayam, Dhanwantharam kuzhambu, Ashtavargam kashayam etc (Sivarajan and Balachandran, 1997).

2.1.4.2. Orchids in Unani

Unani doctors credited the tubers of *Eulophia campestris* with tonic, aphrodisiac and astringent properties. They considered it useful in stomatitis, cough and paralytic conditions. *Vanda tessellata* is considered as laxative and tonic to the liver and the brain. It is used to cure bronchitis, piles, lumbago, toothache and boils. It also lessens inflammation and heals fractures (Singh and Duggal, 2009).

2.1.4.3. Orchids in ethno medicine

As most of the medicinal orchids are generally wild, the tribal people are more knowledgeable about their medicinal uses. Udayan (1999) has observed the use of *Eulophia cullenii* as antidote against spider bite and other poisonous bites among the Kani tribes of Kerala, India.

The order is remarkable for the absence of poisonous properties, however two species are considered toxic, as reported by Caius (1936). The natives of Portuguese believed that a liquid extract of *Monadenium lugardae* was poisonous and caused vomiting and the leaves and stem of the Malayan *Vanilla griffithii* contained irritating latex.

He has also reported the use of four orchids in the treatment of various forms of insanity as described below. Natives in the Mpike district of Northern Rhodesia administered an infusion of leaves and stems of *Ansellia humilis* as a remedy for madness, American Indians gave a decoction of the Swamp orchid, *Simodorum giganteum* to combat manias; on the Malabar coast, the powdered

flowers of *Vanda spathulata* were said to abate frenzy; and in France the fruit of *Vanilla planifolia* was prescribed in order to overcome melancholia.

2.1.5. *Materia Medica*

A few medicinal orchids have been investigated therapeutically.

Dactylorhiza hatagirea (*Salaam panja*) is a terrestrial orchid with fleshy tuberous roots. Tubers are slightly flattened, palmately lobed. It contains mucilage, starch, loroglossin, albumen, volatile oil and ash. Khory (1982) reported it to be aphrodisiac, expectorant and nervine tonic and used for treating diabetes, diarrhoea, dysentery, paralysis, impotence and malnutrition.

Orchis latilfolia (oriental salep) is a terrestrial orchid with fleshy tuberous roots. It contains mucilage. It is reported to have astringent and expectorant properties. The plant is used for treating diarrhea, bronchitis etc. Part used is bulbs (Khory, 1982).

Stuart (1984) found that *Arundina graminifolia* (bamboo orchid) a terrestrial orchid by habit, contained arundinol, stilbenoid and arundinan. Officinal part is rhizome and it is reported to be antibacterial.

Eulophia nuda (salep orchid) is known as mankand in Ayurveda. It is distributed in Himalayas. The tubers are conical, surrounded with circular marks. Officinal part is tuber. Bhandari (1985) reported that nudol which is extracted from the tubers is demulcent and anthelminthic.

Dan and Doan (1989) isolated dendrobine from *Dendrobium nobile*. It is used as sialogue, stomachic and tonic. It is used against tuberculosis, general debility, flatulence, dyspepsia, fever and anorexia.

Warrier *et al.* (1995) have reported *Seidenfia rheedii* to be sweetish, refrigerant, aphrodisiac and febrifuge and also tonic in properties. It is used for the treatment of fever, seminal weakness, burning sensation, emaciation, tuberculosis and general debility.

Aerides multiflorum distributed in Taiwan is reported to contain glycoside kinsenoside and polysaccharides. Part used is tuber and is reported to have anticancer property. Tuber is used in treatment of hepatitis; hypertension and cancer. It also acts as an antioxidant, hepatoprotective and immuno-modulating (Ghanaksh and Kaushik, 1999).

Bletilla striata (urn orchid) is a deciduous terrestrial orchid. The pseudo bulbs contain polysaccharide. It is considered as antibacterial, anti inflammatory, antiphlogistic and demulcent and also used for treating internal hemorrhage (Zheng *et al.*, 2000). Vaidya and Dhumal (2000) have reported *Malaxis rheedii* for its use for ulcer, healing of wounds, as antiseptic, in amoebic colitis, viper bite and dysentery.

Vanda tessellata is an epiphytic orchid with leafy stem. It contains alkaloid, glucoside, bitter principle, tannins, resin, saponin, sitosterols and colouring matter. Ahmed (2001) reported that it is used as aphrodisiac, analgesic and nervine tonic. Whole plant is officinal.

Fossen and Ovstedal (2003) found that *Cypripedium calceolus* (lady's slipper orchid) contained the active ingredient cyprepedin. Officinal part is root. It is reported to be antispasmodic, diaphoretic and hypnotic, nervine, sedative and tonic and used for treating diabetes, diarrhoea, dysentery, paralysis, impotence and malnutrition.

Habenaria edgeworthii, is a fine flowering terrestrial orchid with tuberous roots covered with hairs. It has been proved to promote strength and alleviate all

the three doshas. It is reported to be spermatopoetic, sweet and heavy. *Habenaria latilabris* (Vriddhi), another fine flowering terrestrial orchid with tuberous roots covered with hairs, is reported to help in conception. It is cooling and aphrodisiac. It cures bronchitis (Singh, 2006).

2.1.6. Phytochemicals from Medicinal Orchids

Medicinal orchids are rich in alkaloids, flavanoids, glycosides, carbohydrates and other phytochemical contents.

A large number of alkaloids have been isolated from orchids. The first one was dendrobine isolated from *Dendrobium nobile* in the year 1932. Most of the orchid alkaloids are either pyrrolizine or dendrobine based structures. According to Britto and Senthilkumar (2004), the genus *Dendrobium* contains maximum number of alkaloid rich species. They also reported that the flavanoids in orchids are flavones, flavonols and xanthones.

Anthocyanins which range from yellow to red and magenta in colour play a major role in orchid flower pigmentation. It is present in all the plant organs in the flowers and roots. For their chemical complexity, structural variability, physiological stability, wide spread distribution and comparative ease of isolation and identification, these serve as good chemotaxonomic characters and act as a medicine also (Britto and Senthilkumar, 2004).

Britto and Senthilkumar (2004) have also reported polysaccharides and oligosaccharides from various orchids. Sugars have been separated by TLC technique from orchid nectars. The pseudo bulbs of *Microstylis wallichi*, an Indian drug used in *Ashtavarga* contains sugars besides basic compounds. Several orchids are glycosidal plants. Loroglossin glycoside is an example. *Paphiopedilum javanicum* has saponine glycoside. The vanilla glycosides and related aromatic compounds are found in several species of *Vanilla*.

Miniraj *et al.* (2007) quantified total soluble sugars, phenols, amino acids, proteins and starch in the domesticated crop of *Seidenfia rheedii*. Presence of hexosamine/amino sugars or glycosides was also reported by them in the pseudobulbils of *Seidenfia rheedii*.

2.1.6.1. Phytochemistry and pharmacology of Nervilia species

In'Orilathamara', tuber is considered as the officinal part. Leaves are also used as medicine. The plant is reported to be bitter, acrid, cooling, galactagogue, diuretic and tonic and is used in uropathy, lithiasis, colic, agalactia, mental instability, epileptic fits, haemoptysis, diarrhoea, asthma, cough, vomiting and vitiated conditions of *pitha*.

Rastogi and Mehrotra (1999) have reported phytol, cyclo leucallenol, stigmasterol, linoleic acid, linolenic acid, L-norieucine and glycerine esters in *Nervilia aragoana* Gaud.

In Malaya, the decoction of leaves is used as a protective medicine after child birth (Prajapati and Kumar, 2003).

Hua *et al.* (2006) have isolated five compounds from the petroleum ether extract of leaves of *Nervilia fordii* by silica gel column and recrystallization. Compounds identified through spectral analysis were lupine, 2, 3 lanostene-3-alchohol, octacosamoic acid, hexo decanoic acid etc.

Flavonoids, mineral elements like S, Ca, Mg, Fe, Cl, P, Na and amino acids like alanine, valine, isoleucine, aspartic acid etc were reported from *Habenaria grandifloriformis* and *Nervilia aragoana* by Bhogaonkar and Devarkar (2007). Zhong *et al.* (2007) confirmed the anticancer effect *in vivo* of active fraction from *Nervilia fordii*.

Studies were conducted to compare the antioxidant capacity on *Nervilia plicata* and *Nervilia aragoana* species gathered in October in Taiwan by Chen (2008). The results showed that the phenolic acid content of leaves of *Nervilia plicata* in October was 36.5 micro g/mg, which was better than 25.2 micro g/mg for *Nervilia aragoana*. The scavenging effect of superoxide anions (IC_{50}) was 0.30 and 0.36 micro g/ml, respectively. The inhibition of xanthine oxidase was 29 and 33.6%, respectively. The scavenging effects of OH free radical ability of *Nervilia plicata* were 2.1 folds than those of *Nervilia aragoana* and also six- folds than mannitol.

Reddy *et al*. (2010) reported that ethyl acetate extract of the whole plant of *Nervilia aragoana* Gaud. (Orchidaceae) and ethanol extract of the leaves of *Atlantia monophylla* Linn. (Rutaceae) have antifungal and antioxidant activities. At 5mg/ml concentration of the extracts, the former exhibited more inhibitory activity than the latter, against fungi. The order of MIC values for *Nervilia aragoana* were *Saccharomyces cerevisiae* (1.4mg/ml) >*Aspergillus niger* (1.2 mg/ml) > *Aspergillus fumigates* (0.95mg/ml) > *Cryptococcus neoformans* (0.75 mg/ml).

2.1.7. Botanical identity of Medicinal Orchids

Most of the medicinal orchids, especially the *Ashtavarga* drugs remain unidentified or not properly identified even now. *Ashtavarga* has been assigned various medicinal properties by ancient *Materia Medica* dealing with Ayurveda and is important ingredient of various classical Ayurvedic formulations like chavyanprasha. *Ashtavarga* is subject of rigorous botanical research. Although work has been done on identification of medicinal plants mentioned under *ashtavarga*, still lot is to be done to identify the true representatives. Taking into consideration the medicinal properties of these plants, Singh (2006) opined that

phytochemical and pharmacological investigations are the need of the hour to scientifically validate the claims.

In the study conducted by Miniraj *et al*. (2007) to ascertain the correct botanical identity of the drug *Jeevaka* and *Rishabhaka,* two *ashtavarga* orchids, it was ascertained that *Seidenfia rheedii* Sw. was the source plant for both the drugs and in Kerala, larger bulbils were used as *Jeevaka* (*Jeevakom* in vernacular) and smaller ones as *Rishabhaka* (*Edavakom* in vernacular). Information gathered from tribal herb gatherers also endorsed this view. What is marketed in Kerala as *Jeevakom* and *Edavakom* from Punjab was also the same species, *Seidenfia rheedi* Sw.

2.1.7.1. Botanical identity of Nervilia

Botanical source of this drug is highly controversial and Kerala physicians consider three species of *Nervilia* and *Habenaria diphylla* of Orchidaceae family as the source plants. *Hybanthus enneaspermus* and *Habenaria grandiflora* are also considered as source plants (Sivarajan and Balachandran, 1997).

2.1.8. Threat Status of Medicinal Orchids

The added virtue of medicinal uses of the orchids in fact has made them vulnerable to harvest. Indiscriminate harvest, endemism, illegal trade, deforestation and change in climate have already driven many to go extinct while the survival of many is threatened.

Udayan (1999) has given the threat status of some medicinal orchids as given below.

Table 2. Threat status of some medicinal orchids

Botanical name	**Distribution**	**Status**
Eulophia cullenii	South Kerala	Critically endangered
Eulophia ramentacea	Konkan, Mysore	Critically endangered
Seidenfia rheedii	Himalayas,Nilgiris, Kerala	Vulnerable
Nervilia aragoana	Maharashtra, Kerala	Endangered regionally

He placed *Eulophia cullenii* and *Eulophia ramentaceae* in the critically endangered category, *Seidenfia rheedii* in the vulnerable category and *Nervilia aragoana* as endangered regionally.

Sasidharan (1997) reported that the much needed medicinal plant *Nervilia aragoana* was found restricted in distribution in Kerala forests.

Genus *Nervilia* is listed in the rare/endangered category of medicinal plants by many workers (Vogel *et al.*, 2002). Miniraj (1997) observed that *Nervilia aragoana* was a delicate ground orchid which suffered from the habitat changes without being able to tide over it.

Pandey *et al.* (2003) listed some important medicinal orchids like *Habenaria latilabris, Pholidota articulata, Pogonia gammiena, Eulophia nuda* and *Vanda tessellata* whose natural populations were in threat because of exploitation by human beings for their benefits.

Lin and Yeh (2008) reported that the natural population of *Nervilia plicata* has decreased rapidly due to over harvesting.

Convention on International Trade in Endangered Species of Fauna and Flora (CITES) have enlisted orchids like *Dendrobium cruentum, Paphiopedilum* sp, *Vanda coerulla* and *Phragmipedium* spp in its Appendix I which include the most endangered animals and plants. In Appendix II, they have listed species that are not necessarily now threatened with extinction but that may become so unless trade is closely controlled. Kandavel *et al.* (2004) have reported that the whole Orchidaceae family is placed in the Appendix II.

Miniraj *et al.* (2007) have stated that because of several factors, anthropogenic and otherwise, the natural sources of *Seidenfia rheedii* have been getting depleted day by day and placed it in the rare category.

Xavier and Senthilkumar (2007) have opined that the disappearance of orchids was due to lack of proper documentation of economic potential and biological potential of orchids, besides unawareness of their potential among local inhabitants.

Over exploitation of natural resources by mankind through habitat destruction and frequent occurrence of natural calamities which caused ecological changes in natural habitats were listed as major reasons responsible for depletion of orchid diversity by Satyanarayana (2010).

Performance of *Jeevakom (Seidenfia rheedii)* a medicinal orchid, under the changed rainfall regimes in central Kerala was studied by Miniraj *et al.* (2009). From the rainfall pattern observed over a five year period, it was inferred that an evenly distributed summer showers, followed by timely and good south west monsoon with lesser dry spells, again followed by a timely and good north east monsoon were ideal for the regeneration and growth of *Jeevakom*. The spatial and temporal distribution of rainfall and not the total rainfall was critical for this highly rainfall dependent species.

2.2. NATURAL HABITAT STUDIES ON MEDICINAL PLANTS

Most of the medicinal plants are collected from the wild. Many of the wild medicinal and aromatic plants are highly habitat specific, found only in forest and occupy highly specialized ecological niches with restricted distribution (Pushpangadhan, 1992). Assessment of the habitat status of the species requires a detailed investigation of the ecosystem. Habitat study forms an integral part of domestication experiments.

A study conducted by Miniraj and Nybe (1998) have brought out three distinctly different environments *viz*., optimal, degrading and suppressive environments for *Piper longum* in the moist deciduous forest of Peechi hills, the main contributing components being quantum of radiation flux and herb cover. Suppressive environment suppressed growth and quality and exerted an extinction pressure while degrading environment lead to reduced quality and modified the quality components. Optimal environment ensured maximum quality and ensured genetic conservation. The study indicated the pathway of genetic and environmental conservation in a herbaceous creeper like *Piper longum* as low light medium herb cover, non flowering and vegetative propagation as ensured by low radiation flux and medium herb cover.

Detailed natural habitat studies were carried out in select medicinal plants viz., *Piper longum, Naravelia zeylanica, Sida rhombifolia ssp retusa, Desmodium velutinum, Baliospermum solanifolium and Barleria prattensis* by Miniraj and Nybe (1998). Natural habitat characteristics of these species have been described by them. They observed that all these species were abundant in the moist deciduous forest of Peechi hills at altitude from 180 to 500 meters above MSL.

In another study carried out by Miniraj *et al*. (2007) on *Seidenfia rheedii*, the habitat characteristics have been described. *Seidenfia* was a terrestrial orchid which was found to grow on specialized niches. In all the natural habitats, the

plant was found to grow on extensive spread out rock formations in the openings of evergreen forests. The shade level was approximately 50 per cent. The only exception was Silent Valley forests where the species was found growing in open grass land over scattered wet rocks which received 100 per cent sunlight. In Peechi forest, at all *Jeevakom* habitats, the species colonized on the decomposed organic matter on wet rocks admist moss and grass. Though inside evergreen forest, the plant was abundant in the openings which received at least 50 per cent sunlight. At all locations, the species behaved as typical lithophyte and the natural habitat was described as dripping rock ecosystem.

Ganapathy (2003) studied the variability in St. John'swort (*Hypericum* sp.) in the Western Ghats of Kerala. Habitat characteristics of *Hypericum japonicum* and *Hypericum mysorense* were recorded during his study.

2.3. DOMESTICATION STUDIES ON MEDICINAL PLANTS

Medicinal and aromatic plants in general have very short history of cultivation and artificial selection. History of systematic cultivation of medicinal plants in India is relatively very recent. Experimental cultivation of some of the exotic plants was started in India as early as the beginning of the 18th century. In the government sector, agro-technology of 40 odd species were developed by the ICAR- agricultural system and CSIR. But most of them were aromatic plants used by the modern pharmaceuticals industry. No systematic agro-technology has been developed for the 400 odd species used by the traditional pharmaceutical industry. Much of the propagation and cultivation trials were concentrated in a few crops like *Rauvolfia, Catharanthus, Solanum, Cannabis, Dioscorea, Ocimum, Costus, Opium* and *Senna*.

Cultivation trials were carried out by Karnick (1977) in *Hemidesmus*; Rajagopalan (1983) in *Kaempferia galanga*; Soldati and Tanaka (1984) in *Panax ginseng*; Nayar (1992) in *Holostemma* and *Indigofera*; Meera (1994) in

Holostemma; Menon (1994) in *Plumbago* and Menon (1996) in '*Njavara*'- a medicinal rice.

Propagation studies were conducted in plants like *Alstonia venenata, Coscinium fenestratum, Habenaria latilabris, Rotula aquatic* and *Woodfordia fruticosa*. The natural method of propagation was through seeds in *Alstonia venenata* and seeds and root suckers in *Coscinium fenestratum*. Seeds were the normal propagules in *Rotula aquatica* and *Woodfordia fruticosa* (Kritikar and Basu, 1935). *Habenaria* naturally multiplied through pseudo bulbs.

Nair *et al*. (1992) suggested that depending upon the natural habitats of the plant, various zones at different altitudes should be selected for the cultivation of a particular drug. They stressed the importance of factors like soil, temperature, irrigation and manuring while selecting a drug for cultivation.

Under domestication outside the normal habitat or ecological range, many of the medicinal plants tend to behave differently. In some cases, it becomes difficult to grow them or it may not even survive. In certain other cases, they survive and grow but may not be providing the desired results. Attempts to domesticate the plant *Trichopus zeylanicus* sub sp *travancorensis* through mass multiplication, by tissue culture showed that the tissue culture plants when planted out and analyzed, differed in their properties from those found in the wild (Anon., 1994).

Only less than 20 species of medicinal plants are under commercial cultivation, while over 400 species are used in production by the medicinal plant based industry (Shankar *et al*., 1997).

A study conducted by Miniraj and Nybe (1998) on plant environment interactions in a moist deciduous forest ecosystem in *Piper longum* showed that against the sparse branching, non flowering and creeping habit in the forest, the

plant manifested profuse branching, flowering and erect habit in the domestic environment.

Miniraj and Nybe (1999) conducted domestication studies on select species of medicinal plants like *Piper longum, Naravelia zeylanica, Sida rhombifolia* ssp *retusa, Desmodium velutinum, Baliospermum solanifolium* and *Barleria prattensis* and all the species responded well to domestication. Comparative evaluation of the dry matter production and root yield per plant revealed that the yield was high in all species, except in *Naravelia zeylanica* in the domesticated environment. *Naravelia zeylanica* recorded higher yield in the wild environment.

Positive response of *Seidenfia rheedii* to domestication was reported by Miniraj *et al*. (2007). Both the growth and yield parameters were high in the domestic crop compared to wild plants. Complete domestication /cultivation package were formulated in this species. Pseudo bulbils weighing one gram were the ideal propagule. 50–75 per cent shade level was ideal for cultivation. Growing media containing sand, soil, farmyard manure and leaf compost in equal proportions was the best for maximum growth and yield.

A participatory experiment on domestication of select species of medicinal plants was taken up at Kerala Agricultural University to assess the impact of domestication on their yield and quality and to empower the tribal herb gatherers in the cultivation of medicinal plants. The select species included *Rauvolfia serpentina* and *Pseudarthria visida*. The species were grown under two shade levels: 25 and 50 per cent shade. It emerged from the study that the yield of medicinal part was maximum under 25 per cent shade in *Pseudarthria visida* where as 50 per cent shade was ideal for *Rauvolfia serpentina* in the domestication trial (Miniraj *et al*., 2010).

2.3.1. Growing Media Studies in Orchids

The growing media, nutrition and shade level play a vital role in the cultivation of orchids. Successful cultivation of orchids depends upon selection of an ideal medium with optimal nutrient concentration and shade.

Potting material that can be used for growing orchids include tree bark, osmund fiber, tree fern fiber, block or chunk, charcoal, brick or stone chips, sphagnum moss, polystyrene granules, coconut husk, rock wool, perlite, pumice, vermiculite, peanut, shells, poultry or horse or cowdung manure, Styrofoam leaf mould, top soil, sand, river singles, loam, beech or oak or schima, Wallichi leaves, compost, absorba stone, absorbalite and many others.

The materials used as growing media for epiphytic orchids are entirely different from that used for other plants. An ideal growing media should preferably be inert, porous and resistant to organic decomposition. It should be cheap and easily available (Bose and Bhattacharjee, 1980).

A study conducted by Sudhadevi (1992) showed that pseudo bulbs of *Habenaria latilabris*, remained dormant under the soil for six months and then sprouted into a whole plant. Among the different media tried, potting mixture containing sand, soil and powdered cow dung in the ratio 2:1:1 was identified as the medium best for this plant.

Growing medium that was found best for *Seidenfia rheedii*, a medicinal orchid included sand, soil, well rotten FYM and vermicompost in equal proportions (Miniraj *et al*., 2007).

Coconut fibre is gaining popularity as very good substrate for orchids. Leaf mould is a good choice for terrestrial orchids, it releases lot of nutrients as it

decompose and high in water retention capacity. Cow dung manure is mostly used in Asian countries for growing orchids.

Bose and Bhattacharjee (2008) observed that a combination of equal parts of shredded tree fibre, leaf mould, sandy loam soil and white sand were recommended for good growth and flowering of *Microstylis*.

Conductivity and pH of media were suggested to be very important for salt susceptible plants like orchids. Potting media used for proper establishment and multiplication of orchids at National Orchidarium and Experiment garden Yercaud, for terrestrial orchids included a mixture of two parts of loam and one part of leaf mould and one part of a mixture of equal amount of brick pieces and charcoal (Satyanarayana, 2010).

2.3.1.1. Effect of organic manures in other medicinal species

Application of organic manures to soil, apart from improving the physical properties, improves the availability of nutrients, organic carbon, cation exchange capacity of the soil and increases the yield of the crop. According to Raychaudhuri (1977), incorporation of organic manures at different levels improves the soil physical properties.

Continuous application of coir pith compost resulted in reduction of soil pH. This was probably caused by production of carbon dioxide and organic acid during decomposition of organic matter (Nambiar *et al*., 1978).

Subramanian (1980) found that soil moisture content was higher over control by 1.72 per cent with incorporation of coir pith. The bulk density of the soil progressively decreased with increase in coir pith from 5 to 20 t/ha (Ramaswami and Ramalu, 1983).

Sadanandan *et al.* 1998 obtained the improvement of soil physical properties particularly decreased bulk density by applying FYM, neem cake, leaf compost and vermicompost in six years old black pepper. Application of organic manures, FYM, neem cake, leaf compost and vermicompost resulted in increased availability of nitrogen, phosphorus, potassium and micro nutrients.

Organic manures have been reported to improve the soil physical, chemical and biological properties and also conserved and improved the moisture holding capacity of the soil and resulted in enhanced productivity and quality of East Indian galangal (Maheswarappa *et al*., 1999).

FYM owing to its surplus nutritive content enhances beneficial soil micro flora and it increases plant growth. Since it is cost effective, it can be recommended as best organic manure for medicinal plants.

In galangal, combined application of FYM and vermicompost recorded the highest number of tillers, number of leaves and leaf area index at all stages of growth (Maheswarappa *et al*., 1999).

Rao *et al*. (2003) reported that application of 20t of FYM increased the herbage yield in Bhrami by 18.42 per cent over the control. They also reported that the application of 10 t of FYM could replace 50 per cent of the inorganic fertilizers.

Krishnamurthi *et al*. (2002) obtained 22 per cent increase in yield of turmeric mainly due to application of 10 t of FYM/ha over the unamended control.

The number of tubers, fresh and dry tuber yield and steroidal sapogenin content of safed musli (*Chlorophytum borivilianum*) were significantly increased due to application of FYM at 10 t/ha and vermicompost at 5 t/ha. However no

significant difference in yield was noticed due to FYM and vermi compost application (Patrude *et al.*, 2002).

Krishnamurthi *et al.* (2002) stated that application of coir pith compost recorded higher fresh rhizome yield (16.1 t/ha) in turmeric over the unamended control.

2.3.2. Shade Requirement Studies in Orchids

Duration and intensity of light are important for orchid production. In the natural stands of terrestrials like *Liparis lilifolia, Habenaria clavellat* and *Isotria medeoloides*, light was observed to be a critical factor with respect to flowering and seed production as compared to vegetative growth (Stuckey, 1967).

The light environment of plants as a source of energy has been observed to act in the four dimensions - quality, quantity, direction and periodicity (Harkers and Hemano, 1988).

Plants of *Vanda spathulata* and *Bulbophyllum nilghrrense* naturally adapted to shade conditions have smaller leaf areas, thinner leaves, cuticles and palisade layers and lower concentration of total starch, soluble sugars, protein and amino acid and lipids than that naturally adapted to sunny conditions. Net photosynthesis became saturated at lower light intensities in shade than in sun plants (Radha *et al.*, 1994).

George and Mohanakumaran (1999) reported an increase in plant height, number of leaves and leaf area of *Arachis* orchid c.v Red ribbon at 50 per cent shade level.

In a comparison between open condition and 50 per cent shade level in domestication studies on *Seidenfia rheedii*, crop growth was better under 50 per

cent shade. Shade crop had lengthier roots and larger pseudo bulbils. Single plant yield as indicated by the dry weight of pseudo bulbils was also highest under 50 per cent shade (Miniraj *et al*., 2007).

The best clue about the light requirement of an orchid can be obtained from the knowledge of where it grows and under what situations it grows. More light is not a problem in most of the cases, except the accidental or sudden exposure to the higher intensity of light. However insufficient light seems to be a common problem (Bhattacharjee and Das, 2008).

2.3.3. Impact of Domestication on Medicinal Properties

Environment is the dynamic product of the sum total of interactions of multiple components grouped under soil, weather and flora interacting and modifying among them. Even marginal variations in any one of the components alter the rate and extent of interactions and leads to corresponding changes in the pattern of development in organisms like plants resulting in the structural and functional variability (Nayar, 1992).

Unlike crop plants, the quality and quantity are equally important in medicinal plants. The active principle in these plants are certain secondary metabolites like alkaloids, glycosides, coumarins or steroids which are related with the ecology rather than the normal physiology of the plant. The environmental conditions to which the plant is exposed influence the production of these secondary metabolites and ultimately the efficacy of the drug. In the case of aromatic plants, volatile oil known as essential oils containing different compounds of terpenes or phenols are the active principles. These secondary metabolites are however, not directly involved in the normal growth and reproduction of these plants. These are produced by plants as a biochemical adaptation to prevent illness or as a defense against predators or adaptation to live

in association with other plant or animal communities in the particular ecological, edaphic or climatic niche (Pushpangadan, 1992).

The biosynthesis of these compounds is controlled genetically and the heritability and expression of which are greatly affected by the abiotic and environmental factors. Great emphasis must be given on time, source of collection, preservation, drying and storage of plants as these play an important role for maintaining the efficacy of the drug. Though scarce, there are experimental evidences to strengthen the fact that the secondary metabolite production and the properties of the medicinal plants differ with change in habitat. More simply it is the habit –habitat interactions that decide the quality. So, any improvement method or management practice should be designed in such a way that it is not at the expense of its quality.

Biswas (1955) observed that there was no significant difference in the alkaloid content of roots of *Rauvolfia serpentina* under irrigated agriculture and forestry conditions. Sulochana (1959) noticed that the alkaloid content varied considerably in different geographical regions.

In *Datura stramonium*, Chandrasekharan *et al.* (1984) reported that the scopolamine content of cultivated plants were twice as that of wild samples and fluctuated between 0.126 and 0.309 per cent.

Narayanan (1993) observed variation in the root alkaloid content ranging from 1.38 to 2.05 per cent in *Rauvolfia serpentina* from nine geographical locations of Kerala. He opined that the alkaloid content was a highly complex phenomenon involving environmental factors, species difference and their interaction.

Protein and alkaloid content of *Holostemma annulare* was compared under domestication and wild conditions by Samuel *et al.* (1993). They found that the

protein and alkaloid contents were high in the tubers obtained from domesticated plots when compared to the market samples.

Study conducted by Miniraj and Nybe (1999) on phytochemical evaluation of *Desmodium velutinum* confirmed the presence of nine alkaloids and one terpenoid in both the wild and domesticated crop.

Biochemical characterization of select medicinal plants in the wild and domestic environment was attempted by Miniraj *et al.* (2000). Plants like *Piper longum, Naravelia, Sida, Desmodium, Baliospermum* and *Barleria* were evaluated for the various phytochemicals. Results of the soxhlet extraction with petroleum ether showed that in the cases of *Desmodiun, Baliospermum, Naravelia* and *Piper,* wild samples gave a higher percentage of crude extractables. Reverse trend was observed in *Sida* and *Barleria*. In *Sida, Piper* and *Barleria,* total soluble sugar concentration was high in the domesticated plants. Also high content of total free amino acids were observed in domestic plants.

In the domestication study on *Jeevakom* (*Seidenfia rheedii*), pseudo bulbils from the wild as well as domestic environment were analyzed for selected phytochemical constituents. Higher values of starch, protein and total free amino acids were recorded in the domestic crop compared to the wild (Miniraj *et al.*, 2007).

In the same medicinal orchid, further studies revealed that the wild and domestic pseudo bulbils had the same sugar as well as amino acid profile without much variation in their concentration (Miniraj, 2011). Pharmacological studies carried out in mice brought out the hepatoprotective, antiallergic and immunopotentiating effects of the drug and these properties were expressed in the same magnitude by the wild and domestic samples.

Materials and Methods

3. MATERIALS AND METHODS

The investigations on "Habitat analysis and domestication studies on 'Orilathamara' (*Nervilia aragoana* Gaud.)" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur during 2009–2011. The experimental material and methodology adopted are presented in this chapter. The whole programme was divided into four sets of experiments as described here.

3.1. EXPT. I. EXPLORATION AND COLLECTION OF SOURCE PLANTS OF DRUG 'ORILATHAMARA' FROM FOREST

Secondary data on occurrence and distribution of *Nervilia aragoana* in Kerala was collected. Published floras of the forests of Kerala were scanned and distribution of *Nervilia* genus found out. Based on the results, Thamaravellachal area of Peechi – Vazhani wild life sanctuary was selected for further studies. Tribal herb gatherers in the Thamaravellachal tribal settlement were identified and information regarding the occurrence of *Nervilia aragoana* were collected from them. Forest explorations were carried out along with the tribal herb gatherers and natural habitats of the drug plant identified. Voucher specimens were collected and herbarium made. Herbarium was later identified at Kerala Forest Research Institute, Peechi.

3.2. EXPT. II. NATURAL HABITAT ANALYSIS

Natural habitats of the species were located with the help of tribal herb gatherers in the Peechi forest and the habitat characteristics were recorded.

Observations on altitude, longitude, aspect, forest type, shade level, slope, associated plants, plant density, and soil type of the natural habitat were recorded. Physical properties of soil like pH, EC, structure and texture were estimated,

following standard procedures. Soil aggregate analysis was carried out using Yoder's apparatus. Chemical properties of soil like N, P, K, Ca, Mg, S and micro nutrients were also analyzed using standard procedures as mentioned later in this chapter. At each selected habitat, density of the species was recorded by counting the number of plants in 1 m^2 quadrant.

Litter cover in the forest was also estimated. For this, nets of 1 m^2 area were placed in the natural habitat of the plant to collect the leaf litter. At monthly intervals, observations were taken by recording the weight of litter collected in the collection net.

Complete life cycle of the species in the natural habitat was observed and documented. Growth observations of the plant including tuber characters were recorded at periodic intervals. Flowering season of 'Orilathamara' was ascertained. Plants were tagged and floral characters like time of flowering, days to flowering, length of inflorescence, number of flowers, days to flower opening, number of sepals, number of petals, stamens, stigma, fruit set, seed set and life span of inflorescence were recorded.

3.3. EXPT.III. DOMESTICATION TRIAL

This experiment was laid out as a field trial in the experiment fields attached to the Department of Plantation Crops and Spices, College of Horticulture.

3.3.1. Treatments

There were two shade levels and four growing media combinations as given below. Shade was provided by erecting artificial shade net pandals using 25% and 50% shade nets.

S1 – 50% shade

S2 – 25% shade

T1 – farmyard manure + sand + soil (1:1:1)

T2 – leaf compost + sand + soil (1:1:1)

Plate 1. Preparation of special growing pits

Plate 2. Planting material

Plate 3. Field after planting and mulching

T3 – coir pith compost + sand + soil (1:1:1)

T4 – vermi compost + sand + soil (1:1:1)

Seed tubers of *Orilathamara* were brought from forest. Planting was undertaken in pits of size 0.5 m × 0.5 m × 0.3 m, lined on all the sides with clay tiles. The growing media were replicated five times under two shade levels, giving a total of 40 pits under the trial. The tile lined pits were filled with the growing media to a depth of 30 cm. Tubers of *Nervilia* weighing approximately 3-5 g were planted at the rate of 12 per pit. The tubers were shallowly planted during the second week of June with the onset of south west monsoon and mulched. Crop was raised as rain fed. Weeding was done at monthly intervals. *Pseudomonas fluorescence* application was done three times during the cropping period; both spraying and soil drenching were done. Crop was harvested during December and the observations on days to harvest, yield per plot and yield of single plant were recorded. The tubers were collected cleaned and stored in mud pots.

Mean weekly weather data during the study period were recorded from the Centre for Climate Change Research, Kerala Agricultural University, Vellanikkara. Weather data are presented in appendix 1.

Physical and chemical properties of the growing media were analyzed as per the following procedures.

3.3.1.1. Soil pH

The pH was determined in a 1:2.5 soil water suspension, potentiometrically using a pH meter (Jackson, 1958).

3.3.1.2. Electrical conductivity

Electrical conductivity was estimated in the supernatant liquid of the soil water suspension (1:2.5) used for pH estimation with the help of a conductivity meter (Jackson, 1958).

3.3.1.3. Organic carbon

Organic carbon content of the soil was estimated by wet digestion method (Walkley and Black, 1934).

3.3.1.4. Available phosphorus

Available phosphorus in the soil sample was extracted using Bray No.1 reagent (Bray and Kurtz, 1945) and estimated colorimetrically by reduced Molybdate Ascorbic acid blue color method (Watanabe and Olsen, 1965) using a spectrophotometer (Model: Genesys 20).

3.3.1.5. Available potassium

Available potassium was extracted using neutral normal ammonium acetate and its content in the extract was estimated by flame photometry (Jackson, 1958).

3.3.1.6. Available micronutrients (Fe, Cu, Mn and Zn) in soil

Available micronutrients in soil samples were extracted using 0.1M HCl (Sims and Johnson, 1991). Four gram soil with 40 ml of 0.1M HCl was shaken for 5 minutes. It was filtered through Whatmann No.1 filter paper and the filtrate was collected and analysed for Fe, Cu, Mn and Zn using Perkin Elmer Atomic Absorption Spectrophotometer (Model: Analyst 400).

3.3.1.7. Determination of soil texture

Soil texture was determined using Robinson's pipette method (Robinson, 1922). For the analysis, 20 g air dried soil was taken in a 500 ml tall form beaker, then added 25 ml water, taking care to avoid loss of very fine particles by dusting and swirling. Then added 10 ml of hydrogen peroxide. Covered the beaker with watch glass and set aside overnight. Heated the beaker on a hot plate at 90^0C, added 10 ml portion of hydrogen peroxide at 1 hour intervals, with occasional stirring. Stirring was continued till large bubbles ceased to form, to ensure that all organic matter has been destroyed. Continued boiling for one hour to decompose

excess hydrogen peroxide. Transferred the acid free soil on the filter paper carefully to a shaking bottle, without allowing the volume to exceed. Added 8-10 ml of the dispersing agent (NaOH), until the contents were distinctly alkaline as indicated by the change of red litmus to blue. The mouth of the bottle was closed and it was shaken in a reciprocating shaker for two hours or in a high speed stirrer for 20 minutes to disperse the soil. The contents of the shaking bottle were passed through a 0.20 mm sieve supported over a 1000 ml cylinder. Washed the soil through the sieve with a jet of water until the washings were clear. Ensured that the volume of the sieved suspension did not exceed 100 ml.

The residue in the 0.2 mm sieve (coarse sand) was transferred to a evaporating or china dish, dried, weighed and reported as coarse sand. Then the suspension in the cylinder was made up to 1000 ml. The temperature of the suspension was noted. Covered the mouth of the cylinder and shook the suspension thoroughly without spilling.

The cylinder with the suspension was placed under the Robinson pipette, after noting the time. The suspension was left undisturbed for four minutes 48 seconds, the time for the silt particles to travel a distance of 10 cm. A few seconds prior to the expiry of the time, the pipette was lowered exactly to 10cm depth and 20 ml of the suspension was pipetted out into a china dish which has been previously weighed. Dried the suspension to constant weight at 105^0C in the hot air oven and reported as silt + clay fraction contained in 20 ml of the suspension.

The contents of the cylinder were again shaken and left undisturbed for six hours and 40 minutes, the time for clay particles to settle to a distance of 10cm at the corresponding temperature. At the end of the period, pipetted out 20 ml of the suspension as in the previous case using the Robinson pipette. The suspension was transferred to a china dish, dried and weighed. This represented the clay fraction.

The bulk of the suspension in the jar was siphoned out leaving the fine sand at the bottom. Added water to a height of 10 cm stirred the contents well and allowed to stand undisturbed. After the sand was settled, decanted off the supernatant liquid. The process was repeated until the supernatant was clear. Then the water was completely decanted off, the residue was transferred to a previously weighed china dish, dried and weighed to constant weight. This was reported as fine sand. The percentage of the different size fractions were calculated.

3.3.1.8. Soil aggregate analysis

Aggregate analysis was done using Yoder's apparatus using the method given by Yoder (1936). The graduated sets of sieves were arranged in such a manner that the top one was 5 mm followed by 2, 1, 0.5 and 0.25. Below this, collecting dish was attached. About 50 g of soil clods collected from the field was kept on the top most sieves and the set was fitted to the Yoder's apparatus and oscillated through the water for 30 minutes. This was done by soil on the top sieve and transferring the nest of sieves to the drum of the apparatus and clamping them in screen. Switched on the apparatus and oscillated in water for 30 minutes with a frequency of 30 – 35 cycles per minute through a stroke length of about 3.8 cm and checked that aggregates on the top sieve was moved through water. Taken out the nest of sieves and allowed water to drain and then carefully dried in the oven. Weights were then recorded and percentage of aggregates retained in each sieve was found out. From this Mean Weight Diameter (MWD) was calculated using the following equation.

MWD = $\sum$ CiXi where, Ci is the sieve size in mm and Xi is the percentage retained in the sieve.

Plate 4. Experimental plot

3.3.2. Observations of the Domestic Crop

The following observations were recorded in the domestic crop.

Days to sprouting

Sprouting percentage

Days to leaf emergence

Days to complete unfurling of leaf

Leaf length (cm)

Leaf breadth (cm)

Leaf area (cm^2)

Length of petiole (cm)

Days to flowering

Days to fruit set

Days to senescence

Days to emergence of side sprouts

Number of side sprouts

Days to harvest

Tuber yield of single plant (g)

Yield per plot (g)

3.3.3. Tuberisation and Tuber Development Studies

In order to study the tuberisation and tuber development in the species, tubers were planted in 20 mud pots filled with normal potting mixture. At monthly intervals, plants were uprooted to record the tuber characters.

3.4. EXPT.IV. BIOCHEMICAL ANALYSIS

Biochemical constituents of both wild and domestic crops were analysed using standard procedures with suitable modifications.

3.4.1. Estimation of Chlorophyll

Chlorophyll was estimated using the method given by Sadasivam and Manickam (1992). Weighed 1 g of finely cut and well mixed representative sample of leaf into a clean mortar. Ground the tissue to a fine pulp with the addition of 20 ml of 80 per cent acetone. Centrifuged at 5000rpm for five minutes and transferred the supernatant to a 100ml volumetric flask. Ground the residue with 20 ml of 80 per cent acetone, centrifuged and transferred the supernatants to the same volumetric flask. Repeated this procedure until the residue was colourless. Washed the mortar and pestle thoroughly with 80 per cent acetone and collected the clear washings in the volumetric flask. Made up the volume to 100 ml with 80 per cent acetone and read the absorbance of the solution at 645, 663 and 652 nm against the solvent (80% acetone) blank.

Calculated the amount of chlorophyll present in the extract mg chlorophyll/g tissue using the following equations:

mg chlorophyll a / g tissue $= 12.7 (A_{663}) – 2.69 (A_{645}) \times v/ (1000 \times w)$

mg chlorophyll b / g tissue $= 22.9 (A_{645}) – 4.68 (A_{663}) \times v/ (1000 \times w)$

mg total chlorophyll /g tissue $= 20.2 (A_{645}) + 8.02 (A_{663}) \times v/ (1000 \times w)$

Where A: absorbance of specific wavelengths

V: final volume of chlorophyll extract in 80 per cent acetone

W: fresh weight of tissue extracted

3.4.2. Estimation of Starch

Starch content was estimated by Anthrone method (Sadasivam and Manickam, 1992). Homogenized 0.1 to 0.5 g of the sample in hot 80 per cent

ethanol to remove sugars. Centrifuged and retained the residue, washed the residue repeatedly with hot 80 per cent ethanol till the washings did not give colour with anthrone reagent. Dried the residue well over a water bath. To the residue added 5 ml of water and 6.5 ml of 52 per cent perchloric acid. Extracted at 0^0C for 20 minutes. Centrifuged and saved the supernatant. Repeated the extraction using fresh perchloric acid. Centrifuged and pooled the supernatants and made up to 100 ml. Pipetted out 0.1 ml of the supernatant and made up the volume to 1 ml with distilled water. Then prepared the standards by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and made up the volume to 1 ml in each test tube with distilled water. Added 4 ml of anthrone reagent to each test tube. Heated for eight minutes in a boiling water bath and cooled rapidly and read the intensity of green to dark green colour at 630 nm. Then calculated the content of starch in the tuber.

3.4.3. Estimation of Soluble Sugars

The content of total soluble sugars was estimated using Phenol sulphuric acid method given by Sadasivam and Manickam (1992). For this, standard glucose stock solution was prepared by adding 100 mg of standard glucose to 100 ml distilled water and 10 ml stock solution was diluted to 100ml with distilled water. Then the sample was prepared by homogenizing 500 mg of the fresh plant sample with hot 80 per cent methanol. The extract was centrifuged. Repeated the process and made to 50 ml with 80 per cent methanol. Pipette out 0.2 to 1 ml of the working standards and 0.5 ml of sample extract into a series of test tubes. Made up the volume in each test tube to 1 ml with distilled water. Blank was set with 1 ml distilled water. To each test tube, 1ml phenol solution (5%) was added followed by 5 ml of 96 per cent sulphuric acid. The tubes were shaken well and kept for 10 minutes. It was then placed in a water bath at 25- 30^0C for 20 minutes to develop a light yellowish brown colour and test tubes were cooled and absorbance noted at 490 nm. From this, the content of total soluble sugar was estimated.

3.4.4. Estimation of Protein

Protein was estimated using Lowry's method suggested by Sadasivam and Manickam (1992). For this, standard bovine serum albumin stock was prepared. Then sample was prepared by weighing 500 mg of the fresh plant sample and ground well with 5 ml of Tris buffer. The sample was centrifuged at 4^0C for 10 minutes at 10,000 rpm. Supernatant was used for estimation. 0.2 to 1.0 ml of working standards and 0.5 ml of sample extract was pipetted out into a series of test tubes. Made up the volume in each tube to 1ml with distilled water. Blank was set with 1 ml of distilled water. 5 ml of alkaline copper solution was added, mixed well and allowed to stand for 10 minutes. 0.5 ml of Folin-cio calteau reagent was then added. Mixed well and incubated at room temperature in the dark for 30 minutes. Blue colour was developed. Absorbance noted at 660 nm and content of protein was estimated.

3.4.5. Estimation of Total Free Amino Acids

The total free amino acid content was estimated by the method given by Sadasivam and Manickam (1992). Standard L-leucine stock was prepared by dissolving 50 mg leucine in 50 ml of distilled water. Working standard was prepared by diluting 10 ml stock solution to 100 ml. Sample was prepared by taking 500 mg of the fresh plant sample. It was ground well with 5 ml of 10 per cent isopropyl alcohol. The sample was centrifuged and the supernatant saved. The extraction was repeated and the supernatants were pooled. Pipetted out 0.2 to 1.0 ml of working standards and 0.5 ml of sample extracts into a series of test tubes. Blank was set with 0.1 ml of 80 per cent methanol. To the test tubes, added 1 ml ninhydrin solution and made to 2 ml with distilled water. The test tubes were heated in a boiling water bath for 20 minutes. The test tubes were cooled and added 5 ml diluent solvent and the contents were mixed. Blue colour was developed. After 15 minutes absorbance was noted at 570 nm.

3.4.6. Estimation of Total Soxhlet Extractables

The tubers were cut into smaller pieces and dried till it attained constant weight. The dried tuber pieces were then powdered and used for analysis. Two grams of powered sample was taken for soxhlet extraction. The solvent used was methanol. It took four siphoning for the solvent to become colourless. At this point, extraction was stopped and the extract was then transferred to previously weighed beakers and kept aside till the solvent got evaporated completely. Weight of the beaker and extract was then taken and content of crude extractables calculated.

3.4.7. Thin Layer Chromatography

3.4.7.1. Thin layer chromatography for free amino acids

Thin layer chromatography was done using the procedure given by Stahl (1969).

Sample preparation: About 5 g of the powdered sample was weighed and the extract was taken in ethanol: water (1:1) mixture by boiling for two hours. Filtered extract was taken.

The extract obtained was spotted on silica gel (60 F_{254}) coated MERCK plates using automatic spotting machine. Eleven standard amino acids were also spotted along with the sample. The spotted plates were kept in chromatographic chamber containing the solvent system(butanol: acetic acid: water (6:3:2)). The solvent system was allowed to run $3/4^{th}$ of the plates. The plates were taken out of the chamber and allowed to dry. It was then sprayed with spray reagent (0.1% ninhydrin) and kept in oven (110^0C) for 30 minutes. The spots were purple in colour. Rf value was noted.

3.4.7.2. Thin layer chromatography for sugars

Sample preparation: 5 g of the powdered sample was weighed and the extract was taken in ethanol: water (1:1) mixture by boiling for two hours. Cooled filtered and the extract was taken.

The extract obtained was spotted along with sugar standards (prepared by dissolving 10 mg in 10ml methanol) on silica gel (60 F_{254}) coated MERCK plates using automatic spotting machine. The spotted plates were kept in chromatographic chamber containing the solvent system (ethyl acetate: isopropyl alcohol: acetic acid: water (5:3:1:2). The solvent was allowed to run $3/4^{th}$ of the plates. The plates were taken out of chamber and kept outside. It was then sprayed with spray reagent (anisaldehyde sulfuric acid) and kept in oven (110^0C) for 30 minutes. The spots were grey in colour. Rf value was noted:

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

3.5. ANATOMICAL STUDIES

3.5.1. Staining using Safranin

Thin hand sections of the tubers were taken. Sections were stained using safranin for 10 minutes. Then they were dehydrated using alcohol 75, 90 and 95 per cent progressively. The sections were taken out and washed in acetone and then in xylene for two minutes and then mounted with DPX (Yeung, 1998).

3.5.2. Staining using Acetocarmine

Thin hand sections of the tuber were taken. They were stained with acetocarmine for five minutes. Dehydration was done by washing with alcohol

75, 90 and 95 per cent respectively for two minutes. The sections were taken out and washed in acetone and then in xylene. Then they were mounted on a slide with DPX, covered with coverslip and viewed under microscope.

3.5.3. Double Staining

Thin hand sections of the tuber were taken and then staining done using safranin for about 30 minutes. After this, dehydration was done using series of alcohol solutions from 30, 40, 50, 60, 70, 80, 90 and 100 per cent and it was done by washing with alcohol for about 2-5 minutes. Again it was stained using fast green diluted with clove oil. The sections were immediately taken out after dipping in fast green stain. Then the sections were washed in clove oil for two minutes followed by xylene- clove oil mixture (1:1) for two minutes. Again washed in xylene 1 and xylene 2 and mounted on a clean glass slide with DPX. Coverslip was put and viewed under the microscope. Photographs of these sections were taken.

3.6. STATISTICAL ANALYSIS

The data recorded were subjected to statistical analysis using MSTATC package. Analysis of variance was performed following the procedure by Panse and Sukhatme (1978). The treatments were compared using DMRT.

Results

4. RESULTS

The study entitled "Habitat analysis and domestication studies on 'Orilathamara' (*Nervilia aragoana* Gaud.)" intended to collect the source plants of the drug 'Orilathamara' from wild, carry out the natural habitat analysis of the species and analyse its response to domestication. Results of the study are presented in this chapter, experiment wise.

4.1. EXPT. I. EXPLORATION AND COLLECTION OF SOURCE PLANTS OF THE DRUG 'ORILATHAMARA' FROM FOREST

Scanning of the published forest flora of Thrissur district helped in finding out the distribution of *Nervilia* genus. Based on the results, Thamaravellachal area of Peechi – Vazhani wild life sanctuary was selected for further studies. Tribal herb gatherers in the Thamaravellachal settlement were identified and information regarding the occurrence of *Nervilia aragoana* were collected from them. Forest explorations were carried out along with the tribal herb gatherers and natural habitat of the species identified. Voucher specimens were collected and herbarium made. Herbarium was later identified at Kerala Forest Research Institute, Peechi. Following *Nervilia* species were located in Peechi forests.

1. *Nervilia infundibulifolia* (Blatt. & McCann)
2. *Nervilia plicata* (Andr.)
3. *Nervilia crociformis* (Zoll. & Moritzi)
4. *Nervilia aragoana* Gaud.

Of the four species, the first three were very rare and found in certain pockets only with restricted distribution. *Nervilia aragoana* was the only species available in sufficient number and hence, further studies were confined to this species.

The tribal people in the area seemed to gather only *Nervilia aragoana* for drug purpose. There was no regular collection, only a few people collected it on

demand basis. Pictures of the four species located in Peechi forest are given in plate 5.

4.2. EXPT. II. NATURAL HABITAT ANALYSIS

Two natural habitats of *Nervilia aragoana* in Peechi forests were selected for detailed studies.

4.2.1. Habitat Characteristics

Habitat 1 - Kanjippara

Altitude : 500 m above MSL

Latitude : 10^0 30' 57"

Longitude : 76^0 22' 38"

Slope : very gentle

Aspect : south east

Vegetation : Moist Deciduous Forest

Soil type : sandy clay

Density : 17/m^2

Approximate shade level : 75 %

Nervilia species found : *Nervilia aragoana, Nervilia plicata*

Associated plants : *Piper longum, Curculigo orchioides, Grewia tiliifolia, Helicteres isora, Pterocarpus marsupium*

4.2.1.2. Habitat 2 - Aanakuzhi

Altitude : 180 m above MSL

Latitude : 10^0 30' 57"

(A)

(B)

(C)

(D)

Plate 5. Four species of *Nervilia* reported from Peechi forest

(A) ***Nervilia crociformis***

(B) ***Nervilia infundibulifolia***

(C) ***Nervilia aragoana***

(D) ***Nervilia plicata***

Longitude : 76^0 22' 38"

Slope : very gentle

Aspect : south east

Vegetation : moist deciduous forest

Soil type : forest loam

Litter cover : 140 g/m^2

Approximate shade level : 50 %

Nervilia species found : *Nervilia aragoana*

Associated plants : *Piper longum, Eupatorium odoratum, Calophyllum calaba, Xylia xylocarpa, Tectona grandis, Caesalpinia sappan, Holarrhena pubescence*

From the habitat characteristics recorded at two places, it was observed that the genus *Nervilia* is a delicate ground orchid found in the ground vegetation in Moist Deciduous Forests along with other plants.

Data pertaining to physical and chemical properties of the forest soil are presented in table 3. From the soil analysis data it is clear that the soil pH is towards neutral and electrical conductivity is 105 µs/cm. Mean weight diameter (MWD) is 1.107 which means a good structured soil with good water stability. Soil structure is granular and soil texture is sandy clay. From the estimation of chemical properties of soil in the natural habitat, it was found that the organic carbon, available phosphorous and potassium were high in the soil and available sulphur was sufficient. Micronutrients like zinc, iron and manganese were sufficient. Calcium and magnesium were high and copper deficient.

Plate 6. Forest explorations

(A) **(B)**

Plate 7. Natural habitat analysis

(A) Plants in the natural habitat **(B) Litter cover measurement**

Table 3. Physical and chemical properties of forest soil.

Sl. No.	Parameters	Values
1	pH	6.1
2	Electrical conductivity	105.3 µs/cm
3	Mean weight diameter	1.107
4	Organic carbon	1.78 %
5	Available phosphorous	106.02 kg/ha
6	Available potassium	369.6 kg/ha
7	Available sulphur	5.0 ppm
8	Copper	0.90 mg/kg
9	Zinc	2.10 mg/kg
10	Iron	34.8 mg/kg
11	Manganese	7.83 mg/kg
12	Calcium	0.08 %
13	Magnesium	125 mg/kg

4.2.2 Life Cycle of *Nervilia aragoana* in the Natural Habitat

Complete life cycle of *Nervilia aragoana* was observed in the wild. The observations started in March, 2010, when the plants were in the dormant stage.

4.2.2.1. Flowering

Immediately after the receipt of the summer showers in March, the underground tubers were activated and they put forth flowers. Long inflorescence was visible after one week of receipt of summer rains. Flowering continued during April – May. Fruits were developed in the last week of May. During May – June the inflorescence dried up and withered away. Stages of flowering and fruit set of *Nervilia aragoana* in the natural habitat are presented in Fig. 1 and Plate 2.

Floral characters

Floral characters of *Nervilia aragoana* in the natural habitat are presented in table 4. Inflorescence was a raceme, about 17 cm in length with an average of ten flowers. Flower opening started from bottom upwards and first flower opened after 3-4 days of opening o0f the inflorescence. Flower remained fresh for 5-7 days after which it withered. There was an average of 2-4 fruits/inflorescence. Fruits split on drying, releasing innumerable tiny seeds. Longevity of the inflorescence was 8-10 days after which it got dried up.

Flowers were bisexual, epigynous, trimerous, outer three perianth lobes (calyx) were equal and pale green and linear lanceolate, acute and the inner three lobes (petals) were smaller than the outer ones. Labellum was white, three lobed about the middle and purple tinged with yellow near the base. Androecium consisted of single fertile stamen which on union with the gynoecium formed the gynostegium. Pollen was present in the pollinia. Ovary was inferior, tricarpellary and syncarpous. Fruit was a dehiscent capsule, seeds were innumerable and tiny.

Table 4. Floral characters of *Nervilia aragoana* in the natural habitat

Sl. No.	Parameters	Observations
1	Period of flowering	April – May
2	Average length of inflorescence	17 cm
3	Average number of flowers	10
4	Days to flower opening	3 days
5	Longevity of flowers	5-7 days
6	Average fruit set	2-4
7	Longevity of fruit	7 days
8	Seed set	Innumerable tiny seeds
9	Life span of inflorescence	8-10 days

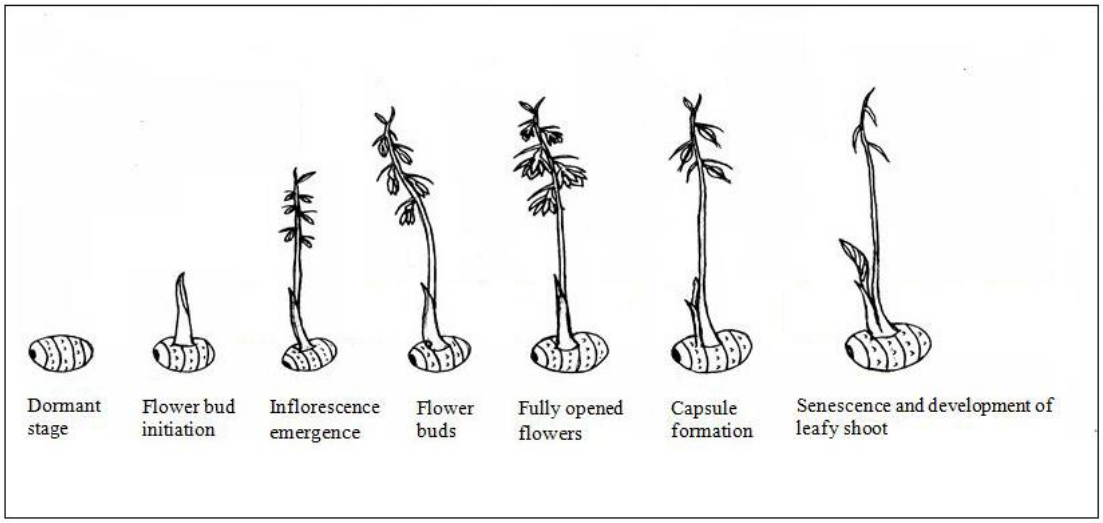


Fig.1. Developmental stages of inflorescence


Plate 8. Flowering in *Nervilia aragoana* Gaud.


Plate 9. Flower structure


Plate 10. Fruit and seed set in *Nervilia aragoana* Gaud.

Table 5. Morphological observations of *Nervilia aragoana* Gaud. in the natural habitat.

Plants	Petiole length (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Tuber weight (g)
1	30	18	16	200.96	22
2	30	15	13	132.66	16
3	28	16	16	200.96	12
4	27	14	14	153.86	14
5	29	12	12	113.04	21
6	28	15	16	200.96	12
7	18	8	8	50.24	10
8	17	5	7	38.465	8
9	26	10.5	11	94.985	8
10	26	6	7	38.465	6
11	25	6	10	78.50	5
12	27	12	13	132.66	16
Mean	25.91	11.46	11.92	119.65	12.5

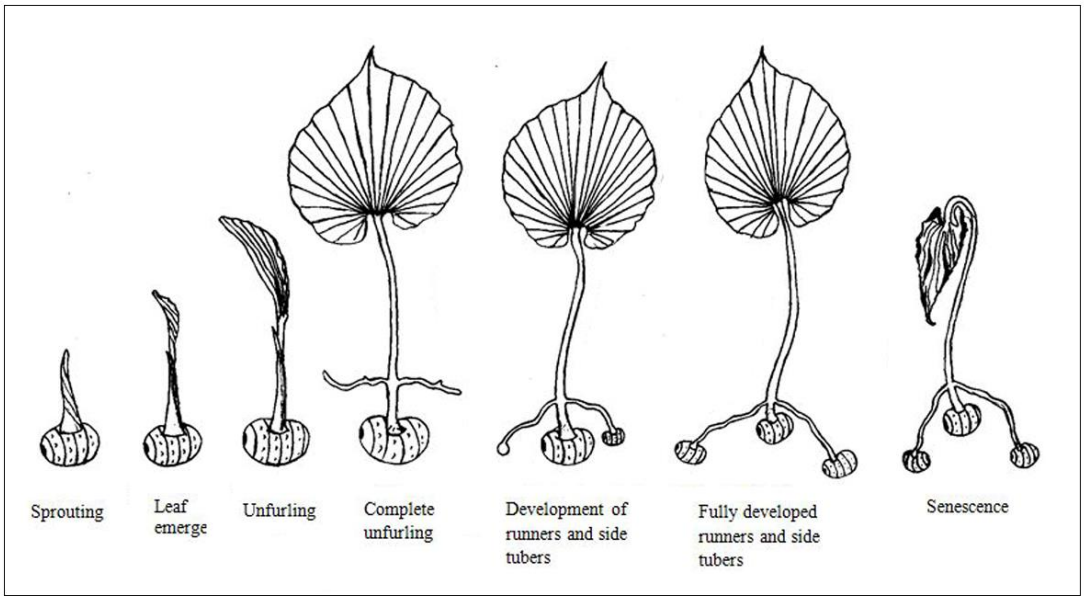


Fig.2. Developmental stages of *Nervilia aragoana* Gaud.

Table 6. Developmental stages of *Nervilia aragoana* Gaud.

Sl. No.	Stages	Period
1	Dormant stage	Dec – March
2	Flower bud initiation	April – May
3	Emergence of inflorescence	April – May
4	Fully developed inflorescence	April – May
5	Fruit and seed set	April – May
6	Senescence and development of leafy shoot	May - June
7	Sprouting	May – June
8	Leaf emergence	June – July
9	Complete unfurling of leaves	July – Aug
'10	Runner and side tuber initiation	Aug – Sept
11	Fully developed runners and side tubers	Sept – Dec
12	Senescence	Dec

4.2.2.2. Regeneration in the forest

After withering of the inflorescence, approximately after one week, leaf started emerging. Single leaf emerged from the tuber. Growth characters of the plant in the natural habitat were studied and morphological observations were recorded. Morphological observations recorded from 12 plants are presented in Table 5. Petiole length varied from 17 cm to 30 cm with an average of 25.92 cm. Leaf length ranged from 5 to 18 cm with an average of 11.46 cm and leaf width varied from 7 cm to 16 cm with an average of 11.92 cm. Leaf area ranged from 38.46 cm^2 to 200 cm^2 with an average of 121.31 cm^2. Average tuber weight was recorded to be 12.5 g/plant.

The fully opened leaves remained green till December. With the receding of North East Monsoon, leaves turned yellow and withered away gradually. Thereafter, the tubers remained dormant during summer. The developmental stages of the plant are represented in Fig. 2. Table 6 shows the developmental stages of *Nervilia aragoana*. The plant was in reproductive phase during April – June and from June to December the plant was in vegetative phase. From December to March the plant remained in the dormant stage as underground tuber.

4.3. EXPT. III. DOMESTICATION TRIAL

This experiment was laid out as a field trial in the field attached to the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara. Planting was done on June 15, with the onset of monsoon.

4.3.1. Weather Data

Mean weekly weather data during the study period was recorded from the Centre for Climate Change Research, Kerala Agricultural University, Vellanikkara. It is presented in appendix 1.

During the study period, the maximum temperature ranged from 21.70C to 370C and minimum temperature ranged from 21.30C to 26.20C. Rainfall ranged

from 000.0 mm to 051 mm. Humidity ranged from a maximum of 97 % to a minimum of 30 % during March 2010.

The crop was planted on the third week of June 2010, when the maximum temperature was 28.1^{0}C and minimum temperature was 23.3^{0}C. Rainfall received was 045.3 mm and humidity ranged from 89 % to 97 %.

4.3.2. Tuberisation and Tuber Development in *Nervilia aragoana*

Along with the domestication trial, sample plants were also raised in mud pots in normal growing medium in order to study the tuberisation pattern of the species. The observations recorded are presented in table 7.

Tuber weighing 3-5 g was the starting propagule in the experiment. One month after planting there was no runner development as also side tuber production. The runners started growing sixty days after planting. There were two runners, left one which grew to a length of two centimetres and right one which grew to a length of three centimetres. At the distal end of the runners which lengthened (16 – 17 cm) as the growth progressed, side tubers started forming around 90 days after planting (4 g and 2 g in weight). The runners and the side tubers increased in size gradually. At the time of harvest *ie.*, six months after planting, the mother tuber and the side tubers attained almost equal size.

4.3.3. Standardisation of Shade Level and Growing Media

Objective of this experiment was to find out the optimum shade level and growing media that supports best growth and tuber production in *Nervilia*. There were two shade levels as:

S1 – 50% shade

S2 – 25% shade

Table 7. Tuberisation and tuber development of *Nervilia aragoana* Gaud. in the domestication trial

Sl. no.	Days After Planting	Tuber weight			Length of runners	
		Main tuber (g)	Side tubers (g)		Left (cm)	Right (cm)
1	30	4	0	0	0	0
2	60	4	0	0	2	3
3	90	4	4	2	8.5	11
4	120	4	4	2.5	9.5	13
5	150	2.52	2.8	0.44	11	12.5
6	180	2.3	3.3	1.07	19	16.5

(I)

(II)

(III)

(IV)

Plate 11. Tuberisation and tuber development in *Nervilia aragoana* Gaud.

Four growing media as:

T1 – farmyard manure + sand + soil (1:1:1)

T2 – leaf compost + sand + soil (1:1:1)

T3 – coir pith compost + sand +soil (1:1:1)

T4 – vermi compost + sand + soil (1:1:1)

4.3.3.1. Physical properties of growing media

Physical properties of growing media used in the study are presented in table 8.

pH of growing media ranged from 5.3 to 5.6 all towards acidic range. Media T1, T3 and T4 were strongly acidic and T2 moderately acidic. EC ranged from 0.317 to 1.097 dS/m, all the values were in the safe range. Mean weight diameter (MWD) ranged from 0.896 to 1.31. An increased MWD value indicates that the soil has good water stability and has bigger sized aggregates which in turn results in good aeration and good moisture retention capacity of the soil. From the MWD values it is clear that the growing medium T1 had good structure and stability (1.31) compared to others, T4 (1.27), T2 (1.22) and T3 (0.896). All the four media were granular in structure. T1 medium was loamy in texture, T2 sandy clay, T3 sandy clay loam and T4 medium sandy.

4.3.3.2. Chemical properties of growing media

Chemical properties of all the growing media were analysed. Both major and minor nutrients were estimated. The results are presented in table 9.

Organic carbon content ranged from 1.08 to 1.99 per cent. In media T1, T2 and T3, organic carbon content was medium and in T4 containing vermi compost it was high. Nitrogen percentage ranged from 0.11 to 0.20 per cent, it was almost equal in the first three media and slightly higher in T4. Calcium content ranged from 80.9 -88.5 mg/kg and Magnesium content ranged from 5.4-9 mg/kg. Both Calcium and Magnesium were low in all the media and Sulphur was

Table 8. Physical properties of growing media

Sl. No.	Growing media	pH	EC (dS/m)	MWD	Structure
1	FYM +sand + soil	5.5	1.09	1.31	Granular
2	Leaf compost + sand +soil	5.67	0.36	1.22	Granular
3	Coir pith compost + sand + soil	5.33	0.31	0.89	Granular
4	Vermi compost + sand + soil	5.42	0.69	1.27	Granular

Mechanical analysis

Sl. No.	Growing media	Sand (%)	Silt (%)	Clay (%)	Texture
1	T1	40	42	23	Loamy
2	T2	50	10	40	Sandy clay
3	T3	55	20	25	Sandy clay loam
4	T4	50	30	20	Sandy loam

Table 9. Chemical properties of growing media

Nutrients	Growing media			
	T1	**T2**	**T3**	**T4**
Organic carbon (%)	1.16	1.1	1.08	1.99
Nitrogen (%)	0.12	0.11	0.11	0.20
Available P (mg/kg)	148	27	31.65	80
Available K (mg/kg)	647	278	201	283
Ca (mg/kg)	80.9	88	81.35	88.5
Mg (mg/kg)	9	5.4	8.2	8.2
S (mg/kg)	156.7	21.7	55	46.7
Cu (mg/kg)	4.38	3.31	2.9	2.86
Zn (mg/kg)	5.24	5.71	11.78	23.1
Fe (mg/kg)	73.64	57.2	51.23	61.95
Mn (mg/kg)	34.5	15	32.4	32.5

(A)

(B)

(C)

(D)

Plate 12. Developmental stages of *Nervilia aragoana* Gaud.

(A) Sprouting (B) Leaf emergence (C) Unfurling of leaves (D) Complete unfurling of leaves

high in all. Micro nutrients like Copper, Zinc, Iron and Manganese were sufficient in all the four growing media tried.

4.3.4. Influence of Shade Level and Growing Media on Sprouting and Biomass Production of *Nervilia aragoana* Gaud.

Two weeks after planting, sprouting was observed in all the treatments. Sprouting percentage of *Nervilia aragoana* in domestication trial is presented in table 10. Data show that neither the shade level nor the growing media had any effect on the sprouting percentage in *Nervilia aragoana*. In all the shade levels and growing media, there was good germination.

Data on the influence of shade level and growing media on total biomass production of *Nervilia aragoana* are presented in table 10. There was no significant difference among the shade level or growing media for total biomass production of this species.

4.3.5. Influence of Shade Level and Growing Media on Growth Parameters of *Nervilia aragoana* Gaud.

There was no significant difference in the days to leaf emergence and days to complete unfurling of leaves in the plant, under domestication

Data pertaining to various growth parameters are presented in table 11. With regard to petiole length, leaf length, leaf width and leaf area it was found that under 50 per cent shade level, treatment T1 (FYM + sand + soil) recorded highest values for all these growth parameters. All other treatments were on par.

4.3.6. Influence of Shade Level and Growing Media on Yield Parameters

Data with respect to the influence of shade level and growing media on yield/plant of *Nervilia aragoana* Gaud are presented in table 12. There was wide variation in the tuber yield /plant and it ranged from 7 g to 27 g per plant.

Results on the influence of shade level and growing media on yield /plot of *Nervilia aragoana* Gaud. are presented in table 13. Data indicate that under 50

Table 10. Influence of shade level and growing media on sprouting percentage, days to leaf emergence, days to complete unfurling of leaves and total biomass production in *Nervilia aragoana* Gaud.

Shade / Treatment	S1 (50% shade)				S2 (25% shade)			
	SP	**DLE**	**DCUF**	**TBM**	**SP**	**DLE**	**DCUF**	**TBM**
T1	83.320[a]	8.160[a]	7.540[a]	18.800[a]	91.640[a]	8.780[a]	8.400[a]	15.600[a]
T2	86.660[a]	8.360[a]	7.240[a]	14.800[a]	88.280[a]	8.130[a]	8.720[a]	15.800[a]
T3	84.960[a]	8.360[a]	7.710[a]	17.600[a]	93.30 [a]	9.140[a]	7.480[a]	19.200[a]
T4	91.640[a]	8.140[a]	7.320[a]	19.200[a]	91.66 [a]	9.120[a]	8.000[a]	16.400[a]
Mean	86.645	8.255	7.452	17.600	91.220	8.793	8.150	16.750

SP : sprouting percentage

DLE : Days to Leaf Emergence

DCUF: Days to Complete Unfurling of leaves

TBM : Total Biomass Production (g/plant)

Table 11. Influence of shade level and growing media on petiole length, leaf length, leaf width and leaf area of *Nervilia aragoana* Gaud.

	S1 (50% shade)				S2 (25% shade)			
	PL	**LL**	**LW**	**LA**	**PL**	**LL**	**LW**	**LA**
T1	18.540[a]	13.580[a]	12.220[a]	123.352[a]	9.200 [bc]	7.260[b]	6.780[b]	38.646[b]
T2	11.880[bc]	7.740[b]	7.180[b]	45.504[b]	10.720[bc]	9.200[b]	8.040[b]	52.250[b]
T3	13.800 [b]	9.840[b]	8.820[b]	64.030[b]	10.960[bc]	7.920[b]	7.440[b]	48.398[b]
T4	14.100 [b]	9.840[b]	8.740[b]	65.940[b]	8.700 [c]	7.880[b]	7.360[b]	45.596[b]
Mean	14.580	10.250	9.240	74.707	9.895	8.065	7.405	46.222

PL: Petiole length (cm)

LL: leaf length (cm)

LW: leaf width (cm)

LA: leaf area (cm^2)

(A) 50 % shade level

(B) 25% shade level

Plate 13. ***Nervilia aragoana*** **under domestication**

Table 12. Tuber yield per plant in *Nervilia aragoana*

Treatments	Tuber yield/ plant (g)					
	R1	**R2**	**R3**	**R4**	**R5**	**Mean**
S1T1	27	12	15	10	13	15.4
S1T2	19	9	10	12	7	11.4
S1T3	10	13	24	15	8	14
S1T4	19	20	13	15	16	16.6
S2T1	13	18	10	12	11	12.8
S2T2	16	4	7	25	15	13.4
S2T3	24	19	12	17	12	16.8
S2T4	7	13	10	25	13	13.6

Table 13. Influence of shade level and growing media on tuber yield (g/plot) of *Nervilia aragoana* Gaud.

Shade level / Treatment	**S1 (50% shade)**	**S2 (25% shade)**
T1	105.200 [a]	61.800 [b]
T2	62.200[b]	80.400 [ab]
T3	63.800 [b]	80.600 [ab]
T4	80.200 [ab]	62.800 [b]
Mean	77.850	71.400

Table 14. Number of tubers of *Nervilia aragoana* under different treatments

Treatments	Small tubers (per 60 plants)	Medium tubers (per 60 plants)	Large tubers (per 60 plants)	Total tubers (per 60 plants)
S1T1	36	32	30	98
S1T2	34	28	20	82
S1T3	29	30	25	78
S1T4	29	31	20	80
S2T1	23	19	18	60
S2T2	51	22	21	94
S2T3	48	25	23	96
S2T4	11	21	21	53

Table 15. Observations on runner length and tuber weight of *Nervilia aragoana* Gaud. in the domestication trial.

Plants	Runner length (cm)		Tuber weight (g)	
	Left	Right	Left	Right
1	18	6	5.0	3.0
2	17.3	9	2.5	8.6
3	16	24	4.7	5.51
4	23	6.2	1.29	0.88
5	23	15.5	4.08	4.78
6	16.5	7	8.8	9.49
7	5.5	14.5	2.15	3.2
8	9	12	5.07	4.5
9	14	12	11.6	9.8
10	5	20	4.7	3.8
11	24.2	16	11.85	9.5
12	12	24.1	7.34	12.0
13	14.5	20.5	1.48	1.46
14	27.3	25.5	3.71	3.2
15	25.5	18	1.28	0.97
Mean	16.72	15.35	5.03	5.38

Plate 14. Tuber yield of *Nervilia aragoana* Gaud. from T1 (FYM + sand + soil) growing medium under two shade levels

per cent shade level, treatment T1 (FYM + sand + soil) recorded the highest tuber yield/plot and was significantly different from other treatments. T4 medium (vermi compost + sand + soil) was found to be the next best medium for tuber yield per plot. Mean tuber yield /plot ranged from 71.4 – 78 g.

Table 14 depicts the number of tubers obtained from different treatments in the domestication trial. From the table it is evident that number of medium and large tubers as well as total number of tubers were more in treatment S1T1 (50% shade level and FYM +sand + soil in equal proportions).

Fifteen plants were observed during the time of harvest for runner length and weight of tubers and data were recorded. The values are presented in table 15. Runner length varied from 15 cm to 16.72 cm. The average side tuber weight varied from 5.03 g to 5.38 g.

4.3.7. Ideal Shade and Growing Media for *Nervilia aragoana*

From the data generated on morphological and yield parameters it could be concluded that the ideal shade level which favours the growth and tuber production of *Nervilia aragoana* was 50 per cent shade compared to 25 per cent shade, which means an average light intensity of 30,000 lux was found ideal for the plant growth (The total light intensity received for a crop on a bright sunny day was taken as 60,000 lux). From the four growing media tried in the domestication trial, medium T1, farmyard manure + sand + soil in 1:1:1 ratio was found ideal and best for the tuber production and yield in *Nervilia aragoana*.

4.4. EXPT. IV. BIOCHEMICAL ANALYSIS

Results of biochemical analysis of the tubers of *Nervilia aragoana* with respect to content of chlorophyll, protein, total soluble sugars, starch, total free amino acid and soxhlet extractables are presented in the following tables 16 to 21. Both wild and domestic samples were analysed for the biochemical constituents.

Table 16. Chlorophyll content of *Nervilia aragoana* Gaud. under different shade levels and growing media

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
S1T1	1.31	0.46	1.78
S1T2	1.35	0.51	1.87
S1T3	1.20	0.42	1.67
S1T4	1.03	0.35	1.39
S2T1	1.21	0.50	1.71
S2T2	1.52	0.60	2.13
S2T3	1.02	0.38	1.40
S2T4	1.08	0.39	1.48
Forest sample	1.00	0.40	1.41

4.4.1. Chlorophyll

Chlorophyll content of *Nervilia aragoana* under various treatments is presented in table 16. Chlorophyll a, b and total chlorophyll were estimated. Data show that the total chlorophyll content was high in the domestic crop. It ranged from 1.390 to 2.130 mg/g in the domestic crop, while in the forest sample it was 1.415 mg/g. There was no significant difference in the chlorophyll content among various treatments of domestication trial.

4.4.2. Protein

Data pertaining to protein content are presented in table 17. With respect to the content of protein, the domestic crop invariably had higher protein content compared to the wild tubers. It was 11.35 mg/g in forest samples and exhibited a wide range of 9.8 to 32.3 mg/g in domestic sample. Among the various treatments in the domestic crop, highest protein content was recorded in S1T4 followed by S1T3.

4.4.3. Starch

Results of starch estimation are presented in table 18. It was found that in forest sample starch content was almost 2-3 times more than the domestic crop. In the domestic crop, S1T2 recorded highest value (222.742 mg/g) followed by S1T1 (212.709 mg/g).

4.4.4. Soluble sugars

With respect to the soluble sugar content, two treatments in the domestic crop recorded higher values compared to the wild crop. They were S1T1 (17.4 mg/g) and S1T2 (14.9 mg/g). In all other domestication treatments sugar content was low compared to forest sample. Data are given in table 19.

4.4.5. Total free amino acids

Data with respect to the content of total free amino acids (Table 20) revealed that the domestic crop had 6 to 7 times more amino acid content when

Table 17. Protein content of *Nervilia aragoana* Gaud. under different shade levels and growing media

Sl. No.	Treatments	Protein content (mg/g)
1	S1T1	9.84
2	S1T2	19.45
3	S1T3	28.65
4	S1T4	32.35
5	S2T1	15.53
6	S2T2	15.99
7	S2T3	24.08
8	S2T4	15.99
9	Forest sample	11.13

Table 18. Starch content of *Nervilia aragoana* Gaud. under different shade levels and under growing media

Sl. No.	Treatments	Starch content (mg/g)
1	S1T1	212.70
2	S1T2	222.74
3	S1T3	80.26
4	S1T4	60.20
5	S2T1	143.14
6	S2T2	142.47
7	S2T3	89.63
8	S2T4	74.24
9	Forest sample	482.27

Table 19. Soluble sugar content of *Nervilia aragoana* Gaud. under different shade levels and under growing media

Sl. No.	Treatments	Soluble sugar content (mg/g)
1	S1T1	17.4
2	S1T2	14.98
3	S1T3	7.04
4	S1T4	8.22
5	S2T1	7.49
6	S2T2	7.70
7	S2T3	3.04
8	S2T4	6.04
9	Forest sample	14.5

Table 20. Total amino acid content of *Nervilia aragoana* Gaud. under different shade levels and under growing media

Sl. No.	Treatments	Total amino acid content (mg/g)
1	S1T1	59.30
2	S1T2	131.19
3	S1T3	119.35
4	S1T4	118.71
5	S2T1	67.42
6	S2T2	105.59
7	S2T3	99.83
8	S2T4	93.75
9	Forest sample	18.34

compared to forest sample. Among various treatments in domestic environment, treatment S1T2 recorded highest amino acid content of 131.199 mg/g followed by S1T3 (119.359 mg/g) and S1T4 (118.719 mg/g).

4.4.6. Soxhlet extractables

From the estimation it was observed that there was not much variation in the soxhlet extractables among the domestic and the wild samples. Results are presented in table 21.

The data pertaining to the various phytochemical constituents in *Nervilia aragoana* were statistically analysed and the results presented in table 22. In comparing the wild and domestic samples, significant differences were found in the content of starch and total amino acid. Starch content was higher in wild sample whereas amino acid content was significantly higher in domestic samples.

While comparing the two shade levels in the domestic crop, there were no significant differences in the contents of biochemical constituents except the content of total soluble sugars. Under 50 per cent shade level (light intensity of 30,000 lux), sugar content was significantly high compared to 25 per cent shade level (light intensity of 15,000 lux).

4.4.7 Comparison of Biochemical Constituents in the Wild and Domestic Samples

A comparison of biochemical constituents of *Nervilia aragoana* tubers in the wild and domestic environment is presented in table 22. The comparison is also made with two shade levels in the domestic environment. With respect to the content of chlorophyll a, b, total chlorophyll and soxhlet extractables there were no significant differences. Soluble sugars differed significantly between the wild and domestic samples and also between the two shade levels. Content of starch, proteins and total amino acids also differed significantly between the wild and domestic situation whereas it was on par with each other between the two shade levels in the domestic crop.

Table 21. Soxhlet extractables of *Nervilia aragoana* Gaud. under different shade levels and under growing media

Sl. No.	Treatments	Soxhlet extractables (%)
1	S1T1	5.9
2	S1T2	8.5
3	S1T3	6.3
4	S1T4	8.2
5	S2T1	8.4
6	S2T2	5.65
7	S2T3	7.4
8	S2T4	8.05
9	Forest sample	8.5

Table 22. Comparison of biochemical constituents of *Nervilia aragoana* Gaud. in the wild and domestic crop.

Sl. No	Biochemical constituents	Domestic sample		Forest sample	t value		
		S1	S2		S1 v/s F	S2 v/s F	S1 v/s S2
1	Chlorophyll a	0.90	1.21	1.00	0.341 NS	1.864 NS	0.967NS
2	Chlorophyll b	0.44	0.47	0.40	0.970 NS	1.192 NS	0.485NS
3	Total chlorophyll	1.67	1.68	1.41	2.519 NS	1.639 NS	0.027NS
4	Total soluble sugar	11.91	6.21	14.5	1.023	7.643 **	2.067 *
5	Starch	143.97	112.3	482.27	7.900 **	20.722 **	0.681NS
6	Total amino acid	107.14	91.65	18.34	5.481 **	8.696 **	0.848NS
7	Protein	22.57	17.90	11.13	2.272	3.275 *	0.859NS
8	Soxhlet extractables	7.2250	7.3750	8.5	1.939NS	1.841 NS	0.167NS

NS – non significant

** - significant at 1% level

* - significant at 5% level

4.4.8. Thin Layer Chromatography for Sugars

In the thin layer chromatographic profile obtained with ten standard sugars, seven sugars were detected from the tubers of *Nervilia aragoana*. Both the wild and domestic tubers had seven sugars, but the profile was different. Wild tubers lacked D- fructose while domestic samples lacked D- mannose. Results are presented in table 23. The tubers of *Nervilia aragoana* were found to contain sugars like dextrose, D-fructose, D-galactose, lactose, D-maltose, D- mannose, D-ribose and sucrose.

4.4.9. Thin Layer Chromatography for Amino Acids

The profile of amino acids taken with eleven standard amino acids showed that five were present in both the wild and domestic samples. L- Asparagine, L- Glutamic acid, Glysine, Histidine and L- Isoleucine were present in both the wild and domestic tuber samples (Table 24).

4.4.10. Conclusion of Biochemical Studies

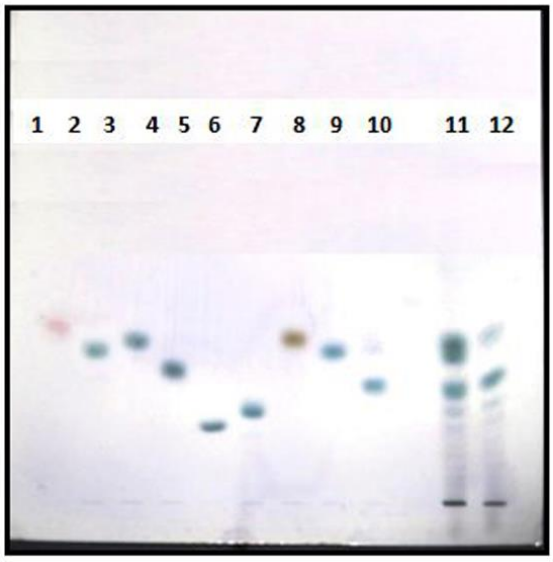
From the biochemical analysis of tubers, it was found that there was significant difference in the content of various biochemical constituents. Soluble sugar, amino acid and protein contents were highest in domestic samples compared to wild samples. There was slight variation in the sugar profile of wild and domestic samples, while the amino acid profiles were the same in domestic and forest samples. Hence it could be concluded that domestication did not affect the biochemical constituents of *Nervilia aragoana*. On the other hand, in certain parameters, the domestic crop recorded high values of the target compound.

4.5. ANATOMICAL STUDIES

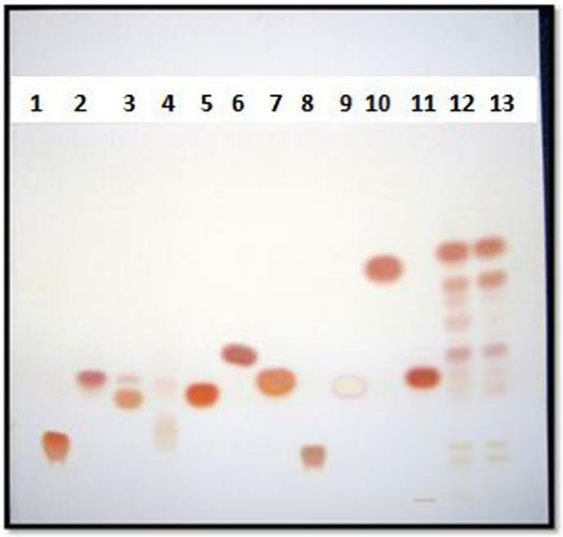
Results of anatomical studies are presented in plate 16.

4.5.1. Staining

Cross section of tuber stained using Safranin, Acetocarmine and double stained using Safranin and Fast green is presented in plate 16. When stained with



(A) Thin layer chromatogram of soluble sugars. 1-10 Standard sugars, 11- Forest sample, 12- Domestic sample



(B) Thin layer chromatogram of total free amino acids. 1-11 Standard amino acids, 12- Forest sample, 13- Domestic sample

Plate 15. Thin layer chromatography

Table 23. TLC profile of sugars in the tubers of *Nervilia aragoana* Gaud.

Sl. No.	Sugars	Rf value	Wild	Domestic
1	Arabinose	0.46	-	-
2	Dextrose	0.39	+	+
3	D – Fructose	0.42	-	+
4	D – Galactose	0.34	+	+
5	Lactose	0.19	+	+
6	D – Maltose	0.25	+	+
7	D – Mannose	0.46	+	-
8	D – Ribose	0.40	+	+
9	Sucrose	0.30	+	+
10	D - Xylose	0.52	-	-

- : absent

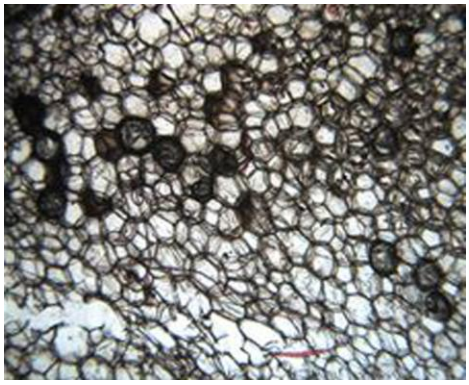
+ : present

Table 24. TL C profile of amino acids in *Nervilia aragoana* Gaud.tubers

Sl. No.	Amino acids	Rf value	Wild	Domestic
1	L – Arginine	0.15	_	_
2	L – Aspartic acid	0.33	_	_
3	L – Asparagine	0.26	+	+
4	L – Cysteine	0.17	_	_
5	L – Glutamine	0.29	_	_
6	L – Glutamic acid	0.38	+	+
7	Glysine	0.31	+	+
8	Histidine	0.13	+	+
9	L - Isoleucine	0.62	+	+
10	Hydroxy L proline	0.34	-	-
11	Serine	0.35	-	-

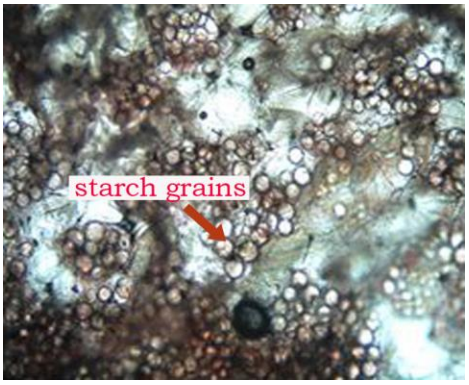
- : absent

+ : present



(4x)

(A)



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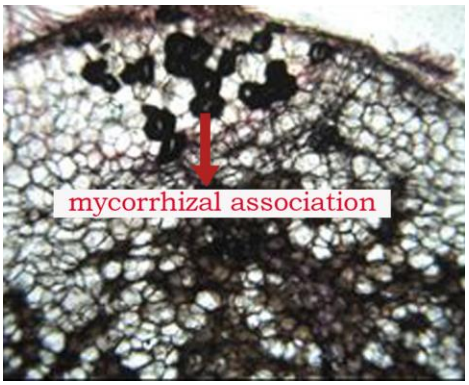
(B)



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(C)

Plate 16. Tuber anatomy (A) Parenchymatous ground tissue (B) Starch grains (C) Mycorrhizal association

safranin it was found that cross section was nearly circular with circular wavy outline. The epidermis was uniseriate occasionally provided with unicellular hairy absorptive extension. The ground tissue was parenchymatous and it was polygonal in shape and was richly packed with starch grains.

When stained using acetocarmine, the starch grains were clearly visible as light purple globules filled in the parenchymatous cells. A few cells contained bundles of calcium oxalate crystals also. Mycorrhizal associations were characteristically evident in some of the cells of the ground tissue as black coloured structures containing mycelial growth. The vascular bundles were seen scattered and did not have a regular pattern. Xylem and phloem cells were visible. Each bundle contained xylem vessels and a small group of phloem cells. No specialized structures were observed when double stained using safranin and fast green.

Discussion

5. DISCUSSION

The results of the study entitled "Habitat analysis and domestication studies on "Orilathamara" (*Nervilia aragoana* Gaud.)" are discussed in this chapter.

5.1. EXPT.I. EXPLORATION AND COLLECTION OF SOURCE PLANTS OF THE DRUG 'ORILATHAMARA' FROM FOREST

In this experiment, forest explorations were carried out to the Peechi – Vazhani wild life sanctuary and source plants of the drug were collected. Information on the species were gathered from the tribal herb gatherers of the area who use to collect it for drug purpose. Miniraj (1997) has reported *Nervilia aragoana* as one of the drug plants collected by the Malayans of Peechi – Vazhani wild life sanctuary. But, now after14 years, the collection has come down substantially, and only very few people gather it from the forest, that too on demand from the traders. The natural population of "Orilathamara" has decreased over years and this, along with other factors like habitat destruction; over collection etc seem to be the main reasons for the limited availability of the drug plant.

Through frequent forest explorations, four species of *Nervilia* could be located in the forest, but all with restricted distribution only. Botanical identification of the species was done by verifying the voucher specimens at Kerala Forest Research Institute, Peechi. Plate 1 shows the *Nervilia* Species collected during the study. In the genus *Nervilia*, four species have already been reported from Kerala forest by Sasidharan (2006). They were *Nervilia aragoana, Nervilia plicata, Nervilia cruciformis and Nervilia infundibulifolia*. Joseph and Mukkuattu (2007) have also reported the distribution of *Nervilia plicata* in the deep forests of Kerala. The four species collected during the study are described below.

***Nervilia aragoana* Gaud.**

Nervilia aragoana was a terrestrial orchid with a single lotus – like leaf raised on a long stalk with subglobose white fleshy tubers which is the officinal part. Leaf appeared after the flowers withered away. Long petioled, leaf was 10 – 20 cm long, orbicular or subreniform, cuspidate, base cordate, 13 or more ribbed, margin wavy, coarsely dentate and 10 – 20 cm diameter. Inflorescence was a many – flowered raceme, sepals pale green, veined with purple. Linear lanceolate, acute, petals narrower, lip white veined with purple, tinged with yellow near the base and three lobed. This description is in conformity with the one given by Sivarajan and Balachandran (1997).

***Nervilia plicata* (Andr.)**

In this species, leaves were ovate – orbicular, rounded at base, margin crenate, pubescent to 8 cm across, green with dark blotches. Scape was 12cm long. Flowers 2.5cm long, bracts subulate, 4mm long, sepals and petals were linear oblong acuminate, 2.2 × 4mm, pale brown. Lip was obscurely three lobed, smaller than petals, white or pale purple with many dark purple veins and a white median ridge. This species has been described earlier by Sasidharan and Sivarajan (1996).

***Nervilia crociformis* (Zoll. & Moritzi)**

In this species tubers were whitish, subglobose to ovoid. Leaves were abaxially green with fine white reticulate venation, cordate or polygonal, slightly fleshy, adaxially sparsely setilose throughout with seven main veins, base cordate and undulate. Apex of leaf was acute. Flowering season and flower characters could not be studied in this species.

***Nervilia infundibulifolia* (Blatt. & McCann)**

It is known as funnel – leaf *Nervilia*. It was a miniature sized, terrestrial orchid with a slightly flattened tuber. The tuber produced tall straight stem with two internodes and linear oblanceolate pointed wide sheath. Leaves were broadly

heart – shaped, funnel shaped with crimped margins, minutely toothed, 5-9 veined, gradually narrowing below into the stalked base. Flowering season and flower characters could not be studied in this species too.

Threat Status

All the four species collected and identified in the study were found to have restricted distribution and hence could be classified as endangered. Over exploitation from the wild and destruction of the natural habitat could be the cause for threat. In one of the natural habitats, presence of *Chromolaena odoratum*, an invasive weed species was recorded which is a clear indication of the disturbance in the natural ecosystem. Moreover, as tubers are the officinal part in *Nervilia*, only destructive harvesting is possible. This could also have contributed to the disappearance of these species from the wild.

The genus *Nervilia* has been reported as rare/ endangered by Pandey *et al.* (2005) and as endangered in western ghat region by Udayan *et al.* (2007). *Nervilia aragaona* was reported as endangered in Peechi forest by Miniraj (1997). The species *Nervilia nipponica* was reported to be in the critically endangered category by Gale and Kuroiwa (2006).

5.2. EXPT. II. NATURAL HABITAT ANALYSIS

Habitat characteristics of two locations, *Kanjippara* and *Aanakuzhi* areas of the Peechi forest where "Orilathamara" was located were studied in detail. The natural habitats were located at an altitude of 180m – 500m above MSL. Approximate shade level ranged from 50 – 75 per cent. Slope was very gentle and soil type forest loam. Moist deciduous forests constituted the vegetation. The species was a lowest canopy component of moist deciduous forests.

Sasidharan and Sivarajan (1996) have reported that *Nervilia aragona* as fairly common in moist deciduous forests and *Nervilia plicata* and *Nervilia prainiana* as occasional in moist deciduous forests of Kerala.

The species observed in the identified natural habitats were *Nervilia aragoana* and *Nervilia plicata* of which further studies were focussed on *Nervilia aragoana* only. The natural habitat was multi storied, with top layer of trees, middle layer of shrubs and the bottom layer of herbs as under growth. Dominant tree species included *Grewia tiliifolia, Pterocarpus marsupium, Calophyllum calaba, Xylia xylocarpa, Tectona grandis* and *Holarrhena pubescence*. *Helicteres isora* was the dominant shrub. Herbaceous plants associated with *Nervilia aragoana* included *Curculigo orchioides and Piper longum*. All these species are common to the moist deciduous forests (MDF) of Kerala (Sasidharan, 1997). *Chromolaena odoratum* was also present which is not natural to moist deciduous forest. This is an invasive weed species and an indication of the degradation of forest ecosystem by alien weeds. *Nervilia*, being a single leaved ground orchid would succumb to these disturbances fast.

5.2.1. Physico – Chemical Properties of Forest Soil

The edaphic factors of the habitat were studied and the results are presented in table 3. Soil type was sandy clay with soil pH towards neutral. Soil aggregate analysis showed that it was a good structured and stable soil. The fertility status of the soil was high as the major and minor nutrients were present in sufficient quantities. This confirms that the ideal soil which supports good growth of the genus *Nervilia* would be a fertile forest loam soil with neutral pH and larger aggregates. Knowledge of the physico – chemical properties of the soil in the natural habitat would be helpful in the selection of growing media for the domestication and cultivation of any species (Nair, 1992).

5.2.2. Life Cycle of *Nervilia aragoana* in the Natural Habitat

Complete life cycle of *Nervilia aragoana* was studied during the study period. A diagrammatic representation of the sequence of flowering and fruit set is presented in Fig.1. The study period started during March 2010 and it extended up to December 2010. In the forest, flowering of *Nervilia* started during April – May. Immediately after receipt of summer showers, it was the inflorescence that

emerged first. It is understood from the weather data presented in appendix 1 that the quantity of summer showers received during this period was 2.4mm. The summer rains received might have triggered the flower bud initiation. Flower buds emerged from the underground tubers which have been lying dormant under the soil. In all similar corm/rhizome forming species, (*Curcuma, Zingiber, Amorphophallus* etc), if not harvested in the first season after the vegetative phase, flowering is seen to occur during April – May after the receipt of pre monsoon showers (Ravindran, 2003). In ginger and turmeric, inflorescence arises directly from the rhizome and in elephant foot yam it arises directly from the corm.

Flowers were exactly like that of Orchidaceae family. They were bisexual, epigynous, trimerous, outer three perianth lobes (calyx) were equal and pale green and linear lanceolate, acute and the inner three lobes (petals) were smaller than the outer ones. Labellum was white, three lobed about the middle and purple tinged with yellow near the base. Androecium consisted of single fertile stamen which on union with the gynoecium formed the gynostegium. Pollen was present in the pollinia, ovary inferior, tricarpellary and syncarpous. Floral characters of *Nervilia aragoana* have been described earlier by Warrier *et al*. (2000).

Floral characters presented in table 4 shows that inflorescence was about 17cm in length with an average of ten flowers. Flowering started from bottom upwards and first flower opened after 3-4 days after the emergence of the inflorescence. Flowers remained fresh for 5-7 days after which it withered. Fruit set started with the last week of May. Fruit was a dehiscent capsule with numerous white powdery seeds.

5.2.3. Seed Germination

The seed germination in orchid is a complex process. A single capsule produces several millions of seeds, however the percentage of germination and

number of plants developed are very low due to lack of any functional endosperm (Nair, 1982).

Like other orchid seeds, in *Nervilia* also seeds were innumerable and tiny. During May – June all the capsules dehisced releasing the powdery seeds. Eventually the inflorescence also dried up and withered. Seeds did not germinate under natural conditions. Various workers have noticed the difficulty in natural regeneration of orchid seeds due to multiple obstacles in germination and development (Gale *et al.*, 2010).

Bernard (1899) reported that the process of germination in orchids proceeds symbiotically in nature, with the association of some root fungus and asymbiotically in aseptic conditions. He found that the fungal infection was necessary for germination. Some of the fungi isolated from orchid roots included *Rhizoctonia, Corticium, Armillaria, Phytophthora, Pencillium, Aspergillus and Trichoderma*. In the present study also, even though seeds were produced in millions, none was found to germinate in the natural condition. Further studies are required to find out the reasons.

5.2.4. Regeneration in the Forest

Diagrammatic representation of the regeneration of the species in the forest is given in Fig. 2 and the monthly sequence in table 6. After withering of inflorescence, approximately after one week, leaf started emerging. Single leaf emerged from the underground tuber. Leaf emergence coincided with the month of June. Quantity of rainfall received during the period as given in appendix 1 is 49.3mm. This indicates that south west monsoon showers triggered regeneration and further growth of the species. The plant grew fast and by the month of December, with the receding of the North East Monsoon, the leaf turned yellow and dried up eventually. Tubers entered into the dormant phase which continued until the next summer rains.

There is also a similar finding by Sudhadevi (1992) who observed that pseudo bulbs of *Habenaria latilabris*, another medicinal orchid remained dormant

under soil for six months and then sprouted into a whole plant. Miniraj *et al*. (2010) reported the same regeneration pattern in the lithophytic orchid, *Seidenfia rheedii* whose pseudo bulbils go to dormant phase in summer and regenerate with the beginning of south west monsoon. This is also the growth pattern of most of other rhizomatous species like *Curcuma, Zingiber* etc when retained as perennials.

In short, the pattern of growth and flowering observed in *Nervilia aragoana* in the present study resembles that of herbaceous perennials like ginger, turmeric, elephant foot yam etc. which are classified as annuals from the cultivation point of view.

Observations on morphological characters of *Nervilia aragoana* revealed that the plants in the natural habitat were larger in size with increased leaf size, leaf area and petiole length compared to those in the domestic environment (tables 5 and 25). It is generally observed that most of the species behave differently in its growth pattern when taken out of its natural habitat. The domestic environment cannot always provide exactly similar situation as that of the wild environment. That could be the reason for enhanced growth in forest. Moreover, the soil analyses data given in table 3 also justify the stability and fertility status of forest soil which also might be responsible for better growth in the forest. Miniraj and Nybe (1999) have reported about the robust growth of select species of medicinal plants viz. *Naravelia zeylanica* and *Barleria prattensis* inside the forest, compared to the domesticated condition.

5.3. EXPT. III. DOMESTICATION TRIAL

5.3.1. Tuberisation and Tuber Development in *Nervilia aragoana*

Tuberisation and tuber development were studied during the cropping season in a separate set of plants maintained in the domestic environment. Results are presented in table 7 and Fig 2. It was observed that after 60 days of planting, runners started developing from the base of the plant. During the subsequent months, their length increased and after 90 DAP side tubers started developing at the distal end of runners. The runners elongated further and side tubers increased

in size. Weight of the side tuber was 4g and 2.5g respectively for the left and right one at 120 days after planting. There was maximum development of the runners (16.5 to19 cm length.), at 180 days after planting, and by this time the side tubers also attained the size of main tuber. At the end of vegetative phase, the leaf also started yellowing and got withered subsequently. This stage was reached 180 DAP and harvesting was done to collect the tubers.

Understanding on the tuberization and tuber development pattern is essential to formulate the package of practices of a tuberous species. Spacing, manuring, earthing up and harvesting packages require prior knowledge on these aspects. In the present study, the total duration of the crop was six months. Tuber formation started three months after planting and hence, top dressing of manures two months after planting would be beneficial to get more tuber yield. There was no additional supply of nutrients as top dressing, in the present experiment. So supply of additional nutrients could still improve the tuber size and yield.

Significance of days to tuberisation has been emphasised by Abraham (2002) and Shinoj (2003) in *Solenostemon rotundifolius* accessions. They found that number of days to tuberization was negatively correlated with tuber yield.

5.3.2. Standardisation of Shade Level and Growing Media

The growing media, nutrition and shade level play a vital role in the cultivation of orchids. Successful cultivation of orchids depends on selection of an ideal growing medium with optimal nutrient concentration and shade. In this study, the attempt was to standardise the shade level and growing media that supports growth and tuber production in *Nervilia aragoana*.

Shade levels tried in the present study were 50 and 25 per cent and the four growing media tried were:

T1 – FYM + sand + soil (1:1:1)

T2 – leaf compost + sand + soil (1:1:1)

T3 – coir pith compost + sand + soil (1:1:1)

T4 – vermi compost + sand + soil (1:1:1) .

5.3.2.1. Physico – chemical properties of growing media

The growing media tried were analysed for the physico- chemical properties and the results are presented in tables 8 and 9. Data revealed that pH of all the media was in the acidic range whereas the forest soil was neutral in reaction (table 3). MWD was maximum for T1, the media containing FYM, closely followed by T4 and T2. MWD value was lowest for T3 media containing coir pith compost. In comparison with forest soil which recorded a MWD value of 1.107 (table 3) the three growing media which recorded higher values of MWD possessed better structure and stability, ideal for tuber forming species like *Nervilia*. Data given in tables 3 and 9 indicate that the macro and micro nutrient status of the media were also comparable with the forest soil. Among the four growing media tried, T4 (vermicompost + sand + soil) recorded the highest values for organic carbon and Nitrogen followed by T1. With respect to P, K, Ca, Mg and S, T1 media recorded highest values. Micro nutrient status was sufficient in all the growing media tried. In short, all the four growing media provided the necessary nutrients required for the growth and tuber development in the plant. Media T1 appeared to be the ideal one taking into consideration both the physical as well as chemical properties. Nair *et al*. (1992) suggested that factors like soil properties, weather, irrigation methods and manuring should be taken into consideration at the time of selecting a drug plant for cultivation.

5.3.3. Influence of Weather Parameters on Growth of *Nervilia aragoana*

Weather data recorded during the study period and presented in appendix 1 was correlated with growth and development in the domestic crop. Planting was done during third week of June and during this period the average rainfall received was 45.3 mm. Sprouting initiated two weeks after planting and during that time rainfall was 49.3 mm. The low, but well distributed rainfall obtained at the time of planting might have triggered the tubers in the dormant phase to put

forth sprouts. Good rainfall was obtained during the whole cropping period till the crop attained full growth. Harvesting was done in the month of December when the plant entered into the senescence phase. Rainfall received during that period was only 5.1mm. From the growth and development it is seen that *Nervilia aragoana* is a rainfall dependent species. From planting to harvest it requires well distributed rainfall coupled with a temperature range of 22^0C to 31^0C and a relative humidity ranging from 55 to 95 per cent. Similar results were obtained in the case of *Seidenfia rheedii* another medicinal orchid by Miniraj *et al*. 2009. They observed that the spatial and temporal distribution of rainfall and not the total rainfall is critical for highly rainfall dependent species like *Seidenfia rheedii*.

5.3.4. Influence of Shade Level and Growing Media on Morphological Characters of *Nervilia aragoana.*

Data presented in table 10 and Figs. 3 and 4 show that there were no significant differences in sprouting percentage, days to leaf emergence and days to complete unfurling of leaves and total biomass production in *Nervilia aragoana* under various treatments. In *Nervilia*, sprouting, leaf emergence, leaf opening etc. happen within one month and this short period is insufficient to have the influence of treatments imposed. Total biomass production seemed to be not influenced by the environmental conditions and the growing media provided.

The information given in table 11 and Fig.5 revealed that significant differences were there in petiole length, leaf length, leaf width and leaf area among various treatments. This implies that the shade level and growing media provided influenced these growth parameters of the plant. Plants grown under 50 per cent shade level and in growing media comprising of FYM, sand and soil in equal proportions recorded higher values in petiole length, leaf length, leaf width and leaf area.

In terms of light intensity received it comes to about 30,000 lux under 50 per cent shade net and 15,000 lux under 25 per cent shade net on the assumption that the total light intensity received on a bright sunny day is 60,000 lux. This

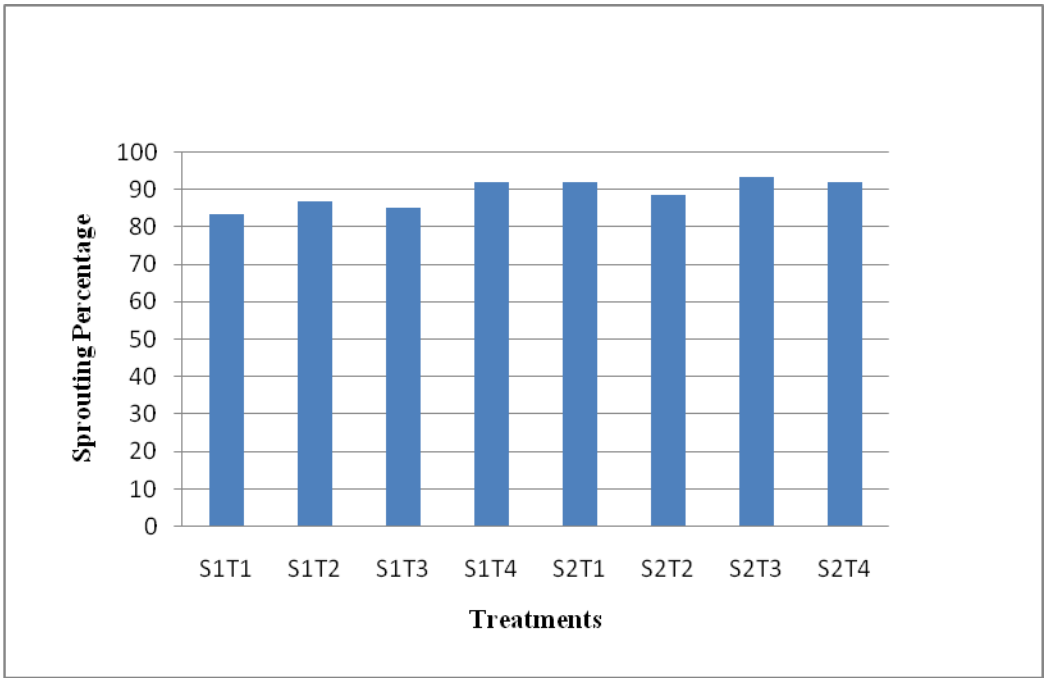

Fig. 3. Influence of shade level and growing media on sprouting percentage

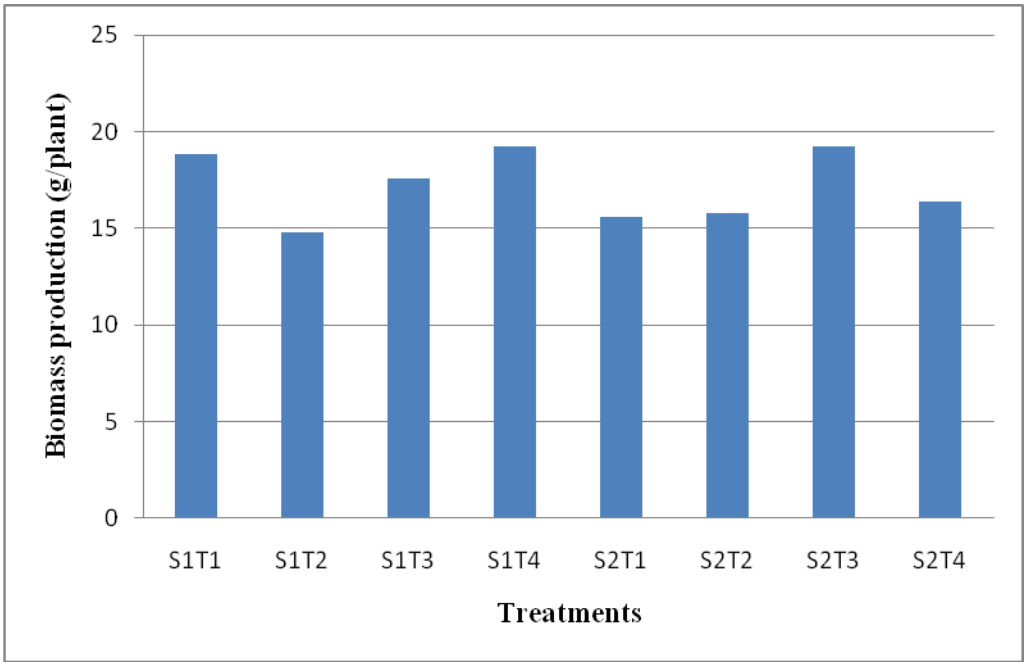

Fig. 4. Influence of shade level and growing media on biomass production

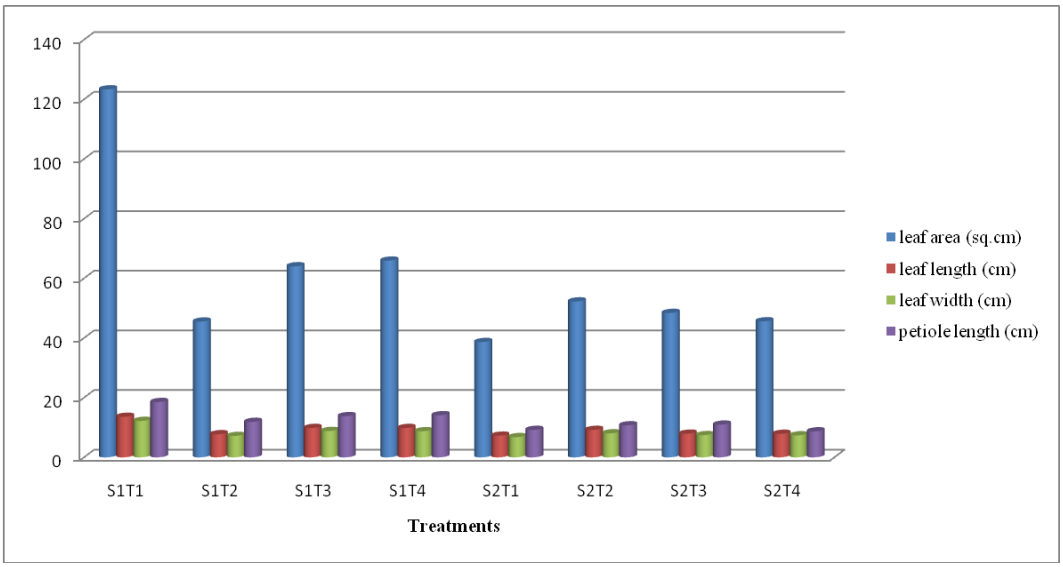

Fig. 5. Influence of shade level and growing media on leaf area, leaf length, leaf width and petiole length of *Nervilia aragoana*

indicates that for terrestrial orchids like *Nervilia*, a filtered light intensity of 50 per cent (30,000 lux) is needed for optimum growth. From the natural habitat analysis also it was understood that the species grew best under 50 to 75 per cent shade level and it was proved true by the response of the crop under 50 per cent shade level in the domestication trial.

George and Mohanakumaran (1998) reported an increase in plant height, number of leaves and leaf area of *Arachis* orchid c.v Red ribbon at 50 per cent shade level.

Miniraj and Nybe (1998) have reported that quantum of radiation flux received is a major contributing component which ensured maximum quality in *Piper longum* in the moist deciduous forests of Kerala.

Similar results were obtained in another medicinal orchid by Miniraj *et al*. (2007). In their study on comparison between open condition and 50 per cent shade level in domestication studies on *Seidenfia rheedii*, crop growth was better under 50 per cent shade level.

In another study conducted by Pavithra (2008) in *Phalaenopsis*, it was observed that there was significant variation in leaf length, leaf width and leaf area at 50 per cent shade level when compared with 25 and 75 per cent shade level and she concluded that the most suitable shade level conducive for growth of this orchid was 50 per cent.

Miniraj *et al*. (2010) had reported that 50 per cent shade was ideal in *Rauvolfia serpentina* for better growth and root yield.

5.3.5. Influence of Shade Level and Different Growing Media on Yield Parameters of *Nervilia aragoana.*

Single plant yield obtained under various treatments is presented in table 12. There was wide variation in single plant yield under various treatments (7 to 27 g). Highest single plant yield was obtained from treatment S1T1, followed by S2T2 and S2T4. Lowest single plant yield was obtained in the treatment S2T2.

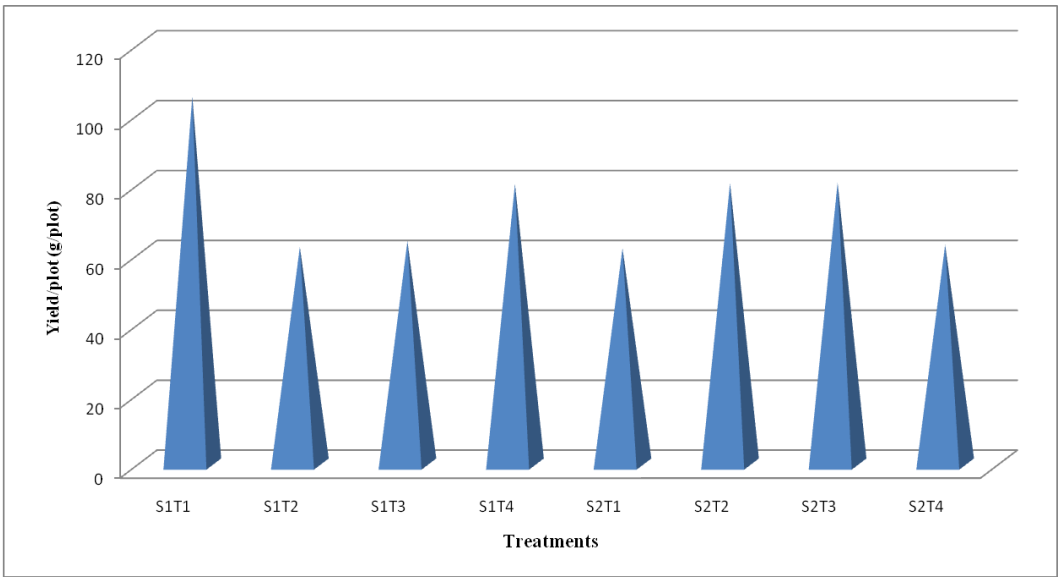

Fig. 6. Influence of shade level and growing media on tuber yield (g/plot) of *Nervilia aragoana*

Per plot yield is a better indicator of yield compared to per plant yield in herbaceous plants and hence the medium which gave highest per plot yield was counted as the best medium. It is evident from table 13 and Fig 6 that 50 per cent shade level and T1 medium containing FYM, sand and soil in equal proportions gave maximum yield of tubers per plot. Number of tubers produced is the main parameter that contributes to final yield and in the study, higher production of medium and large sized tubers was recorded in the same medium. Among the medium tried, the structure, texture, stability and nutrient status were best in T1 media (tables 8 and 9) and all these would have contributed to the better growth and tuber yield. The next best medium was T4 which contained vermi compost + sand + soil in equal proportions. Here again 50 per cent shade level was best compared to 25 per cent shade level.

The results obtained in the study (table 14 and Fig. 6) revealed that number of tubers differed under various treatments and the maximum number of tubers was obtained in the treatment S1T1. Number of medium and large sized tubers was also more in the same treatment. This implies that among the four growing media, the one found ideal for tuber production of *Nervilia aragoana* was T1 ie, FYM, sand and soil in equal proportions.

Similar results were obtained in other medicinal plants by earlier workers also.

Sudhadevi (1992) reported that among the different media tried, potting mixture in the ratio 2:1:1 sand, soil and powdered cow dung was identified as the best for the medicinal orchid, *Habenaria latilabris*.

Miniraj *et al.*(2007) reported that the growing medium that was found best for *Seidenfia rheedii*, a terrestrial medicinal orchid included sand, soil, well rotten FYM and vermi compost in equal proportions.

Bose and Bhattacharjee (2008) reported that cow dung manure is mostly used in Asian countries for growing orchids. It releases lot of nutrients as it decomposes and is good in water retention capacity.

Table 25. Comparison of growth and yield characters of *Nervilia aragoana* in wild and domestic environment

Characters	Wild plants	Domestic plants
Petiole length	25.91cm	13.95cm
Leaf length	11.46cm	10.33cm
Leaf width	11.92cm	9.65cm
Leaf area	119.65cm^2	79.75cm^2
Tuber weight	12.5g/plant	12.29g/plant

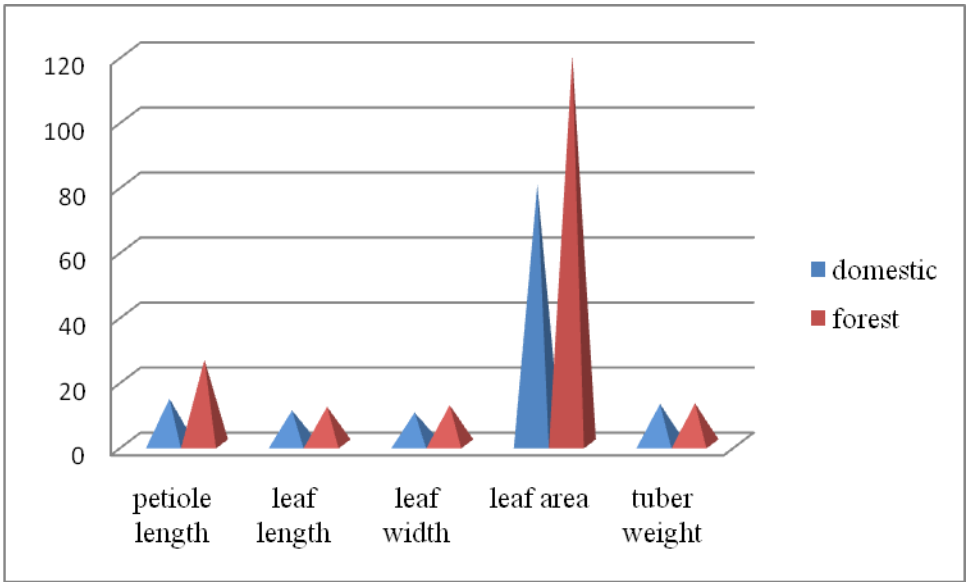

Fig. 7. Comparison of growth and yield parameters of *Nervilia aragoana* in wild and domestic environment

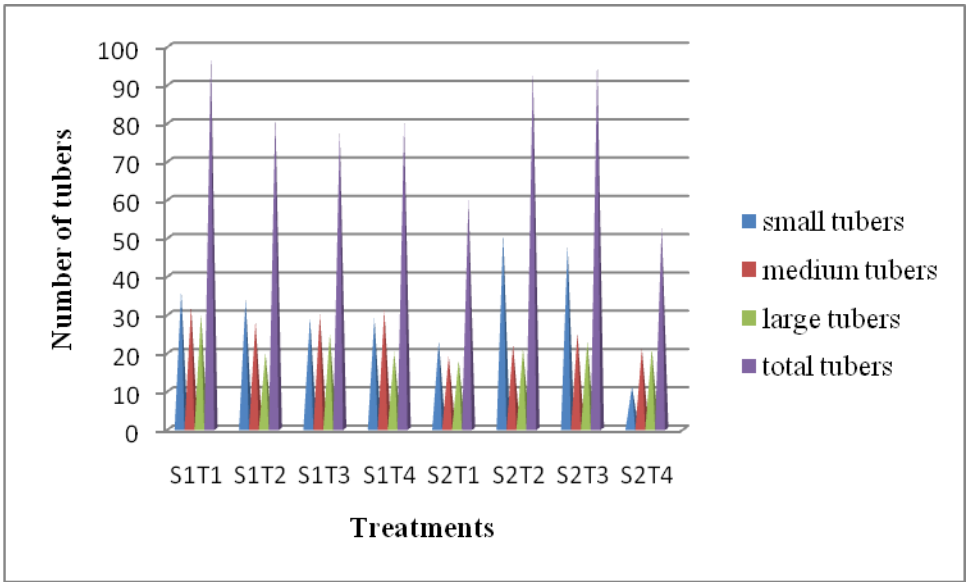

Fig. 8. Number of tubers of *Nervilia aragoana* under different treatments

Satyanarayana (2010) reported that the potting media used for proper establishment and multiplication of terrestrial orchids included a mixture of two parts of loam and one part of leaf mould and one part of a mixture of equal amount of brick pieces and charcoal.

5.3.6. Impact of Domestication on Growth and Yield

The morphological characters viz. petiole length, leaf length, leaf width and leaf area were lesser in the domestic environment compared to the plants observed in the natural habitat (tables 5 and 25). This may be due to the favourable environmental conditions that prevailed in the natural habitat. But the tuber weight was found to be comparable with the forest plants (Table 25).

As in *Orilathamara* underground tubers are the officinal part, interest is in tuber production and the domestication trial gave satisfactory results in the case of tuber production and yield. Approximately about 156 g tubers were obtained from a plot of 1 m^2 area. So it can be concluded that *Nervilia aragoana* responded positively to domestication and can be cultivated successfully under 50 % shade level, in a growing medium comprising of FYM, sand and soil in equal proportions.

5.4. BIOCHEMICAL ANALYSIS

In medicinal plants, quality is equally important as quantity and hence tubers of both wild and domestic crops were subjected to detailed biochemical analysis to assess the impact of domestication on quality. Results are presented in table 16 to 24 and Fig. 9 to 13.

5.4.1. Variation Pattern in Primary and Secondary Metabolites

Fig.10, 11, 12, and 13 depict the variation in content of both primary and secondary metabolites of *Nervilia aragoana* in both the wild and domestic crops.

There was no significant difference observed in the chlorophyll a, b and total chlorophyll content when compared between domestic and wild samples.

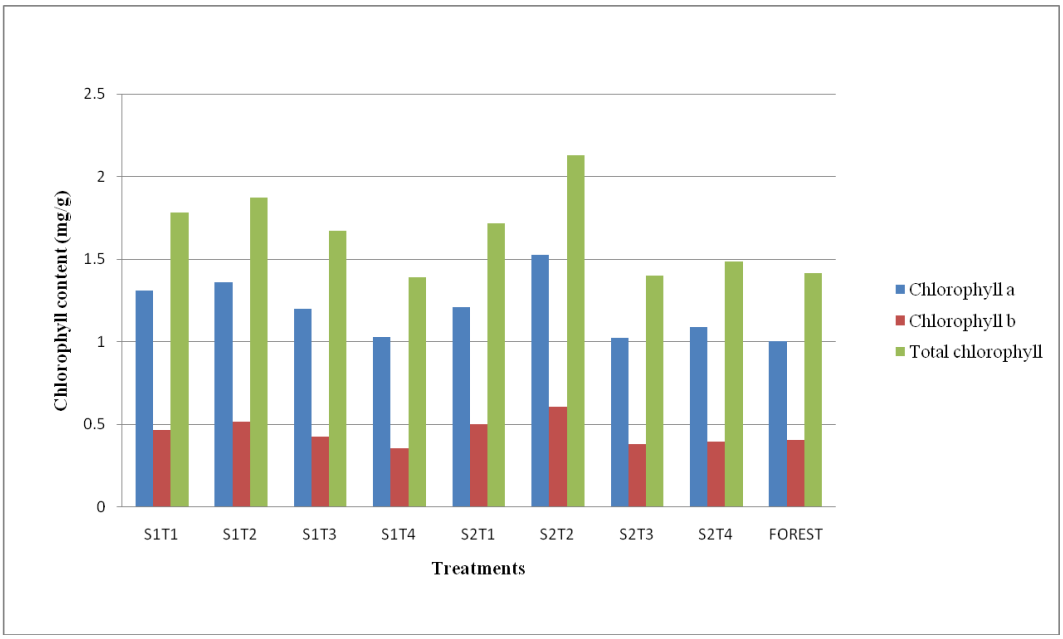

Fig. 9. Chlorophyll content of *Nervilia aragoana* under different shade levels and growing media

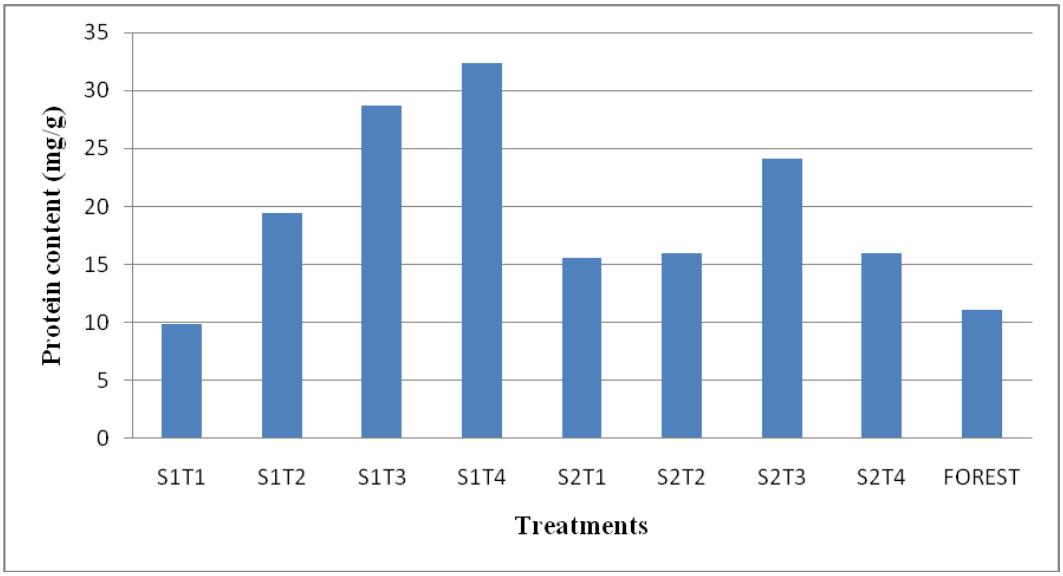

Fig. 10. Protein content of *Nervilia aragoana* under different shade levels and growing media

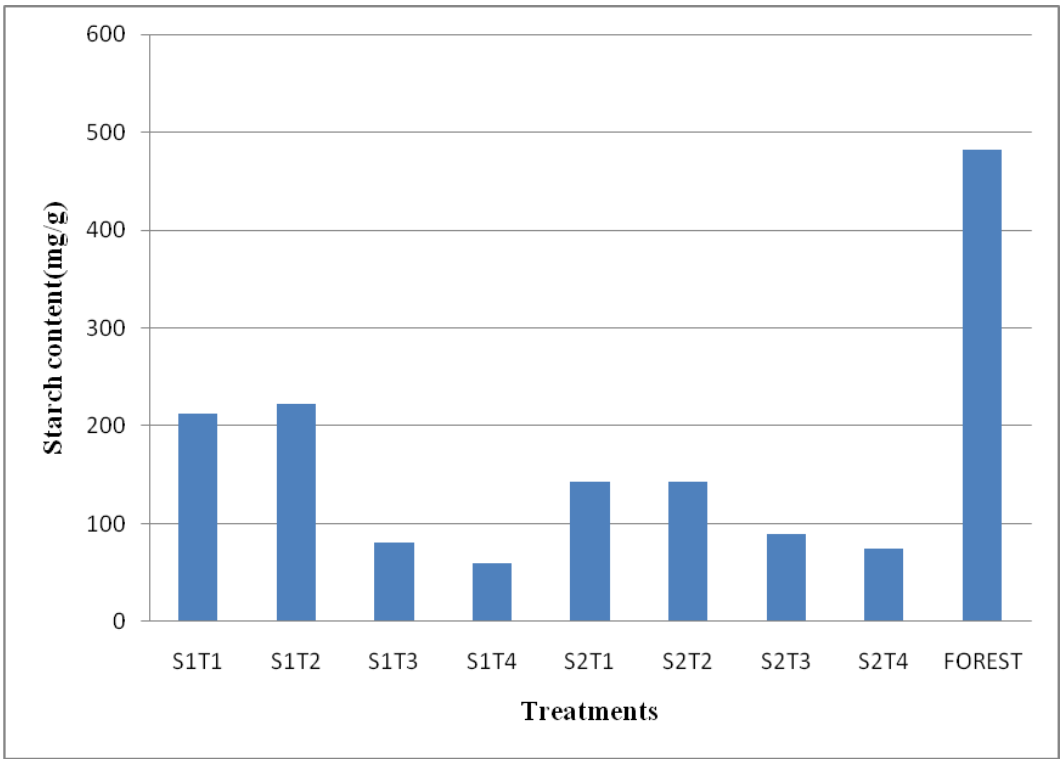

Fig. 11. Starch content of *Nervilia aragoana* under different shade levels and growing media

The domestic crop invariably had high protein content compared to the wild tubers (Fig.10). Among the various treatments in the domestic crop, higher protein content was recorded in treatment S1T4 (vermi compost + sand + soil) followed by S1T3 (coir pith compost + sand + soil). Similarly data given in table 20 revealed that the domestic crop had 6 to 7 times more amino acid content than the forest sample. Among the various treatments in the domestic environment, treatment S1T2 (leaf compost + sand + soil) recorded the highest amino acid content followed by S1T3 and S1T4.

In the case of soluble sugar content, domestic treatment S1T1 (FYM + sand + soil) recorded the highest sugar content which was slightly higher than the wild sample. The next higher value was recorded by treatment S1T2, which was almost same as that of wild sample (Table 19).

On the contrary, starch content of wild tuber was almost 2 -3 times more in the wild sample compared to the domestic crop (Fig.11). In the domestic crop again, treatment S1T2 (leaf compost + sand + soil) recorded highest values for starch content followed by treatment S1T1 (FYM + sand +soil).

There was no significant difference in the soxhlet extractables present in *Nervilia aragoana* under wild and domestic environment conditions which means that the domestication did not influence the content of crude extractables of the species (Table 21).

Soluble sugars are the initial products in photosynthesis. Plants always maintain equilibrium of soluble sugars in the source and whenever the concentration exceeds it is either converted to polysaccharides, starch or interconverted to other primary products or translocated to other organs for the synthesis of secondary metabolites. Once it is in the form of starch further transformations are slow or limited.

Generally sugars are produced in the leaves and accumulated in the roots and tubers. Since sugar is the base for the synthesis of starch, the quantity of sugar has direct relation with the starch content.

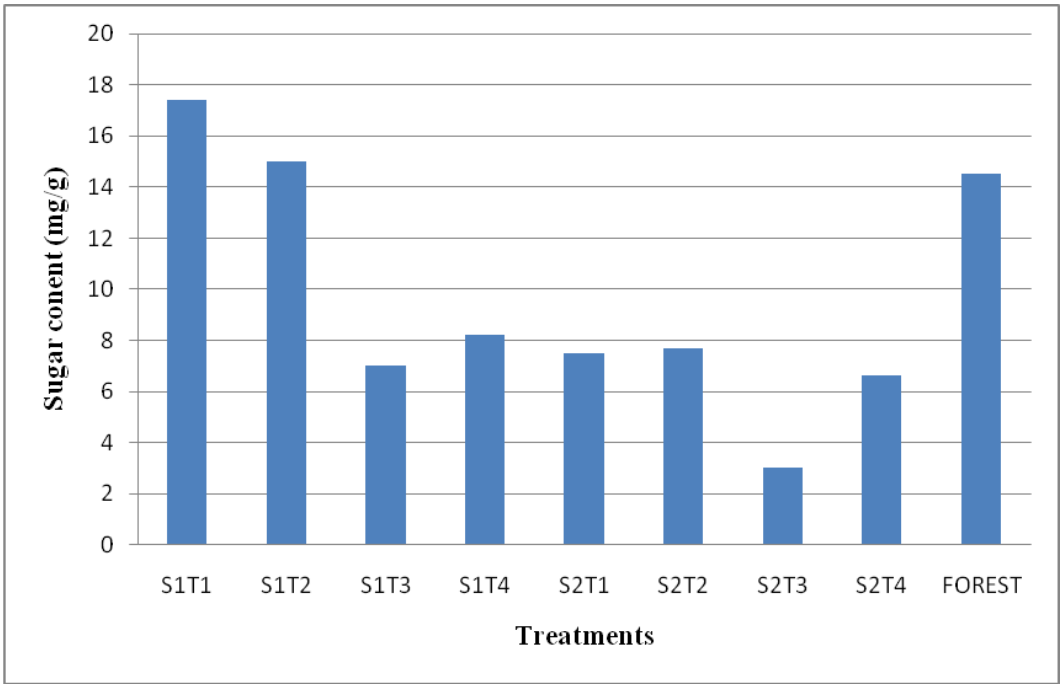

Fig. 12. Sugar content of *Nervilia aragoana* under different shade levels and growing media

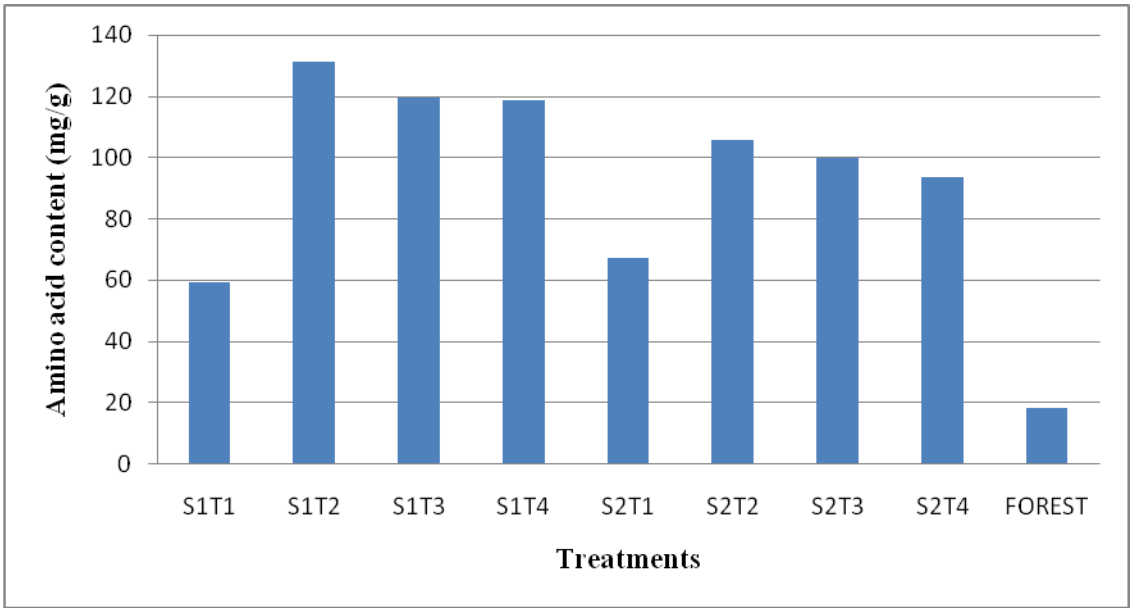

Fig.13. Amino acid content of *Nervilia aragoana* under different shade levels and growing media

In the present study, domestic plants contained more soluble sugars, protein and amino acids than the wild. Status of a monocrop, coupled with plentiful resources including water, light and nutrients, might have contributed to increased photosynthesis, resulting in more production of primary metabolites except starch. In the forest, on the contrary, competition for resources and the prevailing a biotic stresses might have influenced negatively the synthesis of proteins and amino acids. Higher starch content in the wild sample indicates the conversion of sugars to starch.

Similar results were reported in other medicinal plants also. In *Datura stramonium*, Chandrasekharan *et al.* (1984) reported that the scopolamine content of cultivated plants were twice as that of wild samples and fluctuated between 0.126 and 0.309 per cent.

Narayanan (1993) observed variation in the root alkaloid content ranging from 1.38 to 2.05 per cent in *Rauvolfia serpentina* from nine geographical locations of Kerala. He opined that the alkaloid content is a highly complex phenomenon involving environmental factors, species difference and their interaction.

Miniraj *et al.* (2000) found that in select species of medicinal plants-*Desmodium, Barleria, Sida, Baliospermum* and *Naravelia,* the plants in the domestic environment contained more total free amino acids compared to those in the wild.

Samuel *et al.* (2008) compared the protein and alkaloid content of *Holostemma annulare* under domestication and wild conditions. They found that the protein and alkaloid contents were high in the tubers obtained from domesticated plots.

5.4.3. Thin Layer Chromatographic Profile of Sugars and aminoacids

TLC profile of sugars in the tubers of *Nervilia aragoana* (Table 23) showed the presence of seven sugars in the wild and domestic samples. The wild

tubers lacked D- fructose while domestic samples lacked D – mannose. In *Seidenfia rheedii*, a rejuvenating drug, both the wild and domestic samples contained lactose, sucrose, fructose and mannose out of which, sucrose, fructose and mannose were high in the domestic crop compared to the wild (Miniraj, 2011).

TLC profile of amino acids taken with eleven standards (Table 24) showed the presence of five in both the wild and domestic crop. This shows that domestication did not alter the constituents present in the plant significantly.

Bhogaonkar and Devarkar (2007) had also reported the presence of amino acids like D – Alanine, Citruline, Valine, Iso – Leucine, glutamic acid and aspartic acid in *Nervilia aragoana*. Presence of eight amino acids viz. DL-Alanine, DL-Aspartic acid, DL-Methionine, L-Proline, L-Arginine Monochloride, DL-Valine, DL-Serine and DL-Phenyl Analine were reported in the pseudo bulbils of *Seidenfia rheedii* by Miniraj (2011). In this medicinal orchid also domestication did not influence the amino acid profile as well as content.

5.4.5. Conclusion of Biochemical Studies

Under domestication outside the normal habitat or ecological range, many of the medicinal plants tend to behave differently. In the present study however, domestication did not alter the biochemical constituents in a big way. On the other hand, soluble sugars, protein and amino acids were high in the domestic crop. Higher contents of sugars and free amino acids are advantageous to medicinal plants, especially in plants used as rejuvenating drugs, tonics, adaptogens etc. There are no other secondary metabolite reported in the tuber of *Nervilia aragoana*, which is responsible for its medicinal property and hence it could be concluded that the domesticated crop had the same or better quality, with respect to the constituents studied.

5.5. ANATOMICAL STUDIES

Plates depict the cross section of tubers. The cross section was nearly circular with wavy outline. The epidermis is nearly circular with wavy outline. The parenchymatous ground tissues are richly packed with starch grains. In the acetocarmine stained section, the starch grains were clearly visible as light purple globules. A few cells contain bundles of calcium oxalate crystals. Mycorrhizal associations were characteristically evident in some of the cells of the ground tissue as black coloured structure containing mycelial growth. The vascular bundles are seen scattered and do not have a regular pattern.

Warrier *et al.* (2000) and Bhogaonkar and Devarkar (2007) have attempted anatomical studies of *Nervilia aragoana* and have reported the tuber epidermis as single layered with thin cuticle and unicellular trichomes. They also identified vascular bundles as small, a few and scattered and thin walled polygonal parenchymatous ground tissue harbouring microbial colonies. Such mycorrhizal associations shall be helpful in the formulation of manurial package for the commercial cultivation of "Orilathamara".

Summary

6. SUMMARY

The present investigations on "Habitat analysis and domestication studies on 'Orilathamara' (*Nervilia aragoana* Gaud.)" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2009-2011, with the objectives to collect the source plants of the drug 'Orilathamara' from wild, to carry out detailed natural habitat analysis and to analyze the response of the species to domestication.

The study was conducted in four separate experiments as exploration and collection of source plants of 'Orilathamara' from forest, natural habitat analysis. Domestication trial and biochemical studies.

Results of the study are summarised here, experiment wise.

Peechi-Vazhani wild life sanctuary was selected for plant exploration and natural habitat studies. Four species of *Nervilia* were identified from Peechi forests. They were *Nervilia infundibulifolia* (Blatt & McCann), *Nervilia plicata* (Andr.), *Nervilia crociformis* (Zoll. & Moritzi) and *Nervilia aragoana* Gaud. All the four species were categorised as endangered.

The tribal people in the area seemed to gather only *Nervilia aragoana* for drug purpose. There was no regular collection, only a few people collected it on demand basis.

Natural habitats of 'Orilathamara' were located at an altitude of 180m – 500m above MSL. The species occurred as a lowest canopy component of moist deciduous forests. Approximate shade level ranged from 50% to 75%. Slope was very gentle and soil type sandy clay. pH of the soil was in the neutral range and the mean weight diameter (MWD) value indicated that it was a good structured soil. Fertility status of the soil was high as expressed by the content of macro and micro nutrients.

Complete life cycle of *Nervilia aragoana* was studied in the forest. Immediately after the receipt of summer showers in March, the inflorescence emerged from the tubers. Flowering continued during April – May. Fruits were developed in the last week of May. During May – June the inflorescence dried up and withered away.

Flowers were exactly like that of Orchidaceae family. Fruit was a dehiscent capsule with numerous white powdery seeds. Seeds did not germinate under natural conditions. After withering of the inflorescence, approximately after one week, leaf started emerging. The fully opened leaves remained green till December with the receding of the North East Monsoon, leaves turned yellow and withered away gradually. There after the tubers remained dormant during the summer.

The domestication experiment was laid out as a field trial in the field attached to the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, during May – December. Tuberisation pattern in the species was studied separately in the domestic crop.

Two runners started growing from the main tuber, sixty days after planting. At the distal end of the runners which lengthened as the growth progressed, side tubers increased in size gradually. At the time of harvest *ie* six months after planting, the mother tuber and the side tubers attained almost equal size.

The growing media tried were analyzed for the physico-chemical properties and the results revealed that pH of all the media was in the acidic range. MWD value was maximum in the media containing FYM. The macro and micro nutrient status of the medium was also comparable with the forest soil.

Weather data recorded during the study period was correlated with growth and development of the domestic crop and *Nervilia aragoana* seemed to be a rainfall dependent species. From planting to harvest it required well distributed rainfall coupled with a temperature range of 22^0C to 31^0C and a relative humidity

ranging from 55% to 95%. In the domestication trial, there was no significant difference in the sprouting percentage, days to leaf emergence and days to complete unfurling of leaves and total biomass production under various shade levels and growing media tried.

Significant differences were observed in petiole length, leaf length, leaf width and leaf area among various treatments and plants grown under 50% shade level and in growing medium comprising of FYM, sand and soil in equal proportions recorded higher values in petiole length, leaf length, leaf width and leaf area. Number of tubers was also highest in the same treatment.

There was wide variation in single plant yield under various treatments (7 to 27g). 50% shade level and medium containing FYM, sand and soil in equal proportions gave maximum yield of tubers per plot.

Tubers of both wild and domestic crop were subjected to biochemical analysis to assess the impact of domestication on quality.

There was no significant difference in the chlorophyll a, b and total chlorophyll content between domestic and wild samples and among various treatments in the domestic crop.

In the case of soluble sugar content, domestic treatments S1T1 (50% shade; FYM + sand + soil) recorded the highest sugar content which was slightly higher than the wild sample.

On the contrary, starch content of the tuber was almost 2-3 times more in the wild sample compared to the domestic sample. In the domestic crop, treatment S1T2 (50% shade; leaf compost + sand + soil) recorded the highest values for starch content.

The domestic crop invariably had high protein content compared to the wild tubers. Among the various treatments in the domestic crop, higher protein content was recorded in treatment S1T4 (50% shade; vermicompost + sand +soil). Similarly the domestic crop had 6-7 times more amino acid content than the forest

sample. Among the various treatments in the domestic environment, treatment S1T2 (50% shade; leaf compost + sand + soil) recorded the highest amino acid content.

There was no significant difference in the soxhlet extractables from the tubers of *Nervilia aragoana* under wild and domestic environment and among various treatments in the domestic crop.

In the thin layer chromatographic profile obtained with ten standard sugars, seven sugars were detected from the tubers of *Nervilia aragoana*. They were dextrose, D-fructose. D-galactose, lactose, D-maltose, D-mannose, D-ribose and sucrose. Both the wild and domestic tubers had seven sugars, but the profile was different. Wild tubers lacked D-fructose while domestic samples lacked D- mannose.

The profile of amino acids taken with eleven standard amino acids showed that five were present in both the wild and domestic samples. L-Asparagine, L-Glutamic acid, glysine, histidine and L-isoleucine were present in both wild and domestic tuber samples.

Anatomical studies were also carried out in the tuber. Cross section was nearly circular with wavy outline. The parenchymatous ground tissue was richly packed with starch grains. A few cells contained bundles of calcium oxalate crystals. Mycorrhizal associations were characteristically evident in some of the cells of the ground tissue as black coloured structure containing mycelia growth. The vascular bundles were seen scattered and did not have a regular pattern.

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Appendices

Appendix I. Mean weekly weather data during the study period (March 2009 to December 2010)

Period	Temperature		Rainfall (mm)	Humidity	
	Maximum (^{0}C)	Minimum (^{0}C)		Maximum (%)	Minimum (%)
5/3/'09 - 11/3/'09	35.0	24.3	000.0	084	053
12/3/'09 – 18/3/'09	35.3	23.9	003.0	086	049
19/3/'09 – 25/3/'09	35.1	24.3	000.8	088	051
26/3/'09 – 1/4/'09	35.1	25.2	000.0	089	056
2/4/'09 – 8/4/'09	37.0	25.3	001.2	090	055
9/4/'09 – 15/4/'09	33.4	24.5	000.7	091	060
16/4/'09 – 22/4/'09	33.3	26.0	000.4	089	066
23/4/'09 – 29/4/'09	34.2	26.2	000.0	087	056
30/4/'09 – 6/5/'09	34.4	26.0	000.1	085	056
7/5/'09 – 13/5/'09	34.8	25.3	003.3	088	059
14/5/'09 – 20/5/'09	33.0	24.4	001.6	087	063
21/5/'09 – 27/5/'09	30.8	24.1	020.1	094	074
28/5/'09 – 3/6/'09	31.8	24.4	003.7	091	066
4/6/'09 – 10/6/'09	29.8	23.7	033.6	095	076
11/6/'09 – 17/6 '09	31.1	24.0	006.5	092	067
18/6/'09 – 24/6/'09	29.7	23.5	017.3	095	074
25/6/'09 – 1/7/'09	29.0	23.1	022.9	095	080
2/7/'09 – 8/7/'09	27.1	22.5	031.6	096	089
9/7/'09 – 15/7/'09	28.8	23.2	026.4	095	080
16/7/'09 – 22/7/'09	27.6	22.7	051.0	096	084
23/7/'09 – 29/7/'09	30.7	23.0	005.8	094	069
30/7'09 – 5/8/'09	29.7	23.2	019.0	096	075
6/8/'09 – 12/8/'09	30.9	23.5	004.4	095	074
13/8/'09 – 19/8/'09	30.3	23.5	015.4	095	070
20/8/'09 – 26/8/'09	30.3	22.7	027.1	095	082
27/8/'09 – 2/9/'09	29.1	22.9	015.3	096	077
3/9/'09 – 9/9/'09	28.5	23.2	015.8	096	076
10/9/'09 – 16/9/'09	31.1	23.6	0.000	093	068

Appendix I continued

Period	Temperature		Rainfall (mm)	Humidity	
	Maximum (^{0}C)	Minimum (^{0}C)		Maximum (%)	Minimum (%)
17/9/'09 – 23/9/'09	30.2	23.2	010.1	094	072
24/9/'09 – 30/9/'09	30.5	23.1	007.8	094	072
1/10/'09 – 7/10/'09	29.5	22.5	019.9	095	075
8/10/'09–14/10/'09	32.0	22.5	003.8	094	059
15/9/'09-21/10/'09	32.3	23.0	000.1	095	061
22/10/2009-28/10/'09	33.7	23.9	000.0	091	053
29/10/'09 – 4/11/'09	32.7	25.2	000.0	074	056
5/11/'09 – 11/11/'09	30.4	24.0	018.0	087	083
12/11/'09 – 18/11/'09	31.6	23.8	002.0	090	064
19/11/'09 – 25/11/'09	31.9	23.7	005.7	091	062
26/11/'09 – 2/12/'09	31.8	22.6	000.0	070	054
3/12/'09 – 9/12/'09	31.5	23.2	000.0	078	054
10/12/'09 – 16/12/'09	32.1	23.8	000.0	070	049
17/12/'09 – 23/12/'09	32.2	25.0	000.0	072	052
24/12/'09 – 31/12/'09	31.4	23.8	005.3	072	053
1/1/'10 – 7/1/'10	32.2	22.5	000.0	071	042
8/1/'10 – 14/1/'10	32.6	23.5	000.0	084	054
15/1/'10 – 21/1/'10	32.5	22.9	000.0	073	049
22/1/'10 – 28/1/'10	32.5	21.8	000.0	073	044
29/1/'10 – 4/2/'10	33.2	23.3	000.0	063	039
5/2/'10 – 11/2/'10	33.5	23.1	000.0	066	037
12/2/'10 – 18/2/'10	35.5	23.6	000.0	082	040
19/2/'10 – 25/2/'10	35.8	24.4	000.0	091	043
26/2/'10 – 4/3/'10	36.5	24.1	000.0	092	037
5/3/'10 – 11/3/'10	35.8	25.2	000.0	070	030
12/3/'10 – 18/3/'10	37.0	24.8	000.0	083	044
19/3/'10 – 25/3/'10	36.5	24.6	000.0	089	050
26/3/'10 – 1/4/'10	35.0	25.1	000.0	089	054
2/4/'10 – 8/4/'10	35.5	24.8	004.6	090	063
9/4/'10 – 15/4/'10	34.4	25.9	000.9	091	060

Appendix continued

Period	Treatments		Rainfall (mm)	Humidity	
	Maximum (^{0}C)	Minimum (^{0}C)		Maximum (%)	Minimum (%)
16/4/'10 – 22/4/'10	35.4	25.5	001.0	088	050
23/4/'10 – 29/4/'10	35.1	24.6	004.5	088	057
30/4/'10 – 6/5/'10	34.9	25.4	006.5	087	058
7/5/'10 – 13/5/'10	34.0	25.9	003.7	091	066
14/5/'10 – 20/5/'10	33.4	25.4	004.6	092	069
21/5/'10 – 27/5/'10	31.7	25.7	002.1	093	072
28/5/'10 – 3/6/'10	32.7	25.1	007.8	093	066
4/6/'10 – 10/6/'10	31.4	24.2	002.3	093	079
11/6/'10 – 17/6/'10	28.1	23.3	045.3	097	089
18/6/'10 – 24/6/'10	30.8	23.9	020.7	094	067
25/6/'10 – 1/7/'10	30.2	23.1	028.6	096	076
2/7/'10 – 8/7/'10	28.6	22.8	026.6	096	082
9/7/'10 – 15/7/'10	31.2	24.0	004.7	095	072
16/7/'10 – 22/7/'10	27.8	23.0	023.9	097	084
23/7/'10 – 29/7/'10	29.6	22.3	014.8	095	081
30/7/'10 – 5/8/'10	28.6	22.3	019.2	096	074
6/8/'10 – 12/8/'10	30.6	24.1	004.4	095	073
13/8/'10 – 19/8/'10	29.5	23.0	006.2	094	079
20/8/'10 – 26/8/'10	28.7	23.3	001.9	094	083
27/8/'10 – 2/9/'10	28.6	22.8	009.1	095	076
3/9/'10 – 9/9/'10	29.9	23.1	005.6	094	073
10/9/'10 – 16/9/'10	29.8	23.2	021.2	096	072
17/9/'10 – 23/9/'10	30.2	23.0	008.9	095	072
24/9/'10 – 30/9/'10	31.9	23.0	010.8	092	068
1/10/'10 – 7/10/'10	30.6	22.7	041.4	095	078
8/10/'10 – 14/10/'10	29.5	23.3	005.1	094	070
15/10/'10 – 21/10/'10	28.3	21.9	018.6	095	078
22/10/'10 – 28/10/'10	29.3	22.3	013.4	094	076
29/10/'10 – 4/11/'10	30.6	22.2	022.0	095	071
5/11/'10 – 11/11/'10	30.4	22.3	017.0	096	073
12/11/'10 – 18/11/'10	31.3	22.5	008.5	092	067
19/11/'10 – 25/11/'10	30.8	22.5	008.7	091	071

Appendix I condinued

Period	Temperature		Rainfall (mm)	Humidity	
	Maximum (^{0}C)	Minimum (^{0}C)		Maximum (%)	Minimum (%)
26/11/'10 – 2/12/'10	28.1	22.8	001.2	083	071
3/12/'10 – 9/12/'10	31.0	21.8	000.3	089	059
10/12/'10 – 16/12/'10	31.4	21.5	000.4	091	059
17/12/'10 – 23/12/'10	30.9	22.8	002.6	077	055
24/12/'10 – 31/12/'10	30.7	21.8	000.0	076	051

HABITAT ANALYSIS AND DOMESTICATION STUDIES ON 'ORILATHAMARA' (*Nervilia aragoana* Gaud.)

By

ANULAKSHMI SANKAR

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ABSTRACT OF THE THESIS

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Master of Science in Horticulture

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COLLEGE OF HORTICULTURE

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Abstract of the Thesis

ABSTRACT

The present investigations on "Habitat analysis and domestication studies on 'Orilathamara' (*Nervilia aragoana* Gaud.)" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2009 – 2011, with the objectives to collect the source plants of the drug *Orilathamara* from wild, to carry out detailed natural habitat analysis and to analyze the response of the species to domestication.

The study was conducted in four separate experiments as exploration and collection of source plants of *Orilathamara* from forest, natural habitat analysis, domestication trial and biochemical studies.

Forest explorations were carried out in the Peechi – Vazhani wild life sanctuary. Information regarding the occurrence of the plant was collected from tribal herb gatherers, voucher specimens collected and herbarium made. Four species of *Nervilia* were identified from Peechi forests. They were *Nervilia infundibulifolia* (Blatt. & McCann), *Nervilia plicata* (Andr.), *Nervilia crociformis* (Zoll. & Moritzi) and *Nervilia aragoana* Gaud. All the four species were categorised as endangered.

Natural habitat studies were also carried out in the Peechi forests. It was mainly confined to *Nervilia aragoana*, the only species available in sufficient numbers. Complete life cycle of the plant was studied in the natural habitat. The habitat characteristics indicated that *Nervilia aragoana* is a delicate orchid found in the ground vegetation of moist deciduous forest. Physical properties including soil aggregate analysis and chemical properties of the forest soil were analysed and results presented.

Domestication trial was carried out in the field attached to the Department. There were two shade levels (50%, 25%) and four growing media as FYM + sand + soil (1:1:1), leaf compost + sand + soil (1:1:1), coir pith compost + sand + soil (1:1:1) and vermi compost + sand + soil (1:1:1).

All the growing media were analysed for their physical properties, aggregate stability and chemical properties and results presented.

From the study it was observed that there was no significant difference in sprouting percentage, days to leaf emergence, complete unfurling of leaves and total biomass production of *Nervilia aragoana* under different treatments. Significant differences were observed in growth parameters like petiole length, leaf length, leaf width and leaf area and also tuber yield per plot. For all these parameters, performance was better under 50% shade level and in the growing medium containing FYM, sand and soil in equal proportions. Tuberization and tuber development of the species was also documented during the study and the same was correlated with weather data during the period.

Biochemical analysis was also carried out to estimate the constituents like chlorophyll, starch, soluble sugars, protein, amino acids and soxhlet extractables. Both the wild and domestic samples were analysed. The results indicated that constituents like sugars, protein and amino acids were higher in domestic tubers and starch was higher in wild tubers. TLC profile of sugars and amino acids revealed the presence of seven sugars and five amino acids in *Nervilia aragoana*. Anatomical study of the tuber was carried out which revealed the presence of starch grains, Calcium oxalate crystals and mycorrhizal associations in the ground tissue.

In short, the study identified four species of *Nervilia* in Peechi forests and they were categorised as endangered. Natural habitat characteristics of the species have been described and complete life cycle documented. Domestication proved to be successful in *Nervilia aragoana*. 50% shade level and normal potting

mixture containing FYM, sand and soil proved to be the ideal one for growth and tuber production. Domestication also did not affect the quality of the drug in respect of components studied.