Phcog Rev. Vol, 3, Issue 5, 154-169, 2009
Available Online : www.phcogrev.com

PHCOG REV. : Review Article

Chlorophytum borivilianum (Safed musli): A Review

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ABSTRACT

Chlorophytum borivilianum Sant. and Fernand. belongs to family Liliaceae, is a promising medicinal plant with great economic potential. Appreciation of medicinal value of safed musli tubers has been made in ancient Indian medicine literature right from 11th century A.D. The tubers contain saponins and are aphrodisiac, adaptogenic, antiageing, health restorative and health promoting. The plant is cultivated in different parts of India on a small scale at present. However, systematic information on different aspects of this species is not available. In this review, an attempt has been made to present this information.

A. About the genus *Chlorophytum*

Genus *Chlorophytum* earlier included in family Liliaceae has been recently kept in family Anthericaceae of series Coronariae in the class Monocotyledons (1). The name *Chlorophytum* is derived from Greek word, *Chloros* meaning green and *phyton* meaning plant (2). Hooker and Jackson (3), in Index Kewensis have listed more than 300 species of the genus *Chlorophytum* and suggested that its probable centre of origin and diversification lies in tropical and subtropical Africa, where 85% of the species are found. However, Poulsen (personal communication) has informed that listing of the *Chlorophytum* species is still not completed and about 125-150 species might be existing. Most species (perhaps more than 90%) of the genus *Chlorophytum* are tuberous geophytes, well adapted to light exposure and seasonal variations in precipitation found in habitats such as Savanna grassland or open woodland (4). According to Bordia *et al.,* (5), *Chlorophytum* species are distributed predominantly in the tropical parts of the world and some of these species are cultivated for their attractive flowers. In India, about 16 species of *Chlorophytum* have been reported to occur and these are represented in Table **1**. Recently, an addition of one more species of *Chlorophytum* namely *C. kolhapurense* has been made from Kolhapur (Maharashtra) region (6).

Chlorophytum species namely *C. arundinaceum* Baker (7-14), *C. attenuatum* Baker (14), *C. borivilianum* Sant. and Fernand. (15-18) *C. brevescapum* Dalaz. (19), *C. laxum* R.Br. (13) and *C. tuberosum* Baker (14, 20, and 21) are all grouped under one trade name of 'Safed musli'. There are several vernacular synonyms in India for Safed musli. These are –

Languages	Vernacular names
Sanskrit	Shweta musli
Tamil	Taniravi thang
Hindi (U.P.)	Khiruva
Marathi / Hindi	Safed musli
Gujarati	Dholi musli
Telugu	Tella nela tadi gaddalu
Malayalam	Shedheveli

Amongst the species used as Safed musli, *C. arundinaceum* is specifically reputed for the tuber quality in the *Ayurvedic* text (5). However, at present, *C. borivilianum* has been paid greater attention and regarded as a main Safed musli crop by several workers (5, 22-24).

B. Botany

Santapau and Fernandes collected the specimens of a new species of *Chlorophytum* on 14th June 1954 in the plains and lower slopes of the Krishnagiri National Park Salsette Island, Borivali, Mumbai and named it as *Chlorophytum borivilianum* Santapau and Fernandes (25). After its discovery at Borivali (Mumbai) *C. borivilianum* has been reported from several localities in India such as Dang forest in Gujarat, Aravali hills in Rajasthan, along plains and lower hill slopes of Akola, Amaravati, Mumbai, Kolhapur, Pune and Raigad in Maharashtra (15, 26). Nayar and Shastri (27) have considered *Chlorophytum borivilianum* as an endangered species. It is noticed that the plant population is diminishing at an alarming rate due to over exploitation from the wild stands (5, 27). According to Begum and Ved (29) *C. borivilianum* is endemic to India and its IUCN Red list status is 'vulnerable'. It is a small perennial herb with a full crown of radical leaves appearing over the ground (Fig.**1**) with advent of summer rain. Roots (tubers) are fleshy, fascicled and directly originate from the stem disc. The tubers are 5-20 in number, 10-25 cm x 1-2 cm in dimension and cylindrical. These are devoid of any fibrous structure with straw coloured, outer skin and interior white after peeling. Leaves are 6 to 13 in number, 13 to 23 cm x 1.75cm in size, spirally imbricate and slightly narrowed at the base, sessile linear or ovate with acute apex. The leaves spread horizontally and the lower surface of leaf is rough with wavy margins and parallel venation. The plant produces a solitary unbranched scape which is 15-30cm long and terete and it bears flowers on above upper ¾ of its length. The flowers are small, white, bracteate, pedicellate, usually arranged in alternate clusters, each cluster consisting of 3 flowers. The flower clusters are dense on the upper part of the scape. The floral bracts are linear, papery and purplish, 1.0-1.5 cm long; pedicel whitish jointed and kneed at the joint, 6-10 mm long. Tepals are white, 6 in number, arranged in two whorls of three each, linear,

membranous, acute, 3-5 nerved with imbricate aestivation. Stamens are 6, arranged in 2 whorls situated opposite to tepals, united to the perianth, as long as perianth, dithecus filaments are glabrous, anthers are yellow, linear, dehisces by longitudinal slits. Style is slightly longer than the stamens, swollen at the apex. The ovary is green, globose and sessile, three lobed with obtuse angles and has axile placentation. Fruit is a loculicidal capsule, green to yellow, triquetrous to 3-sulcate, almost equal in length and width with 14-16 seeds. Seeds are endospermic, black coloured, angular.

Floral formula:⊕, ♂, ♀, P $_{(3+3)}$, A $_{(3+3)}$, $\underline{G}_{(3)}$.

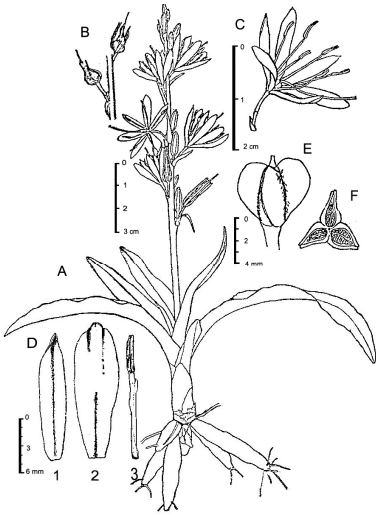

Figure No. 1: Chlorophytum borivilianum *sp. nov.* (after Santapau and Fernandez, 1955).
A. Whole plant; B. A branch with fruit; C. A single flower, magnified.; D. (1) Sepal; (2) Petal; (3) Stamen; E. Outer view of fruit; F. Transverse section of the fruit.

Figure No. 2: *Intercropping in the field of* ***Chlorophytum borivilianum***

C. Morphological variability

The studies performed under All India Coordinated Project on Medicinal Plants at Indore (16, 17 and 30) and Bordia *et* al., (5) on plant collections in different districts of M.P., Rajasthan and Gujarat revealed significant variability among different characters such as fleshy root yield plant^{-1}, dry root yield plant^{-1}, thickness of fleshy roots, leaf length and leaf width, number of fleshy roots plant^{-1} (Table **2** and **3**).Considerable morphological variability was also observed in *Chlorophytum borivilianum* collections maintained at the National Research Centre for Medicinal and Aromatic Plants (NRCMAP). Two distinct plant types were observed, i.e., prostrate / spreading type, in this type, the leaves come out from the stem disc and immediately spread out while in erect type, newly produced leaves grow upwards for a short distance and then spread out. Leaf length, leaf breadth and maturity period also vary a lot in this species. Frequency of variability was seen in the case of root, shape, size and colour. A plant with variegated leaves was also identified. It was observed that the roots having blunt tip had better post harvest keeping quality than the tapering root tips (31). The National Research Centre for Medicinal and Aromatic Plants has recently (1999) undertaken a programme for collection, conservation and cataloging of *C. borivilianum* biodiversity under a World bank funded National Agricultural Technology Project (NATP) (23). Thus there is a great scope for further selection of promising strain of *Chlorophytum borivilianum*.

Besides above variations, a few abnormalities were also observed by Bordia *et al.,*(5) in fasciculated roots of Safed musli like fusion of pair of fleshy roots together throughout the length, fusion of one pair of fleshy root in the upper portion upto half of its length. In other instances, they recorded fusion of two fleshy roots from top part upto 2/3 part of its length and then formation of knot which again bifurcated into two parts or two small roots joined together upto their ends and extension of one root further (32). Aundhe and Deokule (33) studied root morphology of ten species of *Chlorophytum* namely *C. borivilianum*, *C. bharuchae*, *C. orchidastrum, C. arundinaceum, C. glaucum*, *C. attenuatum, C. glaucoides*, *C.breviscapum*, *C. laxum*, *C. tuberosum* and prepared a key based on root morphology.

D. Flowering and seed formation

Not much attention has been paid to the floral biology of *C. borivilianum*. Bordia *et al.,* (5) have noticed a peculiar flowering pattern in Safed musli. When plantlets with fleshy tubers were planted both in nature as well as in the field, inflorescence emerged within 4-6 days of receipt of first shower. When plant was raised in the field from individual fleshy roots, then leaves and inflorescence sprouted only from well developed fleshy roots: Leaves and inflorescence also sprouted from the fleshy root bunches and such inflorescence had higher number of flowers than those emerging from single root. The probable reason for such behavior appears to be presence of comparatively more stored food material in the later. Generally one plant produces only one inflorescence but sometimes 3-4 inflorescence have been observed depending upon the age of the mother plant or thickness and broadness of the stem disc (older and well developed stem disc produced more sprouts/inflorescence). The inflorescence peduncle was round in shape and it originated from the centre of the leaf whorl. Dalal *et al.,* (34) reported that Safed musli is an insect

pollinated crop. Several workers attempted to study various aspects of reproductive biology (5, 35, and 36). Anthesis is not synchronous within the inflorescence. The flowers opening on the same day in an inflorescence were found to be arranged in different directions away from one another and within a flower, the stigma placed away from the anthers favouring cross-pollination. Anthesis occurs at about 4.00 a.m. followed by longitudinal splitting of the anther lobes. Geetha and Maiti, (36) observed that pollen dispersal is by insects, *A. mellifera* Linn. and *A. cerana-indica* Fabr. Maximum activity of pollinating agents is reported between 8.00 and 9.00 a.m. Pollen shows viability of 87.5% at 7.00 a.m. on the day of anthesis and thereafter it gradually reduces to zero at 5.00 p.m. Pollen when stored at about 6 ± 1°C maintains viability (83%) up to 30 h after anthesis. Stigma receptivity is found to be maximum at 9.00 a.m., reducing gradually to zero at 3.00 p.m. Although, the species is cross-pollinated in nature, artificial self-pollination is equally effective, leading to 60% seed set. In this respect *C. borivilianum* differs from African species of *Chlorophytum* which are found to be self- compatible with high autodeposition efficiency (37). Each capsule bears 14-16 seeds (5). The seed collection is very difficult in this crop since all capsules do not mature at the same time and dehiscence of capsule takes place as soon as it turns brown.

E. Anatomy

Anatomical studies of different *Chlorophytum* species with respect to tuber and leaf structure have been performed by Naik and Nirgude (38). But *C. borivilianum* is not included in these studies. Anatomical description of *C. borivilianum* tubers has been obtained from the website (39*)*. A transverse section of tuber shows a circular outline without any appendages. The outermost layer is a yellowish, uniseriate epidermis which consists of cells with very thickened and swollen walls due to siliceous deposition. The cells though appearing mostly rectangular with few squares in shape do not have clear demarcations. In some cells very narrow lumen is noticed. This is followed by a very large zone of cortex. The outermost layer of the cortex, just below the epidermis, consists of cells which are mostly rectangular appearing much longer than wide. Rest of the cortical cells are rounded to polygonal, parenchymatous cells and with little or no intercellular spaces (probably due to swelling). The innermost layer of the cortex is a single layered endodermis. The stellar structure shows that the endodermis is followed by a uniseriate pericycle layer. The vascular tissue is not very elaborate. The xylem is exarch and consists of jointed vessels, 3-5 in number in each group. There are about 30-35 groups of xylem elements. However, xylery fibers are quite abundant, surrounding the vessels and jointed to form a more or less continuous irregular ring. The amount of xylery fibers is not uniform at all places. The phloem is grouped in between the arches of xylery tissue along with parenchyma. The central region is occupied by a fairly large pith region where the cells are closely packed as in cortical region. These are mostly polygonal in shape.

Aundhe and Deokule (40) made comparative histochemical studies of *Cynotis tuberosa* Roxb, *Chlorophytum laxum* Br. and *Chlorophytum borivilianum* Sant. and Fernand. These workers mentioned that the root tubers of Safed musli are very rich in proteins, raphides and sphaeraphides. Among different species of *Chlorophytum* namely, *C. attenuatum*, *C. borivilianum*, *C. capense*, *C. laxum, C. glaucum, C. orchidastrum, C. tuberusum* , *C. comosum* var. C-41, var. C-42 (*variegatum*), var. C-43 (*pictratum*) , var. C-47 (*vittatum*), maximum stomatal index (46.5) was noticed in case of *Chlorophytum borivilianum* leaves (41). This species also exhibited lowest stomatal size with respect to length and breadth.

F. Cytology

There are two basic chromosome numbers in the genus *Chlorophytum* such as x=7 and the other being x=8 (4, 43-47). *C. borivilianum* is a diploid species and the chromosome number in it is 2n=2x=16 with a basic number of x =8 (47, 48). Interestingly, a series of polyploidy in the natural population has been reported in the *Chlorophytum borivilianum*. The polyploidy within the species was attributed to the predominance of vegetative propagation (49). Arora *et al.,* (50) reported that precociously germinated somatic embryos in 6-8 and 18-24 months old cultures of *C. borivilianum* showed a wide range of variation in the ploidy level, ranging from 3x-3 to 4x+4 and 5x+1 to 7x+2. Therefore, these somatic embryos were supposed to be abnormal and thus failed to germinate into a complete plantlet. Further Arora *et al.,* (49) observed chromosomal variation was the least (3x-3 to 3x+3) in regenerants from 1- 4 month-old cultures and increased (5x-1 to 7x) with the age in regenerants from cultures older than 6 months. Geetha and Maiti (51) reported that the chromosome number in this species is 2n=4x=28 which indicating the species as tetraploid with basic number x=7 but an octaploid number 2n=8x=56 is also observed in a few somatic cells of some plant samples. Lavania *et al*., (52) also observed the chromosome number 2n =4x =28 of *C. borivilianum*. They applied the fluorescence in situ hybridization (FISH) technique to elucidate physical localization and measurement of the rDNA sites using two rRNA multigene families homologous to 45S and 5S rDNA in *C. borivilianum* and *C. comosum*. Results revealed the presence of as many as five pairs of 45S rDNA sites in *C. borivilianum*, and three pairs in *C. comosum*, suggesting their multi-genomic origin, and also occurrence of discrete variation in rDNA continuous tandem strings for three rDNA loci corresponding to their respective rDNA locus size. Joshi *et al.,* (53) performed karyomorphological study of seven accessions of *C. borivilianum* collected from the Rajasthan and Madhya Pradesh. They found that all the accessions were tetraploid with 2n=4x=28 chromosomes which were resolved into 7 groups, each comprising 7 homologous chromosomes. All the accessions had submetacentric chromosomes in high proportion while metacentrics, telocentrics and subtelocentrics were very few in numbers. Nucleolar chromosomes were noticed in 5 (PBL-1, PBL-2, PBL-4, PBL-5 and PBL-7) accessions out of the 7 accessions.

G. Cultivation practices and post-harvest processing

At present, tubers of *Chlorophytum borivilianum* are mostly collected from their natural habitats in southern Rajasthan, Western Madhya Pradesh and North Gujarat (5). At the same

time, at some places cultivation of this species is also practiced. Hence, there is a need to standardize the cultivation practices for this medicinal plant.

a) Climate and soil

Safed musli grows satisfactorily in wide range of temperature and rainfall. But high and low temperature extremes are found to affect the growth and tuber yield. The plant can be grown in all kind of soils in India but performs well in the sandy loamy to red loamy soils having neutral pH range (6.5 to 7.5). The soils having good drainage system are reported to promote better tuber growth (54).

b) Preparation of land

Tractor drawn plough is used for deep cultivation followed by disc/ cultivator harrowing to get fine tilth. This operation is generally performed in the months of March-April upto 1.5 to 2 feet depth and sowing of seeds of Sunhemp (boru) or *Sesbania* species (Dhaincha) is done at the rate of 60kg per acre for green manure purpose. Before Sunhemp/ Dhaincha start flowering the plants are cut and mixed into the soil during April. This is followed by irrigation of field for the easy decomposition of green manure. If the area of cultivation is infested with termites then Datura plants are mixed with soil. If soil is of clay nature and requires soil conditioning then soil conditioner mycemeel (Hindustan Antibiotics, Pune) at the rate of 1.5 t $acre^{-1}$ is applied. Deep ploughing is done in the month of mid May and soil is mixed 30-40 t of decomposed FYM $acre^{-1}$ (55, 23). Besides FYM, application of neem cake at 500 kg ha^{-1} is found to enhance the growth of tubers (23). According to Jana (56), 22-25 quintal cow dung manure and 250 kg bonemeal per acre are beneficial for tuber growth. If the soil gets clods, then 3-4 tillers are given for better pulverization of soil. No inorganic fertilizers are recommended for the crop. Singh *et al*., (57) suggested that neem cake supplement for nutrients to keep the crop free from plant parasitic nematodes.

c) Preparation of bed

After preparation of field, beds are prepared. Safed musli is cultivated on raised beds or ridges or flat beds depending upon the location. In places where rainfall is high and the drainage system is poor, the crop is grown on raised beds or ridges. On well-sloped land, flat beds can be adopted. Raised beds are prepared using a double raw planting system. A sowing rate of 333,000 plants per hectare (spacing 30 x 10 cm) is recommended for optimum economic yield (58, 59). About 1000 kg of fleshy roots are needed for planting a hectare. As per the observations of Sushir *et al.,* (54), this tuberous crop performs well on raised beds. The beds with 3.5 feet width and 1to1.5 feet height of suitable length are prepared for plantation of crop.

d) Varieties

According to Manjunatha *et al.,* (23), selections such as RC-2, RC-16, RC-36, RC-20, RC-23, RC-37 and CT-1 are found to be good in terms of yield and saponin content. These varieties have been collected and maintained at Rajasthan Agriculture University, Udaipur. The other varieties of Safed musli which give high yield and exhibit insect and disease resistance are MDB-13 and MDB-14.

e) Sowing

Safed musli being a kharif crop is sown in the month of June after first shower of the monsoon. About 400-500 kg seed tubers or nearly 30,000-35,000 fingers are required per acre (wet tuber with crown). To avoid fungal and bacterial infection the tubers are treated with contact fungicide or bactericide solution at the rate of 0.2% or with *Trichoderma* bio-agent. Positive results have been reported when tubers were treated with '*Gau-mutra*' (cow urine) (54). Such treated tubers are planted on the raised beds at a distance 15-20 cm, plant to plant and row to row.

f) Detopping/ Deflowering

In some cases following sowing in of tubers, the sprouted tubers give rise to inflorescence. The inflorescence arises from the base of the plant and immediate removal of such inflorescence is highly essential to encourage the tuber bulking (23, 54, 60).

g) Irrigation

Water management plays a vital role in Safed musli cultivation. Utmost care is to be taken to keep the soil moist until planting material sprouts. Irrigation is given at 8-10 days interval; however it depends on prevailing weather. Light irrigation is needed until harvest even after drying and falling of leaves. Use of sprinkler or drip method of irrigation is advisable for better results. Attention is needed to be paid while irrigating the land, because standing of water for period affects the crop growth and favour fungal infection and ultimately it hinders production. After senescence of leaves, irrigation is to be given at 15-20 days interval. In case of long periods of no rainfall or rainfall scarcity, irrigation is necessary to maintain moisture in the soil for healthy growth of the plant and the tubers (23).

h) Fertilizers and pesticides requirement

Application of a mixed fertilizer NPK in the ratio 50:40:40 kg $acre^{-1}$ is advisable for the growth of Safed musli. Safed musli crop is generally free from most of the pests and diseases. For control of illi and other pests, a spray of Thirum 5ml l^{-1} every month is recommended as a precautionary measure. *Trichoderma viridae* is found to be effective in checking the attack of fungus '*Fusarium*' on Safed musli (55).

i) Weed management

An efficient weed control ensures satisfactory yield of the Safed musli crop (54, 55). For the control of weed growth two to three weeding are to be done in 30-40 days. Irrigation of the field a day before weeding is advised for easy hand weeding. Singh and Chauhan (61) observed that three manual weedings at 15–20, 25–30 and 50–55 days are sufficient to keep the weed population under check.

j) Intercropping

According to Manjunatha *et al.,* (23), Safed musli can be cultivated as an intercrop with mango, teak, neem, amla and sapota etc. Reports are also available which indicate successful intercropping of Safed musli with crops such as banana and maize. Pigeonpea intercropped with safed musli was reported to give high land equivalent ratio, indicating significant yield advantage (62). In Pattankodali Taluka- Hatkangale, District-

Kolhapur (Maharashtra) attempts to grow safed musli and pigeonpea as intercrops are found successful (Fig.**2**).

k) Harvesting

The plants are harvested for their tubers after 3 to 3.5 month of growth generally in the month of Oct. /Nov., when the leaves of musli start yellowing, subsequently they become dry and fall off and get detached from the tuber disc. A light irrigation is given if the soil is too dry. Some scientists are of the opinion that this is a maturity stage of the crop. When the crop attains this stage then the crop life-cycle can be regarded as completed and the tubers are dug out. But people engaged in the cultivation of musli in the most professional manner differ with this practice. In view of these workers, maturation of tubers and accommodation of medicinal principle in tubers is not completed at the leaf drying and detachment stage. Hence, according to them, even after the drying up of the leaves, tubers of Safed musli should be kept on maturing in the soil for some time. During this period also the adequate moisture level in the soil should be maintained. In the month of January-February the tubers mature and their skin changes its colour and becomes dark brown. This is considered as the right time to dug out the tubers (23, 54, 55). However, the exact mechanism of such soil mediated improvement of tuber quality is not yet understood.

l) Storage of planting material

The harvested bunch of tuberous roots is collected with sand stick and heap in the shade. The heaped is stirred at 3-4 days interval for next 8 to12days. Then tuber bunches are removed from sand and washed with water. Fleshy roots are separated from the bunch by applying slight pressure of the fingers and thumb on each root which leads to separation of the roots from the main bunch. Ventilated racks are used to store musli tubers in controlled temperature of 25-31^{0}C and at a relative humidity of 50-65 percent and these tubers are used as a planting material for the next year (23).

m) Yield

Average Safed musli yield is about 6-8 times of the planting material used. In one acre about 80,000 plants are planted and more than 70,000 plants survive upto harvest. If one plant tubers weigh even 30 g, from one acre area 2100 kg (21 quintal) fresh tubers can be obtained and it gives about 400-500 kg of dry musli (23). Tiwari (63) conducted field experiment to evaluate yield and quality of *C. borivilianum* at Mandsaur (M. P.). He reported significantly higher yield attributes and yield of fresh fasciculated roots of Safed musli var. *MCB-412* (3980 kg ha^{-1}). After application of two doses of NPK fertilizers, N45: P60: K60 followed by N30: P40: K40 yield increased upto 3640 kg ha^{-1}. In other experiment, organic manuring with FYM at the rate of 10-20 t ha^{-1} or vermicompost at the rate of 5 t ha^{-1} were found equally effective in increasing the yield of fresh fasciculated roots (2036 to 2127 kg ha^{-1}, as compared to control 1873 kg ha^{-1}). The closer plant spacing 30 x 10 cm also resulted significantly higher root yield upto 2299 kg ha^{-1}.

n) Processing

According to Manjunatha *et al.,* (23), processing is a part of marketing service. For small scale cultivation manual processing is carried out while large scale cultivation demands semi-automized or fully automized processing. Following steps are involved in the processing of Safed musli tubers.

i) Peeling

The skin of the washed and healthy tubers is removed manually with special types of knives to save the precious part of the tubers. A special skill is needful for this process.

ii) Drying

The peeled fingers are wet and removal of moisture is necessary. They are dried in greenhouses by providing controlled sunlight to obtain better quality of dry musli. This process is carried out for 7 days to ensure complete drying. Due to drying the moisture content is reduced by about 20-25% of the original wet tubers.

iii) Grading

Grading of fully dried fingers in 'A', 'B' and 'C' categories is carried out on the basis of their colour and size.

iv) Packing

Safed musli tubers are packed in airtight polybags to prevent the entry of moisture.

CSIR institutions (chiefly Central Institute of Medicinal and Aromatic plants, Regional Research Laboratory, Jammu) and ICAR Institutions based on their All India Coordinated trials have standardized Agrotechnology Packages for various Indian medicinal species and *Chlorophytum borivilianum* and *C. tuberosum* have been, included in the list of such species (64).

H. Pathology

Infection by *Aspergillus* species and *Fusarium* species of fleshy tubers of *C. borivilianum* during storage has been noticed and as a result the infected tubers became hollow (16). Inoculation of these fungi to the healthy plant in pot trial did not produce disease. Treatment of the fleshy roots with Thiram and Captan at 4g kg^{-1} reduced rotting of fleshy roots during storage and also enhanced sprouting percentage (65). Raghavendra *et al.,* (66) and Ahir *et al.,* (67) reported tuber rot of Safed musli which was caused by *Fusarium solani*. Further, Raghavendra *et al.,* (68) suggested that for preliminary management of tuber rot, the compatibility of Phyton (a Phytononic) with routine fungicides like Captan and GLSTIN (Carbendazim) can be employed. The combination of Phyton with GLSTIN at the rate of 0.3% each was proved to be the best in reducing the growth of the fungus and the same combination was also proved its efficacy in the enhancement of the yield of the crop. Singh *et al.,* (69) reported a collar rot disease in *Chlorophytum borivilianum* which was caused by *Corticium rolfsii*. Pandey *et al*., (70) observed the root – knot nematode disease in *C. borivilianum* caused by *Meloidogyne incognita*. Sattar *et al.,* (71) recently noticed occurrence of leaf blight disease of *C. borivilianum* in Northern India. The causal organism was *Colletotrichum capsici*. Mandal *et al.,* (72) recorded a leaf spot caused by *Macrophomina phaseolina*, at research farm of National Research Centre for Medicinal and Aromatic plants, Anand (Gujarat State). Oudhia (73) observed orange banded Blister Beetle *Zonabris pustulata* Thunb (Coleoptera: Meloidae) on *C. borivilianum*

Chlorosis in foliage and yellowing and whitening of leaves *C. borivilianum* plants in the clay loamy calcareous soils at Udaipur

was found to cause serious losses (65). This may be probably because of water stagnation due to continuous rains. The symptoms resembled those of iron deficiency. Symptoms of bronzing on leaves of Safed musli were also noticeable.

I. Ethnobotany and Economic Importance of *Chlorophytum borivilianum*

The fleshy roots (tubers) of *C. borivilianum* are the source of raw drug. Tribal people in the Indian states of Gujarat, Madhya Pradesh and Chhattisgarh use the leaves of this species as a leafy green vegetable (23, 24).The traditional healers and natives of this region use it as potherb. They collect both fresh and dry leaves of Safed musli by adopting special worship ceremonies and these are used for treatment of various diseases. According to traditional healers of Bastar region of M.P. state of India, the combination of Safed musli leaves with other herbs makes the human body resistant against the attack of sex related disease and it also delays the menopause (23).

Appreciation of medicinal value of Safed musli tubers has been made in ancient Indian medicine literature right from 11thcentury A.D. Thus, there is a mention of medicinal application of Safed musli tubers in old texts such as Sarngadhar samhita, Sodhala nighantu, Madhava dravyaguna, Madanapala nighantu, Bhavaprakasha, Raja nighantu, Raja ballabha nighantu, Priya nighantu, Siddhabhesaja manimala (39).

It is indicated in the above literature that Safed musli tuber is aphrodisiac, adaptogen, antiageing, health restorative and health promoting. Manjunatha *et al.,* (23) have listed fifteen uses of Safed musli. Prominent among these uses are -1) aphrodisiac agent and a general sex tonic helpful in curing impotency through spermatogenic activity; 2) curative for prenatal and post natal problems in women; 3) remedy for diabetes; 4) remedy for arthritis; 5) stimulant of brain development in children; 6) effective agent for increasing general body immunity and 7) a curative for physical weakness and many illness.

The above qualities have made Safed musli an essential ingredient in several *Ayurvedic*, *Unani* and *Allopathy* formulations .It is a constituent of commercial products such as *Paurush, Chavanprash, Kauchapak,* Painex (23), Gujarat state Forest Development Corporation launched a potency drug by name '*Nai Chetna*' which contains Safed musli (The Indian Express 1st Dec,1999). Because of medicinal value, Safed musli tubers have got a great economic potential. Bordia *et al.,* (5) have stated that the estimated foreign demand for tubers is about 300-700 tonnes annually. According to Kothari and Singh (60), the present market price of dry musli is 0.8 to 1.2 million Indian rupees (US $ 0.02-0.03 million) per ton.

J. Pharmacognosy

Thakur and Dixit (74) studied the effect of five vajikaran herbs on pendiculatory activity and *in vitro* sperm count. The lyophilized aqueous extracts of *Asparagus racemosus* Willd., *Chlorophytum borivilianum* Sant. F., *Curculigo orchioides* Gaertn., Dactylorhiza hatagirea (D.Don) Soo and *Orchis latifolia* Linn.(200 mg/Kg body weight) were administered orally to wistar strain male albino rats. Following this the effect of all the five drugs was evaluated for pendiculatory activity. The effect of extract on *in vitro* sperm count was also assessed in a separate experiment where the initial sperm count and count after 30 min of incubation was determined. The results show that the herbs could significantly improve the pendiculatory activity in male rats after 14 days of treatment. Similarly, the extract could also preserve the in-vitro sperm count significantly when compared to control group after 30 min. of incubation.

A comparative study on aphrodisiac activity of some *ayurvedic* herbs the roots of *Asparagus racemosus*, *Chlorophytum borivilianum*, and rhizomes of *Curculigo orchioides* in male albino rats was also performed by Thakur *et al*., (75). Administration of 200 mg/kg body weight of the aqueous extracts had pronounced anabolic effect in treated animals as evidenced by weight gains in the body and reproductive organs. There was a significant variation in the sexual behavior of animals as reflected by reduction of mount latency, ejaculation latency, post ejaculatory latency, intromission latency, and an increase of mount frequency. Penile erection (indicated by Penile Erection Index) was also considerably enhanced. Reduced hesitation time (an indicator of attraction towards female in treated rats) also indicated an improvement in sexual behavior of extract treated animals. The researchers attributed these effects to the testosterone-like effects of the extracts.

Thakur *et* al., (76) conducted an experiment to determine the testicular damage in male rats following exposure to high temperatures and the preventive effect of some of Ayurvedic herbs known for their sexual health benefits (Vajikaran). Scrotal sacs of male rats were subjected to a 15 min exposure by dipping in water bath maintained at 40± 2^{o}C daily for 14 days. The histo-architecture and the overall spermatogenesis profile was considerably prevented in rats treated with lyophilized aqueous extracts of *Asparagus racemosus* Willd., *Chlorophytum borivilianum* Sant. F., *Curculigo orchioides* Gaertn., *Dactylorhiza hatagirea* (D. Don) Soo and *Orchis latifolia* Linn.(200 mg/Kg body weight) as compared to control group animals which were administered vehicle only. The differently treated groups were also subjected to evaluation of sexual behavior. The treatment with extracts results in significant amelioration of sexual behavior and the mount, intromission and ejaculatory latencies were significantly reduced while the frequencies for the same parameter were significantly restored in rats exposed to heat and treated with extracts as compared to heat exposed control group alone. Epididymal sperm count was reduced significantly in heat treated control group animals, whereas the extracts significantly prevented the decrease in sperm count in rats as compared to positive control group, exposed to heat.

Kenjale *et* al., (77) designed an experiment to evaluate the aphrodisiac and spermatogenic potential of the aqueous extract of dried roots of *Chlorophytum borivilianum* (CB) in male Wistar albino rats. They divided the rats into four groups. Rats were orally treated with (1) Control group: distilled water; (2) CB 125 mg/kg/day; (3) CB 250 mg/kg/day; and (4) Viagra® group: 4 mg/kg/day sildenafil citrate and their sexual behaviour were monitored 3 h later using a receptive female.

It was noticed that at 125 mg/kg, CB had a marked aphrodisiac action, increased libido, sexual vigor and sexual arousal. Similarly, at the higher dose (250 mg/kg) all the parameters of sexual behaviour were enhanced, but showed a saturation effect after day 14. On day 60 the sperm count increased significantly in both the CB groups, 125 mg/kg and 250 mg/kg, in a dose dependent manner. They concluded that roots of *Chlorophytum borivilianum* can be useful in the treatment of certain forms of sexual inadequacies, such as premature ejaculation and oligospermia.

Visavadiya and Narasimhacharya, (78) investigated the efficacy of *C. borivilianum* root (powder) in modulating the hyperlipaemic / hypercholesteraemic conditions in male albino rats. The root powder of *C. borivilianum* was administered in two doses (0.75 and 1.5 g root powder/rat per day for 4 weeks) to hypercholesteraemic rats significantly increased high-density lipoprotein-cholesterol levels and decreased plasma and hepatic lipid profiles. They reported significant increase in faecal cholesterol, neutral sterol and bile acid excretion with elevated hepatic 3- hydroxy-3-methylglutaryl coenzyme-A reductase and bile acid production. The hypercholesteraemic rats treated with both doses of *C. borivilianum* also showed increase in the activity of superoxide dismutase and ascorbic acid content. There was no significant variation in lipid or anti-oxidant profiles in normocholestremic animals treated with both doses of *C. borivilianum* root powder. Therefore the herb was significantly effective in ameliorating the lipid metabolism in hyperlipaemic animals which remained normal and unaltered in untreated animals. They reported that root powder of *C. borivilianum* also increased the activities of antioxidant enzymes and vitamin C levels, which may have enhanced the antioxidant capacity of the liver.

Govindarajan *et al*., (79) studied *in vitro* antioxidant activity of ethanolic extract of *C. borivilianum*. Potent antioxidant activity of ethanolic extract was found by it ability to scavenge DPPH (84.51%), hydroxyl radical (48.95), ferryl bi-pyridyl complex (84.53%) along with the inhibition of lipid peroxidation (67.17%) at 100ug/ml concentration. Ethanolic extract also showed significant inhibition of superoxide anion radical generated by photochemiluminescence. They concluded that potent antioxidant activity validates the innumerable therapeutic claims of the plant in the traditional system especially its use as a *Rasayan* drug.

Kenjale *et al*., (80) studied anti-stress and anti-oxidant effects of roots of *C. borivilianum*. It was noticed that the aqueous extract of *C. borivilianum* (250 mg/kg for 7 days) significantly reverted the elevated levels of plasma glucose, triglycerides, cholesterol and serum corticosterone and also reduced the ulcer index, adrenal gland weight more as effectively as the standard drug (diazepam) in rats. At 125 mg/kg, it showed a mild anti-stress activity. Under *in vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and lipid peroxidation assay the extract considerably inhibited, in a dose-dependent manner, the levels of DPPH free radicals and thiobarbituric acid reactive substances, respectively thus showing significant antioxidant property. They suggested that it could be used for the treatment of oxidative stress-induced disorders.

Sreevidya *et al*., (81) reported that root extract and isolated fructo-oligosaccharide from *C. borivilianum* have significant antidiabetic activity with the blood sugar levels being 118.32 ± 3.56 and 110.21 ± 4.22, respectively, as compared to the control value of 231.25 ± 3.03 along with moderate antioxidant activity in streptozotocin-induced diabetic animals.

Ethanolic extract of the roots and sapogenin of *C. borivilianum* were evaluated for immunomodulatory activity (82). The assessment of immunomodulatory activity was carried out by determining the effect of azathioprine induced myelosuppresion and administration of extracts on hematological and serological parameters. Administration of extract greatly improved survival against *Candida albicans* infection. An increase in delayed type hypersensitivity response, % neutrophil adhesion and *in vivo* phagocytosis by carbon clearance method was observed after treatment with extracts. Ethanolic extract was found to have more potent immunomodulatory activity than sapogenin fraction of *C. borivilianum*.

Ethanolic extract of the roots as well as isolated root sapogenin from *C. borivilianum* were studied for effect on sexual behavior and spermatogenesis in albino rats (83). It was noticed that administration of 100 mg/ Kg and 200mg/Kg body weight of the sapogenin and ethanolic extract respectively had pronounced anabolic and spermatogenic effect in treated animals as evidenced by weight gains in the body and reproductive organs and histological studies. The treatment markedly affected sexual behavior of animals as reflected in reduction of mount latency, ejaculation latency, post ejaculatory latency, intromission latency and increase of mount frequency and attractability towards female.

K. Phytochemical studies of *Chlorophytum borivilianum*

The phytochemical analysis of tubers of *C. borivilianum has* been performed by some organizations engaged in Safed musli cultivation and this data is available on Internet. Physicochemical analysis of Safed musli tubers was carried out by Malbar Herbs and Musli Grower's Society (84) and it reveals the following picture.

Total ash	3.02%
Acid-insoluble ash	0.25%
Water soluble ash	50.70% of total ash
Hot extraction	20.48%
Cold maceration	0.35%
Water and volatile matter	7.60%
Foaming index	166.67%

Swelling index	4.60 ml
Mass fraction of tannins	1.20%

The studied carried by Bordia *et al.,* (5) revealed following organic and inorganic constituents of Safed musli tubers.

Organic constituents	**%**
Carbohydrates	42 .0
Proteins	8-9 .0
Fibers	3-4 .0
Saponins	2-17 .0
Inorganic constituents	**mg g^{-1} dry weight**
Sodium	0.040
Potassium	0.800
Calcium	6.600
Magnesium	1.900
Phosphorus	3.200
Zinc	0.002
Copper	0.148

The organic analysis of root powder was performed by some scientists. It reveals following data (84).

Saponins	12-17 %
Stigmasterol	1.9-3.5 %
Sugars	
Arabinose	0.79 %
Galactose	3.80 %
Glucose	0.73 %
Rhamnose	0.78 %
Xylose	0.76 %
Carbohydrates	35-42 %
Reducing sugar	20-25 %
Nonreducing sugar	15-17 %
Protein	8-8.5 %

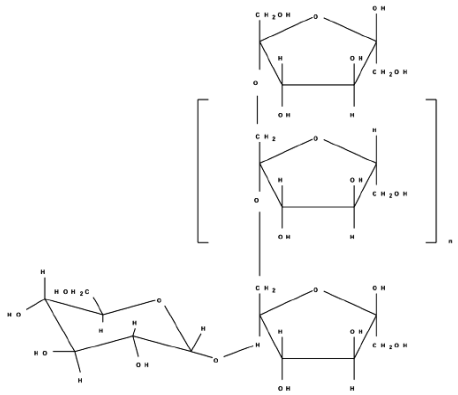

Figure No. 3: Structure of fructans from *C. borivilianum* (n = 5-30)
(after Sreevidya *et al*., 2006).

The author noticed that the tubers contain 0.63% of polyphenols and very low amount of ascorbic acid (0.12%) on fresh weight basis (85). According to Bordia *et al.,* (5), among all the species of *Chlorophytum* in India, *C. borivilianum* produces the highest yield of roots alongwith the highest saponin content. At the same time, these workers have mentioned that the saponin content is influenced by genotype and localizational environment (Table **4** and **5**). When some accessions were collected from forest and cultivated on sandy loam soil at CTAE Udaipur and on clay loam soil at RCA, Udaipur, different response of genotypes with respect to two locations, in terms of saponin content was noticed (Table **5**). Genotype RC-14, yielded saponin content as high as 9.3% while at the same site the other genotype RC-28 yielded only 1.8% saponin. Recently, Tiwari (86) conducted a field experiment to study influence of soil fertility on quality of *C. borivilianum* tubers. He noticed the highest saponin and protein percentages (6.93 and 9.28%, respectively) under the highest fertility level upto NPK in the ratio of 60:80:80. Further he applied vermicompost (5 t ha^{-1}) and found increase in saponin (6.27%) and protein (9.17%) in roots over other organic manuring treatments. The widest plant spacing (30 x 20 cm) resulted in significantly higher saponin (6.17%) and protein (9.21%) contents in roots over the closer 30 x 10 and 30 x 15 cm spacing (86).

Govindarajan *et al*., (79) were successful in isolation of sapogenins and standardization of the isolated sapogenin fraction using HPTLC. Sreevidya *et al*., (81) isolated fructo-oligosaccharide from tubers of *C. borivilianum* and identified as O-β-D-fructofuranosyl-(2→1)-(β-D-fructofuranosyl)n-(2→1)-a-D-glucopyranoside (n = 5-30) (Fig.**3**) using high-pressure anion exchange chromatography, MALDI-MS, NMR, GC, HPTLC and chemical analysis.

Although the chemical composition of *C. borivilianum* tuber has been extensively studied the chemical composition of the leaf is not paid much attention. The leaf analysis has been performed by the author (85) and it is given below.

Organic constituents	mg 100 g^{-1} dry weight
Reducing sugar	308.0
Soluble sugar	459.0
Starch	1316.0
Total nitrogen	1910.0

Inorganic constituents	mg 100 g^{-1} dry weight
Potassium	1988.0
Calcium	1236.0
Magnesium	278.0
Phosphorus	215.45
Sodium	94.0
Sulphur	36.4
Iron	13.56
Manganese	1.61
Copper	0.56
Zinc	2.16

L. Ecophysiological and Biochemical studies

a. Seed germination

Trivedi and Yadav (22) reported a dormancy period of about 9-10 months in the seeds of *C. borivilianum*. Jat and Bordia (87) also observed about 10 month's dormancy in the seeds. Dalal *et al.,* (34) recorded about 13% germination in one year old seed of *C. borivilianum*, while Trivedi and Yadav (22) reported seed germination about 28 – 62 % after 37 days of sowing in the Petri dishes at Indore. Jat and Bordia (87) carried out germination trial of seeds of *C. borivilianum* in the

Table No. 1: *Indian species of Chlorophytum and their distribution*[*].

Species	Distribution
C. acaule Baker	Peninsular India
C. arundinaceum Baker	Northern and eastern peninsular India.
C. attenuatum Baker	Western ghats, southwards to Coimbatore and west peninsula
C. bharuchae Ansari, Raghavan and Hemadri	Peninsular India
C. borivilianum Sant. and Fernand.	Rare in moist places along plains and lower hill slopes of Maharashtra; Dangs forest (Gujarat); Aravali hills, Mount Abu, Mahi (Rajasthan), Bastar forests (M.P).
C. breviscapum Dalz.	Peninsular India
C. glaucum Dalz.	Peninsular India
C. glaucoides Blatt.	Peninsular India
C. heynei Rott. ex Baker	Tamilnadu
C .khasianum Hook.	Central to eastern Himalaya
C. laxum R.Br.	Peninsular India
C. malbaricum Baker	Peninsular India, Karnataka.
C. nepalense (Lindl.)Baker	Subtropical Himalaya
C. nimmonii (Grah.) Dalz.	Peninsular India
C. tuberosum (Roxb.) Baker	Throughout India
*C .kolhapurense*** Sardesai, Gaikwad and Yadav	Rare, sparsely distributed in the dry hilly tracts of Kolhapur district in Maharashtra and Belgum, Dharwar district of Karnataka

* Karthikeyan *et al.,* 1989)

* * Sardesai *et al.,* (2005).

***Table No. 2**: Collection and evaluation of C. borivilianum germplasm at Udaipur[*].*

Characters	Place of collection and accession no. range			Overall range
	Rajasthan (40) Acc. No. RC1 to RC 25, RC 27, RC 28, RC 30 to RC 34, RC 49 to RC 56	Gujarat (13) Acc. No. RC26, RC 29, RC 33 to RC 48	M.P. (3) Acc. No. RC 35 to RC 37	
Fleshy root yield/ plant (g)	2.40- 11.20	2.30- 5.10	3.80- 4.90	2.30- 11.20
Dry root yield / plant (g)	0.61-2.58	1.17	--	0.61- 2.58
Fleshy root index 100 roots (g)	61.10- 174.30	88.70	--	60.10- 174.30
Moisture (%)	63.00- 84.50	77.00	--	63.00-84.50
No. of leaves / plant	6.80-13.40	6.00-8.00	7.10-8.50	6.00-13.40
Leaf length (cm)	13.50- 23.30	15.70-21.10	18.20- 20.60	13.50- 23.30
Leaf width (cm)	1.06-1.75	0.99- 1.41	1.20- 1.26	0.99-1.75
Days to maturity	82.00- 98.00	77.00-99.00	82.00-86.00	77.00- 99.00
No. of fleshy root/plant	1.20-8.20	3.00-5.80	4.50-5.00	1.20-8.20
Length of main fleshy root (cm)	2.50-7.20	3.40-10.30	3.54-4.50	2.50-10.30
Thickness of main fleshy root (cm)	0.48-0.87	0.57-0.74	0.57-0.60	0.48-0.87

* Bordia *et al., (*1995).

***Table No. 3**: Collection and evaluation of Chlorophytum species at Indore [*].*

Characters	Range of variation recorded
Fleshy root yield/ plant (g)	2.00 -100.50
Dry root yield/ plant (g)	0.64-35.20
Plant height (cm)	15.33-92.96
Moisture (%)	63.20-76.00
No. of leaves / plant	6.00-15.33
Leaf length (cm)	15.93-63.18
Leaf width (cm)	0.82-4.62
Flowering shoots / hill	1.33-5.67
No. of fleshy root/ plant	3.00-162.00
Length of fleshy roots (cm)	2.83-10.33
Thickness of fleshy roots (cm)	0.40-1.27

* Trivedi, (1989).

***Table No. 4:** Chemical composition of different samples of C. borivilianum.*

Chemical Parameters	Content %		
	Market Sample [a] (%)	RC-15 [b] (%)	Different genotypes [c] (%)
1.Carbohydrates	42.00	39.10	16.4-65.8
a) Reducing sugar	-	22.20	-
b) Non reducing sugar	-	16.90	-
2.Protein	8.50	8.50	3.8-3.0
3.Saponin	2.00-3.00	4.00	Traces -17.0
a) Sugars	-	3.8	-
i) Galactose	-	0.73	-
ii) Glucose	-	0.76	-
iii) Xylose	-	0.74	-
iv) Arabinose	-	0.79	-
v) Rhamnose	-	0.78	-
b)Sapogenin (hecogenin)	0.17	0.18	-
4.Aqueous extract	40.00	30.00	-
5.Root fiber	3.00-4.00	5.00	-

*Bordia *et al.,* (1995).

a. Roots samples collected from local market ; b: Elite accession of *C. borivilianum ; c:* Different genotypes collected from Udaipur.

Table No. 5:*Effect of environment on saponin content of C. borivilianum* [*].

Collection No.	Saponin %		
	Forest produce	CTAE produce[a]	RCA produce[b]
RC-1	1.8	5.0	2.4
RC-6	-	7.0	3.1
RC-8	-	8.6	1.4
RC-14	-	9.3	--
RC-15	6.3	--	Trace
RC-19	-	5.3	7.3
RC-22	-	2.8	8.3
RC-28	-	1.8	1.5

*Bordia *et al.,* (1995).a. CTAE = College of Agricultural Engineering and Technology Farm, Udaipur. b. RCA= Rajasthan College of Agricultural Farm, Udaipur.

month of July (1990) and they observed about 11-24% germination in different cultures at Gujarat. Shrivastava *et al*., (88) observed 25-30% germination in this species at Madhya Pradesh. Maiti and Geetha (24) also observed a great difference in seed germination in their experiments: seeds from wild habitat of Maharashtra state show up to 50% germination, but from cultivated populations from Gujarat and Madhya Pradesh there was only 5–15% germination. Thus, the poor seed germination capacity of this species is a limiting factor in its direct cultivation from seed.

b. Vegetative propagation

For large scale cultivation of Safed musli, vegetative propagation is at present only practical means. About 7-8 months dormancy in fleshy roots has been noticed by Jat and Bordia (87). Verma *et al.,* (89) studied influence of invigoration treatment of tubers with phytohormones on sprouting percentage and seedling vigour. They selected 400 small unsprouted roots, weighing 0.3-0.5g and dipped the roots for 24 h in 100 ppm GA_3 solution, 100 ppm GA_3 + 50 ppm Kinetin and 50 ppm Kinetin solution. These were placed in petri plates on filter papers moistened with same solutions as used in each of the treatments. The control tubers were dipped for 24 h in distilled water and then kept on filter paper moistened with distilled water. Invigoration treatments with phytohormones increased sprouting percentage, emergence index, speed of germination, mean germination time and coefficient of velocity and among the three treatments 50 ppm kinetin was most effective. Vijayaraghavan *et al.,* (90), planted fleshy roots with crown in raised beds to test their sprouting ability in relation to different treatment such as *'Gomutra'* (cow urine), aqueous leaf leachate of neem, aqueous leaf leachate of *Calotropis gigantea* and 0.2% Bavistin (Carbendazim). The roots treated with aqueous leaf leachate of *C. gigantea* exhibited the highest (87%) sprouting and surpassed all other treatment. They attributed increase in sprouting percentage to presence of phytochemicals such as beta- amyrin, taraxasterol, beta-sitosterol, free sapogenins and resins in *C. gigantea* leaf leachate as these compounds are antifungal and antibacterial in action.

Kothari and Singh (91) observed a large variability in terms of morphological and yield attributing characters and tuber yield among the germplasm. CB/MS-6 gave highest number of leaves per plant (23.8), number of tubers per plant (10.2), tuber thickness (0.8 cm) and fresh (65.3g $plant^{-1}$) and dry (8.5 g $plant^{-1}$) tuber yield. They also observed prostrate or spreading and erect types of plants, plants grown in different locations revealed the feasibility of its cultivation in semi arid areas of Andhra Pradesh, Madhya Pradesh and Maharashtra. Some growth features and mineral nutrition in *C. borivilianum* studied by Author (92). A continuous increase in biomass upto 120 days period was evident and from 45 days onwards the partitioning of biomass towards tuber was noticed. Average leaf area increased upto 75 days growth and thereafter the area declined due to senescence and withering of old leaves. Leaf nitrogen also followed a similar trend. Maximum phosphorus content was recorded in the leaves of young plants at 15DAP and thereafter a decline in P level in leaf tissue was evident. The levels of calcium and magnesium in the leaf and tuber tissue were found to be continuously increased alongwith advancement of plant age. Accumulation of nitrogen, phosphorus and potassium was noticeable in the tuber tissue during the latter stages of plant growth.

c. Tissue Culture studies

Attempts have been made by several workers for *in vitro* propagation of *C. borivilianum*. Using young shoot bases as explants, Purohit *et al.,* (93) have achieved *in vitro* clonal multiplication of Safed musli on Murashige and Skoog's (MS) medium supplemented with 22.2 µM BA. These workers recorded multiplication at four-fold higher rates every 3 weeks. Further, it was noticed that all shoots rooted when transferred to MS medium with ¾ strength inorganic and organic constituents and 9.8 µM IBA and 67% of micropropagated plants were successfully established in pots which produced normal fasciculate storage roots as in wild plants. These studies were continued further (94) and multiplication through somatic embryogenesis from young shoots on the same medium was possible. Also they successfully raised callus from zygotic embryos which produced somatic embryos on MS medium containing 4.53 µM 2,4-D. Somatic embryos were maintained on low concentration (1.13 µM) of 2,4-D. It was noticed that 20% embryos produced plantlets when inoculated on a medium without any growth regulator.

These workers also developed a protocol (95) for somatic embryogenesis and plantlets regeneration in Safed musli. Induction of callus development from immature zygotic embryos took place after inoculation on MS medium containing 1.0 mg l^{-1} 2, 4-D. Initially a slow growing, soft, watery and slimy callus was seen but after six weeks of growth and subsequent subculture on MS medium containing 0.5 mg l^{-1} 2,4-D, development of yellow compact, hard, nodular, shiny, somatic embryos was noticeable. Embryogenic cultures were maintained by repeated subculturing after every four weeks on a medium supplemented with 0.25mg l^{-1} 2,4-D alongwith 100mg l^{-1}ascorbic acid. Ascorbic acid was found to be useful for healthy growth of callus. Reduction of concentration of 2, 4-D to 0.1mg l^{-1} was found to be most suitable for embryo growth under dark conditions. The mature somatic embryos were then transferred on medium without growth regulators for germination and were kept exposed to light. It was noticed that 21% of the inoculated embryos produced shoots and roots alongwith a mixed population of precociously germinating embryos showing profuse rhizogenesis. These germinating embryos were separated and transferred to fresh media for completion of germination and plantlet development. In subsequent subculture, profuse tillering was observed in the developed plantlets. Only a few plants could be established in soil till maturity. These plants showed very poor growth and storage root development.

The work of Suri *et al.,* (96) has made further improvements in a protocol for rapid multiplication of *C. borivilianum* using stem disc explants through *in vitro* organogenesis. These workers used fungicide ((2- methoxy carbamoyl)-benzimidazol) and antibiotics (amoxacillin and cloxacillin) treatment for 24h prior to sterilization to reduce contamination. The stem disc explants produced large number of shoots (15 per explant) during first passage on B_5 medium supplemented with 0.18mM adenine and 22 µM BAP. Further they used the same medium and found increased number of shoots during subsequent passages (58 per inoculum consisting of clusters of 10 shoot buds in third passage). A decline of organogenetic potential was noticed after more than 10 subcultures. The increase in concentrations of $(NH_4)_2SO_4$ and KNO_3 in the medium caused increased shoot proliferation in long term cultures. According to these workers, effectiveness of different cytokinins for shoot proliferation was in the order TDZ>BAP> Kinetin> 2 iP. B_5 medium supplemented with 2880mg l^{-1} KNO_3, 471.4 mg l^{-1} $(NH_4)_2SO_4$, 0.18mM adenine and 22 µM 2 iP was effective in producing 58 shoots per inoculum during the first passage and was considered optimal for regeneration and long term maintenance of organogenetic potential. It was further observed that the rooting of isolated shoots was optimal in B_5 liquid medium containing 0.49 µM IBA, showing *de novo* tuberous roots (3 $plant^{-1}$). Fibrous roots did not turn tuberous. These isolated shoots with tuberous roots showed 100% survival following transfer to the soil. After over-wintering, these plants sprouted in the next season and were comparable to *in vivo* cultivated plant.

Pudake and Dhumale (97) developed an improved method for large-scale multiplication through shoot base and stem disc culture. *In vitro* multiplication was achieved on MS medium supplemented with 2 mg/l BA. Up to 90% of plantlets were established in pots by a hardening treatment, where the plants were first transferred to sterile sand and kept in a mist chamber under high humidity. Gaikwad *et al.*, (98) compared efficiency of seedling, root, stem disc and leaf (basal half) as explants, suggesting leaf base as the best explant followed by stem disc. Debnath *et al.,* (99) also regenerated *C. borivilianum* plants via organogenesis and embryogenesis and studied the influence of auxins and cytokinins on these processes.

Arora *et al.,* (50) obtained somatic embryos on MS medium containing 2.25µM 2, 4-D and 1.15µM Kinetin. The combinations alongwith of KNO_3 and $(NH_4)_2SO_4$ in the medium showed marked effects on the somatic embryogenesis and low total nitrogen level (250 or 500mg N l^{-1}) in the medium was found to be favourable for the growth and somatic embryogenesis. Among the various cytokinins tested by them 2-iP had a maximum stimulatory effect on somatic embryogenesis. The order of effectiveness of various cytokinins incorporated in MS-9 medium (containing 100mg N l^{-1} $(NH_4)_2SO_4$ and 400mg N l^{-1} KNO_3 supplemented with 2.25µM 2, 4-D) was 2-iP>TDZ>Kinetin>BAP. Incorporation of amino acids such as glutamine and proline in the medium promotes embryogenesis. They observed maximum number of somatic embryos (63 $inoculum^{-1}$) on MS-9 medium supplemented with 100 mg l^{-1}proline, 1.12 µM 2, 4-D and 7.42 µM2-iP. Germination of somatic embryos, isolated from young cultures (1-5 months old) was maximum (48%) on MS-9 medium supplemented with17.6µM BAP.

Arora *et al.,* (49) achieved somatic embryogenesis in long-term calluses of seedling and leaf explants, obtained from *in vitro* grown plants of *C. borivilianum*. Large number of such plants were raised in the field and evaluated for variability among the regenerants. The plantlets obtained through seedling derived embryonic callus showed high level of morphological and cytological variations, which increased with the increase in age of cultures. Occasionally they also observed variegated plants along with variations in the leaf size, stomatal number, epidermal cell size and chromosomal number. Finally, they concluded that instability and decline in embryonic potential was a major problem with the seedling derived callus for long term maintenance. According to them this problem can be overcome by the use of callus obtained from the leaf explants that forms a cyclic system of shoots→ callus → embryogenesis→ germinated embryo→ leaf→ callus.

Dave *et al.,* (100) developed highly reproducible field-tested and cost-effective micropropagation scheme for *C. borivilianum*. Shoot multiplication was best achieved on agar gelled MS medium containing 22.2µM BA and 3% sucrose and it lead to production of more than 15,000 plantlets within 20 weeks. Plantlets subjected to hardening under agro-shadenet conditions during the monsoon months of high humidity showed better survival rate and growth compared to plantlets hardened *in vitro* and subsequently transferred to the greenhouse for acclimatization. Rate of plantlet survival was 87% and 90% under open field and agro-shadenet conditions respectively which produced tuberous roots which could be

grown in next season as a secondary propagule. These workers concluded that *in vitro* production of Safed musli was cost-effective compared to conventional propagation and holds great potential for commercial production of this medicinal species.

Dave *et al.,* (101) achieved *in vitro* propagation of *C. borivilianum* using encapsulated shoot buds. They noticed that 4mm long shoot buds encapsulated in 3% sodium alginate matrix polymerized by 100mM solution of $CaCl_2.2H_2O$ yielded best results. They reported that the storage conditions, gel matrix media and period in storage influenced the *in vitro* regrowth potential of shoot buds when transferred on standard multiplication (SM) medium with ingredients of MS salts and 5.0 mg L^{-1} BAP. In case of encapsulated shoot buds started on wet agar-gel and kept under culture room conditions of light and temperature (45µ mol m^{-2} s^{-1} and 28± 2ºC resp.) more than 80% sprouting within 3 weeks was noticeable while dark stored (4ºC) encapsulated shoot buds on agar-gelled wet medium exhibited more than 90% sprouting after 7 days storage. The regrowth potential in terms of percentage declined to 60% when buds were stored for more than 30 days. It was found to be decreased below 20% after another 30 days of storage. These workers further noticed that supplementation of alginate matrix with sucrose and MS salts produced better results as compared to that with MS salts alone or without any of them. All the sprouted shoot buds, irrespective of their storage conditions, produced normal shoots on SM medium and multiplied at a rate of 2.5 fold per subculture of 21 days each. Microshoots could be rooted on the medium containing ¾ MS salts and 2.0 mg l^{-1} IBA, when rooted plantlets were hardened successfully and grown under greenhouse conditions production of normal tuberous roots was observed. These workers emphasized that this method of micropropagation opens possibilities for storage of shoot buds during off-season of production and facilitate transport of germplasm with ease.

Sharma and Mohan, (102) developed a novel method of shoot regeneration from immature floral buds along with inflorescence axis in *C. borivilianum* and established axenic cultures with very less contamination (10%). MS medium containing 2 mg l^{-1} kinetin and 0.1 mg l^{-1} 2, 4-D proved to be the best for multiple shoot induction. They also reported maximum number of shoot production (35) in MS medium with 2 mg l^{-1}BAP. Rooting of shoots (86.7%) with maximum fasciculated roots (5) occurred on Knops medium containing iron and vitamins of MS medium with 2 mg l^{-1}IBA and 0.1% activated charcoal. As much as 80% plant survival was observed in 4 weeks after removal from *in vitro* conditions and thirty four hardened plants were generated per explant within 50 weeks. These workers concluded that this protocol is useful for large scale clonal multiplication from immature floral buds with inflorescence axis and it can be successfully used for germplasm conservation of this rare medicinal herb without destroying the mother plant.

Singh *et al.,* (103) investigated biochemical changes leading to shoot regeneration during *in vitro* culture of *C. borivilianum* derived from bud pedicel on MS medium supplemented with 1.0 mg l^{-1}BAP + 1.0 mg l^{-1} NAA. In the control callus, starch content and reducing sugars were high which further increased significantly in shoot differentiating cultures. The concentration of total soluble sugars, free amino acids, total soluble proteins and total phenols were lower in former and increased in the shoot differentiating cultures. The activities of enzymes like α-amylase, acid-protease, acid phosphatase and peroxidase increased up to appearance of green patches (8-12 days) and reached a peak on 16th day of inoculation that coincided with the appearance of shoots. Contrary to above, the acid invertase activity decreased till the appearance of shoots.

Rizvi *et al.,* (104) achieved an efficient and cost- effective micro- propagation employing liquid medium (MS basal). They established *in vitro* culture from young shoot apices obtained from the field grown tuberous roots. These workers advocated that the application of liquid medium reduces the amount of medium consumed with better shoot growth and multiplication response than in solid medium. About 7.5 fold increase in shoot multiplication in liquid medium against 4.5 fold on agar solidified medium was evident in their experiment. These workers estimated that the use of liquid culture medium resulted in 92.31% reduction in single shoot production cost compared to solid medium. Based on these findings these workers concluded that liquid culture medium can be more useful for large-scale multiplication of *C. borivilianum.*

Mathur *et al.*, (105) investigated biological hardening and genetic fidelity testing of micro-cloned progeny of *C. borivilianum.* They observed that the extent of establishment of micro-cloned plantlets of this species established was more than 95% in soil treated with various bio-inoculants such as *Glomus aggregatum*, *Trichoderma harazianum* and *Piriformospora indica* whereas inoculation with *Azospirullum* sp. (CIM-azo) and *Actinomycetes* sp. (CIM-actin) showed only up to 85% plantlet establishment. The un-rooted shoots were also treated with these bio-inoculants, for *in vivo* root induction and increased survival rate/establishment frequency when transferred to soil. The un-rooted shoots also showed *in vivo* rooting (50%) when treated with mycorrhiza *Glomus aggregatum* (VAM) and *Trichoderma harazianum.* The genetic fidelity testing of micro-cloned, bio-hardened progeny based on a RAPD analysis (using 40 random decamer DNA primers) indicated a strong uniformity in relation to the parent genotype.

d. Growth and yield

A great variability in plant maturity has been recorded by Geetha and Maiti (31) in their germplasm collection. According to them the plant maturity varied from 3 to 6 months. During safed musli cultivation emergence of inflorescence occurs following sprouting of tubers and it is immediately removed. The economically unwanted sink is removed during the initial phase of plant growth. Bordia *et al.*, (5) reported that about 15-30% of dry matter is present in the fleshy roots of *C. borivilianum*. Nikam and Chavan (92) noticed that the dry matter partitioning towards tubers was accelerated after 45 days of plant growth.

The pattern of nutrient uptake during different growth stages of *C. borivilianum* was investigated by Nikam and Chavan (92). The accumulation of nitrogen and potassium took place in the leaf tissue upto 75 days of plant growth and thereafter there was decline. On the other hand calcium and magnesium accumulation in both leaf and tuber tissue occurred during the entire course of plant development. The leaves of young plants were quite rich in phosphorus and thereafter a decline in P level in the leaf tissue was noticeable. The level of all these elements recorded a continuous increase in the tuber tissue alongwith progress of plant growth. A considerable retranslocation of NPK from leaf to tuber tissue was evident after 75 days of plant growth.

Singh *et al*., (106) recently performed a variability and association study of *C. borivilianum* over two microenvironmental conditions on yield and yield contributing morphometric fruits. These workers indicated that musli yield can be improved through an increase in any of the major yield contributing characters viz. root number, root length and root diameter. According to these workers productive root number is the most important selection criterion for improving the musli productivity. Kothari and Singh (61) noticed that removal of inflorescence buds increased fleshy root length (32%) and fresh fleshy root yield (22%). The study of Singh *et al*., (106) revealed that all the yield contributing characters except root diameter had better expression under integrated nutrient management (2 tons of vermicompost with 40:30:25 Kg per hectare) them under organic conditions (5 tons of vermicompost per hectare).

e. Responses to environmental constraints

Although it has been stated that *C. borivilianum* is sensitive to waterlogging the metabolic responses of this species to various environmental stresses has been hardly studied. The author has made attempt to study these aspects (85). The influence of sodium chloride salinity on phosphorus metabolism in *C. borivilianum* was investigated (107). Salt stress caused the lowering of phosphorus content in both leaf and tuber tissue. This was accompanied with decline in alkaline phosphatase and ATPase activities in leaf and tuber tissue and acid phosphatase activity in the tuber tissue. However, leaf acid phosphatase activity was found into be stimulated by salt treatment. The mineral status in this species under water deficit and waterlogged condition has been investigated (108). The analysis of macro element in the leaf tissue revealed that water stress caused a decline in levels of nitrogen, phosphorus and potassium while there was accumulation of calcium, magnesium and sulphur. In the leaf tissue of waterlogged plants reduction in phosphorus and potassium content was noticeable while the levels of nitrogen, calcium, magnesium sulphur were increased. Potassium and sulphur contents were found to be reduced in the tuber tissue by both water stress as well as waterlogging treatments. An increase in phosphorus and magnesium content was noticed in water stressed tuber tissue while similar trend was noticed in case of magnesium under waterlogging stress. The micronutrient composition was also altered due to both kinds of stresses. The contents of copper, zinc and manganese in the leaf tissue were increased by both stresses. Leaf copper content was reduced due to water stress and increased due to waterlogging stress. An increase in iron content and reduction in sodium level was observed in tuber tissue under both kinds of stresses. Prolonged waterlogging (10 days) affected the level of N, P, Ca, Zn and Mn in the tuber tissue.

Epilogue

It is evident from the foregoing account that the agrotechnological practices for *Chlorophytum borivilianum* are very well standardized and attempts to cultivate this species on large scale are underway. The poor seed germination potential in this species can be compensated in future by various by micropropagation protocols developed in different laboratories. Eventhough, number of pharmacological studies have been performed, the phytochemistry of the tuber and leaf is not clearly understood. Similarly a major limitation in this species appears to be a poor knowledge about various physiological and biochemical processes.

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