STUDIES IN STRUCTURAL AND PHYTOCHEMICAL PARAMETERS OF FLOWERED AND NON FLOWERED MANGA BAMBOO *DENDROCALAMUS STOCKSII* (munro).

Thesis submitted to

DR. BALASAHEB SAWANT KONKAN KRISHI VIDYAPEETH,

DAPOLI, DIST. RATNAGIRI, MAHARASHTRA STATE.

In partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE (FORESTRY)

In

FOREST BIOLOGY AND TREE IMPROVEMTNT

By

HIROLE SAGAR KUNDAN

Under the guidance of

Dr. A.D. Rane

Associate Professor
College of Forestry, Dapoli.

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C E R T I F I C A T E

This is to be certified that the thesis entitled **"Studies in structural and phytochemical parameters of flowered and non flowered Manga Bamboo *Dendrocalamus stocksii* (munro)."** is a record of independent bonafied research work carried out by **Mr. Hirole Sagar Hirole.** (Registration No. FDPM-15-53) at this college during the period of study from 2016 - 2017 under our guidance and supervision for the degree of Masters of Science (Forestry) in Forest Biology and Tree Improvement of Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. The said thesis has not previously formed the basis for the award of any degree, diploma, fellowship or any other similar title.

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Countersigned

Associate Dean

College of Forestry,
Dapoli

External Examiner

STUDIES IN STRUCTURAL AND PHYTOCHEMICAL PARAMETERS OF FLOWERED AND NON FLOWERED MANGA BAMBOO *DENDROCALAMUS STOCKSII* (munro).

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Mr. HIROLE SAGAR KUNDAN

B.Sc. (Forestry)

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AUGUST, 2017

DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the thesis entitled **"Studies in structural and phytochemical parameters of flowered and non-flowered Manga Bamboo *Dendrocalamus stocksii* (munro)"** reported in this thesis, except where otherwise indicated, is my original work and has not been submitted for any degree or examination at any other university. This thesis does not contain other persons' writing, unless acknowledged as being sourced from other researchers. Where other written sources have been quoted, then their words have been re-written and the information attributed to them has been referenced.

Place: Dapoli. **(Hirole Sagar Kundan)**

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The source of materials used and all assistance received if any, during the course of investigation have been duly acknowledged.

Place: Dapoli

Date:

Dr. A.D. Rane
Chairman
Advisory Committee

ACKNOWLEDGEMENT

"Cultivation of mind should be the ultimate aim of human existence"

By this thought of Dr.BhimraoRamjiAmbedkar I got inspiration to bring this piece of work into light. By the ignition of light of education, compliment and fight for the right,Dr.Amedkarleadsmy path for each and every step of this research.

After these two years of my research work, I've got a long list of names thatmade significant contribution in some way or other for completion of the study andpreparation of thesis and I owe my deepest gratitude to all of them.

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It gives me great pleasure to express my gratitude and heartfelt respect to my Advisory committee members, **Dr.A. D. Rane** Associate professor, college of forestry, Dapoli,**Dr. S.S. Narkhede**, Associate Dean, College of forestry, Dapoli, **Dr. V. K. Patil**, Associate professor, College of forestry, Dapoli, **Dr. S.K. Jain**, Head of Department , farm structure, C.A.E.T, Dapoli

I thankto **Dr.Meshram,** Associateprofessor, soil science dept.he was always there withinspiration and proper solutions for every problem I had to face from the verybeginning of my researchs and for helping me in laboratory and contributing a lot for timely completion of the work.I owe my deepest gratitude tomy B.Sc. advisor and my motivation Dr**.H. K. Deshmukh**, Associate professor, college of forestry, Akola for their unstinted support. I would like to express my deepest gratitude to for his valuable suggestions and encouragement.

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I express my heartfelt gratitude and humble respect towards my beloved father **Mr.KundanHirole**, my beloved mother **Sou. NaliniHirole**, my parents are only next to my god any and every good qualities which seems in me is belongs to them. I thank god for giving me such honest and sacrificial parents to whom I owe all that is mine.

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Last but not least I thank all the staff of College of Forestry, Dapoliwho helped me during course of work.

Above all, I bow before my Parents for all the blessings!!!!!!!

(Sagar Kundan Hirole)

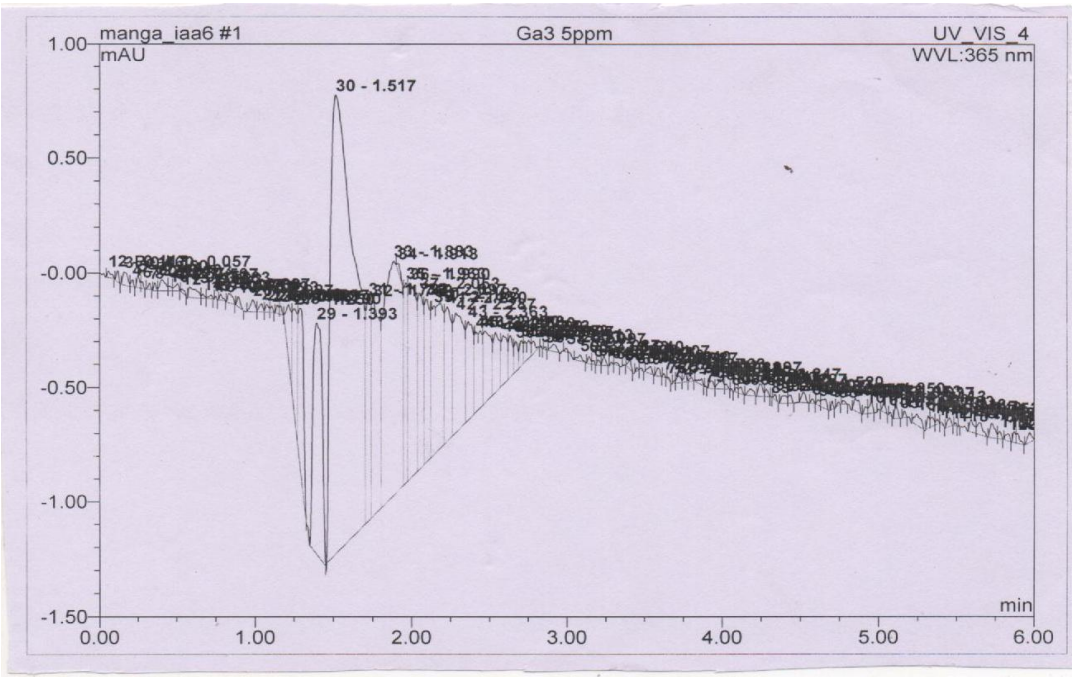


Fig. 16: HPLC chromatogram of standard GA3 5 ppm

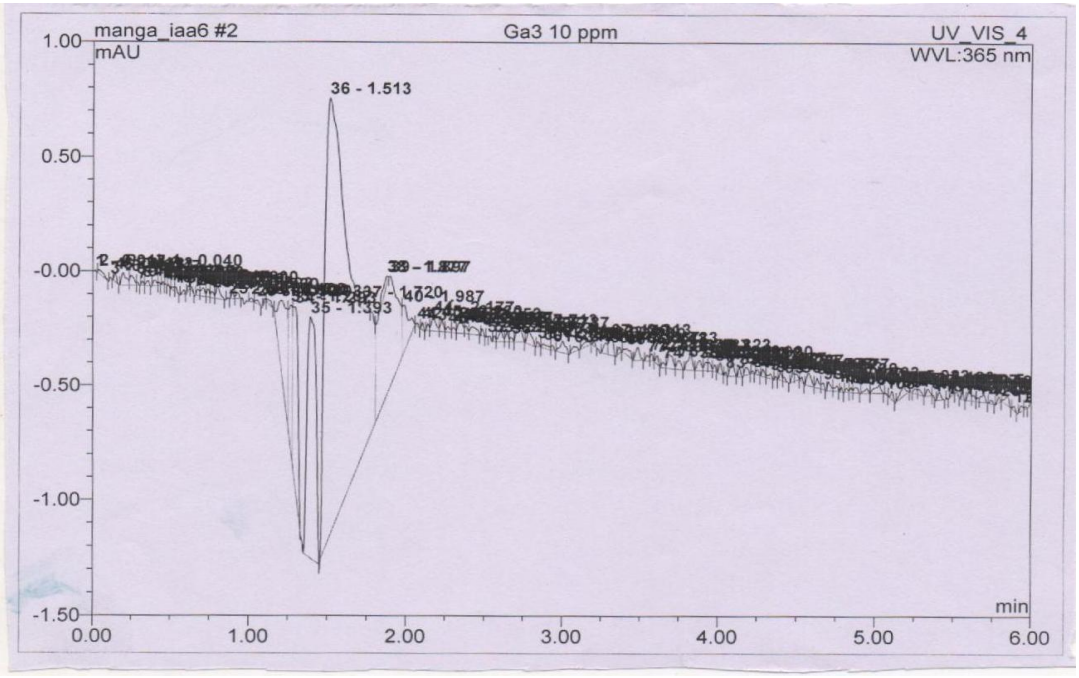


Fig.17: HPLC chromatogram of standard GA_3 10ppm

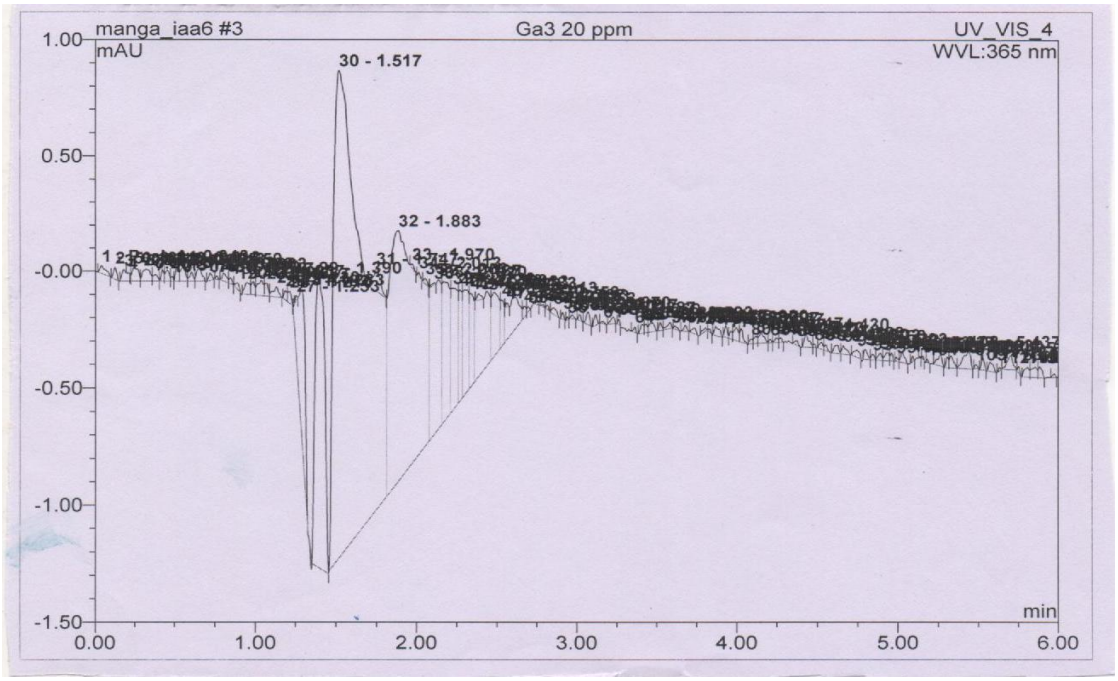


Fig. 18: HPLC chromatogram of standard GA_3 at 20ppm

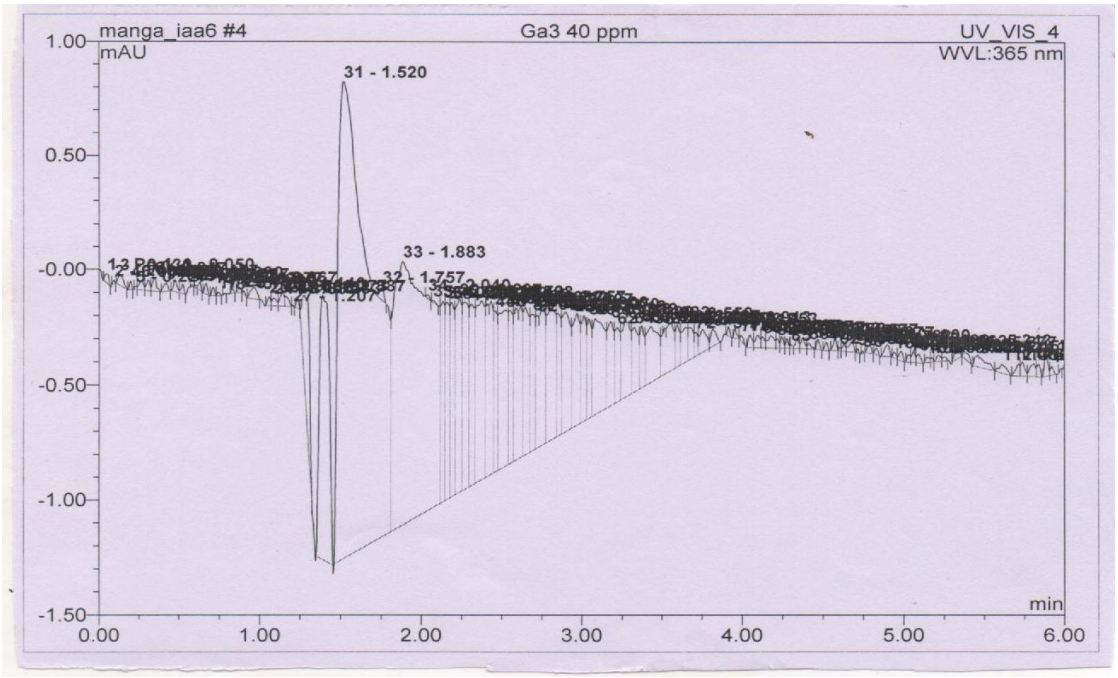


Fig. 19: HPLC chromatogram of standard GA_3 at 40ppm

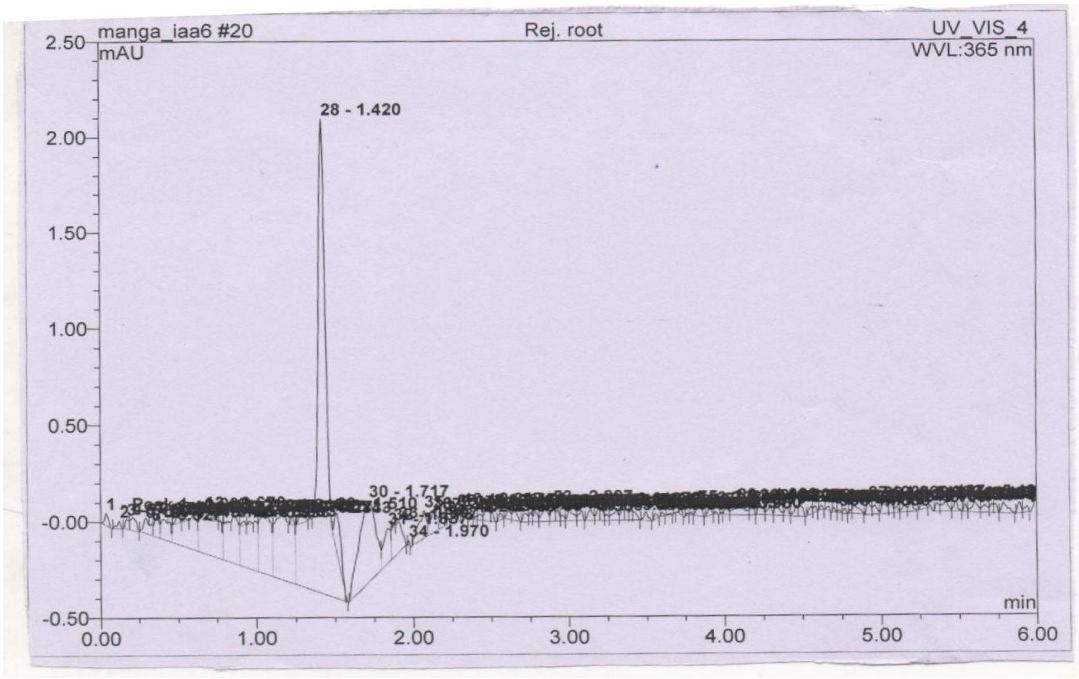


Fig. 20: HPLC chromatogram of root of rejuvenated stage

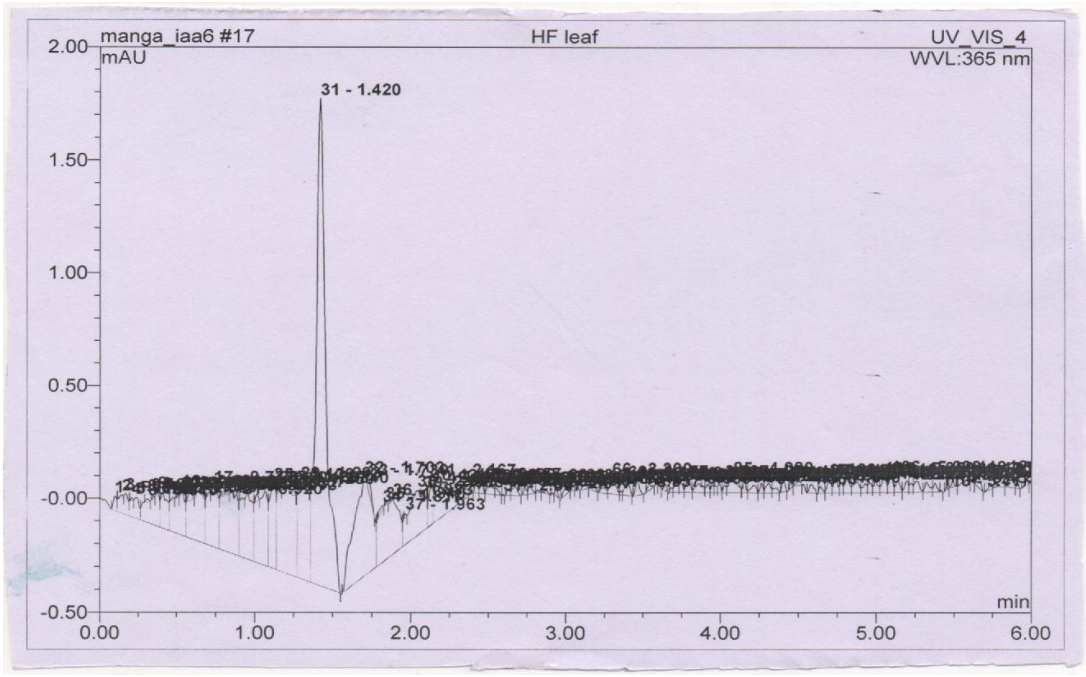


Fig. 21: HPLC chromatogram of leaf of initial flowered

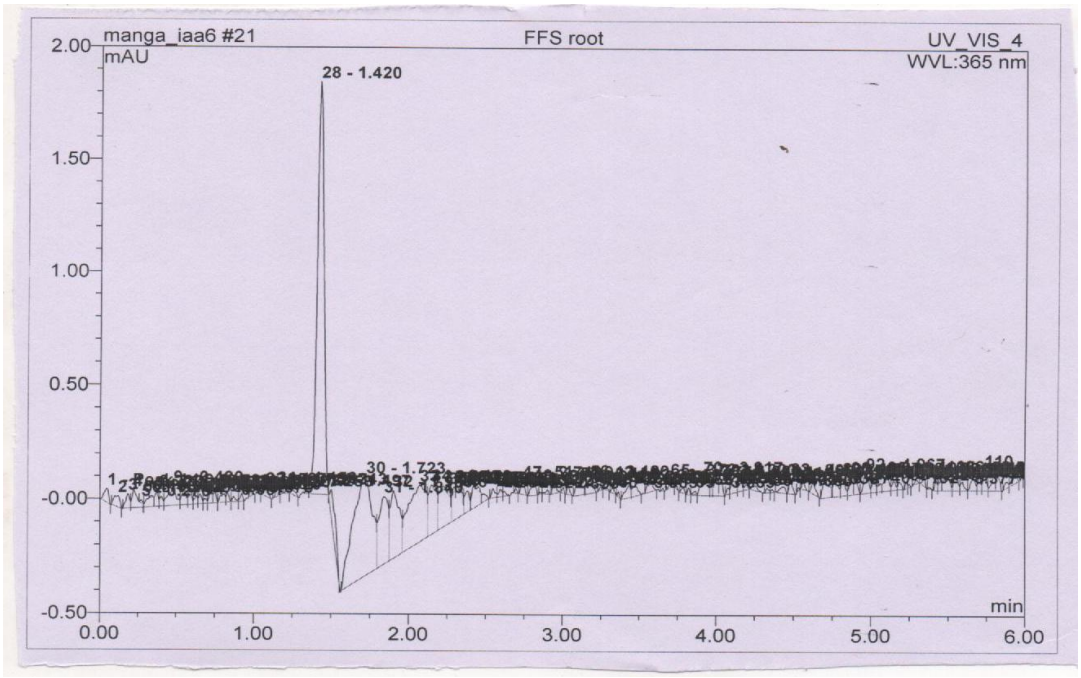


Fig. 22: HPLC chromatogram of root of full flowered from farmer's field

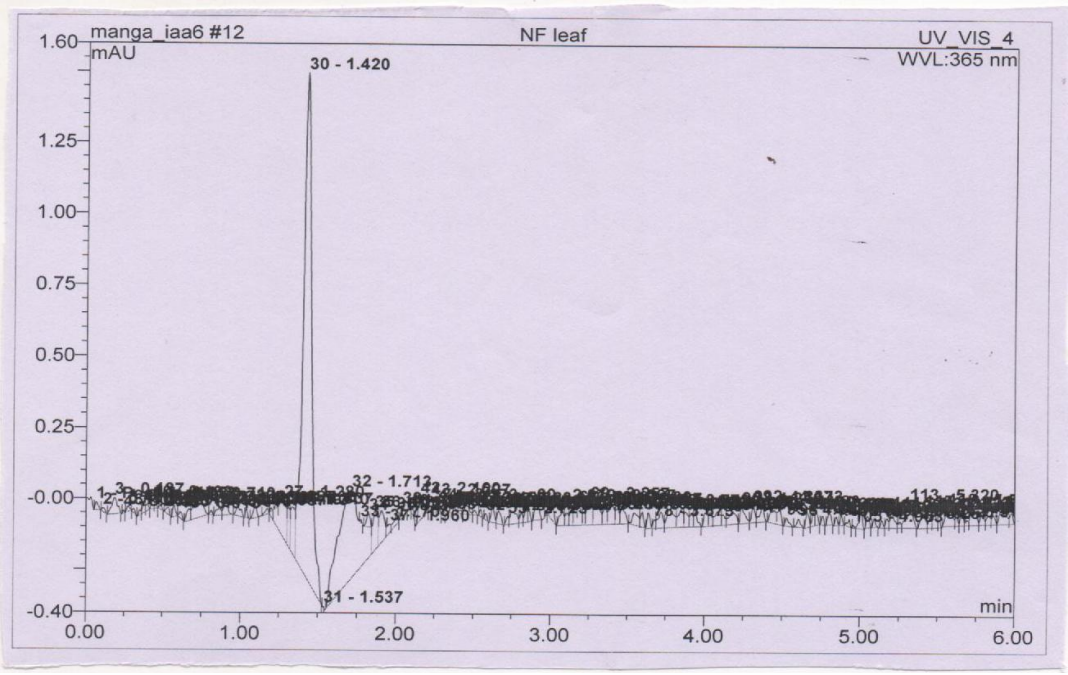


Fig. 23: HPLC chromatogram of leaf of Null flowered

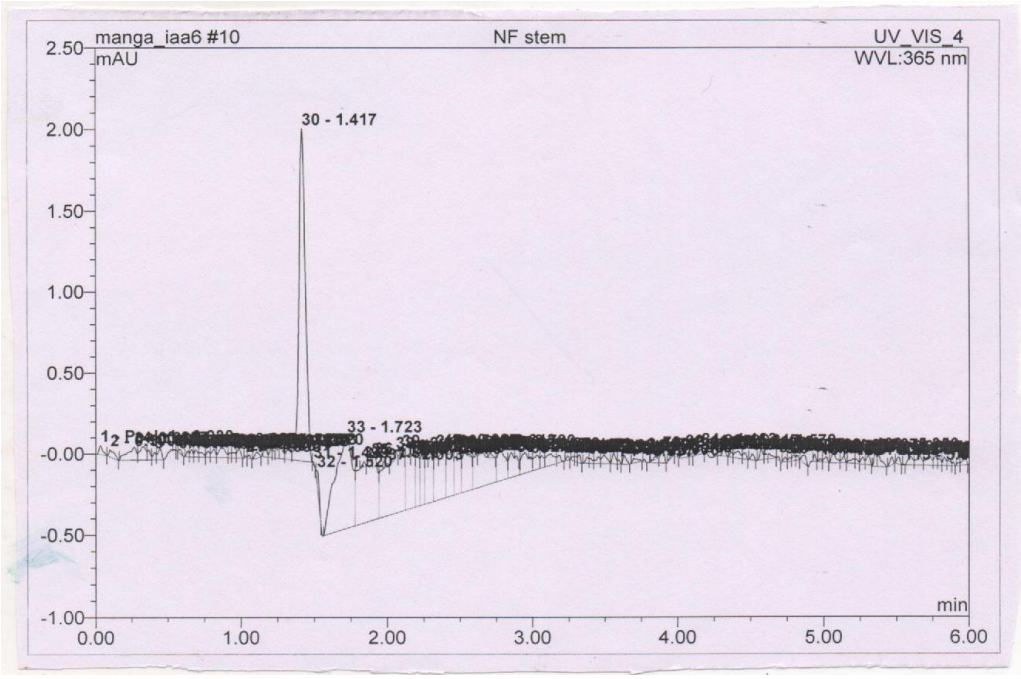
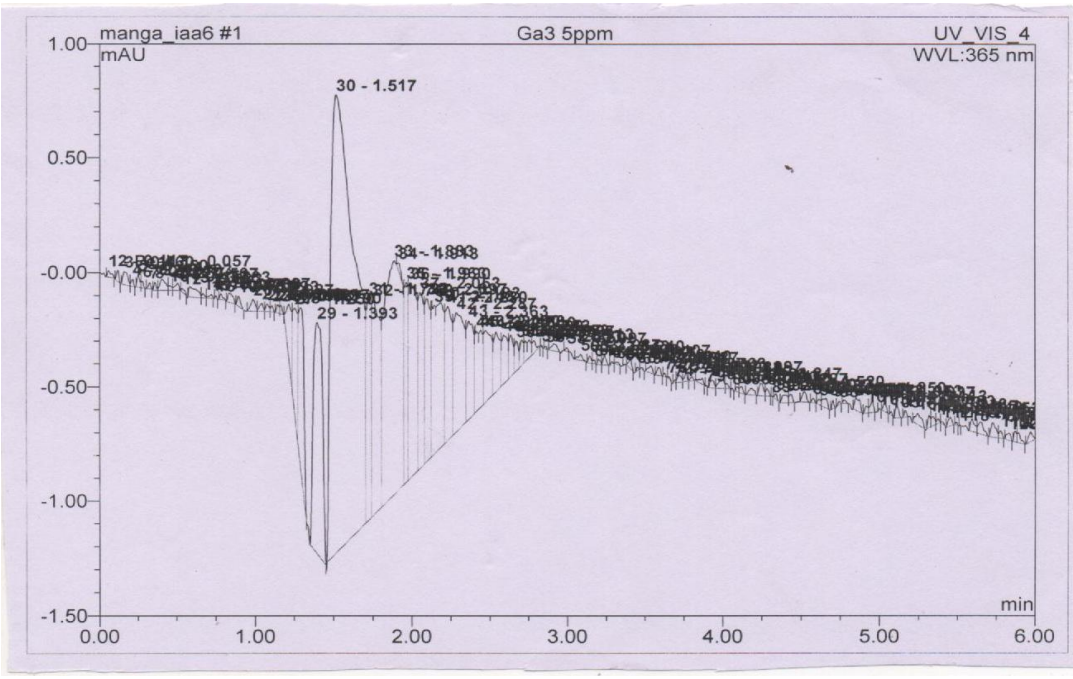


Fig. 24: HPLC chromatogram of stem of Null flowered



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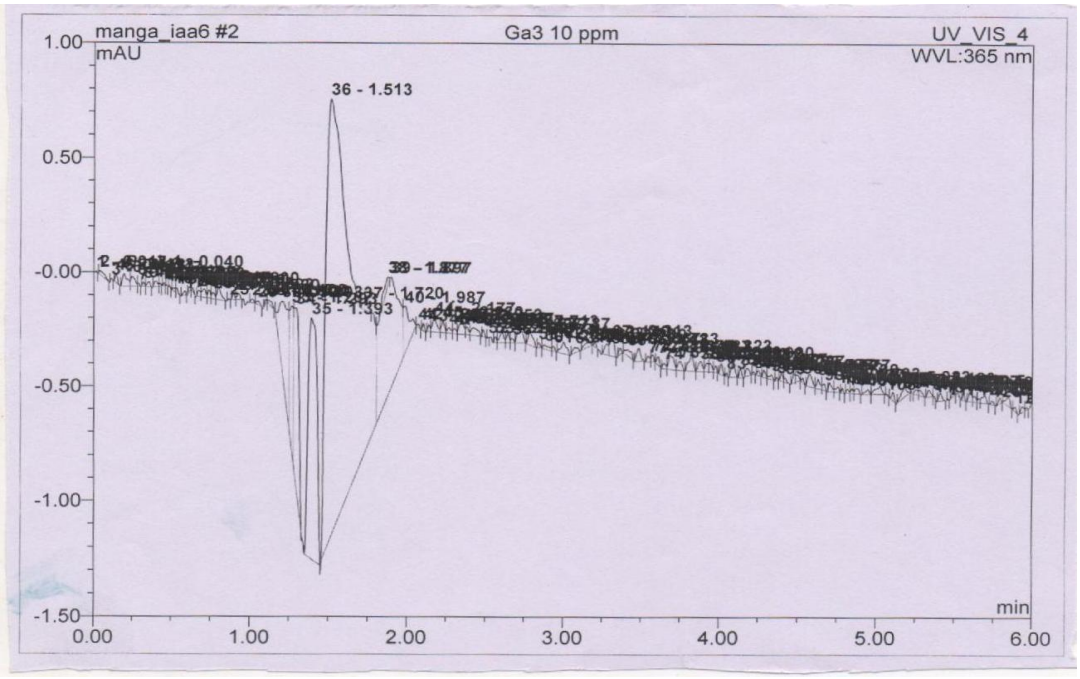

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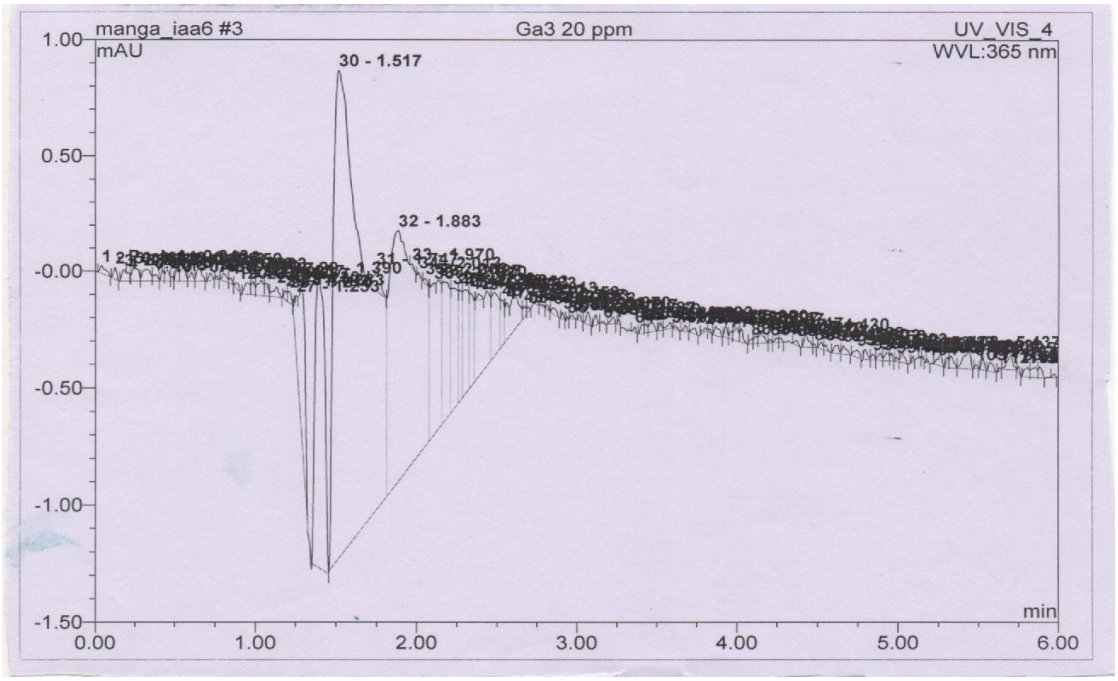

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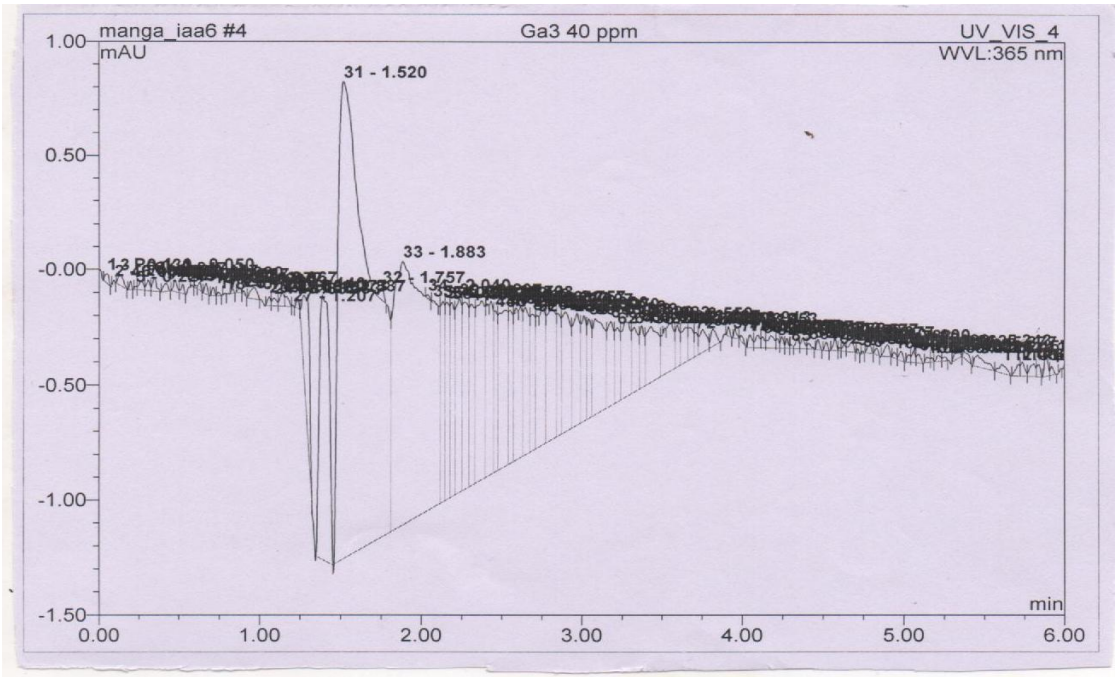

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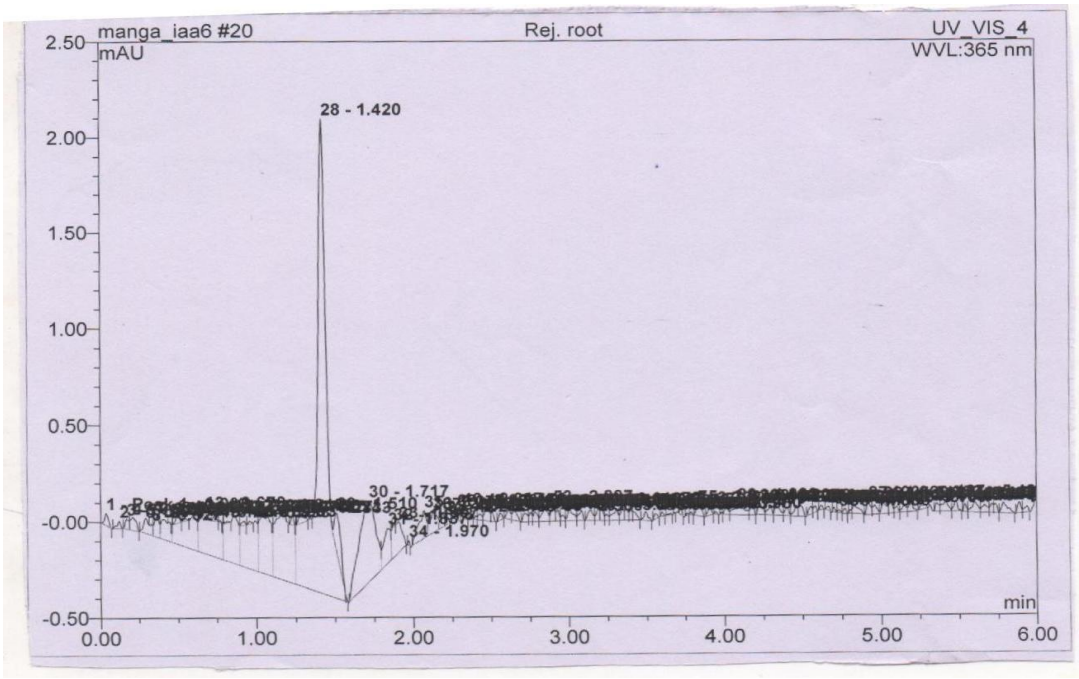

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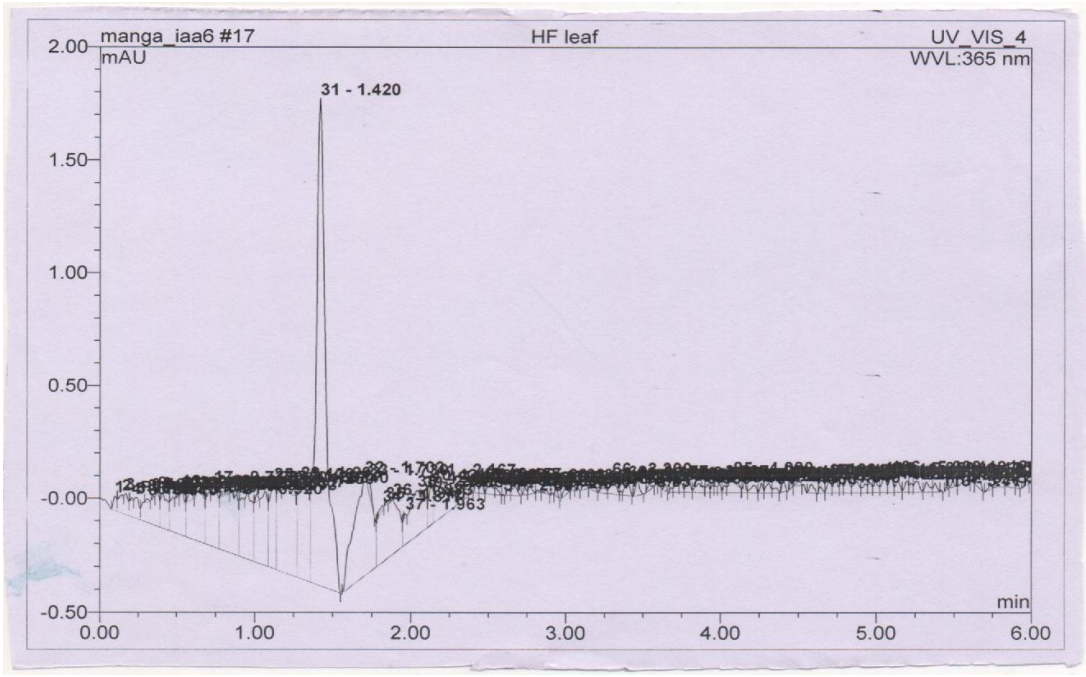


Fig. 21: HPLC chromatogram of leaf of initial flowered

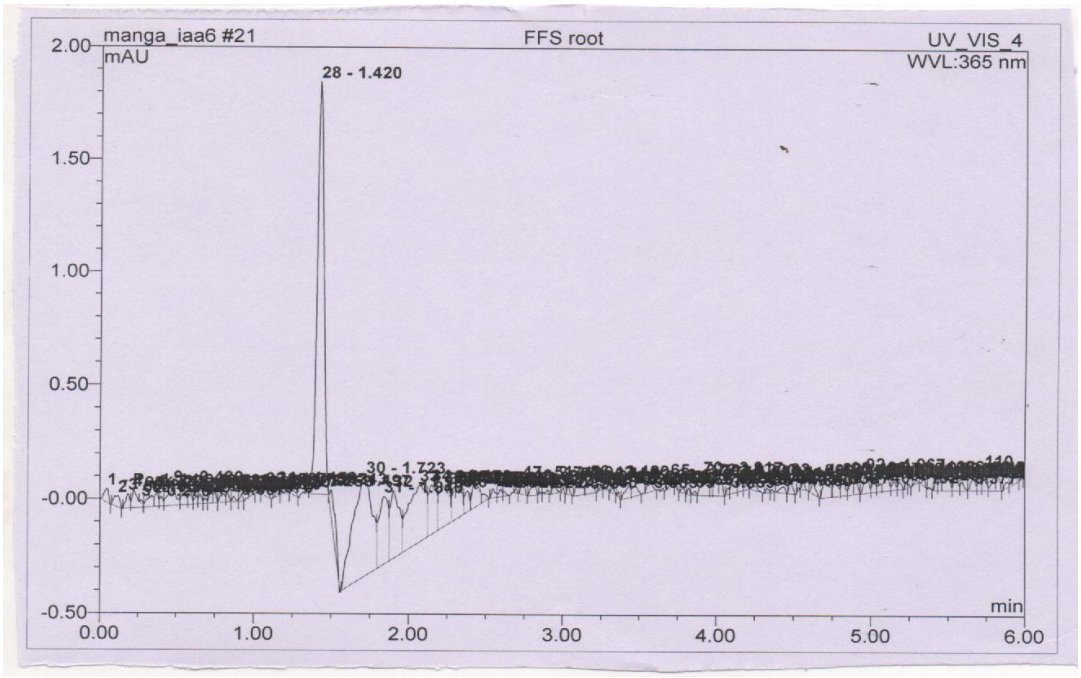
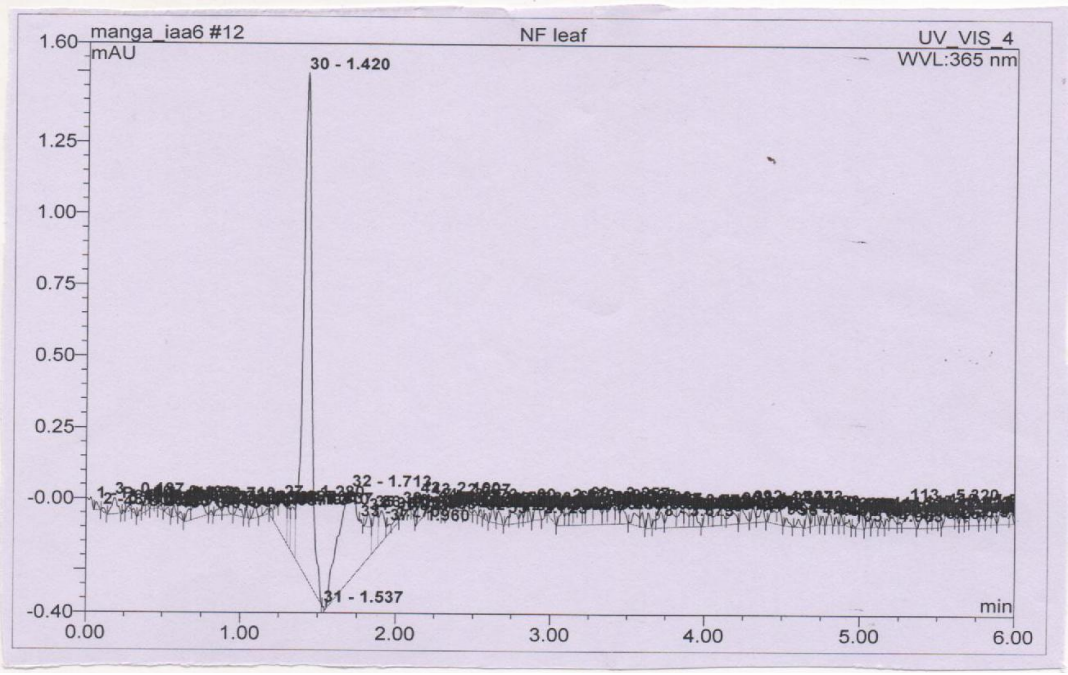


Fig. 22: HPLC chromatogram of root of full flowered from farmer's field



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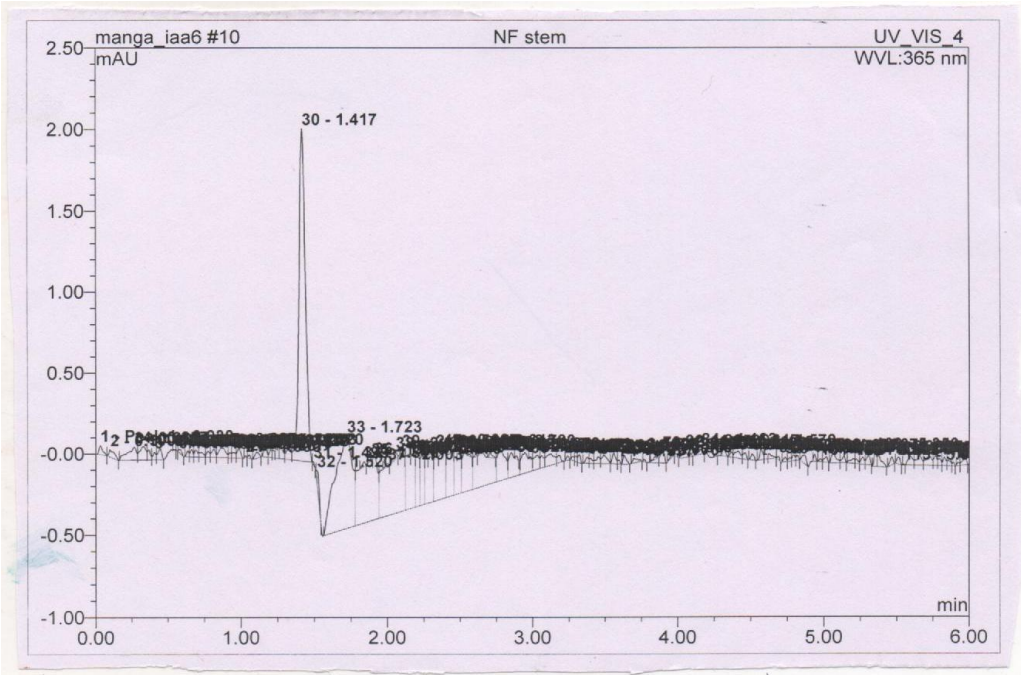


Fig. 24: HPLC chromatogram of stem of Null flowered

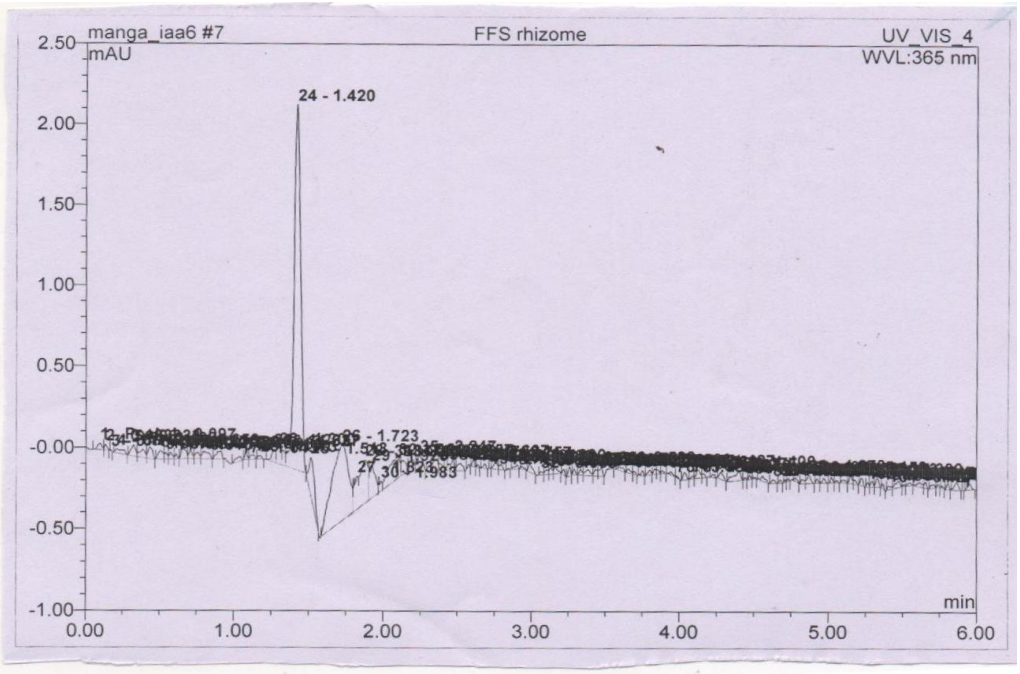


Fig. 25: HPLC chromatogram of rhizome of full flowered from farmer's field

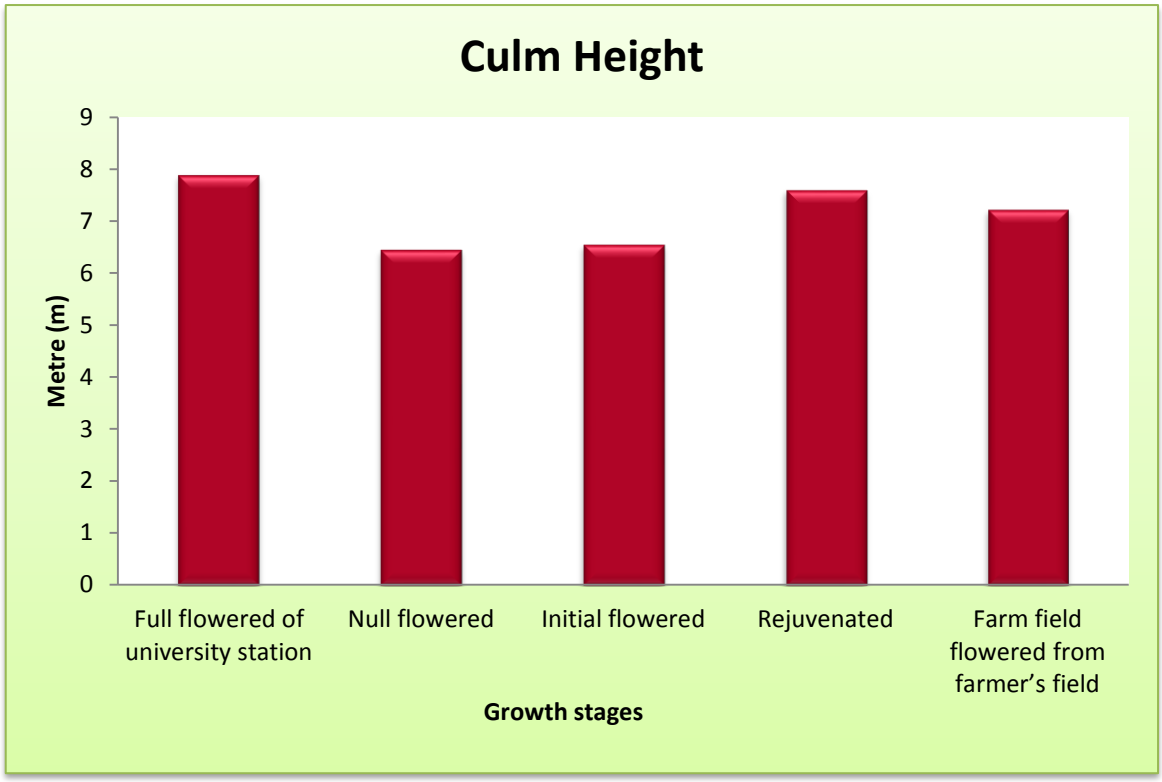


Fig.1:Clum height of *D.stocksii* at different growth stages

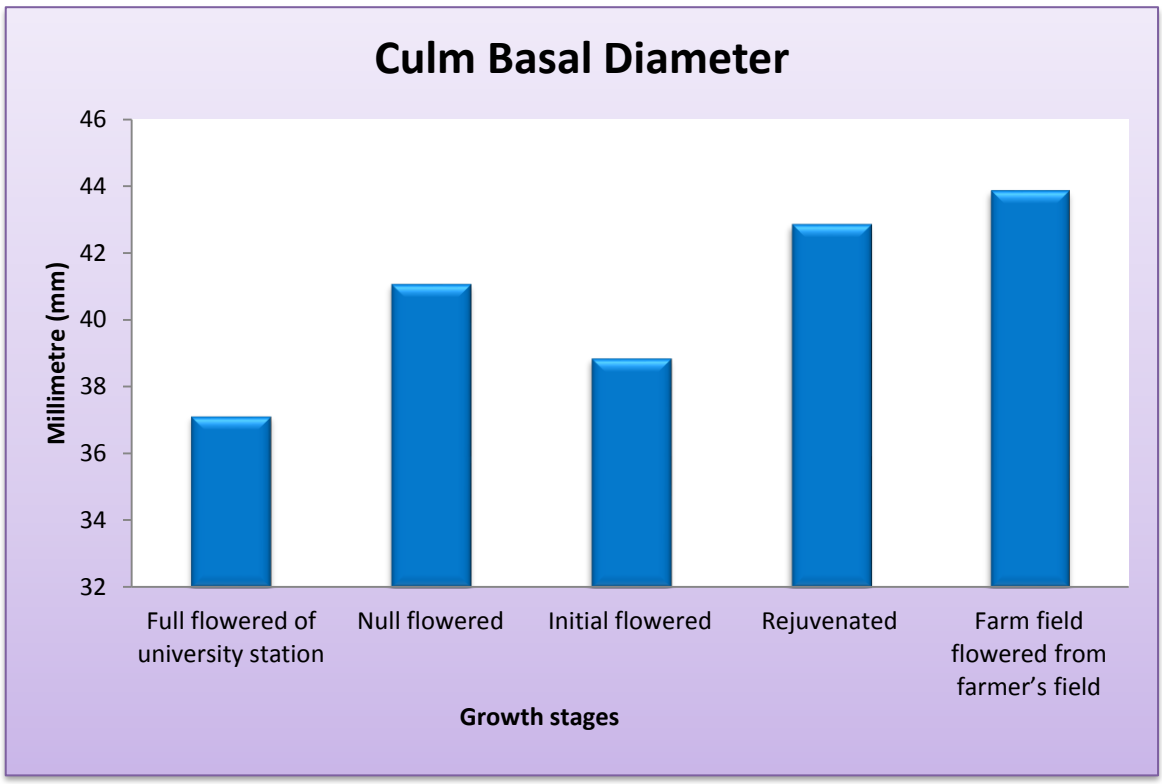


Fig.2:Clum basal diameter of *D.stocksii* at different growth stages

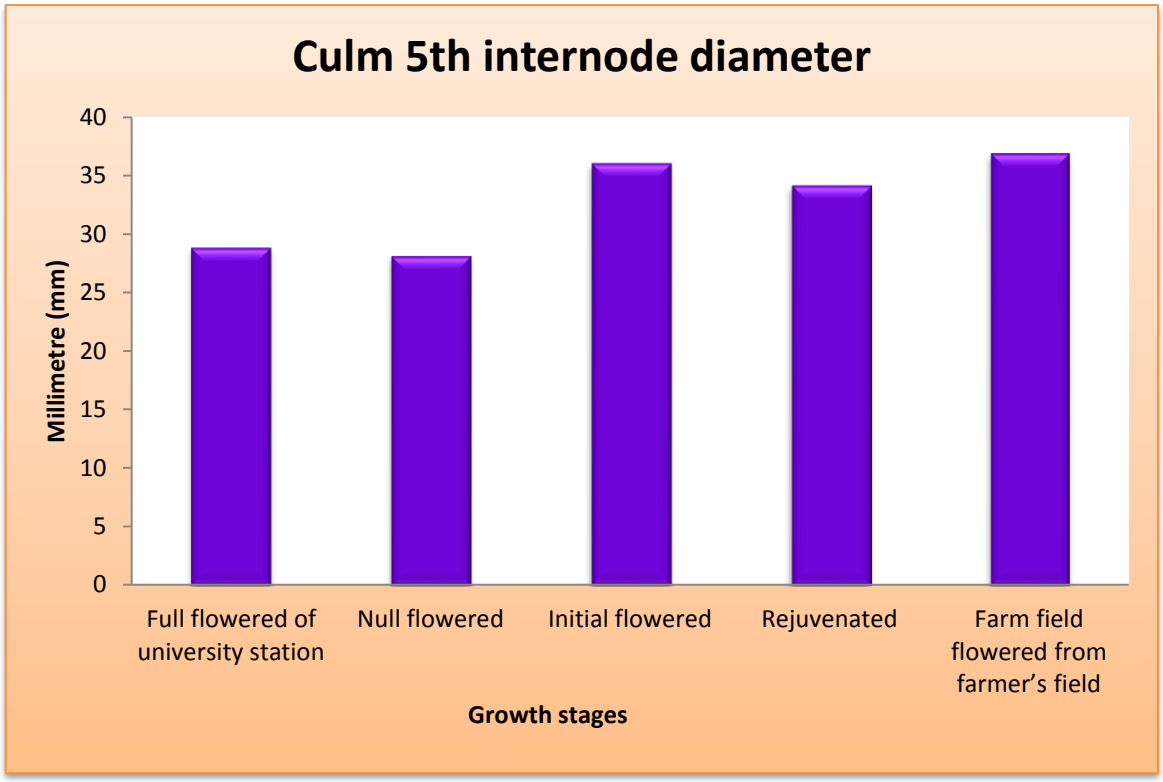


Fig.3:Clum5th internode diameter of *D.stocksii* at different growth stages

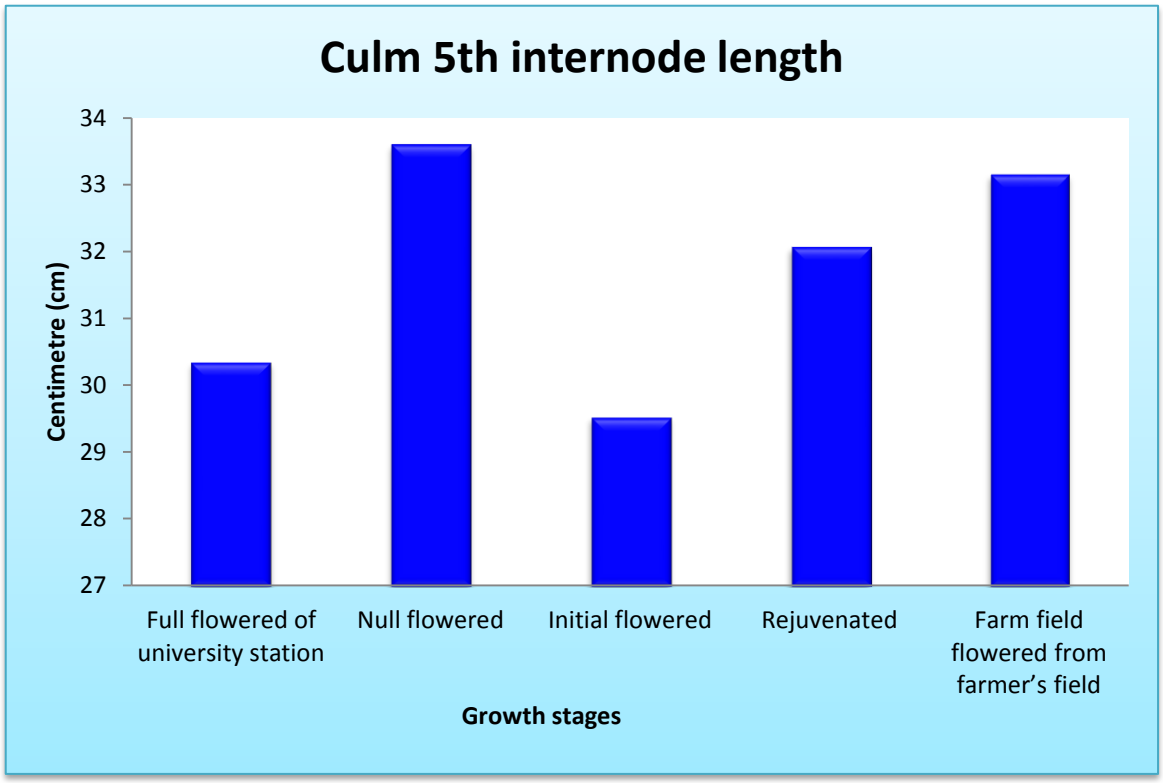


Fig.4:Clum 5th internode length of *D.stocksii* at different growth stages

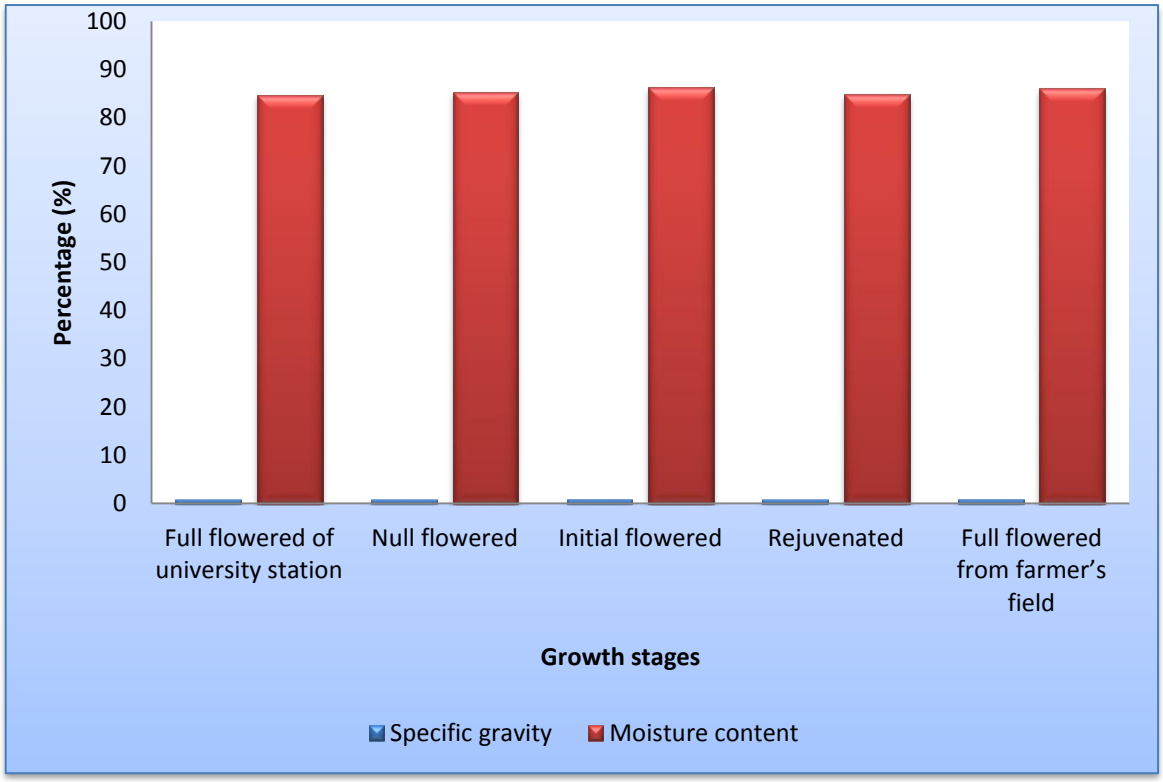


Fig.5: physical parameters of bamboo at different stages

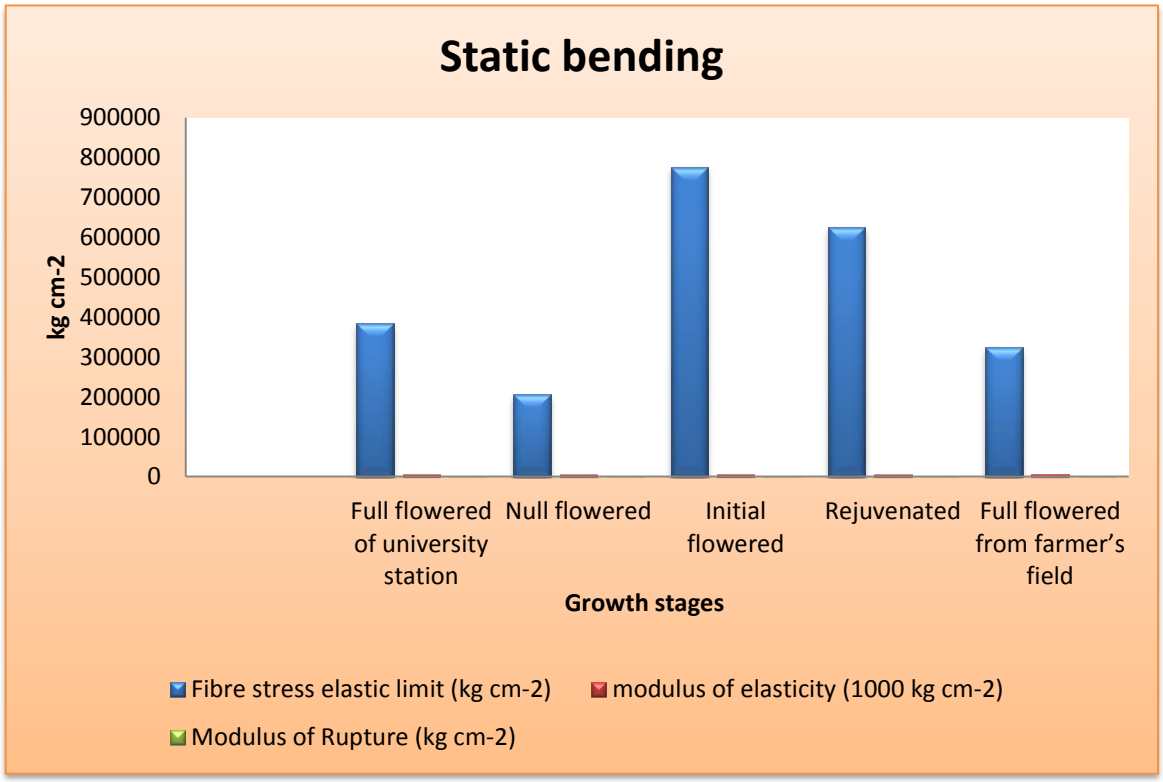


Fig.6: Static bending strength for *D. stocksii* of different growth stages

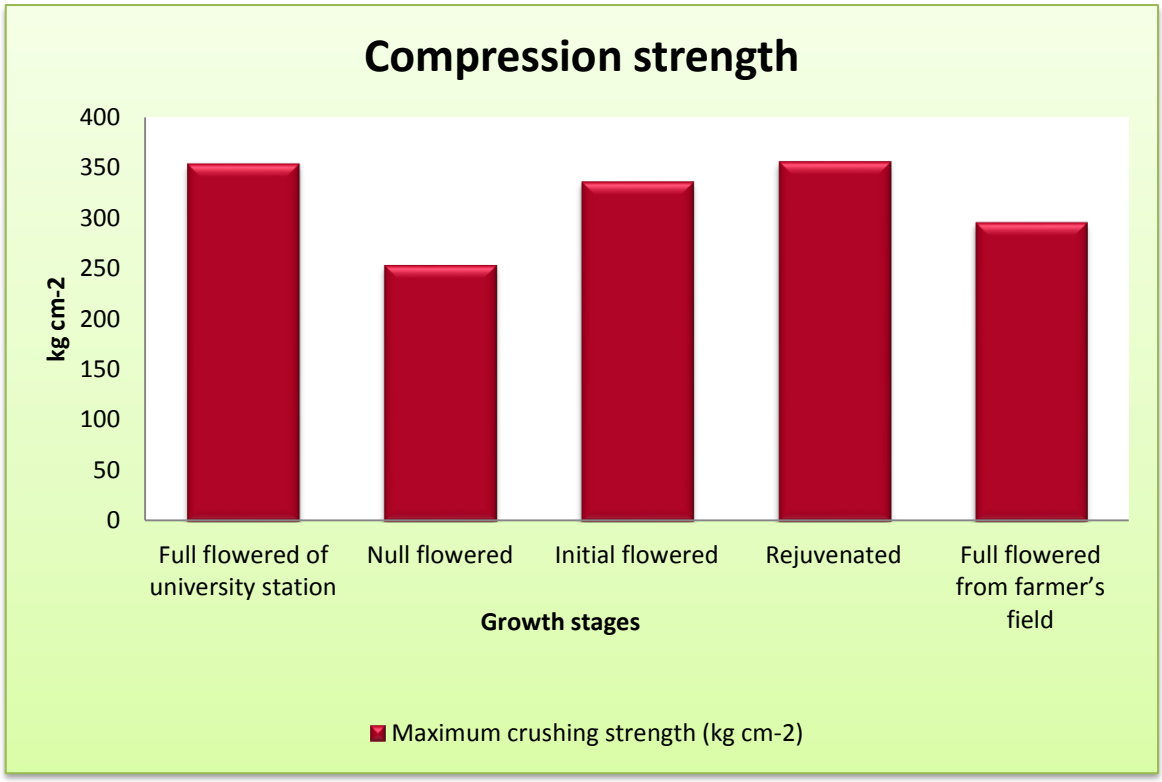


Fig.7: Compression strength preferred for bamboo culms of different stages.

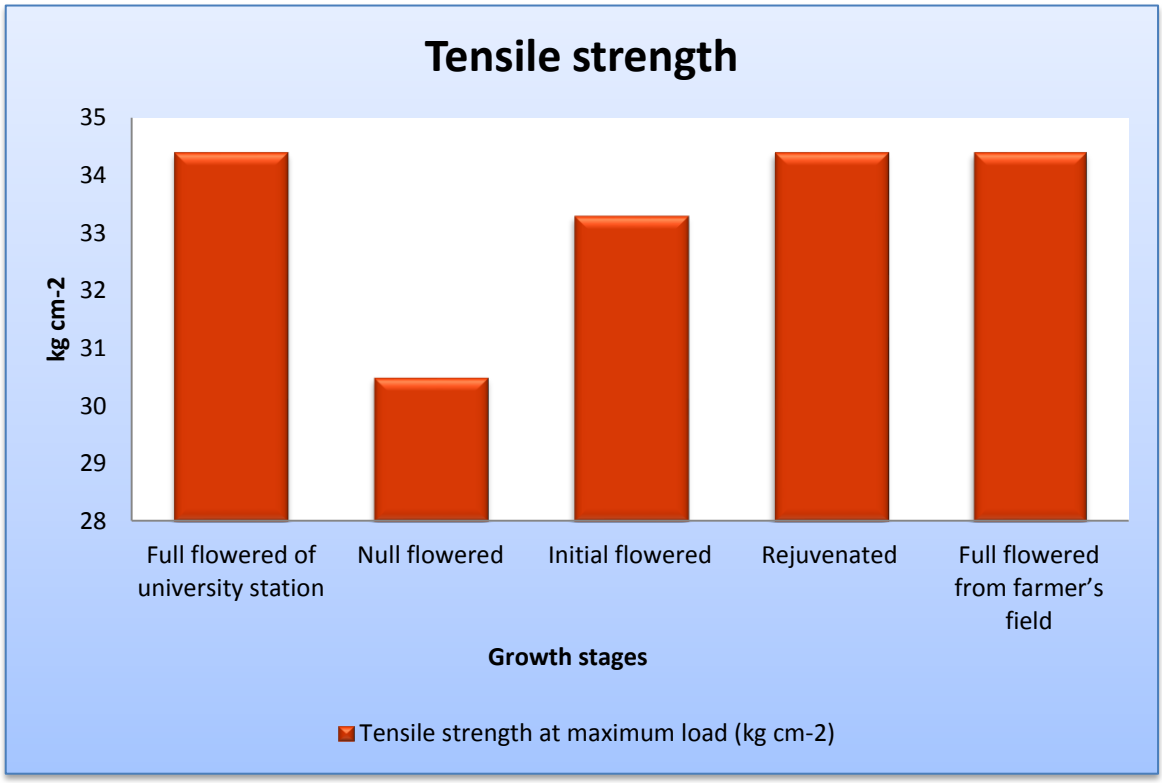


Fig.8: Tensile strength preferred for bamboo at different stages

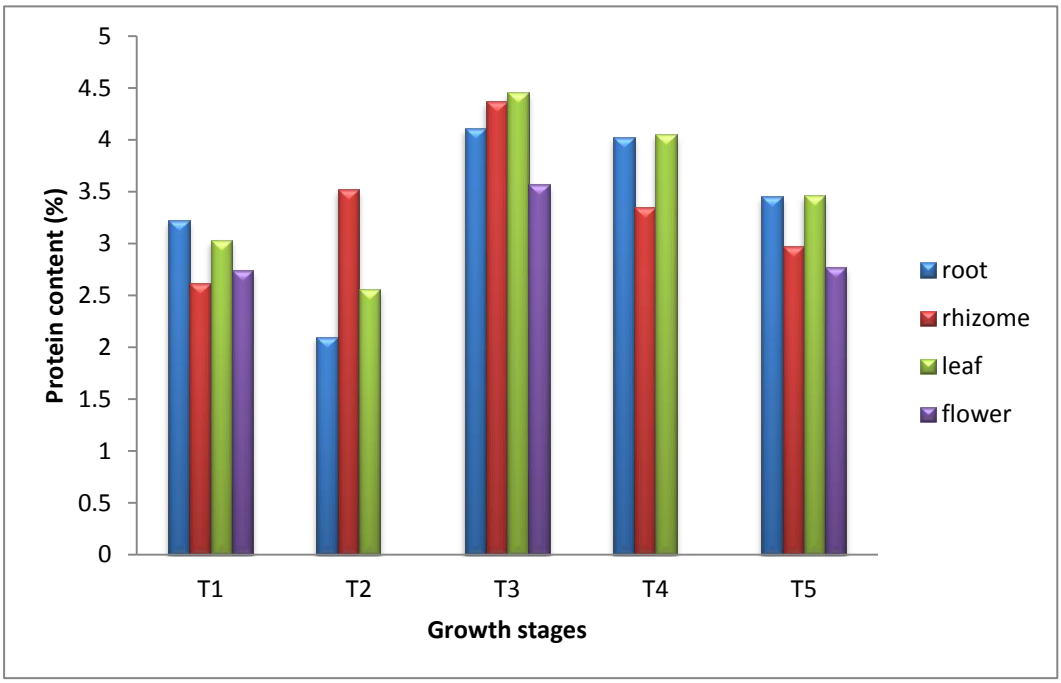


Fig. 9: Protein content of *D.stocksii* in all stages of clums

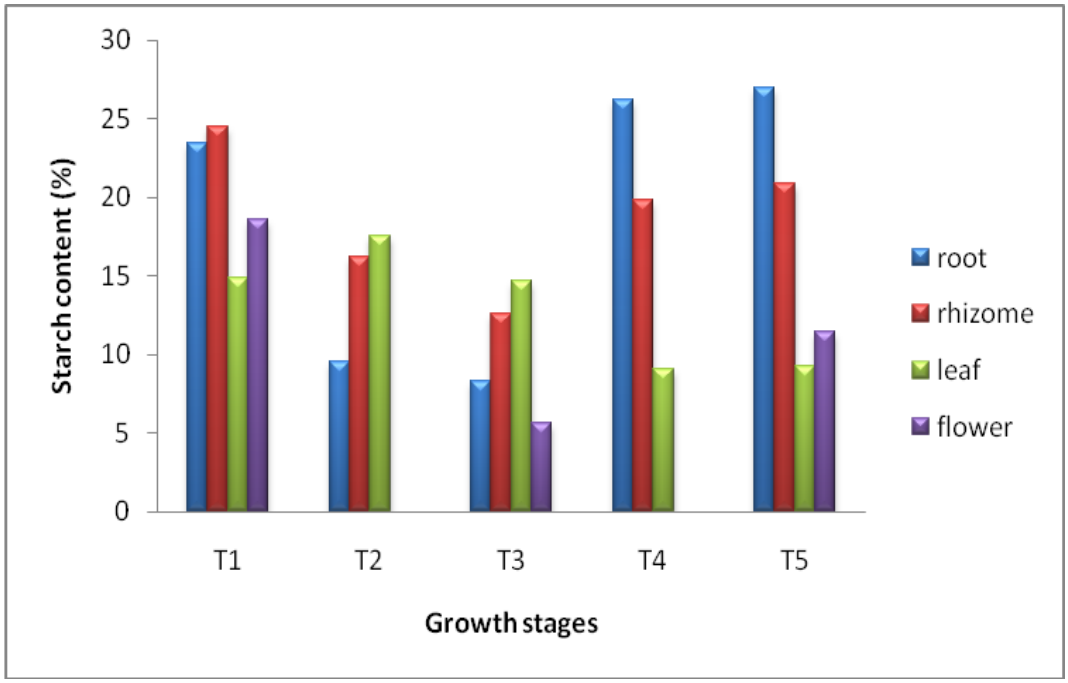
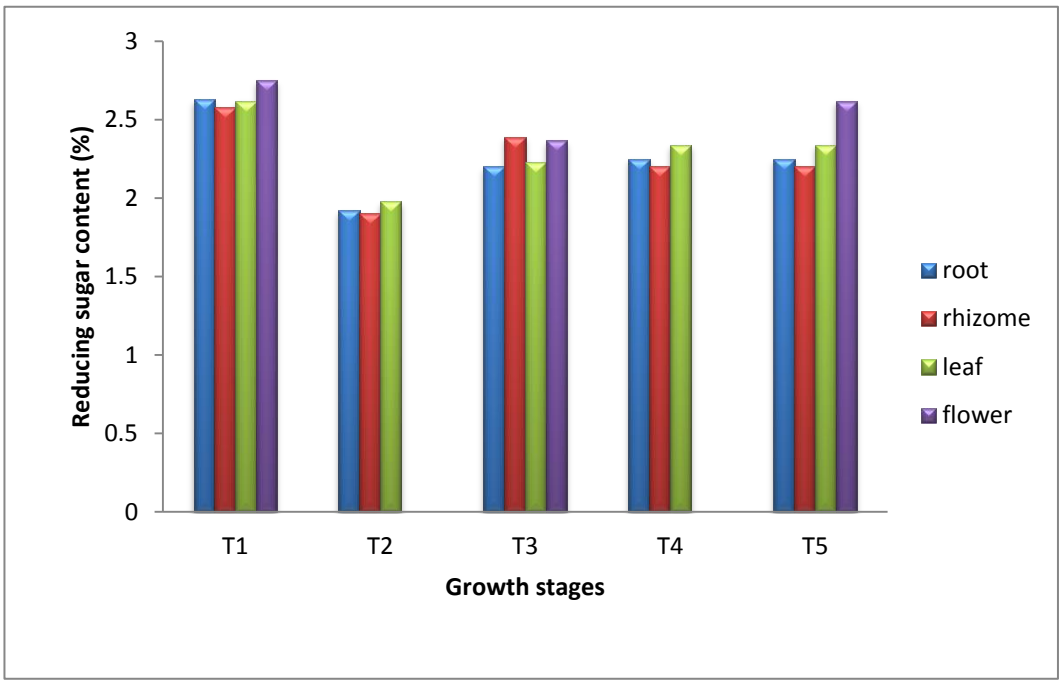


Fig. 10: Starch content of *D. stocksii* in all stages of clums

Fig. 11: Reducing sugars content of *D.stocksii* in all stages of clums



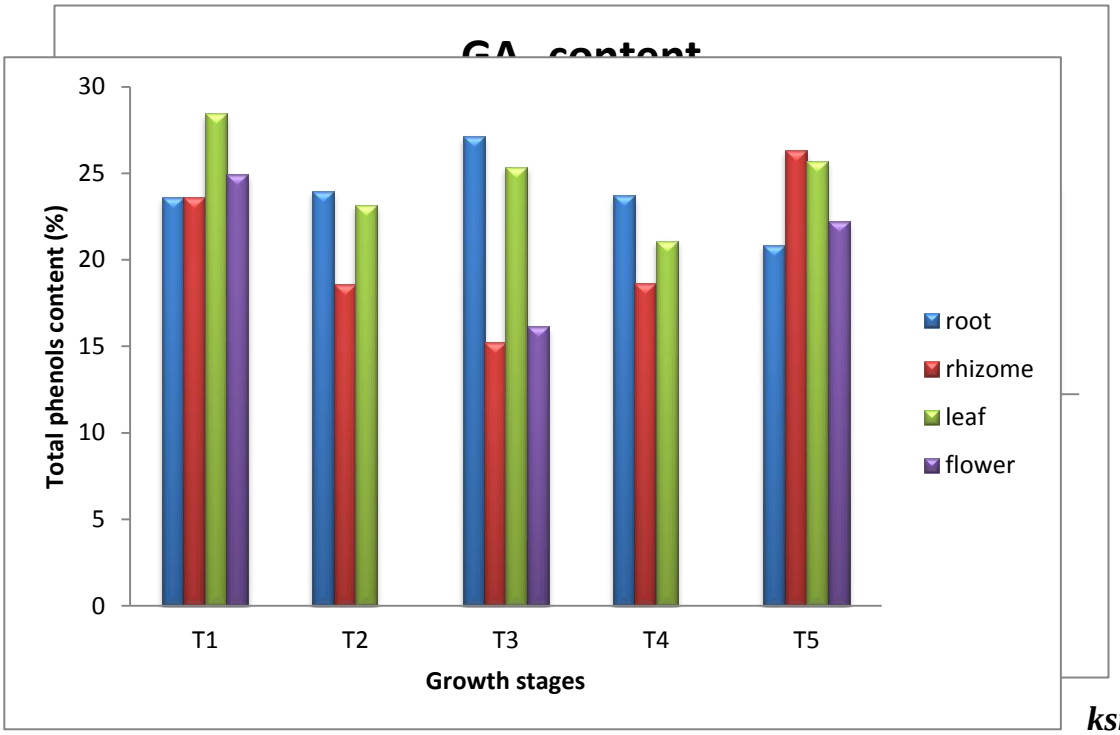


Fig. 12: Total phenols content of *D.stocksii* in all stages of clums

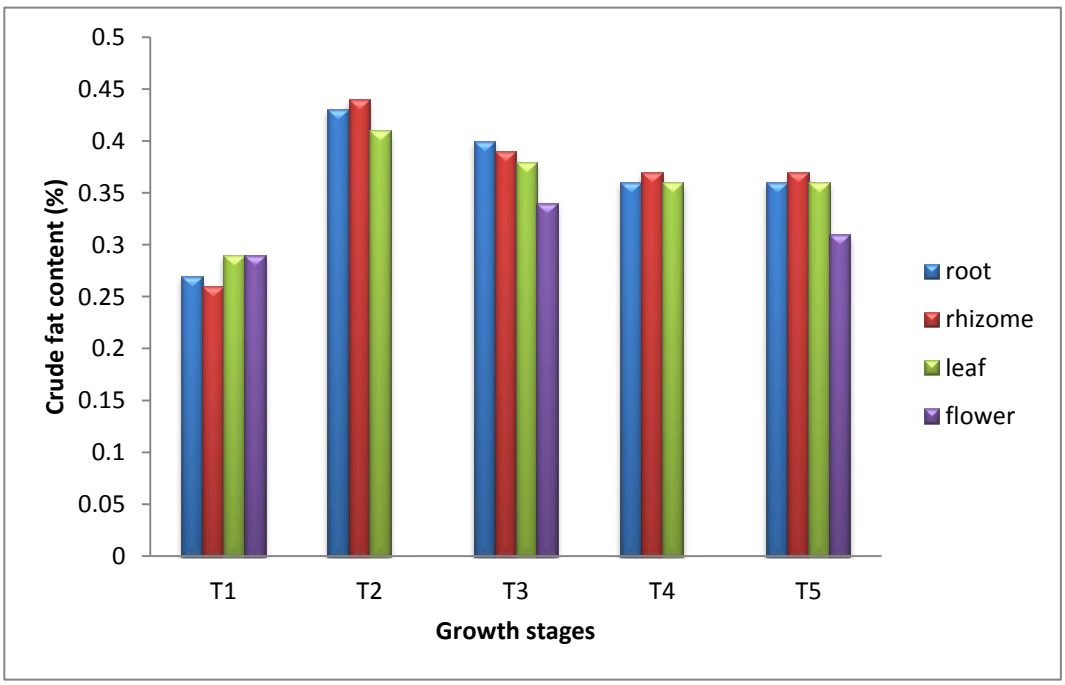
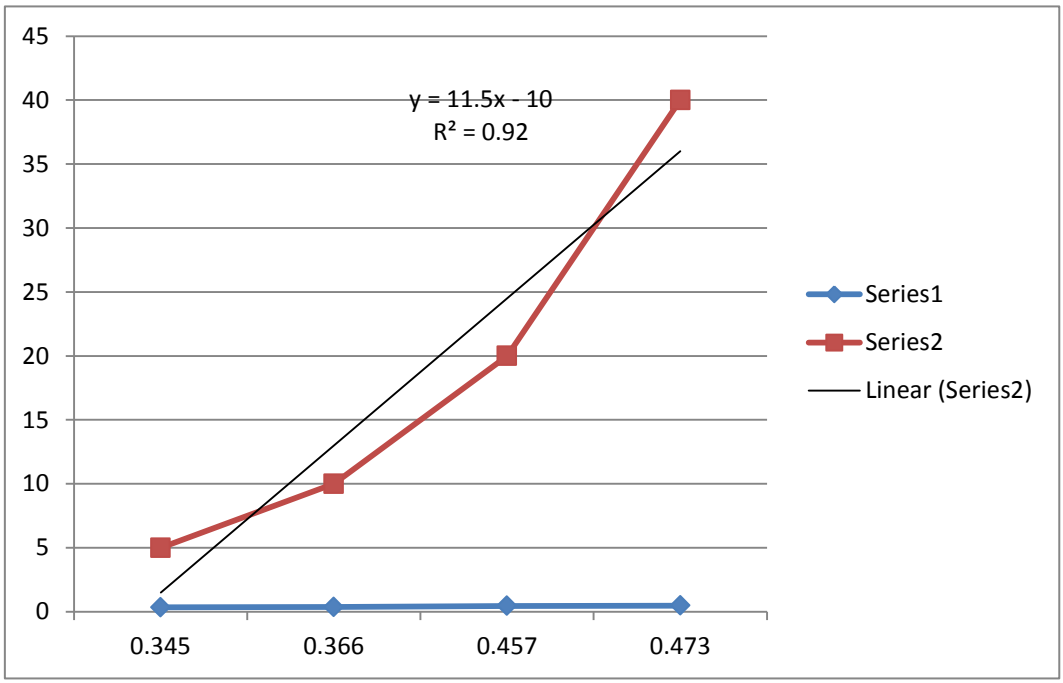


Fig. 13: Crude fat content of *D.stocksii* in all stages of clums

Fig.14 : GA_3 content of *D.stocksii* at different growth stages

Fig .15: Calibration curve of GA_3



DEPARTMENT OF FOREST BIOLOGY AND TREE IMPROVEMENT
COLLEGE OF FORESTRY, DAPOLI

Title of thesis	**: "STUDIES IN STRUCTURAL AND PHYTOCHEMICAL PARAMETERS OF FLOWERED AND NON-FLOWERED MANGA BAMBOO *DENDROCALAMUS STOCKSII* (munro)"**
Name of student	**: Hirole Sagar Kundan.**
Regd. No.	**: FDPM-15-53.**
Name of research guide	**: Dr. A.D. Rane, Associate Professor, College of Forestry, Dapoli**
Major subject	**: Forest Biology and Tree Improvement**
Year of award of degree	**: 2017**
Degree to be awarded	**: M. Sc (Forestry)**

ABSTRACT

The present study was undertaken in Konkan region of Maharashtra to know the differences in structural and phytochemical parameters of flowered and non flowered Manga bamboo at different stages based on morphological, physical, mechanical and phytochemical parameters. *Dendrocalamus stocksii* (*Manga*) is one of the commercially important bamboo species recommended by National Bamboo Mission (Banik, 2008). It is widely distributed in Konkan region of Maharashtra and preferred by farmers for large scale plantation on farm lands and home stead garden. This species, being endemic to Central Western Ghats, is distributed in Karnataka, Goa, Kerala and Maharashtra. The samples of *Dendrocalamus stocksii*were selected from a plantation established in 2007 at Tetawli B Block of Central Experimentation Station, Wakavli under Dr.Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.

Five clumps of different flowering and non-flowering stages were selected from bamboo plantation for study. These five culms from each stage were assess for morphological properties (clum height, fifth internode diameter and length, basal diameter), mechanical properties. (moisture content and specific gravity). And by harvesting each culm are categorized into different parts *i.e.*, rhizome, roots, leaves, flowers and stem. All these plant parts were air dried and then after powdered in (IKA Tube Mill). 10-15 gm powder of each sample was collected. Then each powder bag is labeled by their clump growth stage and used

for phytochemical analysis. (protein, starch, reducing sugars, total phenols, crude fat) and GA_3 content by HPLC.

Among the parameters recorded for bamboo variation stage, height of bamboo, culm basal diameter, diameter at fifth internode and length at fifth internode are presented. The culm height of bamboo of different stages observed significant variation. The highest height of culm was found in full flowered culm of university station (7.89 m), full flowered culm of farmer's showed highest basal diameter (43.87 mm), diameter of fifth internode was found highest in full flowered culm of farmer's field (36.89 mm), the maximum length was found in mm in culm of null flowered stage(33.60 cm).

In physical properties specific gravity did not shown significant variation; however, it was highest in culm of full flowered stage of farmer's field (0.86), Moisture content showed significant variation and it was highest in culm of initial flower stage among all stages of culm.

In mechanical parameters of bamboo culms the modulus of elasticity of static bending did not showed significant variation, Fibre stress at elastic limit was highest in culm of rejuvenated stage (34.49 kg cm^{-2}), Modulus of rupture was found to be highest in culm of null flowered stage (3.98 kg cm^{-2}), In case of compression maximum crushing stress was found to be highest in culm of rejuvenated stage (356.08 kg cm^{-2}) and in tensile maximum force did not varied significantly.

In phytochemical parameters, all the parameters shown significant variation expect crude fat for all the stages of bamboo. It was observed that highest protein percentage was found in leaves of initial flowered clum (4.46 %), highest starch percentage was observed in roots of full flowered culm of farmer's field (26.94 %), highest reducing sugar percentage was observed in flowers of full flowered culm (2.75 %), highest total phenol percentage was observed in leaves of full flowered clum (28.47 %), are higher crude fat percentage was observed in rhizome of null flowered clum (0.44 %) Significant amount of $GA_3$was observed in the underground parts of *D. stocksii* in the rejuvenated clumps(2.28 µg ml^{-1}).

Date:Signature

Place : Dapoli. (Mr. Hirole Sagar Kundan)

CHAPTER I

INTRODUCTION

Bamboos are a group of woody perennial, evergreen to deciduous Plants belonging to the grass family Poaceae, subfamily Bambusoideae, tribe Bambuseae. From the time immemorial, bamboo has been a part of human life due to its versatility which makes it a good raw material for numerous applications. Bamboo stands occupy an area of 36 million hectares worldwide which is equivalent to 3.2 per cent of the total forest area in the world. It is estimated that bamboo occupies over one per cent of the tropical and subtropical forest area - over 22 million ha. Over 80 per cent of the total area covered by bamboo is located in Asia, 10 per cent in Africa and 10 per cent in America. About 30 per cent of bamboo may be classified as forest plantations vs. 3.8 per cent of woody plantations. According to the FAO/INBAR global thematic study, over 63 per cent of bamboo resources are privately owned with 36 per cent bamboo owned by Governmental entities. In comparison 80 per cent of all World forests are on public lands (Lobovikov, 2007).

In Asia, India is the one of the major bamboo producing countries (almost 11.4 Million hectares) which accounts for roughly half the total area of bamboo reported for Asia. There are different reports on the number of genera and Species of bamboos in India. As per the latest compilation 18 genera and 128 species has been reported (Seethalakshmi and Muktesh Kumar, 1998). The 18 genera found in India are 1. *Arundinaria,* 2.*Bambusa*, 3.*Chimonobambusa,* 4.*Dendrocalamus,* 5.*Dinochloa,* 6.*Gigantochloa, 7.Melocanna*, *8.Ochlandra, 9.Oxytenanthera, 10.Phyllostachys,* 11.*Pleioblastus,* 12.*Pseudosasa*, 13.*Pseudoxytenanthera,* 14.*Racemobambos,* 15.*Schizoztachyum,* 16.*Sinarundinaria*, 17.*Thamnocalamus*and 18.*Thyrsostachys*. Another major bamboo producing country is China having highest bamboo diversity in Asia. With on over 500 species, covering 5.4 million ha or about 3% of its total forested area. *Dendrocalamusstocksii* (Munro),

Synonym to *Oxytenantherastocksii* is *Pseudoxytenantherastocksii* is naturally distributed in central Western Ghats. It Marihal bamboo' in Karnataka; 'Manga' in Goa.*D. Stocksii* has medium sized, stout solid and strong culms. Though the natural distribution of this species is in humid tropics with on lateritic soil type, this species has a wide adaptability and comes up well in tropical humid, sub humid and semi –arid conditions under black and red soils as well. This species, being endemic to central Western Ghats, is distributed in Karnataka, Goa, Kerala and Maharashtra. Its natural distribution is mostly confined to the banks of streams as it requires well drained deep soil. *D. stocksii* is cultivated in the coastal belts of Karnataka (Seethalakshmi and Mukteshkumar, 1998) and is primarily planted around

the arecanut gardens and paddy fields (Devar, 2000). 11.4 mha one third of the world surface covered by bamboo and 17% of the country's Bamboos are tall, perennial, arborescent grasses, belonging to family Poaceae (Gramineae).

Bamboo is a viable replacement for wood and is one among the strongest and oldest building materials ever used. Application of scientific and engineering skills on bamboo has led to an extended diversity of products ranging from domestic household items to industrial applications and generates income and employment. It contributes substantially to the ecological, economic and social development. Ecologically, bamboo plays a critical role in soil and water conservation, the balance of oxygen and carbon dioxide in the atmosphere, lowers light intensity and protects against ultra violet rays. The inherent ability of different bamboo species to grow on marginal and wastelands makes it one of the preferred crops for greening the wastelands and degraded sites resulting in conservation of soil moisture and resulting in carbon sequestration.

The demand for bamboo is growing more than its production and no doubt cultivation of bamboo in large scale is an immediate priority to support the developing industries with sufficient raw material. Lack of seed and/or other types of propagules could be a limiting factor for establishment of large – scale commercial plantations of the desired species. Some species *viz., Dendrocalamusstocksii*, *Bambusabalcooa*and *B. vulgaris* do not produce any seeds even after profuse flowering (sterile). Flowering of bamboo has been a botanical enigma since time immemorial. Although three types of flowering (annual or continuous flowering, gregarious or periodic flowering, sporadic or irregular flowering) have been reported among bamboos, most of the woody bamboos that are commercially useful belong to the gregarious flowering group. Since gregarious flowering leads to the death of entire population, this may lead to the extinction of the sterile species. Attention is needed to find out the problems associated with sterility in bamboos and take necessary measures to promote seed production. Conventional methods of vegetative propagation like rooting of culm (stem) cuttings and rhizomes that are used in the absence of seeds have several limitations such as availability in limited number, transport problems and synchronous flowering (simultaneous flowering of the parent and offspring originated from the same clump). It adds to the necessity of having planting materials of bamboo from seed origin.

Generally, the flowering of plants is associated with biochemical changes in the vegetative parts as more food resources are required for the seed production. In bamboo during flowering, biochemical changes occur in the culms and it is imperative to record the

biochemical changes during and after flowering. The information on chemical composition during flowering, and subsequent death need to be studied to find out whether any leading to reversion. There is only limited literature available on such studies on bamboo. *Dendrocalamusstocksii* is an important bamboo species in Konkan region & due to its solid & thorn less nature is widely used for industrial agricultural usage. The clump formation in the species is attained in after 3-4 years after plantation. The usual protocol of the species is attained in after 3-4 years, however there is need to observed the physiological, chemical & strength properties of the culms under different age culms so as to recommend its exact harvest age. Profuse flowering is found in *D. stocksii*but seed production is not yet reported (Seethalakshmi and Muktesh Kumar, 1998). In this species, the flowered clumps do not die and is observed to revert back to vegetative phase. With this background present study was undertaken with following objective.

1) To assess the growth of culms of different flowering conditions in *D. stocksii.*
2) To compare the biochemical constituent of flowered, non- flowered and rejuvenated culms of *Dendrocalamusstocksii.*
3) To compare the strength properties of flowered and non-flowered clumps of *Dendrocalamusstocksii* .

CHAPTER- II
REVIEW OF LITERATURE

A number of research studies have been undertaken in the field of physical, mechanical and phytochemical properties of bamboo species. The information pertaining to the present study has been reviewed in the light of work done on various bamboo species in India and abroad. The important information/investigations related to present work has been described in this chapter under the following headings:

1. Physical and mechanical properties of bamboo
2. Phytochemical properties of bamboo
3. Hormonal study by HPLC.

1. Physical and Mechanical properties of bamboo and other tree species

Purushotham (1963) reported the both round and split bamboos are used as structural components for buildings houses and other structures. Half split bamboos are carefully scooping out the inner nodal portion, have been used as corrugated roofing. Bamboo has been used as reinforcement for mud walls in rural and tribal areas in many developing countries such as India and Mexico.

Rajput *et al.* (1996) studied timber mechanics: strength, classification and gardening of timber and he reported highly desirable mechanical strength properties of *Dendrocalamusstrictus* from Uttar Pradesh and Andhra Pradesh.

Shekhar and Rawat (1996) studied physical and mechanical properties of teak from different localities in India and neighbouring areas reported that data on testing of teak for its physical and mechanical properties from different localities in continental India and Burma are tabulated.

Shekhar and Gulathi (1973) reported that Bamboo possess excellent strength properties, especially tensile strength. Most of the properties depend upon the species and climatic conditions under which they grow. An increase in tensile and compressive strength up to six years and bending strength up to six and up to eight years is known to occur. Strength properties are reported to decrease in older culms (Zhou, 1981). They also increase from the central to the outer part and from bottom to top.

In general, specific gravity and the properties of bamboo dropped from the top portion to the bottom. The increase in weight is cumulative and directly related with age. Strength properties are reported to decrease (Zhou, 1981). Limaye , (1952) found that older culms of *Dendrocalamusstrictus* became 40-50 % stronger and stiffer than young ones. Maximum values were found in 3-6 year old culms. Shekhar*et al*. (1962) found highest values in 3-4 year old culms of *Bambusanutans.*

Lakkad and Patel (1981) studied the detailed mechanical properties of bamboo. Typically, species like *Dendrocalamusgiganteus*have tensile strength of about 120 Mpa, compressive strength of 55 Mpa and Young's modulus of 14 Gpa. These figures do not compare badly with mild steel which has an ultimate strength of 410 Mpa, yield strength of 250 Mpa and Young's modulus of 200 Gpa. It was observed that concrete has much lower strength than those of bamboo reported here. In addition, the low density of bamboo, which is

typically 700 kgm^{-3} , results in much higher strength to weight ratio as compared to steel (density = 7800 kgm^{-3}) and concrete (density = 2400 kgm^{-3}).

Gadgil and Prasad (1984) studied ecological determinants of life history evolution of two Indian Bamboo species and he reported that almost all (70 out of 72) of the Indian bamboo species possess a long period of vegetative growth followed by a single suicidal out of reproduction. Only about eight of these 70 monocarpic species exhibit synchronized seeding over an area of several hundred square kilometres. Amongst the two commonest species, *Bambusaarundinacea,* which forms dense stands along water courses in areas of less variable rainfall, belongs to this minority of synchronized seeders and *Dendrocalamusstrictus,* occurs in scattered numbers in areas of more variable rainfall, exhibits sporadic seeding behaviour. Individuals of both species grow exponentially during the vegetative phase. Observations on the synchronized seeding of *B. arundinacea*show it to be spread over 5-6 years, with flower and seed production by any individual clump being completed over the dry season from December to April. An average clump produced 100 kg of seed, corresponding to 30 % of its above-ground biomass. One major cohort of *B. arundinacea* widely distributed over peninsular India seeded in 1868-72, 1912-16, and 1958-62; while a number of minor cohorts have seeded at other times. There is no defined direction over which seeding progress in time. The bamboo seeds are subject to high levels of predation, and we propose that the exponential nature of clump growth in conjunction with predator swamping has led to the evolution of a long pre-reproductive period and monocarpy, which characterize most Indian bamboo species. Synchronized mast seeding is a further independent adaptation favoured in a few species occurring in dense stands in more constant environments.

Jindal (1984)studied the mechanical properties of *Dendrocalamusstrictus* and found that specific ultimate tensile strength of bamboo specimens is nearly six times than that of mild steel. This led to the use of bamboo fibres for reinforced plastic composites (Jindal, 1986). In these composites, bamboo fibres were all aligned only in one direction. Though the maximum ultimate tensile strength achieved was 425 Nmm^{-2}, these composites have useful strength only in one direction.

Espiloy*et al*. (1986) reported the mechanical properties of six bamboo species namely, *Bambusablumeana, Bambusa vulgaris, Dendrocalamusmerriliamus, Gigantochloaaspera, Gigantochloalaevis*and*Schizostachyumlumampo*. Various scientists (Espiloy and Sasondoncillo, 1976, 1978; Espiloy*et al.,* 1979; Espiloy and Robillos, 1985) have observed a

general increase in strength properties towards the top portion of the Culm. This trend could be attributed to the corresponding increase in specific gravity and fibro vascular bundle frequency.

There is also variation in strength properties along the culm height as well. compressive strength tends to increase with height (Liese and Hamburg, 1987). The strength increases from the central to the outer part. There is more than 100 % variation in strength from the inner to the outer layers of bamboo (Narayanamurti and Bist, 1947).

Liese (1987) reported that there is also variation in strength properties along the culm height as well. Compressive strength tends to increase with height. The strength increases from the central to the outer part.

Bhat*et al.* (1992) studied selected mechanical properties such as static bending, tensile and compression strengths (parallel to grain) of ten south Indian rattan species representing large, medium and small diameter categories. They reported that the two strongest rattans, *vizCalamusnagbettai* and *C. gambleicome* under the class I while the weakest rattans, *C. lacciferrus* and *C. metzianus*, which are commercially less important species, fall under the class III and majority of the species come under class II. Furthermore, they reported that within the stem, strength decreases' from the periphery to the centre and from the base to the top correlating with fibre proportion and specific gravity.

Abd.Latif*et al.* (1993) studied the effect of anatomical characteristics on the physical and mechanical properties of B. *bluemeana*. According to this study, age and height did not significantly affect moisture content. The range of green moisture content was 57 to 97 per cent. Younger bamboo showed higher moisture content compared to an older bamboo.

Acosta *et al.* (1995) studied the physical and mechanical properties of hybrid *Eucalyptus grandis*x *Eucalyptus tereticornis*. They determined the values of density, hardness, static bending (module of elasticity and rupture), tension in the limit, parallel cut, and compressive strength parallel and perpendicular to the grain, shrinkage, nails and screw withdrawal. They reported that these properties of the hybrid showed intermediate values between the *Eucalyptus grandis*and *Eucalyptus tereticornis.*

Annon. (2000) studied Mechanical and Physico-chemical properties of Bamboos and he reported highly desirable physical properties *Bambusabambos.*

Chauhan*et al.* (2000) reported that ageing of a bamboo culm influences physical, chemical, and mechanical properties and consequently its processing and utilization. Further

they reported that physical and mechanical properties of bamboo vary with age of the bamboo and the height of the culm.

Shanavas and Kumar (2006) evaluated the wood properties of three locally important fast growing tree species (*Acacia auriculiformis*, *Acacia mangium*, and *Grevillearobusta*) from agricultural lands of Kerala. Species and sample positions exerted a profound influence on the physical and mechanical properties of wood. Basic wood density of *A. auriculiformis* was greater than that of *A. mangium* and *G. robusta*, while moisture content followed a reverse sequence: *G. robusta>A. magnum>A. auriculiformis*.

Yu *et al. (2008)* studied the selected physical and mechanical properties of 4-6 year old moso bamboo (*Phyllostachyspubescens)* grown in Zhejiang, China at different vertical and horizontal positions. Height affected on all selected properties expect for tensile strength. Relative density, tangential shrinkage, tensile modulus of elasticity (MOE) and tensile strength of bamboo increased greatly from the inner layer outwards. However, longitudinal shrinkage decreased greatly from the inner layer outwards. Relative density, tangential shrinkage and tensile MOE at 1.3 m were less than those at 4.0 m from the base.

Kelemwork (2009) evaluated the effects of anatomical characteristics of *Oxytenantheraabyssinicaon*selected physical and mechanical properties. He reported that age and culm height has significant effect on both properties of this solid culm bamboo. The density and moisture content were highly affected by age and culm height variation. Density increased with an increase of culm age and moisture content decreased with an increase in age. The higher values of Modulus of rupture (MOR) and Modulus of elasticity (MOE) were obtained in the middle portions of the culm. Both values show decreasing trend towards the top portion. Compression strength of culms decreases with increase in age and culm height. The results of correlation analysis revealed that fibre length, fibre diameter and fibre wall thickness positively correlated with density and negatively correlated with moisture content. The results further revealed strong positive correlation of density with MOR and negative correlation with MOE. The fibre length also showed strong positive correlation with MOE. Fibre diameter and vascular bundles size showed positive correlation with compression strength. However, moisture content negatively influenced MOE.

Malanit*et al.* (2009) studied the mechanical properties of *Dendrocalamus* as per and its stability as a raw material for manufacture of composite panels. The mean values of Modulus of rupture (MOR), Modulus of elasticity (MOE), compression strength

perpendicular to grain and shear strength parallel to grain are 198.52 Mpa, 15,363 Mpa and 11.91 Mpa, respectively. They vary with the position in culm and dependent on the specific gravity. The values slightly differ along the culm length. It is concluded that *D. asper* had superior mechanical properties which are comparable to certain softwood and hardwood species. It should therefore be promoted as a substitute for wood in the manufacture of structural composite timber like oriented strand board or oriented strand timber.

Bhattacharya *et al.* (2009) studied morphological and molecular characterization of *Thamnocalamusspathiflorus* subsp. *spathiflorus* at population level and he reported that comprehensive morphological characterization and an incidence of gregarious flowering in *Thamnocalamusspathiflorus* (Trin.) Munro subsp. *spathiflorus* are described. Twenty-eight key vegetative characters as well as reproductive morphology were studied. They are in gross agreement with prior taxonomic descriptions, yet more elaborate. Statistical analyses of the quantitative vegetative characters revealed significant high variability existing between populations, but not within populations. However, DNA fingerprinting analyses by applying highly polymorphic random primers could not detect any polymorphism either between populations or within populations. Insignificant within-population variability indicates possibility of clonal propagation from the donor(s) possessing similar genetic background and thus reducing genetic variability.

Terai and Minami (2011) demonstrated use of bamboo for reinforcement of cement concrete. Bamboo is reported to yield strength of 1400 $kgcm^{-2}$ in tension as compared to steel which is 4980$kgcm^{-2}$ in cement concrete reinforcement.

Gattoo (2011) studied that physical and mechanical properties of wood used for mango packaging and he reported that as far as the bamboo species concerned, *Dendrocalamusstrictus*and *Bambusabambos* recorded highest values for mechanical properties.

Rane*et al.* (2014) studiedculm emergence and soil properties in *Dendroclamusstocksii* under different land use systems in Central Western Ghats and he reported among the five land-use types evaluated, homestead, farm boundaries and mixed forest component exhibited production of higher new culms per clump. Available soil nitrogen and organic carbon was better among bamboo clumps present as mixed forest component. It may be concluded that this species is widely accepted by the farmers in this region and is luxuriantly present in different land use systems.

Rane*et al.* (2015) studied morphological variation in culm and clump characteristics in natural populations of *Psuedoxytenantherastocksii* (Munro) T.Q Nguyen (Marihal bamboo) in Central Western Ghats and he reported that there was significant variation in most of the culm parameters between the accessions. Coefficient of variation of all the culm parameter was < 30 per cent expect for commercial culm height and culm wall thickness to diameter ratio (CW: CD). Culm diameter at fifth internode varied from 6.25 to 57.32 mm with a mean of 37.39 mm while culm height varied between 16.2 to 5.4 m with an average of 10.70 m and height of culm solidness from the culm base ranged between various accessions of *P.stocksii* in central western Ghats and among the populations indicate that there is tremendous scope for selection and improvement within the species.

2. Phytochemical Properties of bamboo:

Mac Donald (1969) and Liese*et al.* (1985) reported the chemical composition variation according to the individual characteristics of the species, age and position of the culm, its geographical position and other related factors. The chemical analysis during flowering and death brings out the consumption of the reserved food in the rhizomes of the clump leading to death. Since there is no seed setting consumption of necessary materials is less and new shoots are produced leading to rejuvenation of flowered clumps in *D. stocksii*. Even though biochemical changes during these stages play an important role, studies regarding this are scanty. Gregarious flowering often followed by death leads to accumulation and wastage of culms. The present study gives information of the variation in chemical changes.

Srinivasa*et al.* (1974) studied bamboo flowering and he reported that the accumulation of the chemicals like starch in the rhizomes, sugars and other substances in the plant tissues cause flowering.

Janzen (1976) reported that bamboo is different from other perennial plants in its typical flowering behaviour where it flowers only once in its lifetime and most of the species die after the gregarious and synchronous blooming. The well-defined flowering cycles which vary from 3 to 120 years is supposed to be a genetic trait. The long flowering intervals and the factors that trigger flowering in bamboo are still largely a mystery to scientists. Many causes have been recognized to trigger gregarious flowering in bamboo but it has not been possible to establish their consistency.

Varmah and Bahadur(1980) reported that when the bamboo flowers gregariously, the flowering is so profuse that the whole plant is transformed into a gigantic inflorescence.

Hsiung*et al.* (1981) undertook experiments on *Phyllostacysvivax*, which flowered in China from 1969 to 1976showed some positive results with regard to artificial rejuvenation and he reported that nitrogen fertilizer does not necessarily stop the flowering of bamboo stands; sometimes it tends to retard their rejuvenation.

Campbell (1985) Bamboo flowering although amazing to scientists from time immemorial, detailed observations are limited, since flowering is rare in many species, historical records are fragmentary and not dependable as adequate verification is not possible.

Wang and Shen(1987) evaluated vigorous groves of bamboo in china over 200 years old, maintained by carefully controlling their flowering behaviour. Whenever old culms show first signs of flowering, they are immediately cut off and large quantities of nitrogen are applied to the underground parts to circumvent flowering.

Adarshkumar*et al. (*1990) Growth regulators such as Coumarin and NAA treatments enhance the sprouting and flowering of sprouts developed from flowered bamboo cuttings. Although the age of the rhizome seems to have major influence, a short rainy season followed by a spell of severe drought also stimulated flowering.

Liese and Weiner (1996) studied Ageing of bamboo culms. A review and he reported that properties and utilization of bamboos are influenced by structural changes due to ageing. During the few months growth of a culm only minor anatomical changes result in the meristematic tissue within one internode and along the culm length. Culms originating from a seedling or rhizome cutting show in subsequent years an age-related development until their full size is attained. The life cycle of an individual culm is of special interest. Investigations of culms up to the age of 12 years from phyllostachysviridiglaucescens have shown definite anatomical changes during the maturation period, but also in later years. They appear as a cell wall thickening of the fibres. Tyloses and depositions in vessels and sieve tubes develop as age-related factors.

Garg*et al.* (1998) studied some insight on the death of bamboo after flowering and he found that after the vegetative growth from rhizomes for a period of 50-80 years, clumps of bamboo, *D. strictus*, in a particular area undergo flowering, setting free enormous quantities of seeds and die synchronmisly. Changes in major constituents of bamboo (α-cellulose, hemicellulose, reducing sugars, starch, lignin, moisture and ash) at different stages of the

above process are monitored in the light of supporting evidence form literature; some light is thrown relating to bamboo death. It seems that bamboo death may have been caused by excessive deprivation of reducing sugars and moisture content, leading to loss in vitality and osmotic shock along with toxicity generated due to enormous increase in lignin content.

John and Nadgauda(2002) reported that clear-cut evidences are not available to substantiate any of the above hypotheses. Death of flowered bamboos in large populations is a cause of concern due to ecological, social and economic crises that set forth.

Ramanayake (2006) observed that during flowering, there is a switching of resources from vegetative to reproductive parts when stored nutrients are used up in the production of vast quantities of flowers and seeds.

Corbesier*et al.* (2007) studied that FT protein movement contributes to long-distance signalling in floral induction of Arabidopsisreported that In plants, seasonal changes in day length are perceived in leaves, which initiate long-distance signalling that induces flowering at the shoot apex. The identity of the long-distance signal has yet to be determined. In Arabidopsis, activation of flowering locus (FT) transcription in leaf vascular tissue (phloem) induces flowering. We found that FT messenger RNA is required only transiently in the leaf. In addition, FT fusion proteins expressed specifically in phloem cells move to the apex and move long distances between grafted plants. Finally, we provide evidence that FT does not activate an intermediate messenger in leaves. We conclude that FT protein acts as a long-distance signal that induces Arabidopsis flowering.

Beena (2011) analysed biochemical components of *Dendrocalamusstocksii*and *Ochlandratravancorica*and itindicated the utilization of starch, reducing sugars, crude fat, amino acids, fattyacids and vitamins during flowering and post-flowering stages.

3.Harmonalstudy by HPLC:

Cih*aet al.* (1976) studied that rapid separation and quantification of ABA from plant tissues using HPLC he reported that Abscisic add (ABA) was purified from soybean (Glycine max [L.]) Seed extract using a preparative high performance liquid chromatography (HPLC) procedure. The preparative procedure was rapid (70 minutes per sample), required no prior partitioning for purification and was quantitative as demonstrated with an internal standard of [2-'4CJABA, of which 98.9% was recovered. Following purification by the preparative HPLC procedure, the ABA in a soybean seed extract was quantified using either GLC with an electron capture detector (GLC-EC) or by analytical HPLC with a UV detector. For soybean

seed extracts, two analytical HPLC column packing materials were found adequate: jtPorasil and jaBondapak-NH2 (Waters Associates). However, with complex tissue extracts, such as soybean leaf and nodule tissues, only GLC-EC had the necessary selectivity and sensitivity.

Guardiola*et al.* (1982) studied inhibitory effect of gibberellic acid on flowering in Citrus and he reported that the application of gibberellic acid (GA_3) at any time from early November until bud sprouting resulted in a significant inhibition of flowering in the sweet orange sinensis (L.) Osbeck and the Satsuma (*C. unshiu Marc*.) and Clementine (*C ticulata Blanco*) mandarins. Two response peaks were evident: the first occurred when the application was timed to the translocation of an unknown flowering signal from the leaves to the buds. The second occurred during bud sprouting, at the time of the flower primordia were differentiating. From the pattern of flowering, it appears that the mechanism of inhibition was similar irrespective of the timing of GA_3 application. There was an initial reduction in bud sprouting affecting selectively those bulb originating leafless inflorescences. An additional inhibition resulted in a reduction of the number of leafy inflorescences with an increase in the number of vegetative shoots, suggesting the reversion of a floral to a vegetative apex. The inhibited bulb sprouted readily in vitro but invariably vegetative shoots were formed. A continuous influence of the sustaining branch is necessary to keep the flowering commitment the buds; irreversible commitment occurs when the petal primordia are well differentiated.

Araki and Sako (1987) studied determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection and they found that the total homocysteine in plasma consists of free homocysteine (*i.e.*, reduced plus oxidized homocysteine in the non-protein fraction of plasma) and protein-bound homocysteine. The thiol compounds in plasma, which are reduced or liberated from plasma proteins with tri-n-butylphosphine, are derivatized with a thiol-specific fluorogenic reagent, ammonium 7-fluorobenzo-2-oxa-1, 3-diazole-4-sulphonate. The derivatives are separated by reversed-phase high-performance liquid chromatography. The concentrations (mean +/- S.D.) of free and total homocysteine in plasma from 35 normal subjects were 1.94 +/- 0.46 and 6.18 +/- 1.19 nmol/ml, respectively.

Mustafa *et al.* (2004**)** studied the pH and polarity of the mobile phase was taken into consideration to optimize the mobile phase for the chromatographic separation of 3 important plant hormones: abscisic acid (ABA), indole-3-acetic acid (IAA) and gibberellic acid (GA3). pK*a*values of ABA, IAA and GA3 were determined using retention factors. These 3 hormones were extracted from 99 R (*Vitisberlandieri*x *Vitisrupestris*) and rose oil (*Rosa*

*damascena*Mill.) and the chromatographic method developed was used for the separation of these hormones.

Kim *et al*. (2006) the study of auxin action by GC-MS is technically the best method to measure IAA, because of high sensitivity and specificity. However, its high cost for setting and maintenance makes it difficult for daily use in ordinary laboratory. Therefore, we established a standard method to quantify IAA based on HPLC, adopting fluorescence detector as the monitoring device to ensure the specificity and sensitivity. By applying this protocol, we quantified IAA from the tip of maize (*Zea mays*) primary root.

Nakurte*et al.* (2012**)** developed a simple, sensitive, precise, and specific reverse HPLC method and validated for the determination of plant hormones in barley (*Hordeumvulgare*L.). This method includes extraction in aqueous organic solvent followed by solid-phase extraction, sample evaporation, and reversed-phase HPLC analysis in a general purpose UV-visible (abscisic acid (ABA)) and fluorescence detection (indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPA)), high-performance liquid chromatography system. The separation was carried out on Zorbax Eclipse XDB C8 column (150 × 4.6 mm I.D) with a mobile phase composed of methanol and 1% acetic acid (60: 40 v/v) in isocratic mode at a flow rate of 1ml min^{-1}. The detection was monitored at 270nm (ABA) and at 282nm (Ex) and 360nm (Em) (IAA, IPA). The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification, and robustness. The determined validation parameters are in the commonly acceptable ranges for that kind of analysis.

Dobrev (2005) studied purification and determination of plant hormones auxin and abscisic acid using solid phase extraction and two-dimensional high performance liquid chromatography he reported that A method for separation and purification of plant hormones auxin and abscisic acid based on mixed mode reversed-phase anion-exchange solid phase extraction and two-dimensional HPLC was developed. As reported elsewhere we developed a protocol for extraction and purification of the plant hormones cytokinins IAA and ABA using a mixed mode reversed-phase cation exchange polymeric sorbent Oasis MCX (Waters). It allows retention of hydrophobic analytes, including weak carboxylic acids when their ionization is suppressed, as well as cationic species.

Marilia *et al.* (2014) studied validated method for phytoHormonale quantification in plants he reported that Quantifications of phytoHormonale levels in plants are typically

carried out using GC or LC-MS/MS systems, due to their high sensitivity, specificity, and the fact that not much sample preparation is needed. However, mass spectrometer-based analyses are often affected by the particular sample type (different matrices), extraction procedure, and experimental setups, i.e., the chromatographic separation system and/or mass spectrometer analyser (Triple-quadrupole, Iontrap, TOF, Orbitrap). None of them was performed under the regime of a fully-validated method. Therefore, we developed and established such validated method for quantification of stress-related phytoharmonal such as jasmonates, abscisic acid, salicylic acid, IAA, in the model plant *Arabidopsis thaliana* and the fruit crop *Citrus sinensis*, using an Iontrap mass spectrometer.

Dunming*et al.* (2015) studied that simultaneous determination of 21 plant growth regulators in various fruits using QuEChERS coupled with HPLC-MS/MS technique reported that the simultaneous detection of 21 plant growth regulators in fruits by QuEChERS combined with an HPLC-ESI-MS/MS technique based on our previous work. The samples were initially extracted with acetonitrile containing 1 % acetic acid, followed by cleanup using C18 sorbent in the presence of magnesium sulphate. All 21 compounds showed a linear dynamic range of 2–3 orders of magnitude in the 0.10–1,000 μg/L range for the examined matrixes of apple, pear, strawberry, grape, and orange, with correlation coefficients above 0.99. The limits of detection (LOD) and the limits of quantification (LOQ) of the method ranged between 0.020 μg/kg–6.0 μg/kg and 0.10 μg/kg–15.0 μg/kg, respectively. For all the compounds, the average spiked recoveries ranged from 73.0 % to 111.0 %, and the relative standard deviations (RSDs, n = 6) were in the range of 3.0-17.2 %.

CHAPTER III
MATERIALS AND METHODS

Dendrocalamusstocksii (Munro) M. Kumar, Remesh&Unnikrishnan is a commercially important bamboo species recommended by National Bamboo Mission (Banik,2008). It is widely distributed in Konkan region of Maharashtra and preferred by farmers for large scale plantation on farm lands and home stead garden. Though the natural distribution of this species is in humid tropics with lateritic soil type, this species has a wide adaptability and comes up well in tropical humid, sub humid and semi-arid conditions under black and red soils well . This species Being endemic to Central Western ghats, it is distributed in Karnataka, Goa, Kerala and Maharashtra. Its natural distribution is mostly confined to the banks of streams as it requires well drained deep soil. *D. stocksii* is cultivated in the coastal belts of Karnataka (Seethalakshmi and Mukteshkumar, 1998) and is primarily planted around the arecanut gardens and paddy fields (Devar, 2000)

3.1 Morphological and Phenological Characteristics of *D. stocksii*

3.1.1 Culm:

Culm attains a height of 9 m with a basal diameter ranging from 2.5-5.8 cm with internode length of 15-30 cm. The matured culm is smooth and without hairs, however, when young it is covered with dense white or grey deciduous closely matted fine hairs. The culms are light green, loosely spaced and thorn less. The culms are usually solid at the base (upto 6^{th} or 7^{th} node) and even upto more than half the culm height unlike culms of other bamboo species. Hollowness may be more pronounced towards the tip of the culm.

3.1.2 Culm sheath:

Culm sheath is 15-22 cm long and 7-17 cm broad, striate and silvery. The outside portion of the sheath is usually covered with reddish hairs mixed with small white hairs. The shape of the culm sheath is broad, black and bulbous (like a bulb) at the base and gradually tapering upwards and is somewhat concavely truncated (shorten up or cutting off) at apex. Again it expands with a rounded base and tapers isa sharp point with a long fringed auricle. Fine ligules (projections) of 7 mm length are present at the point where the blade meets the sheath.

3.1.3 Leaf and branching habit:

The leaves are 10-20 cm long and 1-2 cm broad, linear-lanceolate and rounded or attenuated at the base with a short petiole (2 mm). Leaf blade is glabrous with scabrous (rough to touch) margins. The culm produce small and few branches at the node.Self-pruning is noticed in the lower portion of the culm upto 5^{th} to 6^{th} node from the base.

3.1.4 Flowering and fruiting

3.1.4.1 Floral morphology

This species usually has a sporadic flowering pattern, fregarious nature is rarely noticed in the species unlike it close associates like *Bambusabambos*or *D. strictus*in Western Ghats region. The inflorescence of *D. stocksii* is a large panicle with sessile closely packed spinousspikelets. Each globose head of the inflorescence has an average diameter of 2.5 cm and is supported by chaffy bracts. The spikelet are 1 to 1.2 cm long, narrow, glabrous, mucronate containing both fertile and few sterile florets. Glumes are ovate, mucronate, 5-7 nerved with 2 hermaphrodite flowers. So also lemmas are ovate, sub-acute, mucronate at the back. Plea of the lower flower is long as lemma, 2-keeled and ciliate on keels. Stamens are long, exserted while anthers are short, acute, ovary ovoid, hairy having a long style. The stigma is a single plumose. Anthers emerge out from 6 A.M in the morning and dehisce by 11 A.M. It is observed that only 15 per cent of anthers dehisce longitudinally and others dry out without shedding pollens. A possibility of anemophilous mode of pollination was predicted (Beena, 2011).

Seed formation is not known in this species. This may be due to feature of dichogamy and protogamy was present in the species, where the gynoecium matures 3-4 days before androecium and this prevents self-pollination. So also a very low pollen viability and germination may be observed in the species.

3.2 Study area

The clump of *Dendrocalamusstocksii* were selected for this study from a plantation established in 2007 at Tetawli B Block of Central Experimentation Station, Wakavli with coordinates at 73^{o} 12‘E and 17^{o} 45’N with an altitude of the 280 m above sea level. The location has a lateritic soil with sandy to sandy loam and acidic type with a mean annual rainfall of about 3000 to 3500 mm, mostly occurring during June to September. The climate is humid throughout the year. The average weekly data during the study period obtained from Meteorological Observatory of the Dr.BalasahebSawantKonkanKrishiVidyapeeth, Dapoli is presented in (Table 1).

3.3 Methodology

Five clumps of different flowering and non-flowering stages were selected from bamboo plantation for study the details are as follows:

(T_1) Full flowered from university station	**(T_2) Null flowered**	**(T_3) Initial flowered**	**(T_3) Full flowered from farmer's field**	**(T_4) Rejuvenated**
All clumps fully matured and all the clumps contained flowers.	In this, the clumps were in vegetative phase and no clump/clums contained flowering.	When flowering was start in clump *i.e.,* in one or few culm showed maturity and had bud initiation stage.	All clumps fully matured and completed vegetative phase and all the clumps are flowered.	Flowered clumps were clear felled from base four years before and had regained the vegetative phase.

All these clumps are sampled by harvesting *i.e.,* each culm is uprooted and detached from main rhizome. Then these are categorized into different parts i.e, Rhizome, Roots, leaves, Flowers, stem. All these plant parts were air dried and then after powdered in (IKA Tube Mill). 10-15 gmpowder of each sample were collected. Then each powder bag is labelled by their clump stage names *i.e.,* Root of full flowered stage, leaves of null flowered stage *e.t.c*.and this powdered sample of five different culm stages were assess for Phytochemical properties *viz.* starch, protein, reducing sugar, total phenol, crude fat.

Table.1: Meteorological weekly weather data of Dapolicentere year 2016-2017.

Weekly Weather Data Dapoli Centre Year 2016 – 2017										
Period (Date)	MW	T max	T min	RH-I	RH-II	Wind speed	Rain	RD	BSS	Epan
		(^{o}C)	(^{o}C)	(%)	(%)	(Km h^{-1})	(mm)	day	(h)	(mm)
04.06-10.06	23	34.0	24.8	91	70	6.0	40.0	2	3.2	3.7
11.06-17.06	24	31.4	24.9	91	76	6.9	93.5	5	4.7	3.6
18.06-24.06	25	29.6	23.1	98	93	4.9	298.7	7	1.4	1.8
25.06-01.07	26	27.2	22.6	98	97	6.2	792.5	7	0.0	0.0
02.07-08.07	27	28.5	23.6	94	92	8.5	462.6	7	0.2	0.7
09.07-15.07	28	28.4	23.3	95	89	10.3	256.8	7	1.4	2.0
16.07-22.07	29	27.3	22.4	99	95	4.5	403.0	7	0.6	0.3
23.07-29.07	30	28.8	22.4	98	85	3.3	268.0	6	0.7	1.4
30.07-05.08	31	27.6	22.7	97	94	11.8	481.5	7	0.6	2.3
06.08-12.08	32	27.7	23.6	94	92	10.5	182.7	7	0.4	2.4
13.08-19.08	33	29.3	24.3	91	84	8.5	39.0	4	1.8	2.6
20.08-26.08	34	28.9	23.4	95	87	5.9	73.6	6	2.0	2.5
27.08-02.09	35	28.4	22.6	95	88	3.5	138.4	7	1.1	1.5
03.09-09.09	36	29.4	21.6	94	72	3.7	36.9	3	5.3	3.9
10.09-16.09	37	29.9	22.2	93	82	2.9	55.8	2	3.3	2.7
17.09-23.09	38	27.0	22.5	98	94	7.7	582.4	7	1.0	1.6
24.09-30.09	39	29.1	22.6	96	82	5.1	88.1	2	3.5	2.2
01.10-07.10	40	27.7	21.4	97	83	3.6	189.1	6	2.4	2.6
08.10-14.10	41	30.4	22.3	94	83	3.6	10.2	1	5.8	3.8
15.10-21.10	42	33.7	21.4	93	81	2.1	0.0	0	6.7	3.4
22.10-28.10	43	32.6	18.8	96	72	2.0	0.0	0	8.2	3.2
29.10-04.11	44	34.5	15.7	92	69	1.5	0.0	0	8.8	3.9
05.11-11.11	45	33.0	13.5	95	63	0.7	0.0	0	8.3	3.3
12.11-18.11	46	33.2	16.5	92	63	1.6	0.0	0	6.9	2.8
19.11-25.11	47	33.1	13.1	91	72	2.1	0.0	0	8.1	3.9
26.11-02.12	48	33.4	13.3	91.3	67.9	1.7	0.0	0	7.7	3.5
03.12-09.12	49	32.7	15.3	93	50	1.9	0.0	0	7.2	3.5
10.12-16.12	50	32.2	14.7	90	41	2.4	0.0	0	7.0	3.0
17.12-23.12	51	32.1	14.2	94	38	1.5	0.0	0	7.6	3.4
24.12-31.12	52	32.0	11.9	94	51	1.6	0.0	0	8.0	3.4
							4492.8	100.0		
(2017) Period	MW	T max	T min	RH-I	RH-II	Wind speed	Rain	RD	BSS	Epan
		(^{o}C)	(^{o}C)	(%)	(%)	(Km h^{1})	(mm)	day	(h)	(mm)
01.01-07.01	1	31.6	11.2	93	60	2.4	0.0	0	7.7	3.0
08.01-14.01	2	28.8	10.9	92	54	3.3	0.0	0	8.9	3.1
15.01-21.01	3	31.0	14.4	94	51	2.8	0.0	0	8.8	3.0
22.01-28.01	4	33.7	14.4	90	54	3.4	0.0	0	9.3	4.2
29.01-04.02	5	33.8	14.4	92	56	3.0	0.0	0	8.9	4.1
05.02-11.02	6	31.0	13.6	90	55	2.8	0.0	0	9.0	4.2
12.02-18.02	7	33.8	13.9	88	55	2.8	0.0	0	8.3	4.7
19.02-25.02	8	34.3	13.3	81	67	2.4	0.0	0	8.7	5.0
26.02-04.03	9	36.1	13.6	86	56	3.3	0.0	0	8.3	5.4
05.03-11.03	10	31.3	13.6	90	68	3.8	0.0	0	8.7	4.4

3.4 Phytochemical properties

3.4.1 Starch:

Take (0.5g) dried sample in 50 ml ethanol. Centrifuge the sample and residue it. Take 80% ethanol 5 to 10ml for washing. Remove it and add 2-5ml of anthrone for colour change. But colour of solution should not be changed and remove the anthrone solution slightly. Dry the residue in oven or waterbath at 40-50^{o}c. Take that residue and add 5ml water then add 52% perchloric acid 6.5ml extract it on 0^{o}c

For 20 min then centrifuge it at 5000 rpm. Take out extract and make volume 100ml. Read the absorbance at 630nm in spectrometer. (Hodge and Hofreider., 1962)

$$\text{Starch \%} = \frac{\text{graph factor x sample absorbance}}{\text{wt. of sample}} \text{x} \frac{\text{dilution factor}}{\text{aliquote taken}}$$

3.4.2 Total phenols:

Weigh exactly 0.5 to 1g of the sample and grind it with a pestle and mortar in 10-time volume of 80% ethanol. Centrifuge the homogenate at 10,000 rpm for 20 min. save the supernatant. Re-extract the residue with five times the volume of 80% ethanol, centrifuge and pool the supernatants. Evaporate the supernatant to dryness. Dissolve the residue in a known volume of distilled water (5ml). Pipette out different aliquots (0.2 to 2ml) into test tubes. Make up the volume in each tube to 3ml with water. Add 0.5ml of Folin-ciocalteau reagent. After 3 min, add 2 ml of 20% Na2Co3 solution to each tube. Mix thoroughly, place the tubes in boiling water for exactly one min, cool and measure the absorbance at 650nm against a reagent blank. Prepare a standard curve using different concentrations of catechol (Sadasivam and Manickam, 1996).

3.4.3 Reducing sugars:

Take 1gm sample in 250ml conical flask. Add 40ml of distilled water then boil it and cool it. Add 3-4 drops of phenolphthalein indicator. Add 1n NaoH to neutralised it (pink colour appear). Add 2 ml lead acetate (45%) shake it and stand it for 10 min. Add 2.5 ml potassium oxalate (22%) to nitrify lead acetate and mix it. Make final volume 250ml with distilled water and filter it. Take 5ml feling's A and 5ml feling's B in 250 ml conical flask. Keep it on gas and allow to boil. Add 4-5 drops of methylene blue indicator. Fill the filtrate in 50ml burette, add drop wise in conical flask while boiling. At the end point brick red colour appears. Note down burette reading. (Ranganna, 1986)

Mg of invert sugar X dilution X 100

Reducing sugars % = $\frac{\text{Titrate X wt. of sample x 100}}{}$

3.4.4 Protein:

Take 0.5 gm of powdered sample in jeldar flask put 30 ml sulphuric acid in it. Add catalyst mixture in it i.e, 1:10 Copper Sulphate + Sodium Chloride + Magnesium Sulphate) then digest it on hot plate by adding distilled water and make up the 100 ml of extract having brilliant greenish colour solution .Then take 10 ml extract in 150 ml tube present in Kel plus, add 10 ml boric acid and 10 drops of mix indicator in it. Set the time 9 min then titrate with 0.02 normal Sulphuric Acid. Estimated per cent value of total nitrogen multiply by standard factor value 6.25 and calculated protein content in plant samples (Jackson, 1973).

3.4.5 Crude fat:

Weigh 2 to 5 gm of powdered sample .prepare a small packet of sample with Whatman no. 1 filter paper .Take weight of empty dry extraction flask. Plug the bottom of thimble by putting cotton or glass wool to avoid the possibility of passing out the sample particles in extraction flask. Connect the rubber tube, water tap to condenser. See that water supply to the condenser is constantly flowing. Put the packet of sample in thimble and pour organic solvent to 2/3 capacity of thimble .Take extraction flask containing 2/3 organic solvent. Connect these extraction flask and thimble to the condenser unit with heating coil. Put the apparatus on heating mantle and start water supply to the condenser. Regulate the rate of heating to allow continuous volatilization of solvent, its simultaneous condensation .Continue heating slowly till 6-8 siphoning collected in extraction flask. And stop heating. Take out extraction flask from the extraction unit. This contains Crude fat with little ether. Evaporate excess ether on water bath. Keep the flask in the oven at 105 °Cfor 1 hour and evaporate remaining spirit. Cool to room temperature and weigh it accurately to know the quantity of crude fat extracted. (Helman and Towerbridge., 1915)

Formula-

$$\text{Crude fat \%} = \frac{(W2-W1)}{X} x\ 100$$

Where,

X =Wt. of sample taken

W_1=Wt. of empty flask

W_2=Wt. of flask + fat

3.5 Mechanical properties

To evaluate the mechanical properties, fifteen samples were collected from five different culm stages. The samples were air-dried to a moisture content of approx. 12 per cent. After drying, the culms were split into different shapes and dimensions as subjected to analysis on a Universal Wood Testing Machine (Model-'Shimadzu').

3.5.1 Tensile strength

Specimens used for the determination of tensile strength had length of 20cm, width of 3 cm and thickness of 0.6 cm. The tensile strength parallel to the grains (TS at LP in $kgcm^{-2}$) was calculated by the equation (Rajput *et al.,* 1996):

$$\text{Tensile stress at maximum Load (TS at LP)} = \frac{\text{P max}}{\text{A}}$$

Where,

Pmax = maximum load required for failure perpendicular to grain (kg) and

A= area of the specimen on which force was applied (cm^2).

3.5.2Static bending strength:

Specimens used for the determination of static bending strength had length of 12 cm, width of 3 cm and thickness of 0.6 cm with a span length of 10 cm. The loading was applied at a constant rate of 1.0 mm/min on the tangential surface of the sample. Three different static bending strength parameters, fibre stress at elastic limit (FS at LP in $kgcm^{-2}$), modulus of rupture (MOR in $kgcm^{-2}$) and modulus of elasticity (MOE in $kgcm^{-2}$) were computed using following equations (Rajput *et al.,* 1996):

(i). Fibre stress (FS) at limit of proportionality (LP) = $\frac{3 \text{ x P x l}}{2 \text{ x b x h}^2}$

(ii). Modulus of Rupture (MOR) = $\frac{3 \text{ x Pmax x l}}{2 \text{ x b x h}^2}$

(iii). Modulus of elasticity (MOE) = $\frac{\text{P x l}^3}{4 \text{ x D x b x h}^3}$

Where,

P = load at the limit of proportionality (kg);

Pmax = maximum load (kg),

l= span of the test specimen (cm),

b = breadth of the test specimen (cm),

h = depth of the test specimen (cm) and

D = deflection at the limit of proportionality (cm).

3.5.3 Compressive strength:

Specimens used for the determination of compressive strength had length of 8 cm, width of 3 cm and thickness of 0.6 cm and the rate of loading was 0.6 mm/min. The compressive strength parallel (MCS in $kgcm^{-2}$) to the grain was calculated by the equation (Rajput et al., 1996):

Maximum Crushing Strength (MCS) = $\frac{\text{P max}}{\text{A}}$

Where,

Pmax = maximum crushing load at break point (kg) and

A = area of cross section of the specimen on which force was applied (cm^2).

3.6 Physical and morphological properties

Five culms of different stages i.e., full flowered from university experiment station, null flowered, initial flowered, rejuvenated and full flowered from farmer's field. These five culms of each stage were accessed for morphological properties *viz.* Culm height, 5^{th} internode diameter, 5^{th} internode length and physical properties *viz.* moisture content and specific gravity.

3.6.1 Moisture content:

For determination of moisture content, the instruments used were digital balance and aerated hot air oven to keep a constant temperature. After weighing the specimens were placed in the oven at $103 \pm 2^{\circ}C$ until their weight becomes constant. (Tsoumis, 1991) The time period after which weight became constant ranged from 2 to 4 days. The moisture content was then determined by the formula as given below (Tsoumis, 1991):

$$\text{Moisture content (per cent)} = \frac{Wm - Wo}{Wo} \times 100$$

Where,

Wm = Initial weight of the specimen.

Wo = oven-dry weight of the specimen.

3.6.2 Specific gravity:

For determination of specific gravity, the instruments used were digital balance and hot air oven. The specimens were placed in the oven till their weight became constant. The time period after which weight became constant ranged from 3 to 4 days. The specimens were weighed using digital balance. The specimen volume was determined by water displacement method (Shanavas and Kumar, 2006). Thus the Specific gravity of the specimen was calculated by following formula (Anon, 1970):

$$\text{Specific gravity} = \frac{\text{oven dry weight of the specimen}}{\text{oven dry volume of the specimen}}$$

3.7 Morphological properties

3.7.1 Culm height:

The culms were individually harvested and the total height upto the tip was measured using the measuring tape in metre.

3.7.2 Culm basal diameter:

The diameter of the culm was measured at the base using vernier callipers in centimetre.

3.7.3 Fifth internode diameter:

The diameter of the culm was measured at the centre of the 5^{th} internode (between 4^{th} and 5^{th} node) and expressed in centimetre.

3.7.4 Fifth internode length:

The internode length of the fifth internode was measured using a measurement tape in centimetre.

3.8 Hormonal studies of *D. stocksii*

To understand variation in Gibberellic acid (GA_3) content of *Dendrocalamusstocksii* of different stages.

3.8.1 Sampling of plants:

*Dendrocalamusstocksii*ofdifferent flowering and non-flowering stages were harvested from Tetavli station and one full flowered from farmer's field for the present research work. Roots, leaf, rhizome, flower, stem were selected of each stages. All these parts of bamboo was separated and thoroughly washed under running tap water to remove dust particles and air dried under shade at room temperature and fined powder was used for the evaluation of Gibberellic acid (GA_3) content.

3.8.2 Standards and Reagents:

Standard GA_3 were procured from Sigma-Aldrich and distilled water used in present research work was obtained using a water purifying system (Wasseraufberetiunj&Regenerierstation, Germany). All solvents of HPLC grade; Acetic acid, Methanol, and analytical grades; Propanol, HCL were used.

3.8.3 Methodology:

The roots, leaf, rhizome, flower, stem were used for analysis for GA_3 content using standard procedure with HPLC system.

3.8.3.1 Extraction from all parts of *Dendrocalamusstocksii*:

Root, leaf, rhizome, flowers, stem of *D.stocksii* in different stages were dried and powdered. Quantity of 100 mg of powder accurately weighed and 15 ml Methanol (100%) was dissolved in it along with IPA (10 Nmol) 30ml were added as internal standard. Extract was cleared by centrifugation (16000 rpm x 10min) at 4°C. The resulting supernatant was transferred to new tube and concentrated in a speed vacuum (IKA Rotatory evaporator) at 50

rpm, 45 temperature and 72 bar vacuum pressure until the volume decreased to less than one-tenth of initial.

3.8.3.2 Preparation of stock solution of GA_3 (10µg/ml)

The stock solution of GA_3 was prepared in 50 ml standard volumetric flask by dissolving 1 mg of GA_3 standard in 10 ml of methanol (100%) to prepare it.

Now serial dilution were prepared using methanol 5 ppm, 10 ppm, 20 ppm, 40 ppm, 80 ppm.

Table No. 4: Stock Solution prepared by various quantities of GA_3:

Concentration of GA_3	Stock taken	Methanol added
5 ppm	0.1 ml	1.9 ml
10 ppm	0.2 ml	1.8 ml
20 ppm	0.4 ml	1.6 ml
40 ppm	0.8 ml	1.2ml
80 ppm	1.6 ml	0.4 ml

3.8.3.3 Preparation of working standard solution of Gibberellic Acid (GA_3):

Into a series of 10 ml standard volumetric flask, a standard solution of GA_3 was prepared in methanol at the concentration of 1mg/ml. Calibration sample was prepared in the range of 1-10 and 1-80 µg/ml, for GA_3. The sample solution was prepared by dissolving 1 mg of extract in 10ml. Both the standard and sample solutions were filtered through Whatman NYL 0.45µm syringe filter. The responses were measured as peak areas and plotted against concentration.

3.8.3.4 Preparation of Mobile Phase for quantitative analysis of GA_3:

The mobile phase was prepared by using methanol as solvent A and 1% acetic acid as solvent B in the ratio of 60:40 (v/v). Both the solvent was filtered through 0.45 mm pore size (Millipore) membrane filter followed by ultra-sonication to de-gas the solvent.

3.8.3.5 Quantitative Analysis by HPLC:

3.8.3.5.1 Chromatographic Conditions:

The suitable chromatographic conditions used for quantification of Gibberellic acid (GA_3) content in *Dendrocalamusstocksii*species are given below:

Table 5: Chromatographic conditions suitable for *Dendrocalamusstocksii.*

Species	Standard	Mobile phase	Elution	Flow Rate
Dendrocalamusstocksii	Gibberellic acid (GA_3)	Methanol: 1% Acetic acid (60:40) v/v,	Isocratic	1 ml/min

3.8.3.5.2 Quantification of Gibberellic acid (GA_3) content:

The Gibberellic acid (GA_3) are quantified ($\mu g\ ml^{-1}$) by using following formula.

$$\text{Response Factor} = \frac{\text{Peak Area}}{\text{Standard Amount}} = \text{Analyte}$$

$$\text{Amount of Analyte} = \frac{\text{Peak Area}}{\text{Response Factor}} = \text{Sample Amount}$$

3.9 Statistical analysis:

The entire data was subjected to statistical analysis using the SAS 9.3 Statistical package (License No. IARSRI, Site 1160/386). The Variation for Morphological, physiological, mechanical, and phytochemical and HPLC analysis of different treatments of various flowering and non-flowering stages were estimated. The data was further analysis to estimate the clum height, clum basal diameter, 5^{th} internode diameter, 5^{th} internode length, moisture content, specific gravity, tensile strength, static bending, starch, protein, reducing sugar, total phenol, crude fat and GA_3were subjected to statistical analysis and ANOVA was constructed for interpretation of data using SAS 9.3.

CHAPTER –IV
RESULT AND DISCUSSION

The results obtained in the present investigation entitled "Studies in structural and phytochemical parameters of flowered and non-flowered Manga Bamboo (*Dendrocalamusstocksii)"*. The selected culms of different growth stages were evaluated for following parameters.

1. Morphological parameters.
2. Physical parameters.
3. Mechanical parameters.
4. Phytochemical parameters.
5. GA_3 content by HPLC.

4.1 Morphological parameters of the selected culms.

Among the parameters recorded for bamboo culms of five different stages *viz.* completely flowered, null flowered, initial flowered, rejuvenated and full flowered culm from farmer field as their height of the bamboo, culm basal diameter, diameter at fifth internode and length of fifth internode are presented in Table 2.

4.1.1 Height of culm

Culm height of bamboo was observed to have significant differences among different selected culms. Height of culms ranged between 6.45 m in Null Flowered culm to 7.89 m *i.e.,* full flowered culm of university station with the average height of 7.14 m. The full flowered culm of university station has the maximum height of 7.89 m than all other stages of bamboo.

4.1.2 Clum Basal diameter

The clum basal diameter showed significant range of variation among the culms of different stages. The clum basal diameter ranged from 37.14 to 43.87 mm, with the mean of 40.76 mm. Full flowered culm of farmer's field recorded maximum culm basal diameter *i.e.,* 43.87 mmand minimum in full flowered culm of university station *i.e.,* 37.14 mm. As observed in case of culm height the bamboo culm with full flowered stage shown better diameter than other stages.

4.1.3 Diameter of fifth internode

The selected bamboo culms of different stages were recorded for the diameter at fifth internode and it was ranged significantly between 28.16 and 36.89 mm, full flowered culm of farmer's field produced comparatively better 5^{th} internode diameter than that of other growth stages.

4.1.4 Length of fifth internode

The length of fifth internode varied significantly among the culms of different stages. The average 5^{th} internode length of 31.74 mm was recorded among different treatments. The minimum 29.53 mm was recorded in culm at initial flowered stage and maximum was 33.60 mm in culm of null flowered stage.

4.2 Physical properties

Fifteen samples from freshly cut bamboo sticks from five different stages *viz.* Full flowered of university station, Initial flowered, Null flowered, Rejuvenated and Full flowered culm from farm field were subjected to the determination of physical properties. The physical properties were determined by converting the bamboo sticks into the specimens of 8 cm length, 3 cm width and 0.6 cm thickness.

Table 2: Morphological properties of *D. stocksii* at different growth stages

Culm stage	Culm height (m)	Culm basal diameter (mm)	Culm 5^{th} internode diameter (mm)	Culm 5^{th} internode length (cm)
(T_1)Full flowered of university station	7.89	37.14	28.86	30.35
(T_2) Null flowered	6.45	41.08	28.16	33.60
(T_3) Initial flowered	6.55	38.86	36.06	29.53
(T_4) Rejuvenated	7.60	42.86	34.17	32.07
(T_5) Farm field flowered from farmer's field	7.22	43.87	36.89	33.15
Mean	7.14	40.76	32.83	31.74
SEm(±)	0.17	0.45	0.13	0.34
CD at 5%	0.54	1.42	0.42	1.06

4.2.1 Moisture content:

All culms were air dried before the evaluation of physical and mechanical properties. All the culms were found to have moisture content near 12 per cent. The moisture content of the bamboo culms evaluated ranged from 84.71 per cent to 86.38 per cent. The moisture content was found to be highest in culm of initial flower stage and lowest in culm of full flowered stage of university station (Table 3).

4.2.2 Specific gravity:

The specific gravity did not varied significantly among the culms evaluated, however the mean values ranged from 0.74 to 0.86. It was found Non significant variation.

Table 3: Physical properties of *D. stocksii* culm of different growth stages

Culm stage	Specific gravity(g/cc)	Moisture content (%)
(T_1)Full flowered of university station	0.78	84.7
(T_2) Null flowered	0.74	85.3
(T_3) Initial flowered	0.85	86.3
(T_4) Rejuvenated	0.76	84.8
(T_5) Farm field flowered from farmer's field	0.86	86.1
Mean	0.80	85.4
SEm(±)	0.04	0.34
CD at 5%	NS	1.07

4.3 Mechanical properties

4.3.1 Static bending

The values of fibre stress at elastic limit (FS at EL), Modulus of Elasticity (MOE), Modulus of Rupture (MOR) were calculated for all growth stages of bamboo. There was a statistically significant variation found among various stages of bamboo in all parameters of static bending.

There was no significant difference among five treatments for MOE and it ranged from 5284 to 6957 kg cm^{-2}. The MOR values significantly ranged from 2.43 to 3.98 kg cm^{-2}(Table 4). The MOR was found to be highest in culm of null flowered stage (3.98 kg cm^{-2}), followed by culm of initial flowered stage (3.14 kg cm^{-2}) and lowest in culm of rejuvenated stage (2.43 kg cm^{-2}). The fibre stress at elastic limit (FS at EL) significantly varied and it ranged from

30.56 to 34.49 kg cm^{-2} (Table 4).The FS at EL was found to be highest in culm of rejuvenated stage (34.49 kg cm^{-2}) and lowest in culm of null flowered stage (30.56 kg cm^{-2}).

4.3.2 Tensile strength

In tensile strength parallel to grain, TS at ML (tensile stress at maximum load) was estimated. TS at ML did not vary significantly among all different growth stages culms and it ranged between 30.565 kg cm^{-2} and34.498 kg cm^{-2} (Table 4).

4.3.3 Compression strength

A statistically significant variation was found among all the growth stages of culms evaluated. The maximum crushing strength (MCS) ranged from 253.48 to 356.08 kg cm^{-2} (Table 4). The MCS was found to be highest in case of culm of Rejuvenated stage (356.08 kg cm^{-2}) and lowest in culm of Null flowered stage (253.48 kg cm^{-2}).

Table 4: Mechanical properties of *D. stocksii* at different growth stages

	Static Bending			**Compressive strength**	**Tensile strength**
Culm stage	Fibre stress elastic limit (kg cm^{-2})	Modulus of elasticity (1000 kg cm^{-2})	Modulus of Rupture (kg cm^{-2})	Maximum crushing strength (kg cm^{-2})	Tensile strength at maximum load (kg cm^{-2})
(T_1)Full flowered of university station	383548	5992	2.65	353.98	34.4
(T_2) Null flowered	206642	5486	3.98	253.48	30.5
(T_3) Initial flowered	776380	5873	3.14	335.98	33.3
(T_4) Rejuvenated	626199	5284	2.43	356.08	34.4
(T_5) Farm field flowered from farmer's field	323861	6597	2.44	295.73	34.4
Mean	463325	5846	2.93	319.0	33.4
SEm(±)	65006.02	914.56	0.29	15.89	2.22
CD at 5%	204838.15	NS	0.91	50.08	NS

4.4 Phytochemical parameters

4.4.1 Protein

In *D. stocksii*, significant variation of protein was observed. The highest per centage of protein was found in leaves of initial flowered clum (4.46 %) followed by flowers of initial flowered culm 3.57 %, and lowest in roots of null flowered (2.10 %) (Table5). The protein content was highest in leaves of all the stages of culms (3.51 %) followed by roots (3.38 %)

and rhizome (3.37 %) of all stages and it was lowest in flowers (1.81 %) of all growth stages of bamboo.

4.4.2 Starch

Starch has also shown the significant variation in all parts of selected culms of bamboo of different growth stages (Table 6). The highest per cent of starch was observed in roots of full flowered culm of farmer's field (26.94 %) followed by rhizome of full flowered from farmer's field (20.84 %), and lowest in flower of initial flowered (5.63 %) in (Table 6). The starch content was highest in roots (1.88 %) of all stages, followed by leaves (13.07 %) and lowest in flowers (7.12 %) of all stages.

4.4.3 Reducing sugars

Reducing sugars has shown the significant variation in all parts of different growth stages (Table 7). The highest per centage of reducing sugar was observed in flowers of full flowered culm (2.75 %) followed by root of rejuvenated culm (2.25 %), and lowest in rhizome of null flowered (1.90 %) (Table 7). The reducing sugars content was highest in roots (2.25 %) and rhizome (2.25 %), followed by leaves (2.23) and it was lowest in flowers (1.55) among all stages of growth studied.

4.4.4 Total phenols

Total phenols showed significant variation among all parts of different growth stages of bamboo culm and it ranged from 15.25 to 28.47 per cent (Table 8). It was observed highest in leaves of full flowered clum (28.47 %) followed by rhizome of full flowered culm of university station (23.62 %) lowest in rhizome of initial flowered clum (15.25 %). The total phenols content was highest in leaves (24.76), followed by roots (23.85) and it was lowest in flowers (12.66) of all stages of growth in bamboo culm.

4.4.5 Crude fat:

Crude fat did not show significant variation in all parts of culms having different growth stages;however its values ranged from 0.26 to 0.44 per cent. The crude fat content was highest in roots (0.37 %) of all stages, followed by rhizome (0.36 %) and leaves (0.36 %) of all stages and lowest in flowers (0.19 %) of all stages.

Table 5: Protein content in various parts of culms of *D. stocksii* at different growth stages

Protein content (%)				
Stages/parts of culm	Root	Rhizome	Leaf	Flower
(T_1)Full flowered of university station	3.22 (10.34)	2.62 (9.31)	3.03 (10.02)	2.74 (9.49)
(T_2)Null flowered	2.10 (8.34)	3.52 (10.82)	2.56 (9.20)	−
(T_3)Initial flowered	4.11 (11.70)	4.37 (12.07)	4.46 (12.19)	3.57 (10.89)
(T_4)Rejuvenated	4.02 (11.53)	3.35 (10.46)	4.05 (11.59)	−
(T_5)Full flowered from farmer's field	3.45 (10.71)	2.97 (9.77)	3.46 (10.60)	2.77 (9.55)
Mean	3.38 (10.52)	3.36 (10.48)	3.02 (10.72)	1.81 (5.98)
SEm(±)	1.38	1.38	1.38	0.51
CD at 5%	4.35	4.35	4.35	1.62

Note- The values in parenthesis are arc sin values.

Table 6: Starch content in various parts of culms of *D. stocksii* at different growth stages.

Starch content (%)				
Stages/parts of culm	Root	Rhizome	Leaf	Flower
(T_1)Full flowered of university station	23.40 (28.92)	24.51 (29.67)	14.85 (22.65)	18.56 (25.52)
(T_2)Null flowered	9.53 (17.98)	16.23 (23.75)	17.53 (24.75)	–
(T_3)Initial flowered	8.26 (16.70)	12.57 (20.76)	14.7 (22.54)	5.63 (13.72)
(T_4)Rejuvenated	26.25 (29.18)	19.83 (26.09)	9.06 (17.46)	–
(T_5)Full flowered from farmer's field	26.94 (29.62)	20.84 (26.69)	9.23 (17.62)	11.44 (19.76)
Mean	18.87 (24.48)	18.79 (25.39)	13.07 (21.0)	7.12 (11.8)
SEm(±)	4.34	4.34	4.34	3.43
CD at 5%	13.66	13.66	13.66	10.80

Note- The values in parenthesis are arc sin values.

Table 7: Reducing sugars content in various parts of culms of *D. stocksii* at different growth stages.

Reducing sugars content (%)				
Stages/parts of culm	Root	Rhizome	Leaf	Flower
(T_1)Full flowered of university station	2.63 (9.33)	2.58 (9.24)	2.62 (9.31)	2.75 (9.55)
(T_2)Null flowered	1.92 (7.98)	1.90 (7.93)	1.98 (8.08)	–
(T_3)Initial flowered	2.20 (8.53)	2.39 (8.87)	2.23 (8.60)	2.37 (8.86)
(T_4)Rejuvenated	2.25 (8.62)	2.20 (8.52)	2.34 (8.78)	–
(T_5)Full flowered from farmer's field	2.25 (8.61)	2.20 (8.51)	2.34 (8.79)	2.62 (9.32)
Mean	2.26 (8.44)	2.26 (8.46)	2.31 (8.57)	1.55 (4.55)
SEm(±)	1.17	1.17	1.17	0.27
CD at 5%	3.69	3.69	3.69	0.86

Note- The values in parenthesis are arc sin values.

Table 8: Total Phenols content in various parts of culms of *D. stocksii* at different growth stages.

Total phenols content (%)				
Stages/parts of culm	Root	Rhizome	Leaf	Flower
(T_1)Full flowered of university station	23.62 (29.08)	23.62 (29.08)	28.47 (32.24)	24.94 (29.96)
(T_2)Null flowered	23.96 (29.30)	18.60 (25.55)	23.17 (28.77)	–
(T_3)Initial flowered	27.11 (31.37)	15.25 (22.98)	25.38 (30.25)	16.15 (22.24)
(T4)Rejuvenated	23.71 (29.14)	18.64 (25.48)	21.09 (27.29)	–
(T_5)Full flowered from farmer's field	20.85 (27.05	26.31 (30.85)	25.67 (30.42)	22.23 (28.08)
Mean	23.85 (29.19)	20.49 (26.79)	24.76 (29.80)	12.67 (16.06)
SEm(±)	3.73	3.73	3.73	1.62
CD at 5%	11.77	11.77	11.77	5.11

Note- The values in parenthesis are arc sin values.

Table 9: Crude fat content in various parts of culms of *D. stocksii* at different growth stages.

Crude fat content (%)				
Stages/parts of culm	Root	Rhizome	Leaf	Flower
(T_1)Full flowered of university station	0.27 (3.00)	0.26 (2.92)	0.29 (3.09)	0.29 (3.12)
(T_2)Null flowered	0.43 (3.78)	0.44 (3.81)	0.41 (3.70)	–
(T_3)Initial flowered	0.40 (3.66)	0.39 (3.60)	0.38 (3.53)	0.34 (3.38)
(T_4)Rejuvenated	0.36 (3.46)	0.37 (3.47)	0.36 (3.44)	–
(T_5)Full flowered from farmer's field	0.36 (3.46)	0.37 (3.46)	0.36 (3.44)	0.31 (3.20)
Mean	0.37 (3.47)	0.37 (3.45)	0.36 (3.44)	0.19 (1.94)
SEm(±)	0.48	0.48	0.48	0.13
CD at 5%	NS	NS	NS	0.42

Note- The values in parenthesis are arc sin values.

4.5 To understand variation in Gibberellic Acid (GA_3) content in various parts of *D.stocksii* at different growth stage.

In the present investigation, determination of GA_3in various parts *viz.*, rhizome, root, stem, flower; leaf of *D. stocksii* at different growth stage was conducted by HPLC. Mobile phase for the detection of the GA_3 compound was used by two solvents- A: Methanol (100%) and B: Acetic acid (1%) in volume ratio (60:40). The solvent system of methanol and Acetic acid was settled upon based on the separation and retention time (resolution), height of peak value and area produced. Flow rate and injection volume were set at 1.0ml/min and 20 µl respectively and Peak was detected at 365nm. Column temperature was kept at an ambient temperature 30^0C. The retention time of GA_3 standard was 1.52, 1.51, and 1.53 min as shown in Fig. 14 respectively. The calibration equation of gibberellic acid was Y = 11.5x - 10 and the correlation factor (R^2) was 0.92 (Fig. 15)

Table 10: HPLC solvent gradient for separation of GA_3.

Substance	Ratio of solvants		Wavelength (nm)	Retention Time (min)	Flow Rate (ml/min)
	Solution A (methanol)	Solution B (1% acetic acid)			
Gibberellic Acid (GA_3)	60	40	365	1.51	1

The extraction method was used for the estimation of GA_3; therefore roots, rhizome, leaves, flowers, stem of different stages of Manga bamboo were dried under shade and pulverized by using a mechanical grinder to make a course powdered. Then powdered roots, rhizome, leaves, flowers; stem (100 mg) was extracted with 100% methanol at room temperature (25^0C).

Retention times was detected in rhizome of full flowered from farmer's field as well as from leaves of null flowered was 1.42 min (Fig 23 and 25) and followed by stem of null flowered 1.41 min (Fig 24). Detection wavelength for the sample and standard was 365nm.

The retention time was as expected for the peak of GA_3 in Manga bamboo extract of various parts. Retention times were detected in roots of full flowered stage from farmer's field 1.42, in rhizome of rejuvenated stage 1.42, and in leaf of initial flowered stage 1.42, in root of rejuvenated stage 1.42, in rhizome of full flowered 1.42. The content GA_3 in root of Manga bamboo was determined to be 0.89 µg ml^{-1} in roots of Full flowered stage from farmer's field (Fig 22), in leaf of initial flowered stage 1.59 µg ml^{-1} (Fig 21) and in root of rejuvenated stage 2.28 µg ml^{-1} (Fig 20) by RP-HPLC analysis (Table 11).

The results presented in (Table 11) revealed that GA_3 content as influenced by different growth stage *viz.* Full flowered, Null flowered, initial flowered and rejuvenated. Present data shown in (Fig 14).

Numerically higher value for GA_3 was recorded in root of rejuvenated stage 2.28 µg ml^{-1}, followed by stem of full flowered stage of university station 1.19 µg ml^{-1}. The minimum value for GA_3 was recorded 0.74 µg ml^{-1} in flower of full flowered stage of university station.

Table 11: HPLC quantification of GA_3 (µg ml^{-1})in various parts of *D. Stocksii*.

Stages/parts of culm	Rhizome	Root	Stem	Flower	Leaf
Full flowered from farmer's field	1.07	0.89	1.59	1.76	1.18
Full flowered of university station	2.12	0.89	1.19	0.74	1.63
Null flowered	1.31	1.38	0.92	_	1.01
Initial flowered	1.31	0.9	1.3	1.64	1.59
Rejuvenated	2.1	2.28	0.93	_	1.89
Mean	1.58	1.26	1.18	0.82	1.46
SEm(±)	0.27	0.27	0.27	0.27	0.27
CD at 5%	0.85	0.85	0.85	0.85	0.85

Irrespective of different plant parts, rejuvenated stage of culm recorded maximum GA_3 (1.80 µg ml^{-1}), followed by initial flowered stage (1.35 µg ml^{-1}). However, null flowered culm recorded the least GA_3 content (1.15 µg ml^{-1}).

4.2 Discussion

4.2.1 Morphological parameters of selected culms:

The clumps selected for study are of 5 different growth stages *viz.* Full flowered from university experimental station, null flowered, initial flowered, rejuvenated and full flowered from farmer's field. Among the various morphological parameters the clum height, diameter and diameter at fifth internode diameter of culms was high in few growth stages. It was observed that full flowered culms was taller than other culms indicating that the growth pattern is dynamic and it increase when they complete their vegetative phase and get matured. The culm height ranged between 6.45 (initial flowered culm) and 7.89 m (full flowered culm of university station).The culm height and culm diameter parameters in this species varied moderately (Rane, 2015). The culm growth parameters in *D. stocksii* at different stages reveal that growth parameters are not indicative of their flowering stage in bamboo (Gadgil and

Prasad 1984).Culm growth parameters does not predict flowering in bamboo and only the clonal history of the species can help in the prediction.(Bhattacharya 2009). Interestingly, rejuvenated culms achieved the required culm growth in *D. stocksii*reported earlier (Rane*et. al.*2014).

4.2.2 Physical parameters of selected culms

Specific gravity of bamboo species did not vary significantly among the different growth stages culms and the average specific gravity was 0.80 for all the selected culms. This is in line with the earlier finding by Gattoo(2011) in *D.strictus*(0.73) and *Bambusabambos* (0.73). Rajput *et al.* (1996) reported that specific gravity of *D.strictus* was 0.626 from Uttar Pradesh. Furthermore, Annon (2000) reported the specific gravity of *Bambusabambos* to be 0.71, which is in confirmation with present study. Moisture content ranged from 84.7 to 86.3 per cent which is within the limits of adjustment factor *i.e.* 10-14 per cent (Shekhar and Rawat, 1968).

4.2.3 Mechanical parameters of selected culms

The mechanical properties *viz*. static bending, compressive strength and tensile strength were studied for different stages of bamboos. MoE (modulus of elasticity) and tensile strength TSML (tensile strength at maximum load) did not vary among the different growth stages. Further, there was a significant variation in fibre stress at elastic limit (FS at EL), MoR (modulus of rupture) and MCS (maximum crushing strength) among the growth stages. This variation is expected due to the strength of bamboo as influenced by age (Zhou, 1981) and the region of the bamboo from where the specimens are evaluated. The MoR of fully flowered bamboo culm was lower than the rest of the stages. The influence of ageing on maturation, especially on strength properties, is well proven (Liese and Weiner, 1996). Interestingly the strength property of the rejuvenated culms has in line with the null flowered and initial flowered culms.

4.2.3.1 Static bending

In static bending, features *viz.* Modulus of Elasticity (MOE), Modulus of Rupture (MOR) and Fibre stress at elastic limit (FS at EL) were assessed.

Modulus of elasticity (MoE) was found to be highest in culms with full flowered stage from farmer's field (6597 kg cm^{-2}), followed closely by null flowered stage (5486 kg cm^{-2}). Rajput *et al.* (1996) reported that MOE of *D.strictus* was 186,000 kg cm^{-2} from Andhra Pradesh, which is in accordance with the present study. Furthermore, Anon (2000) reported

that the MOE of *Bambusabambo*s was 141,000 kg cm^{-2}, which is in confirmation with present study.

Modulus of rupture (MOR) was found to be highest in Null flowered culm (3.98 kg cm^{-2}), followed by Full flowered culm from farmer's field(2.44 kg cm^{-2}). The strength values reported by Anon (2000) as 143 kg cm^{-2} are in line with the present investigation. Rajput *et al.,* (1996) reported that MOR for *D.strictus* was 1239 kg cm^{-2} from Andhra Pradesh, which is in consonance with present investigation.

Fibre stress at elastic limit (FS at EL) was found to be highest in initial flowered culm (776830 kg cm^{-2}), followed by rejuvenated culm (626199 kg cm^{-2}). Rajput et al., (1996) reported that FS at EL for *D.strictus* was 798 kg cm^{-2} from Andhra Pradesh, which is in consonance with present investigation.

4.2.3.2 Tensile strength parallel to grain

The TS at ML was found to be highest for Rejuvenated bamboo as well as culm, Full flowered of university station and farmer's field (34.4 kg cm^{-2}), followed closely by initial flowered culm (33.3 kg kg cm^{-2}). Lakkad*et al.,* (1981) reported that tensile strength of *Dendrocalamusgeganteus* was 1200 kg cm^{-2}. The lesser value in present study may be due to the species and growth stage.

4.2.4 Phytochemical parameters of selected culms of different growth stages.

4.2.4.1 Protein:

The protein content was found to be highest in leaf of initial flowered culm (4.46 %), followed by rhizome of null flowered culm (3.52 %) and lowest in root of null flowered culm (2.10 %).Beena*et al. (*2011) reported that the protein content of *D.stocksii* was 13.5 per cent and protein content increased in non-flowered and flowered culms. The protein content of roots, rhizome, leaf and flower was more during the initial flowered stage and rejuvenated culms. However, the null flowered culms contained comparatively less proteins in all the parts of bamboo culm. Corbesier*et al.,* (2007) has provided facts indicating protein movement contributing to long-distance signalling in floral induction. The rejuvenated culms had protein content in various parts of the culms and were in accordance with those present in the flowered culms, indicating that these culms may flower in future. Beena (2011) also reported that protein content in the flowering culms of *D. stocksii* was lower when compared to the fresh culms originated after reversion to vegetative phase. The highest per cent of protein was observed at rejuvenated stage (4.66 %). In *O. travancorica*per cent protein was highest during flowering and decreased during seed set and death. She also observed that, the highest protein content was observed during the onset of flowering *i.e.*, the first stage (2.647) and the lowest was observed after complete death of the culms (1.969 %).

4.2.4.2 Starch

The starch content was found to be highest in full flowered culm of farmer's field (26.94 %), followed by full flowered clum of university station (24.51 %) and lowest in flowers of initial flowered culm (5.63). Beena(2011) reported that the starch content of *Dendrocalamusstocksii* was 53.9 per cent. It was observed that maximum amount of starch was found in the flowered culms and according to Srinivasa*et al.*, (1974) accumulation of the chemicals like starch in the rhizomes, sugars and other substances in the plant tissues cause flowering.The starch content in the rejuvenated plants was more compared to the non-flowered clump. Increase in the per centof starch was observed during the reversion from reproductive phase to vegetative phase (Beena, 2011).

4.2.4.3 Reducing sugars

The reducing sugars was found to be highest in flower of full flowered culm of university station (2.75 %), followed by rhizomes of full flowered culm of farmer's field and root of initial flowered culm (2.20 %) and lowest in rhizome of null flowered culm (1.90 %). Beena*et al. (*2011) in *D. stocksii*the per cent of reducing sugars ranged from 1.916 to 2.824. The observations made on reducing sugars, wherein, it increased among the flowering clumps clearly indicate the utilization of more food reserves in seed forming in *D. stocksii* (Beena, 2011).Garg*et al.*(1998) studied the chemical changes during flowering and death in *D. strictus* and revealed increase in the level of reducing sugars and total carbohydrates during flowering stage than death stage.

4.2.4.4 Total Phenols

The total phenols was found to be highest in leaves of full flowered clum (28.47 %) followed by rhizome of full flowered culm of university station (23.62 %) lowest in rhizome of initial flowered clum (15.25 %). Beena*et al. (*2011) reported that the total phenols content of *D.stocksii* was 24.8 %. The phenols in roots and rhizomes of *D. stocksii*as more during flowering stage and decreased in non-flowered and rejuvenated stage, and this was confirmed by studies of Beena (2011)

4.2.4.5 Crude fat

The crude fat was found to be highest in rhizome of null flowered clum (0.44), followed by leaves (0.29) and flowers (0.29) of full flowered stage of university station and lowest in rhizome of full flowered clum (0.26). Beena*et al.* (2011) reported that the Crude fat content of *D.stocksii* was 49.3 per cent.

4.2.5 To understand variation in Gibberellic Acid (GA_3) content in various parts of D. *stocksii* at different growth stage.

The extraction method for *D. stocksii*that produced better recovery of GA_3 was by using methanol and 10 nano mole IPA, centrifuged at 4 ^{O}C (16000 rpm) for 10 min. The extract was evaporated on rotary until 1/10 extract was left out. This method was in line with that mentioned by Kim *et al.* (2006).

The retention time was observed for standard GA_3 peak in *Dendrocalamusstocksii* root extract was 1.42 min and that of standard was 1.52 min. Araki and Sako, (1987) made similar observations for SBD-homocysteine in plasma samples where the difference between sample

and standard was 0.7 to 1.0 min. This is acceptable in HPLC studies because the retention time is governed by various factors..

The content GA_3 in *D.stocksii* was determined to be 0.89 µg ml^{-1} in roots of Full flowered stage from farmer's field, in leaf of initial flowered stage 1.59 µg ml^{-1} and in root of rejuvenated stage 2.28 µg ml^{-1} by RP-HPLC analysis. Comparatively more $GA_3$was observed in the underground parts indicating presence in the resistance to the conversion of reproductive phase and retention of vegetative phase. A significant amount of $GA_3$was observed in theunderground parts of *D. stocksii*in the rejuvenated clumps, and this may be critical factor for inhibiting these to regain reproductive phase (Guardiola*et al.,* 1982).

CHAPTER V
SUMMARY AND CONCLUSION

The present study was undertaken in konkan region of Maharashtra to study the structural and phytochemical properties of flowered and non-flowered Manga bamboo at different growth stages based on physical, mechanical, phytochemical and hormonal parameters. The salient findings of the present investigation are summarized below.

5.1 Summary

5.1.1 Morphological parameters of *D.stocksii*.

Among the parameters recorded for bamboo of various stages, height of bamboo, culm basal diameter, diameter at fifth internode and length of fifth internode are presented. The clum height of bamboo with different stages observed significant variation. The highest height of culm found in full flowered stage (7.89 m). In case of culm basal diameter, diameter showed significant range of variation among the culms of different stages. Full flowered culm of farmer's field shown highest basal diameter (43.87 mm) as compare to remaining other stages. Among the bamboo culms, diameter of fifth internode was found maximum in full flowered culm of farmer's field (36.89 mm) comparatively better 5^{th} internode diameter than that of the culms of other stages. The diameter of fifth internode varied significantly difference. Length of fifth internode varied significantly among the bamboo culms of different stages. The maximum length was found in null flowered culm (33.60 cm).

5.1.2 Physical and mechanical parameters of bamboo culms.

Considering the physical properties of all the stages of bamboo culms**,** initial flowered culm was found highest moisture content (86.3 %) and full flowered culm of farmer's field found highest specific gravity (0.86) than other stages of bamboo culm.

The bamboo culms did not show significant variation with respect to all mechanical parameters except MoE and tensile strength. It was observed in bending modulus of elasticity highest in full flowered culm of farmer's field (6597 kg cm^{-2}) and modulus of rupture found highest in null flowered culm (3.98 kg cm^{-2}), fibre stress at elastic limit total work done was highest in initial flowered culm (776380 kg cm^{-2}). In case of compression maximum crushing stress were found to be highest in case of rejuvenated culm (356.08 kg cm^{-2}) and tensile strength at maximum load was found highest in rejuvenated and full flowered of both university station and farmer's field (34.4 kg cm^{-2}).

5.1.3 Phytochemical parameters of bamboo culms.

Considering the phytochemical parameters of all the stages of bamboo culms**,**leaves of initial flowered clum was found highest protein content (4.46 %), roots of full flowered culm of farmer's field was found highest starch content (26.94 %), flowers of full flowered culm was found highest reducing sugars content (2.75 %), leaves of full flowered clum was found highest total phenols content (28.47 %) and rhizome of null flowered clum found highest crude fat content (0.44 %).

5.1.4 Gibberellic acid (GA_3) content of bamboo culms.

GA_3 content was found highest in root of rejuvenated stage (2.28 µg ml^{-1}). Irrespective of different plant parts, rejuvenated stage of culm recorded maximum GA_3 (1.80 µg ml^{-1}), followed by initial flowered stage (1.35 µg ml^{-1}). However, null flowered culm recorded the least GA_3 content (1.15 µg ml^{-1}).

5.2 Conclusion:

The full flowered clum showed the highest clum height, culm basal diameter, diameter at fifth internode better than other flowering stages. However, in case of length of fifth internode culm it was highest in null flowered stage. As per the bamboo culms of different stages are concerned, rejuvenated and full flowered culms showed highly desirable mechanical properties among all the bamboo culms evaluated. protein content was high in leaf of initial flowered clum, starch was high in full flowered culm, reducing sugars was high in flower of full flowered clum, total phenol was high in leaves of full flowered culm, crude fat was high in rhizome of null flowered culm. In case of HPLC quantification of GA_3 it was found to be more in underground parts of rejuvenated clump.

CHAPTER VI
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