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ORIGINAL CONTRIBUTION

Study of Edaphic factors of the location for the growth of white sandal (*Santalum album* L.) in Indo-Nepal Border

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Abstract: Environment plays an important role for growing plants in any location in any country. White sandal (*Santalum album* L.) cultivation is also dependent on some specific edaphic factors along with host-parasite relationship etc. Soil environment factors play an vital role for the growth of white sandal was unknown to the farmer's community though it grows in wild condition since ancient civilization. It is common belief to people that white sandal plant cannot survive without proper environment and specific host plants surrounding its root zone. Keeping all these views in mind an experiment was undertaken in some areas (Rampur, Malangwa, Kamalpur, Bardibas) in Indo-Nepal Border. Soil samples were taken from each and every forest garden and analysed in the laboratory to assess the soil nutrient status. The aims and objects of the study were to observe the role of soil nutrients of the locations in favour of the growth and development of *Santalum album* L.

Key words: edaphic factors, environmental factors, root zone, nutrient status.

1. INTRODUCTION

Sandalwood (White Sandal) is the fragrant heartwood of some species of genus *Santalum*. The widely distributed and economically important *Santalum* genus belongs to the family Santalaceae which includes 30 genera with about 400 species, many of which being completely or partially parasitic (John,1947). The word Sandal has been derived from Chandana (Sanskrit), Chandan (Persian), Savtador (Greek) and Santal (French). There are references of Sandalwood in Indian mythology, folklore and ancient scripts. 'Chandana' the Sanskrit name ascribed to *Santalum album* L. was known and used in India from the earliest historic times and is frequently mentioned in the ancient Sanskrit writings, some of which dated before Christian era. Kautilya's Arthashastra (320 B.C.) considered Sandal as one of the important forest products to increase royal revenue. CharakaSanhitha, the major text book of internal medicine in Ayurveda (300 B.C.) quotes uses of Sandal over 160 time in the entire text. In treatment of major diseases like fever, piles, hemorrhagic conditions, diabetes, dropsy, mental disorders, management of poisons & skin disorders wide spread uses of sandal is seen. SusruthaSamhita (150 B.C.) a great text on Indian wisdom on surgical procedures, equally preferred sandal for the management of wounds. Sandal fumigation is indicated in warding off evils and organisms, which contaminate the wounds. Such fumigations hasten the wound healing & surgical wards remain aseptic. Dusting of wounds with sandal for early healing is common. In the Amarkosha (Lexicon 3rd or 4th Century A.D.) sandal is mentioned and it is said that 'Vina-malayamanyathra-chandanamvivarditha' [Majumdar, 1941]. The extraction and disposal of sandal came under the Forest Department in 1864 in Mysore state [Adkoli, 1977]. In Karnataka (formerly Mysore) the forest working plan for sandal extraction were prepared for HunsurTalik in 1910, Heggadadevanakote in 1920 and Narasimharajapura in 1926. In 1871,

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the parasitic nature of sandal was reported by John Scott. Watt (1893) described the technique of raising sandal seedlings in tile pots in the nurseries and planting in the field. McCarthy (1899) first noticed the spike disease of sandal in Coorg. Brandis (1903) suggested that though sandal is a root parasite, it may derive part of its nutrition from the soil as well. Barber (1905) noted that haustoria formation occurred only on certain roots of sandal and not on all of them.

Ecologically sandal has adapted various agro - climatic and soil conditions for *in situ* regeneration with an exception of waterlogged areas and very cold places. In India, 8 Sandal growing areas have been identified as potential provenances of Sandal on the basis of population density, phenotypic characteristics, latitude, longitude and eco-climate (Jain *et al*., 1998). The provenances vary in climate and edaphic preference since they are located in different localities of South and Central India. The state of West Bengal is cited in the map of occurrence and distribution of *Santalum album* in India (Srinivasan*etal*., 1992).

Some hindrances and problems for its propagation were observed by various workers in these areas. Keeping all these views in mind we are going to undertake the venture for its mass propagation through seeds and its cultivation with their agronomical maintenance properly.

2. MATERIALS AND METHODS

2.1. Materials

(i) Sandalwood seeds: Seeds *Santalum album* L. were collected from

Table I: Temperature regime of the experiment site (in degree Celsius)

Months	2015		2016		2017	
	Max	Min	Max	Min	Max	Min
JAN	27	09	27	9	27	9
FEB	32	11	34	9	32	9
Mar	39	14	38	15	37	16
APR	43	20	42	9	38	18
MAY	44	24	45	22	43	21
JUN	39	24	37	22	41	23
JUL	38	24	37	25	35	25
AUG	35	23	34	24	35	25
SEPT	34	24	36	24	35	22
OCT	34	16	35	20	34	18
NOV	32	11	32	13	32	16
DEC	29	10	29	7	27	9
IN YEAR	44	9	45	7	43	9

North India and Nepal Border Area during the month of November-December and May-June of 2015 and 2016 for experimentation.

Simultaneously, seeds of *Santalum album* L. were also procured from Institute of Wood Science & Technology, Bangalore in the month of February, 2015 for the same experimentation purposes.

Table II: Monthly rainfall of the experiment site (in degree Celsius)

Months	Normal	2015	2016	2017
JAN	17	20	3	7
FEB	12	13	40	15
Mar	19	7	9	4
APR	27	4	57	66
MAY	65	52	76	77
JUN	198	213	247	183
JUL	272	331	255	471
AUG	293	332	445	386
SEPT	246	358	139	384
OCT	122	18	95	0
NOV	15	99	00	0
DEC	03	6	00	0
IN YEAR	1289	1453	1366	1687

(ii) Chemicals : Gibberelic acid(GA_3)

(iii) Apparatus : Container, Markin cloth, Polypots, Hycopots**.**

(iv) Miscellaneous : Sand, bricks, Seive, FYM, Water, etc.

(v) Meteorological Informations.

(vi) Information on Sites

Abbrevi-ation	***Full name***	***Total***
L	Location	4
P	Plantation	3
T	Treatment	9
Y	Year	3

(vii) Name of Location

No.of Location	***Name of Location***
L_1	Rampur
L	Malangwa
L_3	Kamalpur
L_4	Bardibas

(viii) Information of Treatments

No. of Treatment	***Composition [Host(S)]***
T_0	No Host
T_1	Arhar
T_2	Tulsi
T_3	Arhar+Tulsi
T_4	Akand
T_5	Arhar+Akand
T_6	Ghantu
T_7	Arhar+Ghantu
T_8	Nayantara
T_9	Tulsi+Nayantara

(ix) Information of Plantation

No. of Plantation	***Year***
P_1	2015
P_2	2016
P_3	2017

(x) Growth Parameters, viz. plant height (cm) and basal girth (cm), were measured out of all planted saplings year-wise from each and every forest garden. *Santalum album* L.

2.2. Methods

(a) Germination Study in Nursery :

(i) Pretreatment by soaking in water:

Sandalwood seeds are soaked in water for 24 hours before sowing. Seeds are sown in sand bed (6 mm deep). Germination starts after 60 days. In-between 61 to 100 days, only 3-4% germination is obtained.

(ii) Pre-treatment by boiling water:

Sandal seeds are pretreated with boiling water (10 parts of boiling water with one part of seeds) for 1 minute and then kept in normal water overnight for soaking. Treated seeds are sown in sand bed. Germination starts after 50 days. In-between 51 to 100 days, hardly 8% germination is obtained.

(iii) Pre-treatment by alternate wetting & drying:

Sandal seeds are exposed to alternate wetting & drying for 12 hours wetting followed by 12 hours drying in sun. This process is repeated for 7 days and then the seeds are sown in sand bed (6 mm deep). Germination starts after 40 days.

In-between 41 to 100 days, 8 to 9% germination is obtained. Little germination is found even after 100 days upto 150 days.

(iv) Pretreatment by Gibberellic Acid:

Matured seeds were collected from the sandalwood trees of Hirbandh Block in November- December, depulped, dried in sun and stored in polybags for germination test. 6 samples each of 300 sandalwood seeds were taken and tied in markin cloth. 3 containers (1 liter each) were taken for 3 different concentrations (0.0125%, 0.025%, 0.05%) of gibberelic acid. 2 seed samples were dipped in each container for 16 hours & 24 hours soaking. The treated sandalwood seeds were then sown in the sand bed. The sand beds were watered twice daily in the morning & afternoon. First germination was started after 24 days of seed sowing. The number of seeds germinated in each treatment is recorded and the germination is continued upto 90 days after sowing. The germinated seedlings were transplanted in polypot (8″x4″ & 9″x 5″) and 300 cc hycopots in nursery at 3 to 4 leaf stage.

Sandal seeds have a post drop dormancy of two months due to presence of hard seed coat or due to presence of chemical substances in the seed coat which are impervious to water and gases. Germination of Sandal seeds are found profuse from the bird droppings in the forest floor as well as in the village yards and bunds of the agricultural fields. Sandal is also found growing wild in some farmlands, homesteads and wastelands in Hirbundh block of Bankura District and Arabari forests of Midnapur District. This indicates the potential of growing the tree in the farmlands. However, germination of seeds are very low (10-15% within 60 days) when the seeds are sown in mother bed (sand beds) after hot and cold water treatment or alternate wetting & drying due to its hard seed coat and dormancy. Sandal seeds have been found to germinate faster when the seed coat is completely removed, or seeds are soaked in 0.05% gibberelic acid for

Table – III: Plantation of Sandal plant in different forest gardens in North India and Nepal Border Area

Sl. No.	Location	Year of Planting	No. of seedlings Planted
1	Rampur	2015	100
2	Malangwa	2015+2016	150+ 200
3	Kamalpur	2016	30
4	Bardibas	2016	100

12-16 hours (Nagaveni and Srimathi, 1981). In sandal seeds, the duration of germination is much prolonged after the dormancy period. It starts in 25 days and reaches hardly 50% in 90 days with 0.05% GA_3 soaking for 16 hours (Das and Tah, 2013). Germination study conducted in Hirbundh & Kamalpur nurseries of Bankura (South) Division in 2011, 2012 & 2013 where seeds are soaked in different concentration (0.0125%, 0.025%, 0.05%) of gibberelic acid for 16 hours & 24 hours respectively and sown in sand beds of nursery. The germinated seedlings are pricked out from sand bed and planted in polypot&hycopot beds of nursery at 3 to 4 leaf stage and kept in nursery without & with host (*Cajanuscajan*).

B) Soil Testing Methods:

(i) Soil pH

The pH of the soil was determined with the help of a pH meter in 1: 2.5 soil: water suspension ratio as described by Jackson (1973).

(ii) Oxidizable Organic Carbon

Organic carbon was determined by oxidizing soil with 1 (N) potassium dicromate $K_2Cr_2O_7$ in presence of concentrated H_2SO_4 and back titrating the remaining $K_2Cr_2O_7$ with ferrous ammonium sulphate solution using diphenylamine indicator, following the wet digestion method of Walkley and Black as described by Jackson (1973).

(iii) Available nitrogen (N)

The available nitrogen (N) of the soil was estimated through the hot alkaline potassium permanganate method as suggested by Subbiah and Asija (1956).

(iv) Available phosphorus (P)

Available phosphorus (P) of soil is determined by using Olsen's method. In this method, the extractant is 0.5M NaHCO3 solution adjusted to pH 8.5 with 10% NaOH.

(v) Available potassium (K)

Available potassium content of the soil was determined by flame photometer after extraction with neutral normal ammonium acetate solution as described by Jackson (1973).

(vi) Available Zinc(Zn), Copper(Cu), Manganese(Mn) & Iron(Fe)

The method developed by Lindsay and Norvell (1978) using DTPA (DiethyleneTriaminePenta Acetic Acid) for separating soils into deficient and non-deficient categories for Zn, Cu, Mn& Fe is useful and adopted in this Laboratory analysis.

a. Extracting Solution

To prepare 1 litre of DTPA extracting solution, dissolve 13.1 ml reagent grade TEA, 1.967 g DTPA (AR grade) and 1.47 g of $CaCl_2$ in 100 ml of glass distilled water. Allow sometime for the DTPA to dissolve and dilute to approximately 900 ml. Adjust the pH to 7.3 + .05 with 1:1 HCl while stirring and dilute to 1 litre. Addition of approximately 4 ml of 1(N) HCl will bring the pH of the solution to 7.3. This solution is stable for several months.

b. Extraction and Determination

Weigh 10 grams of air dried soil in a 125 ml conical flask or polypropylene bottle. Then add 20 ml of the DTPA extracting solution. Cork the bottles or flask and place them upright on a horizontal shaker. Shake for two hours with a speed of 120 cycles per minute. Filter the suspension through Whatman no 42 filter paper. Keep the ilrate in polypropylene bottles to be analysed for Zn, Cu, Mn and Fe with an atomic absorption spectrophotometer. Analyse the sample as described above under standard curve. When samples need dilution before measurement, they should be diluted with DTPA solution to maintain a constant matrix. Experimental condition such as shaking time. DTPA concentration, pH and temperature during shaking influence the amount of Zn, Cu, Mn and Fe extracted by DTPA. The most suitable pH of extracting solution is 7.3, shaking time 2 hours and temperature during shaking 25 $\pm 1^0$C. The values of the nutrient extracted will change if these precautions are not followed. Increase in shaking time and temperature markedly enhance the extractability of Zn, Cu, Mn and Fe. The increase in pH from neutral (7.0) to 7.9 had no effect on Zn and Cu while extractability of Fe and Mn was increased markedly.

c. Zinc Standard Solution :

Dissolved 0.439 g AR grade Zinc sulphate $ZnSO_4 \cdot 7H_2O$ in 200ml of glass distilled water in a beaker. Added 5 ml of 1:5 H_2SO_4. Transfer to a litre measuring flask and made volume to the mark to have a standard solution of 100 ug Zn/ml (100 ppm). Transfer 10 ml of this standard solution to 100 ml volumetric flask and diluted to the mark with DTPA extracting solution to have a stock solution of 10 ug Zn/ml (10ppm). For preparing working standard, transfer 1,2,4 and 6 ml of stock solution(10 ug Zn/ml) to a series of clean 100 ml volumetric flask and each to the mark with DTPA extracting solution.

Volume of stock Zn solution taken	0	1	2	4	6	ml
Concentration of Zn in solution	0	0.1	0.2	0.4	0.6	ug/ml (ppm)

d. Iron Standard Solution:

Dissolved 0.702g of AR grade Mohr's salt; $(NH_4)_2SO_4FeSO_4 \cdot 6H_2O$, in 300 ml deionized or glass distilled water in a beaker. 5 ml of 1:5 H_2SO_4 is added to it. Transfer to a litre measuring flask and made volume to the mark.

This is a standard solution of 100 ug Fe/ml (100 ppm). To prepare working standards, transfer 1,2,4 and 6 ml of stock solution and diluted each to the mark with DTPA extracting solution.

Volume of stock Fe solution taken	0	1	2	4	6	ml
Concentration of Fe in solution	0	0.1	0.2	0.4	0.6	ug/ml (ppm)

e. Manganese Standard Solution:

0.288g of potassium permanganate ($KMnO_4$) AR grade, is dissolved in 300 ml deionized water in a breaker. Added 20 ml concentrated H_2SO_4 warm to about 60^0 C and added oxalic acid solution dropwise to make the solution colour less. Cool and transfer to a litre measuring flask and made volume to the mark. This solution contains 100 ugMn/ml (100 ppm). To prepare working standards, transfer 1,2,4,6 and 8 ml of the standard solution to a series of clean 100 ml volumetric flask and diluted each to the mark with DTPA extracting solution.

Volume of stock Mn solution taken	0	1	2	4	6	ml
Concentration of Mn in solution	0	0.1	0.2	0.4	0.6	ug/ml (ppm)

f. Copper Standard Solution:

Dissolved 0.392 Copper sulphate ($CuSO_4.5H_2O$) of AR grade in 400 ml glass distilled water in a beaker. Transfer to a litre measuring flask and made volume to the mark with glass distilled water. This is a standard solution containing 100 ug Cu/ml. To prepare the working standards, transfer 1,2,4 and 6 ml of stock solution to a series of clean 100 ml volumetric flask and diluted each to the mark with DTPA extracting solution.

Volume of stock Cu solution taken	0	1	2	4	6	ml
Concentration of Cu in solution	0	0.1	0.2	0.4	0.6	ug/ml (ppm)

(vii) Analysis for Available Molybdenum (Mo)

Ammonium oxalate (pH 3.3) or Grigg's reagent is considered to be the best one for the determination of available Mo. Further, this extractant is easy to prepare, have sufficient buffering capacity to prevent any material change in the pH of the soil extract and forms stable complexes with Mo ($MoO_3 \cdot C_2H_2O_4$ and $(MoO_3)_2 \cdot C_2H_2O_4$) Molybdate absorbed on soil colloids and clay is presumably replaced by the oxalations. This exchange is made irreversible by the formation of strong Mo-oxalic acid complexes to make a single extraction effective.

a. Extracting Solution:

To prepare ammonium oxalate (pH 3.3), dissolve 24.9 g AR ammonium oxalate and 12.6 g oxalic acid per litre of solution, adjusted to pH 3.3.

b. Standard Solution

Prepare a solution containing 100 ug Mo/ml by dissolving 0.150 g AR grade MoO_3 in 100 ml of 0.1N NaoH solution rendering it acidic with HCl and make the volume to 1 litre. Take 10 ml of this stock solution in one litre volumetric flask and make the volume to the mark to get a working standard of 1 ug Mo/ml. To make working standards, transfer 0.5,1.0,2.0,3.0 and 4.0 ml of 1 ug Mo/ml solution to series of clean 125 ml separatory funnels.

c. Extraction and Determination of Molybdenum

Twenty five gram soil is taken in a 500 ml corning or pyrex glass conical flask. To this 250 ml ammonium oxalate of pH 3.3 is added. The contents of the flask are shaken over an end to end horizontal shaker for 10 hours and filtered through Whatman filter paper no 50. Then, 200 ml of the filtrate is taken in a 250 ml breaker and evaporated to dryness on a water bath. The contents of the breaker are headed at high temperature (500^0C) in a furnace for 5 hours to destroy organic matter and oxalates. Contents of the breaker are then digested with 5 ml of diacid (HNO_3 – $HclO_4$ 4:1) after keeping it overnight, then with 10 ml of 4N H_2SO_4 and H_2O_2 every time taking to dryness. Then 10 ml of 0.1N HCl is added to the breaker and filter paper washed with another 10 ml. The filtrate is stored in corked plastic reagent bottles for estimation of available Mo by the procedure described above under standard curve.

(viii) Analysis for Available Boron (B)

Out of several methods devised to assess the level of available B in soil, the hot water soluble B method of Berger and Troug (1939) has been most widely accepted. Recent description of this method include some changes (Keren and Bingham 1985, Sippola and Ervco 1987), but the basic procedure remains the same. Throughout boron analysis, use of borosilicate glassware should be avoided even for storage of chemicals. Plastic containers or corning pyrex glassware should be used.

a. Standard Solution

To prepare the standard stock solution, dissolve 0.570 g boric acid (H_3BO_3)AR grade in a litre distilled water to obtain a stock solution of 100 ug B/ml. Take 5 ml of the stock solution in a 100 ml volumetric flask and dilute to the mark. This solution contains 5 ug B/ml.

b. Extraction and Determination of Boron

A 25 gm soil sample, 50 ml of water and about 0.5 g of activated charcoal is boiled for 5 minutes in a quartz flask and filtered immediately through Wahtman filter paper No. 42. 5 ml of the extract is taken in a 25 ml volumetric flask and 4 ml of buffer masking solution and 4 ml of azomethine –H regent solution is added. The colour is allowed to develop for 1 hour, and the volume is made to the mark**.** Intensity of colour is measured spectrophotometrically at 420 mm, and the B concentration read off from standard curve described above.

C) Statistical Models:

Statistical Models and methods were done as followed by Singh & Chaudhary (1995) and Panse & Sukhatme (2005).

3. RESULTS AND DISCUSSION

Six soil samples were collected from the different forest gardens of North India and Nepal Border Area. The soil samples were tested for pH, Organic carbon (OC), available N, P and K and presented in Table-IV. The result show that Rampur, Malangwa, has highest macronutrient content and lowest is Kamalpur. Micronutrient analysis was done for 4 soil samples, viz. Rampur, Malangwa, Kamalpur and Bardibas and reflected in Table-V. It is evident from the results that the micronutrient (Cu, Zn, Mn, Fe, Mo & B) content is lowest in Kamalpur, Cu content is maximum in Malangwa, Zn & Fe content is highest in Kamalpur, Mn content is highest in Rampur. In all the cases B content is below the critical level (0.3 ppm) and Mo content is below dection level (BDL). Sandal requires good drainage and does not stand waterlogging. Best growth of sandal trees are found on rich fairly moist soil such as garden loam and well drained deep alluvium on the river banks (Troup,1921). A significant relataionship between available nitrogen content in 'A' horizon and annual growth increment was observed in the soils of Talamalai Range (Krishnamurthy *et al.*,1983). In a study carried out by Jain *et al.*,(1988) on soil properties and their relationship to the growth of sandal in three areas, it was

Table - IV: Soil Test Reports of Macronutrients

Location	pH	OC(%)	Available N (Kg $ha^{-1)}$	Available N (PPM)	Available K (PPM)	Available P (PPM)
Rampur	5.71	0.51	234.6	106.63	57.6	8.2
Malangwa	5.70	0.47	221.3	100.59	51.8	7.9
Kamalpur	5.57	0.34	198.8	90.36	49.8	5.7
Bardibas	4.95	0.35	211.3	105.51	50.5	5.6
Rampur	5.66	0.44	217.7	98.95	51.7	7.8
Malangwa	5.30	0.42	210.8	95.82	52.1	5.5

Table - V: Soil Test Report of Micronutrients

Sl. No	Test Parameters	Critical levels of micronutrients ($mgkg^{-1}$or PPM)	Results			
			Hirbandh	Kamalpur	Rangamati	Bagaldhara
1	Copper (Cu)	0.2	0.67	1.08	1.38	0.50
2	Zinc (Zn)	0.6	0.32	0.86	0.46	0.19
3	Manganese (Mn)	2.0	73.72	45.89	69.50	25.89
4	Iron (Fe)	4.5	8.66	17.15	7.96	2.25
5	Molybdenum (Mo)	0.05	BDL	BDL	BDL	BDL
6	Boron (B)	0.3	0.17	0.14	0.17	0.14

observed that lime status, water holding capacity, pore space, volume expansion on wetting, exchangeable calcium and magnesium and available potash, exert positive influence on the increment in girth and height. Requirement of host for proper growth of sandal was demonstrated in a field study by Ananthapadmanabha*et al.,(*1984). Further analysis of soil and leaf samples from this trial had shown that sandal depands on its host for K, P and Mg (Rangaswamy*et al.,* 1986b). Sandal can draw other nutrients directly from soil because its roots have good cation exchange capacity (Parthasarathi*et al.,* 1971).

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