World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Santalol oil of Sandalwood (White) grown in different edaphic factors in west Bengal, India

Roshan Kumar Yadav [1], Subrata Mukhopadhyay [1] and Jagatpati Tah [2,*]

[1] *Department of Chemistry, Jadavpur University, 188 Raja Subodh Chandra Mullick Road, Jadavpur, Kolkatta- 700032, India.*
[2] *Department of Botany, The University of Burdwan, Burdwan (UGC NAAC)- 713104, West Bengal, India.*

World Journal of Advanced Research and Reviews, 2023, 19(01), 1404–1413

Publication history: Received on 29 March 2023; revised on 07 May 2023; accepted on 10 May 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.19.1.0838

Abstract

Santalol means oil of white sandalwood (*Santalum album* L.) which is most valuable and importantfor different medicinal and cosmetic uses. The quality of this santalol is very much dependent on soil factor as well as climatic factor which is called edhapic factors as a whole. The concept of most of the people is sandalwood plant doesn't grow in any atmospheric conditions and cannot produce quality sandalwood oil.

Keeping all these views in mind we have undertaken a venture of producing sandalwood plants in different locations prevailing varied edaphic factors. A uniform agro-measure and techniques were provided for the proper growth and development in for all the plants in all locations. After attaining its heartwood maturity, we collected the heartwood samples from each location and estimated the santalol following standard distillation method.

The aims and object of this experiment were to study the growth index i.e., quantitative assessment and determining the percentage of santalol present in the heartwood for its qualitative assessment. All these peculiarities and the recorded data have been put forth in this paper for a clear explanation.

Keywords: Edhapic factors; Quantitative assessment; Qualitative assessment; Heartwood; Standard distillation method

1. Introduction

Santalum album is a hemi parasitic plant with worldwide distribution. It is also known as East Indian sandalwood plant. Though ecologically sandal wood plants have adopted various soil condition but they can tolerate water logged condition. They generally prefer elevated land with water flow system. This plant has been used form the ancient era in various wood, pharmaceutical, cosmetic and perfumery industries. Presence of the essential oil like alfa santalol, beta santalol make the plant so costly, important and valuable. Several other components like santyl acetate, santalene are also present but are not so valuable as alfa and beta santalol. These essential oils are mainly present in the heart wood and older large roots. Heart wood formation start after attained the age of five years and is rapidly formed after the age of ten years. Huge amount of heartwood can be obtained from 30 to 60 years old plants (Fallick, 2009). The sandal wood materials are procured from different parts of West Bengal for analizing the santalol quantity as well as quality. Sandal woods of newly grown plants were procured from Hirbandh Beat office campus, Bankura (S) Forest Division, Bagaldhora garden, Hirbandh Range, Bankura (S) Forest Division, Khandari Beat office campus, Panagarh Range, Burdwan Forest Division, Guskara Range, Burdwan Forest Division. Because, the concept of general peoples of the state

* Corresponding author: Jagatpati Tah

is that there is a hidden cause not to develop adequate quality and quantity of santalol in the white sandal tree while it is grown in this environment of West Bengal.

What does the term global environment mean. The global environment refers to the environmental factors that impact the entire planet. This might leave you wondering about the components of global environment. Well, in other words, the global environment is the sum total of all local, regional, and national environments in the world. With this in mind, global environmental factors and issues are those that a single community or nation cannot control or contain, such as natural disasters, air quality, water quality, soil quality, and climate change. These environmental factors play an important role in sandalwood plants specially for the production of quality as well as quantity of santalol.

West Australian sandalwood (*Santalum spicatum*) belongs to the genus of hemi-parasitic Santalum (Santalaceae) trees widely exploited for their fragrant heartwood which is used in perfumes, pharmaceuticals, incense and ornamental carvings. The fragrance contained in the heartwood of mature sandalwood trees (>10 years) consists of a complex mixture of sesquiterpenoids, with unique compositions apparent across, and often within species (Jones et al., 2006; Moretta, 2001, Page et al., 2010). Historically, Indian sandalwood (*S. album*) has provided the bulk of sandalwood products; however, *S. spicatum* is frequently used as a supplement to incense feedstock and to a lesser extent as extracted fragrance oil. The international standard for S. album oil requires 41%–55% of the sesquiterpene alcohol α-santalol and 16%–24% β santalol (Howes et al., 2004; ISO:3518, 2002). At present, S. spicatum extracts do not meet these industry standards for two reasons; the combined santalol content is too low, and levels of E,E-farnesol, a suspected allergen, are too high (Brand and Pronk, 2001; Hostynek and Magee, 1997; Schnuch et al., 2021).

Extracts of *S. spicatum* are generally considered less valuable than those of S. album due to the lower overall content and more variable sesquiterpene composition (Brand and Pronk, 2001). S. spicatum is traditionally harvested from natural stands of un-domesticated tree populations throughout south-western Australia. To reduce pressure on wild stands, plantations have been established on former grazing and marginal agricultural land.

Substrate	CYP76F39v1 [%]	CYP76F37v1 [%]
α-santalene	99.8	17.3
β-santalene	100	17.7
α-curcumene	0	0
α-zingiberene	0	0
β-bisabolene	0	0
β-sesquiphellandrene	0	0
α-bisabolol	9.4	0
trans-β-farnesene	0	0
trans-nerolidol	0	11.3

Figure 1 Chemical structures of different santalol components of *S. album*

Significant potential exists for the improvement of S. spicatum plantations through selection of trees with desired growth and sesquiterpene characteristics. By first quantifying the extent and nature of sesquiterpene variation throughout the tree's area of distribution, key selection parameters may be specified. Moreover, by better understanding the process of sesquiterpene formation in sandalwood, relevant molecular markers may be developed to speed up the selection process. Continued development of *S. spicatum*, a species naturally adapted to the arid-zone of Western Australia, will further conservation goals as well as improving its commercial potential. The heartwood extract of S. spicatum contains over 100 different sesquiterpenes (Adams et al., 2017; Baldovini et al., 2011; Brophy et al., 1991; Valder et al., 2003). This complex mixture of sandalwood sesquiterpenes is likely to serve as protection against wood-rotting fungal pathogens, given its highly-enriched localization specifically in the innermost heartwood (Jones et al., 2008). The most abundant compounds of the S. spicatum heartwood extract are the sesquiterpene alcohols E, E-farnesol, α- and β-santalol, lanceol, nuciferol and α-bisabolol, as well as a variety of sesquiterpene olefins such as the santalenes, bergamotene, and several curcumenes (Brophy et al., 1991; Valder et al., 2003). Moretta (2001) studied the heartwood-cores of 87 *S. spicatum* trees from 12 geographic sites in Western Australia and, in the form of a PhD thesis, reported the levels of α- and β-santalol to vary 3%–67%, and E, E-farnesol to range 5%–30% across the entire distribution (Figure 1). Although variation in major components has been reported for S. spicatum, there have been no further investigations into the sesquiterpene composition across populations within its natural distribution. A more comprehensive evaluation of the extent and nature of variability of wild *S. spicatum* trees is needed to inform efforts to increase total santalol content through selection. Figure 1. Biosynthesis of major sesquiterpene alcohols in *S. spicatum*. Proposed formation of farnesol from farnesyl-diphosphate (FPP) via a terpene synthase (TPS) or phosphatase activity, and the known pathway for santalol biosynthesis in sandalwood via TPS and cytochrome P450 (P450) activities. Chemical variation in S. spicatum might be explained by genetic differences amongst populations, while the species is also growing across a range of diverse environments. Discrete chemical differences in heartwood composition appear to exist throughout the species' natural distribution (Moretta, 2001) and several populations have been shown to bear strong genetic similarities (Byrne et al., 2003; Byrne et al., 2003;).

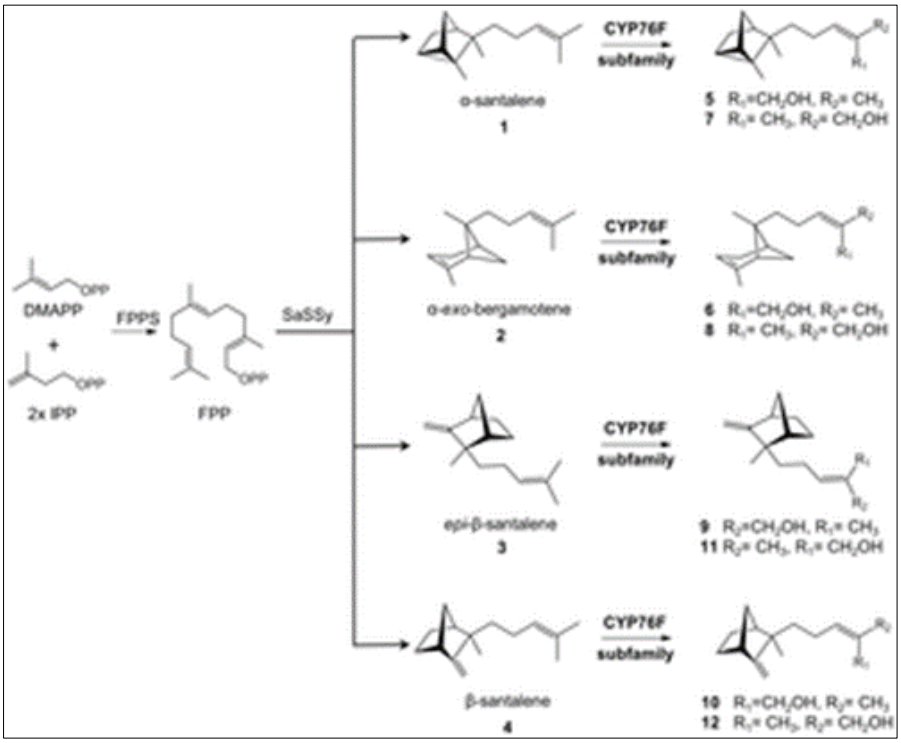

Figure 2 Major components and biosynthesis of *S. album*

Sandalwood is the general name for woody perennials of the *Santalum* genus (Santalaceae), which are exploited for their fragrant heartwood. Sandalwoods are slow growing hemiparasitic trees distributed throughout the tropical and temperate regions of India, Indonesia, Australia and the Pacific Islands (Harbaugh and Baldwin, 2007; Page et al, 2010). The oil extracted from the stems and roots are highly sought after by the fragrance and perfume industry. *Santalum album*, also known as tropical or Indian sandalwood, is the most valuable of the commercially used species due to the high heartwood oil content (6–10% by dry weight) and desirable odor characteristics. Approximately 90% of *S. album* essential oil is composed of the sesquiterpene alcohols α-, β-, and *epi*-β-santalol and α-*exo*-bergamotol (Figure

1). The α- and β-santalols are the most important contributors to sandalwood oil fragrance (Adams et al, 1975; Baldwin et al., 2011). Lanceol and α-bisabolol are also found in modest concentrations (Jones et al., 2007). While the demand for sandalwood oil is increasing, disease, grazing animals and unsustainable exploitation of sandalwood trees has led to the demise of many natural populations. Plantations provide a more sustainable alternative to wild harvesting; however, slow growth rates, high potential for disease and substantial variation in oil yield hamper productivity. Alternatively, chemical approaches to synthesize the santalols have been attempted (Christenson and Willis, 1980; Brocke et al., 2008), but multiple low-recovery steps make chemical synthesis uneconomical at an industrial scale.

2. Material and methods

2.1. Materials

100 g of sandal wood sample from each location, Gas chromatography instrument, pen, Laboratory record book etc.

2.2. Methods

The sample collected from Hirbandh beat office campus, Bankura (S) forest division is denoted as SA001, sample collected from Bagaldhora garde, Hirbandh Range, Bankura (S) Forest Division is denoted as SA002, sample collected from Khandari Beat office campus, Panagarh Range, Burdwan Forest Division is denoted from SA003.

Number and quantity of particulars present in the newly grown sandal wood are analysed through quantification process of gas chromatography of essential volatile oil (GC analysis), and identification process of GC based peak analysis using reference/control.

2.3. Result of the analysis

The result of analysis is given below in tabular form:

Table 1 Analysis of α and β santanol and oil contents

Sample	Particulars	Quantity (%)
SA001	α- santalol	58.2
	β- santalol	29.2
	Total santalol	87.9
	Oil contents	4.1
SA002	α- santalol	59.1
	β- santalol	28.7
	Total santalol	87.8
	Oil contents	4.2
SA003	α- santalol	60.3
	β- santalol	29.9
	Total santalol	90.2
	Oil contents	4.3

SA001= Sample-1 i.e. Hirbandh Beat office campus, Bankura (S) Forest Division, SA002= Sample-2 i.e. Bagaldhora garde, Hirbandh Range, Bankura (S) Forest Division SA003= Sample-3 i.e. Khandari Beat office campus, Panagarh Range, Burdwan Forest Division, SA004= Sample-4 i.e. Guskara Range, Burdwan Forest Division

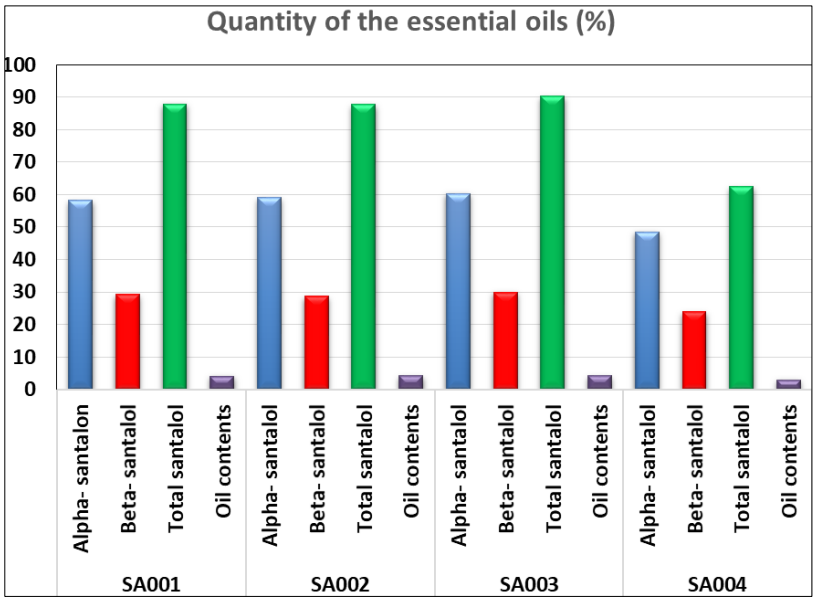

Figure 3 Quantity of essential oil

2.4. Materials

Materials

- Viable seeds of *Santalum album* L.,
- Distilled water,
- Petri dishes,
- Mercuric chloride,
- Tap water,
- Incubator for germination,
- Field for plantation,
- All agronomic facilities in the farming site etc.

2.5. Methods

- A Randomized Block Design Field (RBD) having 12 'X 15' plant spacing,
- Provide uniform agronomic cultures in all the forest gardens,
- collection of data (Plant height, branch number, leaf number) in regular manner,
- biometric calculations followed by Tah, 2018.

Study area This study was conducted in Khandari (Burdwan), Bagaldhara (Bankura), and Hirbandh (Bankura)

2.6. Data collection

Data collection included morphological characteristic of sandalwood tree, trees composition, edaphic factor, number of leaves, number of branches, tree height etc. Size-class structure data were collected from the assistance of supervisor and colleagues. A comprehensive account of growth parameters of *Santalum album* L. in terms of plant height, girth and number of branches per plant have also been presented here to reflect the differences (if any) in its growth pattern across these locations.

2.7. Data analysis

Quantitative data of sandalwood were obtained by calculating the CF, TSS, RSS, TRSS and ESS for all locations with the variation in leaves number, plant height and number of branches.

3. Results

3.1. Leaf Number

Table 2 Total number of leaves of S. album in different study sites

Location	R-I	R-II	R-III	R- IV	∑
L -1	1780,17900,350, 280,16600 ∑=52930 x̄= 10586	19200,800,1200, 13600,19500 ∑=54300 x̄= 10860	18800,16900,6000, 8600,17500 ∑=62400 x̄=12480	14800,16200,10900, 9300,14100 ∑=65300 x̄= 13060	46986
L -2	12500,14600,10800 8600,16500 ∑=63000 x̄=12600	11300,13400,6000, 9600,12000 ∑=52300 x̄=10460	7600,9800,4200, 8100,9600 ∑=39300 x̄= 7860	6300,8100,460, 9000,8600 ∑= 36600 x̄= 7320	38240
L -3	20200,16400,18900, 4200,4600 ∑= 64300 x̄=12860	10200,9000,12500, 16900,14600 ∑= 62700 x̄= 12540	10700,6000,14100, 7200,7800 ∑= 45800 x̄=9160	9300,8200,710, 4600,3800 ∑= 33000 x̄=6600	41160
L -4	16700,8500,3200, 4500,7100 ∑=40000 x̄=8000	16800,9700,7400, 9000,9500 ∑=52400 x̄= 10480	8900,7200,8300, 7600,9600 ∑= 41600 x̄= 8320	8200,9100,9600, 6100,7500 ∑= 40500 x̄= 8100	34900
∑	44046	44340	37820	35080	GT= 161286

L = Location, L1 = Khandari (Bardwan), L2 = Basudevpur (Bankura), L3 = Bagaldhara (Bankura), L4 = Rangamati (Bankura)

[**Calculations:** CF = (161286)2 /80 =325164672.45 TSS = 10094500900- 325164672.45 = 9769336227.55 RSS = (6567044516/ 4)- 325164672.45 = 1316596456.55 TRSS = (6582137396/4)- 325164672.45 = 1320369676.59 ESS = 9769336227.55- (1316596456.55+1320369676.59) = 7132370094.45]

Table 3 ANOVA (Leaf Number**)**

SV	df	SS	MS	F
REP	3	1316596456.55	438865485.516	0.5537
TREAT	3	1320369676.59	440123225.516	0.5553
ERROR	9	7132370094.45	792485566.05	

3.2. Plant Height (cm)

Table 4 Plant height (cm) of S. album in different study sites.

Location	R-I	R-II	R-III	R- IV	∑
L -1	30,35,5,5.8,3,30, 35.2,5.9,5.9,30.6 ∑=107.6 x̄= 21.5	30.5,5.9,6.9,24., 33,20,18,22,26,28 ∑=105.3 x̄= 21.06	23.8,28.7,9.8,12,30. 6 ∑= 104.9 x̄= 20.98	23,25.7,16.9,14,18 ∑= 97.6 x̄= 19.5	83.06
L -2	10.8,13.6,8,12.1 ,25.3 ∑= 69.8 x̄=13.96	18,16.7,9,8.8,10.9 ∑= 63.4 x̄= 12.68	9.7,11.4,8.6,12.4, 12.6 ∑= 54.7 x̄= 10.94	10.8,12.7,9.8,11.8, 12.1 ∑= 57.2 x̄= 11.44	49.02
L -3	10,10,12,12,11,10. 9, 0.7,12,14.6,16.7 ∑= 64.9 x̄= 12.98	8,7.7,10.8,14.1,11. 2 ∑= 51.8 x̄= 10.36	13.7,9.9,18,11.3,10. 7 ∑= 63.6 x̄= 12.72	14.3,15.2,11,10, 4.9 ∑=59.9 x̄= 11.98	48.04
L -4	10.6,9.9,8.6,9,14.6 ∑=52.7 x̄= 10.54	12.3,10.9,11,14,10. 3 ∑= 58.5 x̄= 11.7	10.9,11.2,12,11,14. 4 ∑= 59.5 x̄= 11.9	11.6,12.3,12.2,9.8,1 0 ∑= 55.9 x̄= 11.18	45.32
∑	58.98	55.8	56.54	54.12	GT=225.44

L = Location, L1 = Khandari (Bardwan), L2 = Basudevpur (Bankura), L3 = Bagaldhara (Bankura), L4 = Rangamati (Bankura)

[Calculations: F= (225.44)2 /80 = 635.28 TSS = (30)2 + (3.5)2 + (5.9)2 + (5.9)2 + (30.6)2 ……..+ (10)2 - CF = 19361.08 – 635.28 = 18725.8 RSS = {(58.98)2 + (55.8)2 + (56.54)2 + 954.12)2 }/ 4– 635.28 = (12718.02)/4 – 635.28 = 2544.42 TRSS = {(83.06)2 + (49.02)2 + (48.04)2 + (45.32)2 }/ 4- CF = 3415.9-635.28 = 2780.635 ESS = TSS-(RSS + TRSS) = 18725.8- (2544.22 + 2780.63) = 13400.95]

Table 5 ANOVA of Plant Heifht(cm)

SV	df	SS	MS	F
REP	3	2544.22	848.07	0.5695
TREAT	3	2780.635	926.87	0.6224
ERROR	9	13400.95	1488.99	

3.3. Total No. of Branches

Table 6 Total number of branches of S. album in different study sites

Location	R-I	R-II	R-III	R- IV	∑
L -1	85, 27,15,14,18 ∑=159 x̄= 31.8	44,10,8,12,19 ∑= 93 x̄= 18.6	15,18,7,21,14 ∑= 75 x̄= 15	21,33,25,6,18 ∑= 103 x̄= 20.6	86.0
L -2	8,9,12,23,10 ∑= 62 x̄= 12.4	12,8,6,12,7 ∑= 45 x̄= 9	6,28,12,14,18 ∑= 78 x̄= 15.6	27,19,11,7,16 ∑= 80 x̄= 16	53.0
L -3	16,25,30,22,24 ∑= 120 x̄= 24	4,4,11,9,7 ∑= 35 x̄= 7	22,18,25,19,17 ∑= 101 x̄= 20.2	11,25,19,8,18 ∑=81 x̄= 16.2	67.4
L -4	8,2,4,15,25 ∑= 54 x̄= 10.8	19,9,6,12,8 ∑= 54 x̄= 10.8	19,23,15,6,18 ∑= 81 x̄= 16.2	34,10,6,9,14 ∑= 73 x̄= 14.6	52.4
∑	79.0	45.4	67.0	67.4	GT= 258.8

[Calculations: CF= (258.8)2 /80 = 837.218 TSS = 30934- 837.218 = 30096.782 RSS = (17333.92/4) - 837.218 = 3496.262 TRSS = (17493.52/4) - 837.218 = 3536.162 ESS = 30096.782 – (3496.262+3536.162) = 23064.35]

Table 7 No. of Branches/ plant

SV	df	SS	MS	F
REP	3	3496.262	1165.42	0.4547
TREAT	3	3536.162	1178.72	0.4599
ERROR	9	23064.35	2562.7	

4. Discussion

From the above analysis it found that the quantity of total santalol and other oil contents in the sample of Hirbandh Beat office campus, Bankura (S) Forest Division and Bagaldhora garde, Hirbandh Range, Bankura (S) Forest division is more or less equal. In the sample of Khandari Beat office campus, Panagarh Range, Burdwan Forest Division the quantity of total santalol and other oil contents is comparatively more than that of other three samples. In case of sample collected from Guskara Range, Burdwan Forest Division the quantity of total santalol and other oil contents is little bit lower than the other three samples.

On the basis of quality of □- santalol and β- santalol, it was found from the analytical data which seems to be upto the mark of good quality. No one sample was measured the total santalol oil below 80%.

The average number of leaves found in different geographical regions of our study showed L1 (46986), L2 (38240), L3 (41160), L4 (34900). The average height of plants as in the study site was found to be 83.06, 49.02, 48.04 and 45.32 feet respectively for L1, L2, L3 and L4. Similarly, the average branch number were in the order of 86,53, 67.4 and 52.4 as shown in the table above. Sandalwood (*Santalum album* L.) is a partial root parasite, small evergreen tree attaining a height of 12 to 15 metres and a girth of 1 to 2.4 metres with slender drooping as well as erect branching. FAO, 1995 published a bulletin on Flavours and Fragrances of Plants origin which is very much related tantalum album L. This plant has been rendering its performance properly for the sake of human beings since ancient time of civilization. There was no existence of sandal plant in West Bengal in national map. Recently, Das and Tah (2014) reported its existence in West Bengal in an international forum. A few plants were grown by forest executive in undivided Bankura Forest Division. It was felt by present workers that there is certainly some scientific lacuna for its seed propagation and also adaptation due to specific edaphic factors and less germination percentage. Keeping all these views in mind, this venture was undertaken to find out any reason behind this problem.

Some Photographs from the field

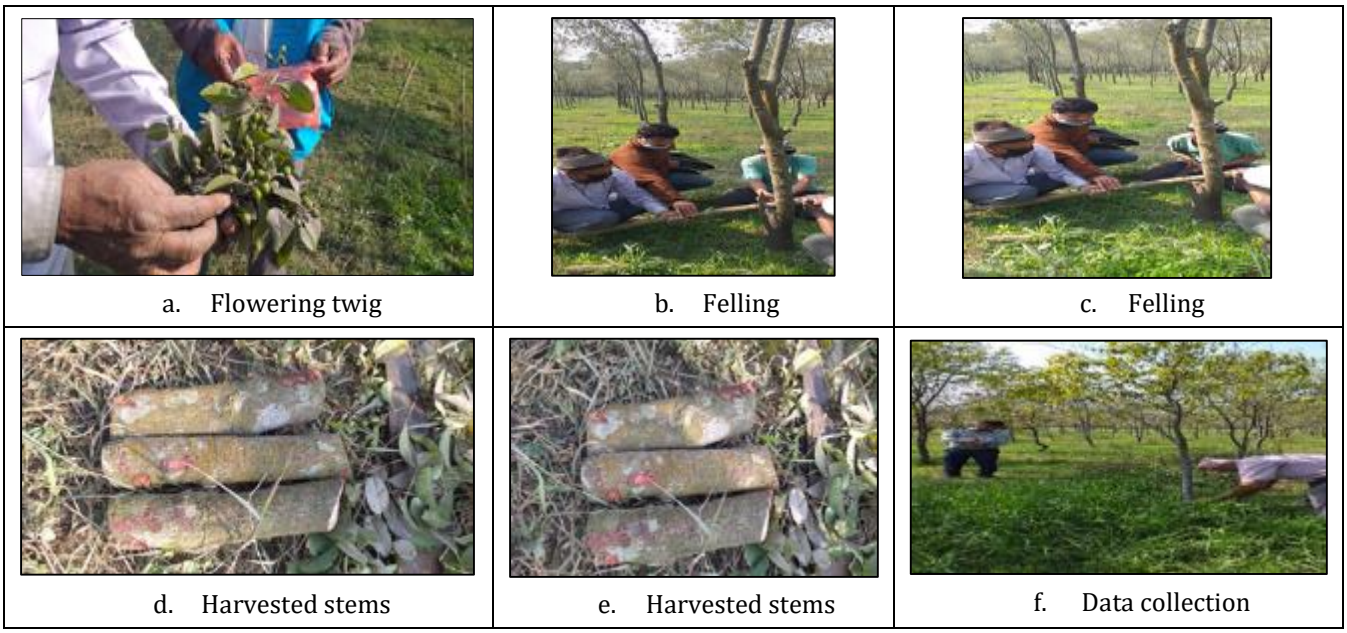

Figure 4 Photographs taken in the crop field

5. Conclusion

It has been found that the edaphic factors are congenial for the physiological growth and development of white sandal (*Santalum album* L.) in all these areas where the those experimental plants were grown. Indeed, it might be better in special private farming technology than forest areas where to take any special care is never possible in general.

The productivity of timber plants is measured by analysing the plant height and breast height girth (b.h.g) in specific unit area. In this case four locations are distributed in both the Forest Divisions, Bankura (South & North). The plant height and basal girth were measured critically as much as it was possible out of 700 of S. album plants, grown in Forest gardens. The yield of heartwood varies from locality to locality and with the age of the tree. In India, trees of 100cm girth have been reported to yield between 85 kg and 240 kg of heartwood according to the area from which they come (FAO,1995). Timber wood production is expressed by its timber volume. It is measured by the plant height and basal girth. Jahan and Rahman, 2014 explained that sandal dissolves inflammation and tumours and stabilizes palpitation. Sandalwood is used as a disinfectant in bronchial and genitourinary tract infection. Das and Tah (2013) observed the effect of concentration of GA3 on seed germination of sandal (*Santalum album* L.). Das and Tah (2014) vividly experimented on Silvicultural practices for its adaptability with different host species and natural regeneration in south-West Bengal in different forest gardens in Bankura and Burdwan Forest Divisions in South Bengal, Das and Tah (2016) studied the soil nutrients for the growth of white sandal (*Santalum album* L.) in southern part of West Bengal. Batabyal Karmakar et al., (2017) experimented on different seed-sources on germination parameters by means of artificial seed germination of *Santalum album* L., different seed bed materials and GA3 on seed germination of *Santalum album* L. and Characterization of Bacillus cereus Symbiotic to Hemi-parasitic Plant *Santalum album* L. Yadav et al., 2018 took up a

venture on the Study of Edaphic Factors of the Location for the Growth of White Sandal (*Santalum album* L.) in Indo-Nepal Border. A paste of the wood is applied in burns, fever and headache. It relieves thirst. Sandalwood is often used for rituals or ceremonies in Hinduism. Sandalwood is considered in alternative medicine to bring one closer with the divine.

Compliance with ethical standards

Acknowledgments

Authors deeply acknowledge with thanks to the Department of Science and Technology, Government of West Bengal, India for financial support of the project .

Disclosure of conflict of interest

Roshan Kumar Yadav worked in this context as PhD scholar. Subrata Kumar Mukhopadhyay the then Dean, Sc., was attached as Joint Supervisor of the PhD program as per university rules and regulations. Jagatpati Tah was attached as Visiting Professor and Subject guide of this PhD thesis program.

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