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Research Article

MORPHO-PHYSIOLOGICAL AND HISTOLOGICAL EXAMINATION OF ORGANICALLY AND NON-ORGANICALLY CULTIVATED ANTIDIABETIC MEDICINAL PLANTS

PRAKASH R. ITANKAR, MOHAMMAD TAUQEER*

Department of Pharmacognosy and Phytochemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur - 440 033, Maharashtra, India. Email: mohdtauqeer06@gmail.com

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ABSTRACT

Objective: The present research work was designed to compare the morpho-physiological and histological characters of *Trigonella foenum graecum* and *Ocimum sanctum* cultivated by organic and non-organic farming.

Methods: The open deforested area of the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, was selected for the cultivation. For the organic cultivation, biodynamic manure and organic pesticides (neem seeds extract and fermented garlic extract) were utilized, whereas in case of non-organic cultivation, the conventional manures (urea, superphosphate, and potash) and chemical pesticides (monocrotophos and zineb) were utilized. Different morpho-physiological (growth) traits were studied at the pre- and post-harvest conditions. Selected parts of the plants were also compared for the histological characters.

Results: Both the research plants with organic treatment comparative to non-organic crop showed 3 days early initiation of flowers/inflorescence followed by early legumes (organically cultivated *T. foenum graecum* [OTF]) and fruiting (organically cultivated *O. sanctum* [OOS]). OTF found with higher mean values of height, secondary branches, flowers, secondary roots, leaves, and root nodules, whereas non-organically cultivated *T. foenum graecum* showed the higher mean values of weight of plants and ultimately higher overall yield. Similarly, OOS showed the greater mean values of all the traits (height, secondary branches, roots length, and secondary roots), except the weight of the whole plant that was found with the greater mean value of 279.61 g/plant in non-organically cultivated *O. sanctum*. The microscopical examination revealed similar type of cellular arrangements in both the crops.

Conclusion: The conclusive evidence proved the betterment of organic crop in terms of their growth and sustainability.

Keywords: Cultivation, Histological, Morpho-physiological, Non-organic, Organic.

INTRODUCTION

The global revolution to improve the people safety is gaining momentum; hence, the food and drug safety for the subject becomes even more prominent in the present day scenario. The population of India is predicted to cross 150 crores by 2025 when the main challenge before the country would be not only food and nutrition but also the health security. Hence, the programs of the agriculture sector, in general, needs a clear vision to meet the challenges ahead while addressing the present day demand. The present situation on future challenges call for systematic and continued accelerative efforts in research of medicinal and aromatic plants (MAP) directed toward sustainable quality production for maintaining the socio-economic and ecological balance. Medicinal plants have been incorporated in seamless fabric of diet in our traditional food habit and medicines which are often connected through a comprehensive traditional theory of disease control. Currently, commercialization of this concept has started in the form of developing a new line of products called "functional food" which contains medicinal plant as ingredients added to health foods. Furthermore, the interest in collection, production, and marketing of MAPs as phytochemicals, pharmaceuticals, nutraceuticals, herbal remedies, food supplements, perfumes, cosmetics, and food flavoring agents, etc., has increased many folds in recent years [1].

Though a lot of ayurvedic and herbal industries are available, the details regarding the source of herbs are obscure and untraceable. Ignorance about the safety measures of plants like the level of heavy metals, mycotoxines, and inorganic pesticides in the cultivated plants and also about its overall quality which definitely affects the predetermined potential of that herb or medicine is a matter of concern. However, Biological Diversity Act (2002) and Rule (2004) enforced the noble

thought of protecting our biodiversity, especially crude drugs from plant origin [2]. Therefore, it has become essential for all the herbal industries and those working on medicinal plants, to source them by producing these crude drugs by cultivation in the fields to meet the growing demand. However, the conventional practice of heavy agricultural reliance on synthetic chemical fertilizers and pesticides is having serious impacts on public health and the environment [3]. Cultivation of medicinal plants with laboratory-generated species with better chemical composition is likely to be used in increased manner for commercial purposes. These changes may have profound impact on the safety, quality, and efficacy of the Ayurveda drugs in the market. A series of food scares and the controversy surrounding genetically modified crops also have prompted heated debate about the safety and integrity of our food and herbal medicines. Against this background, demand for organically grown food has been growing rapidly [4]. The demand for organic food is steadily increasing both in the developed as well as developing countries with an annual average growth rate of 20-25% [5]. Until now, this perception that organically grown food is "better for you" appears to have been largely based on intuition rather than conclusive evidence. Therefore, the present study was designed to scientifically validate the growth pattern (morpho-physiology) and histological examination by taking Trigonella foenum graecum and Ocimum sanctum, as experimental medicinal plants.

METHODS

Parallel organic and non-organic cultivation of selected medicinal plants

The present investigation was carried out in the open deforested area of the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, situated in the center of the Indian

peninsula 79° 7′ East longitude and 21° 7′ North latitude and is at a mean altitude of 310.5 m above sea level. The average annual rainfall of the area is 1205 mm. The average maximum temperature of the city is 33.53°C; the average minimum temperature is 20.37°C, and the relative humidity ranges from 20% to 70% [6].

Experimental land and soil analysis

The land for the organic treatment was selected and converted (period 2.5 years before first harvest) as per the recommendations of the National Centre for Organic Farming, India [7], and the parallel area with marked buffer zone was selected for non-organic treatment (Fig. 1). The soil of experimental site was comprised black clay soil with a pH of 6.8, rich in humus, potash, and trace elements (Cu, Fe, Mn, and Zn) (analyzed by "Soil Survey and Soil Analysis Department" Government of India, Nagpur, MS, India) (Table 1a).

Source of seeds and stalks

T. foenum graecum seeds - label no. 01150, Vijay seeds Co. Ltd., Jalna; and *O. sanctum* seeds - Organic India Pvt. Ltd., Lucknow.

Manures

Organic manure was prepared using standards given by Bio-dynamic Association of India [8]. It was prepared using a mixture of protein-rich materials including animal manures, fresh green grass, leaves and shoots of leguminous plants or trees (*Cassia* species); and carbon-rich materials including wood chips, dry leaves, and grasses in a proportion of 60% protein and 40% carbonaceous. The heap was made by a layering method, of about 2 m wide at the base, 0.5 m high and 3 m

Table 1a: Soil properties

Soil contents	Observed values	Standard values	Remarks
рН	6.8	-	Neutral
Salt (EC) Mscm ⁻¹	0.34	-	Common
Carbon (% w/w)	1.237	-	Very high
Nitrogen (kg/ha)	325	-	Normal
Phosphorus (kg/ha)	18.687	-	Normal
Potassium (kg/ha)	895.1	-	Very high
Copper (ppm)	2.36	0.2	Very high
Iron (ppm)	8.36	4.5	Very high
Manganese (ppm)	11.65	2.0	Very high
Zinc (ppm)	1.73	0.65	Very high

Analyzed by Soil Survey and Soil Analysis Department, Nagpur District, (MS) India (Report No. 30S20120033001, 30M20120020001)

Table 1b: Standard limit of macronutrients

Remark	Organic carbon (%)	Nitrogen (kg/ha)	Phosphorous (kg/ha)	Potassium (kg/ha)
Very less	Up to 0.2	<141	< 7.0	<100
Less	0.21-0.40	141-280	8.0-14	101-150
Normal	0.41-0.60	281-420	15-21	151-200
Normal high	0.61-0.80	421-560	22-28	201-250
High	0.81-1.00	561-800	29-35	251-300
Very high	>1.00	>801	>36	>301

Data provided by Soil Survey and Soil Analysis Department, Nagpur District, (MS) India

Table 1c: Standard limit of salt

Remark	Salt (EC) Dsm ⁻¹
Common	<1.00
Harmful to crop	1.01-2.00
Harmful to salt susceptible minerals	2.01-3.00
Dangerous to crop	>3.00

Data provided by Soil Survey and Soil Analysis Department, Nagpur District, (MS) India

long, comprised first stiff/hard layer of woody stems as a base, later the carbonaceous layer about 10 cm deep, alternating with the loose proteinaceous layer which was about 15 cm deep. The animal dung was made into slurry using cow urine, jaggery (liquid manure), and watered onto the carbon layer. Very little amount of hydrated lime and rock powder were sprinkled over the pile. Heap was turned regularly after about 3 weeks and brown, crumbly humus was ready at 3 months (Fig. 2). Non-organic fertilizer, *viz.*, urea granules (N), superphosphate (P_2O_5), and potash (K_2O) per hectare were applied according to the treatment schedule [9].

Representing preparation of green manure, liquid cow dung with jaggery and actual layering process for the heap.

Pesticides

Organic insecticide and fungicide

Fully dried *Azadirachta indica* L. (neem) seeds of about 500 g were pulverized and macerated for 24 hrs in 10 L of Milli Q (MQ) water (BioAGE Direct Ultra, Punjab, India). Filtrate was utilized as potent insecticide (neem extract). Fresh *Allium sativum* L. (garlic) paste was prepared and about 250 g paste was fermented for about 15 days in 05 L of MQ water. The fermented filtrate was diluted again with 05 L of MQ water and sprinkled over organic crop as fungicide (garlic extract) [10].

Non-organic insecticide and fungicide

Monocrotophos (MC) 36% (Monocil, Insecticide India Ltd., India) as an insecticide and Zineb 75% W.P. (Indofil Z-78, Indofil Industries Ltd., Mumbai, India) (Indofil - IF) as fungicide were purchased from local market and utilized in a ratio of 3:2 in MQ water, respectively, as per product manual [11].

Cultivation, harvesting, and morpho-physiological study (growth) Both the plant seeds were sown in the month of October with the implementation of good agricultural practices by adopting the randomized block design in 12 replicates of each treatment in the year 2012. Two different treatments were utilized, organic, and non-organic (in terms of fertilizer and insecticide/fungicide). About 5 m³/acre organic



Fig. 1: Experimental land in the Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University,
Nagpur



Fig. 2: Preparation of (biodynamic) organic manure

(biodynamic manure) was utilized for the organic crop. Non-organic manure (urea, superphosphate, and potash) was utilized in a ratio of 50:00:00 and 120:105:105 for methi and tulsi, respectively [9,11]. Half of the N and entire P were applied at basal stare, and the remaining of N was applied in two splits at vegetative and flowering stage. About 10 L per acre of each organic and non-organic pesticide were sprayed. Almost all the morpho-physiological traits of plants (pre- and post-harvest) were examined using statistical package PAST (Version 2.03).

Aerial parts of *T. foenum graecum* (organically cultivated *T. foenum graecum* [OTF] and non-organically cultivated *T. foenum graecum* [NTF]) and *O. sanctum* (organically cultivated *O. sanctum* [OOS] and non-organically cultivated *O. sanctum* [NOS]) were harvested at the time of maturity (flowering and/or inflorescence) [11] (Figs. 3 and 4).

Authentication of plant materials

The plants were botanically authenticated by Dr. Alka Chaturvedi, Head, Department of Botany, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur. Voucher specimens (methi - 9783, tulsi - 9784) have been deposited for future reference.

Histological examination

The histological comparisons of plant parts were carried out with the help of MOTIC IMAGE PLUS 2.0 Microscope.

RESULTS AND DISCUSSION

Both the research plants with organic treatment comparative to nonorganic crop showed 3 days early initiation of flowers/inflorescence followed by early legumes (OTF) and fruiting (OOS). Plants experienced a number of developmental phases during their life cycles. The transitions start from germination to juvenile vegetative stage, when the plants are insensible to environmental inductive signals. It is followed by an adult vegetative phase where plants are receptive to external inductive cues for flowering. The shift to reproductive stage is marked by a transition to flowering [12]. Flowering is an important step which shows adaptability of plants to seasonal changes and decides subsequent reproductive success [12,13]. Floral development is controlled by both internal and external cues [14] and elongation of juvenile stage in plants due to any exogenous and endogenous factor may be a constraint in production and growth rate of plants [15].

Different morpho-physiological traits for both the plant were examined, and all the mean values in organic treatment were higher except the overall yield that was higher in non-organic crop. Interestingly, OTF not only found with higher mean values of height, secondary branches, flowers, secondary roots, and leaves but also root nodules, which indicate the more rhizobia and may cause more nitrogen fixation in soil. As both the crop shown the same date of germination but surprising results were found at the time of harvesting that OTF initiated the early transformation of flowers to legumes while till date the NTF was showing flowers only. However, NTF showed the higher mean values of weight of plants and ultimately higher overall yield (Fig. 3 and Table 2). OOS revealed the bigger height, root, more number of secondary branches, and secondary roots, except only the weight of whole plant that was found with the greater mean value of 279.61 g/plant in NOS (Fig. 4 and Tables 4 and 5). The higher mean values of the organic crop may be attributed to the presence of plenty of "beneficial soil microbes" in organic manure which helps in "soil regeneration" and "fertility improvement" and protect them from degradation while also promoting growth in plants [16]. The late commencement of adult vegetative phase might be responsible in the non-organic crop for their low mean values in the majority of morpho-physiological traits [15].

Histological examination

T. foenum graecum

Load

Anatomical section showed the presence of thin layer of cutin on the upper epidermis and lower epidermis. Section showed the presence of unicellular covering and short-stalked glandular trichomes. Mesophyll

was differentiated with 2-3 layers of palisade cells and spongy parenchyma. A prominent central vascular strand was present and it showing closed collateral arrangement of phloem and xylem. Xylem having the annular vessels and epidermis represented the appearance of anisocytic stomata (Fig. 5).

Stem

Microscopy revealed the single layered epidermis covered with thin cuticle followed by 2-3 layers of collenchyma and 3-5 layers of parenchymatous cortical cells. The whole epidermis showed very



Fig. 3: The performance of *Trigonella foenum graecum* in terms of trait (height). Where, OTF: Organically cultivated *Trigonella foenum graecum* and NTF: Non-organically cultivated *Trigonella foenum graecum*



Fig. 4: The performance of *Ocimum sanctum* in terms of trait (height). Where OOS: Organically cultivated *Ocimum sanctum* and NOS: Non-organically cultivated *Ocimum sanctum*

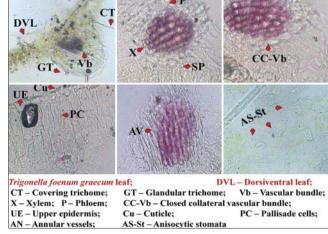


Fig. 5: Transverse section of Trigonella foenum graecum leaf

Table 2: Eight morpho-physiological traits at pre- and post-harvest conditions in 12 T. foenum graecum replicates under organic cultivation

Traits of OTF	Days after germination	Range Minimum Maximum		Mean	SE	Variance	SD
Height (inches)	15 th day	1	2	1.54	0.09	0.11	0.33
	20th day	3	4	3.58	0.14	0.26	0.51
	25 th day	4	5	4.16	0.11	0.15	0.38
	30 th day	6.5	7	6.83	0.05	0.03	0.18
	35 th day	9.5	10	9.75	0.06	0.05	0.22
Number of leaves	15 th day	6	9	7.25	0.44	2.38	1.54
	20th day	7	10	8.86	0.35	1.51	1.23
	25 th day	12	18	13.25	0.67	5.45	2.33
	30 th day	28	45	33.50	2.32	64.63	8.03
	35 th day	42	69	59.25	2.81	95.11	9.75
Secondary branch	30 th day	1	2	1.66	0.14	0.24	0.49
,	35 th day	3	4	3.16	0.11	0.15	0.38
Number of flowers	30 th day	0	1	0.83	0.11	0.15	0.38
	35 th day	1	2	1.33	0.14	0.24	0.49
Post-harvest	,						
Height (inches)	40 th day	9.7	11.3	10.17	0.15	0.05	0.55
Weight of individual plant (g)	40 th day	3.5	6.4	6.3	0.15	0.34	0.66
Number of leaves	40 th day	75	93	86.25	4.33	226.02	15.03
Secondary branch	40 th day	4	8	5.91	0.41	2.08	1.44
Number of flowers	40 th day	4	6	5.08	0.22	0.62	0.79
Number of legumes	40 th day	0	2	1.16	0.20	0.51	0.71
Secondary roots	40 th day	15	24	18.75	0.90	9.84	3.13
Number of root nodules	40 th day	2	6	4.08	0.43	2.26	1.50

T. foenum graecum: Trigonella foenum graecum, SE: Standard error, SD: Standard deviation, OTF: Organically cultivated T. foenum graecum

Table 3: Eight morpho-physiological traits at pre- and post-harvest conditions in 12 *T. foenum graecum* replicates under non-organic cultivation

Traits of NTF	Days after germination	Range		Mean	SE	Variance	SD
		Minimum	Maximum				
Height (inches)	15 th day	1	1.2	1.08	0.02	0.01	0.10
	20 th day	1.5	3	2.77	0.14	0.24	0.49
	25 th day	2.8	3	2.96	0.02	0.006	0.07
	30 th day	4.8	5.2	4.98	0.03	0.01	0.13
	35 th day	8	8.5	8.20	0.06	0.05	0.23
Number of leaves	15 th day	3	6	5.25	0.39	1.84	1.35
	20 th day	7	10	8.75	0.27	0.93	0.96
	25 th day	12	17	13.25	0.56	3.84	1.95
	30 th day	27	35	32	0.80	7.81	2.79
	35 th day	33	66	51.83	3.27	128.33	11.32
Secondary branch	30 th day	0	1	0.5	0.15	0.27	0.52
•	35 th day	2	5	3.08	0.33	1.35	1.16
Number of flowers	30 th day	0	0	0	0	0.15	0
	35 th day	0	1	0.83	0.11	0.24	0.38
Post-harvest	•						
Height (inches)	40 th day	9	10.9	9.9	0.13	0.22	0.47
Weight of individual plant (g)	40 th day	4.9	8.8	8.9	0.12	0.82	0.42
Number of leaves	40 th day	72	90	80.5	1.94	45.54	6.74
Secondary branch	40 th day	6	7	6.33	0.14	0.24	0.49
Number of flowers	40 th day	3	4	3.33	0.14	0.62	0.49
Number of legumes	40 th day	0	0	0	0	0	0
Secondary roots	40 th day	10	16	12.08	0.60	4.44	2.10
Number of root nodules	40 th day	3	5	3.66	0.28	0.96	0.98

 $^{{\}it T. foenum\ graecum: Trigonella\ foenum\ graecum, SE: Standard\ error, SD: Standard\ deviation, NTF: Non-organically\ cultivated\ {\it T. foenum\ graecum}}$

long unicellular clothing trichomes along with stomata. Vascular bundles contained secondary phloem followed by xylem consisted of protoxylem and metaxylem, represented prominent spiral vessels. Broad central spongy parenchymatous pith was present (Fig. 6). Organic and non-organic crop showed the similar pattern of cellular arrangements.

O. sanctum

Leaf

The transverse section of leaf had a pot shape midrib and a thin lamina covered with wavy upper and lower epidermis. Beneath each

epidermis having a layer of palisade cells making it an isobilateral leaf. Midrib consists of thin single cellular epidermis followed by spongy parenchyma having a radiating arc of xylem and phloem. Both upper and lower epidermis showed simple, covering, uniseriate trichomes as well as sessile short-stalked, glandular trichomes. Lamina also showed the presence of lignified pitted vessels and epidermis represented the number of diacytic stomata (Fig. 7).

Petiole

In cross-sectional view, the petiole was circular along with a shallow adaxial groove having long hairy and even surface. The ground tissue

Table 4: Five morpho-physiological traits at pre- and post-harvest conditions in 12 0. sanctum replicates under organic cultivation

Traits of OOS	Days after germination	Range		Mean	SE	Variance	SD
		Minimum	Maximum				
Pre-harvest							
Height (inch)	30	2.5	7	4.86	7.42	11.52	0.10
	40	7.5	19	11	0.11	12.22	0.16
	50	14	26	19.70	0.099	7.62	0.14
	60	17	26	22.08	0.058	4.26	8.20
	70	20	30	24.45	0.071	4.93	0.10
	80	23	32	27.08	0.083	5.53	0.11
	90	23.5	34	29	0.11	7.12	0.15
	100	27	37.5	32	0.12	7.56	0.17
Secondary branch	40	11	22	17	0.86	21.16	1.22
	50	17	25	20.66	0.79	15.77	1.12
	60	12	28	20.83	2.29	39.39	3.24
	70	14	30	21.83	1.44	23.64	2.03
	80	15	36	23.33	1.76	26.89	2.50
	90	18	30	23.75	1.41	21.07	2
	100	18	38	27.5	1.22	16.32	1.72
Post-harvest							
Height (inch)	105	32	39.5	35.70	6.71	3.91	9.50
Secondary branch	105	19	33	26.16	2.03	27.44	2.88
Weight (g)	105	156	455	244.63	39.87	52.69	56.39
Roots length (inch)	105	14	19.5	17.83	0.49	10.13	0.69
Secondary roots	105	8	15	10.83	0.51	16.97	0.73

O. sanctum: Ocimum sanctum, SE: Standard error, SD: Standard deviation, OOS: Organically cultivated Ocimum sanctum

Table 5: Five morpho-physiological traits at pre and post-harvest conditions in 12 O. sanctum replicates under non-organic cultivation

Traits of NOS	Days after germination	Range		Mean	SE	Variance	SD
		Minimum	Maximum				
Pre-harvest							
Height (inch)	30	3	5.5	4.20	0.33	25.48	0.47
	40	7	13	9.79	0.80	26.91	1.14
	50	16	25	20.25	0.89	15.60	1.27
	60	18	24	21.54	0.54	8.70	0.77
	70	20	25	24.41	0.71	10.12	1.01
	80	23	30	27	0.87	11.21	1.23
	90	22	33.5	28.70	1.19	14.30	1.68
	100	23.5	37	31.12	1.37	15.09	1.94
Secondary branch	40	10	23	16.41	0.10	9.94	0.15
	50	16	25	20.25	0.093	7.68	0.13
	60	15	30	19.83	0.25	19.28	0.35
	70	17	24	20.41	0.15	11.74	0.22
	80	12	28	22.25	0.18	13.50	0.26
	90	12	36	22.75	0.14	10.09	0.19
	100	12	36	24.33	0.12	8.81	0.18
Post-harvest							
Height (inch)	105	28	37	34.04	0.78	7.78	1.10
Secondary branch	105	15	40	25.25	0.19	13.50	0.27
Weight (g)	105	148	750	279.61	1.04	22.85	1.48
Roots length (inch)	105	9	19.5	15.70	6.30	5.27	0.089
Secondary roots	105	6	14	10.33	8.20	8.62	0.11

was differentiated into outer four or five layers of collenchyma and inner parenchyma. Vascular bundles of the petiole occur as a large median arc consisted of xylem elements arranged in radial rows; the outline of xylem was surrounded by phloem on both sides; vascular strands were bicollateral. Epidermis had thick, long multicellular uniseriate hairs along with unicellular head and stalk containing glandular trichomes (Fig. 8).

Stem

The histology of stem represented cylindrical arrangement with multiple vascular stands. Stem was covered with thin cuticle layer and epidermis having hairy surface. The cortex of stem had multiple layers of spongy parenchyma followed by phloem patches to xylem tissue and finally central pith. The xylem represented several spiral vessels (Fig. 8). The microscopic examination of *O. sanctum* leaf, petiol, and stem reveal the similar results in the organic and non-organic crop.

CONCLUSION

It can be concluded that the scientific study executed with abovementioned objectives is successful in providing a scientific justification of the common perception that organically grown foods or medicinal herbs are "better for you" in terms of nourishment, sustainability, and better quality. This study, therefore, can be a good model in justifying the importance of organic over non-organic medicinal plant species and can be utilized as a training manual for all the stakeholders

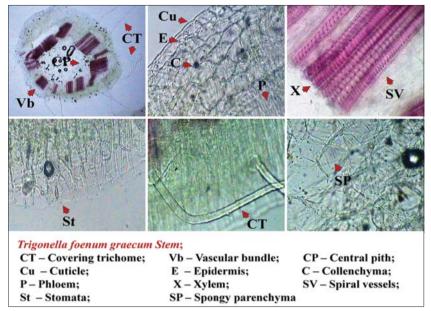


Fig. 6: Transverse section of Trigonella foenum graecum stem

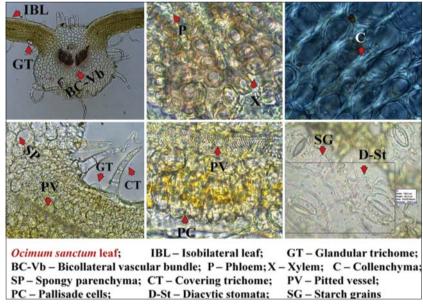


Fig. 7: Transverse section of Ocimum sanctum leaf

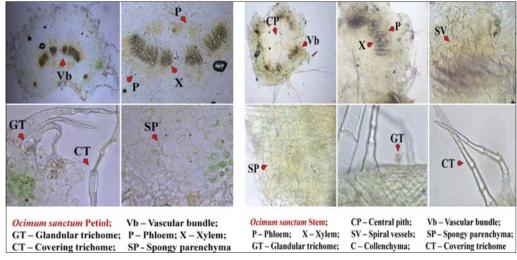


Fig. 8: Transverse section of Ocimum santum petiol and stem

who are dealing with the nutraceuticals and medicinal plants based medicines.

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