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MORPHOLOGICAL AND PHYTOCHEMICAL ANALYSIS OF *OCIMUM GRATISSIMUM* LINN. – A POTENTIAL SOURCE OF NATURAL EUGENOL

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ABSTRACT

This article deals with how different morphological, meteorological and edaphic parameters are correlated with the oil production in *Ocimum gratissimum,* the most potent eugenol producer among the available species of *Ocimum* growing naturally in the agro climatic conditions of West Bengal (India). This correlation has been established through extensive survey on this species growing in eight districts of West Bengal in respect of its various agro morphological traits. Emphasis should be given on some specific traits and parameters for cultivation of this species as discussed in this article with the view to produce more essential oil as well as eugenol in a cost effective manner.

KEY WORDS: ***Eugenol, Ocimum gratissimum, genotypic and phenotypic coefficient of variation, essential oil, agroclimatic parameters.***

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INTRODUCTION

Nature is the donor of all sorts of elixir of life. It contributes different life saving drugs for human beings through its plant resources. Among numerous, *Ocimum* a plant belongs to the family Lamiaceae is an important genus which yields volatile oil of high commercial value used in pharmaceutical industry because of its diverse array of medicinally important constituents. In Indian agroclimatic condition the genus is represented by six species such as *O. sanctum, O. americanum, O. gratissimum, O. kilimandscharicum, O. basilicum and O. adscendens.*[1] The volatile oil of all species contain the active ingredient eugenol The biosynthesis of eugenol resembles the initial step of biosynthesis of lignin which involve conversion of hydroxycinnamic acid to the corresponding coenzyme esters by 4- Coumarate: CoA ligases[2]. Eugenol in addition to other phytochemical constituents of essential oil has antioxidant activity[3]. In recent times, it has been investigated that bioactive principles obtained from plants provide toxicity against multiple drug resistant microorganisms[4]. The antimicrobial property of essential oil of *Ocimum* has also been proved largely due to the presence of eugenol in addition to other compounds[5] In nature, eugenol synthase genes have pivotal role in the generation of diversity among the species which could be substantiated by the fact of the derivation of variation in floral fragrance in *Gymnadenia sp*[6].The ethno botanical, pharmacological, chemical and toxicological properties of different species of *Ocimum* have been thoroughly reviewed[7]. Eugenol as one of the most important constituents in the essential oil has high commercial value because of its modern uses in different fields. It is chemically a phenylpropene used in perfumes and flavours. It has local antiseptic and anesthetic property[8]. In dentistry it has uses in prosthodontic applications such as root canal sealing in the form of compound known as zinc oxide eugenol[9].

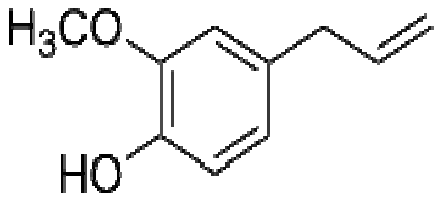

Figure 1
Structure of 4-Allyl-2-methoxyphenol(Eugenol)

As a food preservative eugenol could be applied since it reduces the growth of *Listeria monocytogenes* and *Lactobacillus sakei*[10].The pharmacology of the anticancerous activity of the constituents of essential oil has also been worked out[11]. Eugenol also could be transformed biologically with the aid of microbial activity into aroma compound of high economic value called vanillin[12]. In this regard eugenol as a constituent of essential oil of different species of *Ocimum* is very important because such enriched source might have enormous potentiality for its exploitation as a substrate for industrial production of vanillin. Natural eugenol is mostly obtained from *Eugenea caryophyllata* and *Cinnamomum zeylanicum*. Despite of the availability of high percentage of eugenol in the essential oil in both the species it needs prolonged time to achieve desirable quantity of the oil, as the plants have to mature sufficiently to produce it. In this context highest eugenol yielding species of *Ocimum* could be an ideal alternative and cheaper source of the compound because of its very significant herbage begetting. The high rate of perpetuation in collaboration with the annual to biennial habit of this species imparts sufficient potentiality to produce oil vis a vis eugenol in a high magnitude. In fact very scanty or no report is available as a path finder for the cultivators of West Bengal which agronomic and agro climatic parameters should they emphasize for cultivation of the plant species with the view to enhance the production of essential oil and eugenol[13,14]. Therefore our present study was aimed to determine: i) The highest eugenol producing species of *Ocimum* growing naturally in the agroclimatic regions of the state West Bengal (India). ii) The role of agro morphological parameters in addition to meteorological and edaphic factors on oil yield of the best eugenol producing species. Data were collected from eight districts of West Bengal [24 Parganas North, 24 Parganas South, Howrah, Bankura, Coochbihar, Nadia, Midnapore (East) & Malda] in respect of such parameters or factors through extensive survey on naturally growing plant population of the most efficacious eugenol producing species. The data were subjected to a number of statistical analyses to understand the effect of such parameters on oil retention. To reach a definite conclusion phenotypic, genotypic and agronomic correlation study and their path analyses were performed. Efforts have been made to establish the strength of individual parameters in a cost effective relationship in plant system on the basis of the aforesaid statistical methods and their analytical interpretation.

MATERIALS AND METHODS

Collection of Plant Materials

Germplasms of different species of *Ocimum* were collected from cultivated field located at different sites of eight districts of West Bengal during their vegetative phase of growth to estimate the eugenol content in their oil.

Study of Agronomic Parameters

The agronomic parameters were studied in all the plants grown in different agroclimatic conditions. The age of the plants was estimated on the basis of comparison of their appearances with the cultivated ones. The total number of leaves and branches per plants were counted manually. The height was measured with the ordinary graduated tape. The area of the leaves was measured

simply by graphical method. The fresh weight of 100 leaves was taken immediately after collection and the same were allowed to oven dried at 600 C for two consecutive days. The dry weight of such leaves was then measured. Oil samples were collected from 100 gm of fresh leaves of each species with the help of Clevenger's apparatus, using petroleum ether as solvent (B.P.400–600 C). Before extraction the leaves with their petiole were properly weighed and chopped with the sharp scalpel and placed inside the container of Clevenger's apparatus. Following hydro-distillation method, the oil samples were collected in a clean specially made glass vials with stopper. The difference between empty vials and oil containing vials is expressed in terms of percentage of oil content (w/w).

Analysis of soil samples

The soil samples were collected from different sites of eight different districts of West Bengal. The surface layer of the soil of the cultivated field was removed mechanically and the random soil sampling was done. The soil sample from different districts was then brought to the laboratory for the analysis of the following parameters:

Soil Texture

The textural analyses of soil samples were made following the hydrometer method. It is a new method for mechanical analysis of soils [15].

Soil pH

The pH of soil samples was determined by Jackson's method[16].

Soil Nitrogen

The available nitrogen content of the soil was determined by the standard method. [17].

Organic Carbon

The variability of the organic carbon in the soil samples of different districts was measured by Degtijareff's methods for determination of soil organic matter[18].

Organic Phosphorus

The organic phosphorus content of the soil was determined by ascorbic acid method.[19].

GC analysis of oil sample

GC analysis of oil samples of different species of *Ocimum* was made with the help of a CE-8000 top model chromatogram using liquid nitrogen as a carrier gas. The oven temperature of the chromatogram was raised from 600 C to 2200 C at the rate of 50 C/ min. The holding time of the final temperature in the oven was 10 min. The injector and detector temperature was 2200 C for each. The column used for GC analysis was DB-5 MS type of capillary column of 30 mts length. The film thickness and internal diameter of the column was 25 micrometer. The concentrated essential oil samples were diluted properly up to a particular concentration using n-hexane as a solvent and one microliter of diluted sample was injected into the chromatogram for analysis. The authentic samples were also diluted similarly and the same volume was injected into the column. The peaks produced by authentic samples were compared to the peaks obtained from the test samples with respect to their retention time (RT) in order to the identification as well as quantification of the constituents present in the oil samples. Quantitative estimation of eugenol content in the essential oil of different plants of the species grown in different agroclimatic conditions. The percentage (w/w) of eugenol content was measured after detecting the existence of the same in the test sample by comparing the retention time of GC peaks of the test sample and that of the standard authentic ones. Before GC analysis a stock concentration was prepared both for the authentic sample and test sample. For example, the stock concentration of authentic sample 'X' was prepared as M ppm and the concentration of test sample was prepared as N ppm. Both the stock concentration was prepared by dissolving them into HPLC, n- hexane. If the injected volume for test sample and authentic sample being 1 microliter, then the (%) of 'X' available in the test sample could be calculated by the following formula:

$$\text{The concentration (\%) of X in the test sample} = \frac{M \times A_2 \times 100}{A_1 \times N}$$

Where M = Standard stock concentration (ppm)
A_1 = Area of the standard authentic sample (obtained from chromatogram)
A_2 = Area of the test sample (obtained from chromatogram)
N = Stock concentration of the test sample (ppm)

STATISTICAL ANALYSIS

The different plants of the species was evaluated for parameters like age of the plants, plant height, number of leaves per plant, number of branches per plant, dry and fresh weight of leaves, available macronutrients in soil and its texture for their replicated source at 8 districts. Only the morphological traits such as age of the plant, height, number of leaves per plant, number of branches per plant, dry and fresh weight and oil content were considered for estimation of genotypic variance, co-variance study. The genotypic, phenotypic and environmental correlation coefficients between the traits were calculated and respective correlation matrices were formed. GCV, PCV and heritability in broad sense were also calculated for each trait of the species. Path analysis technique was followed for genotypic and phenotypic correlation matrix to get an idea about the direct and indirect effects of all morphological traits on oil content. Sometimes it may happen that all traits could not be incorporated for genotypic path analysis because of near zero score of estimated genotypic variances of those traits. Similarly, agronomic correlation coefficients between agronomic traits were calculated and respective correlation matrices were made. Agropath analysis technique was followed for agronomic correlation matrices in order to get an idea about the direct or indirect influential effects of such traits on the oil content.

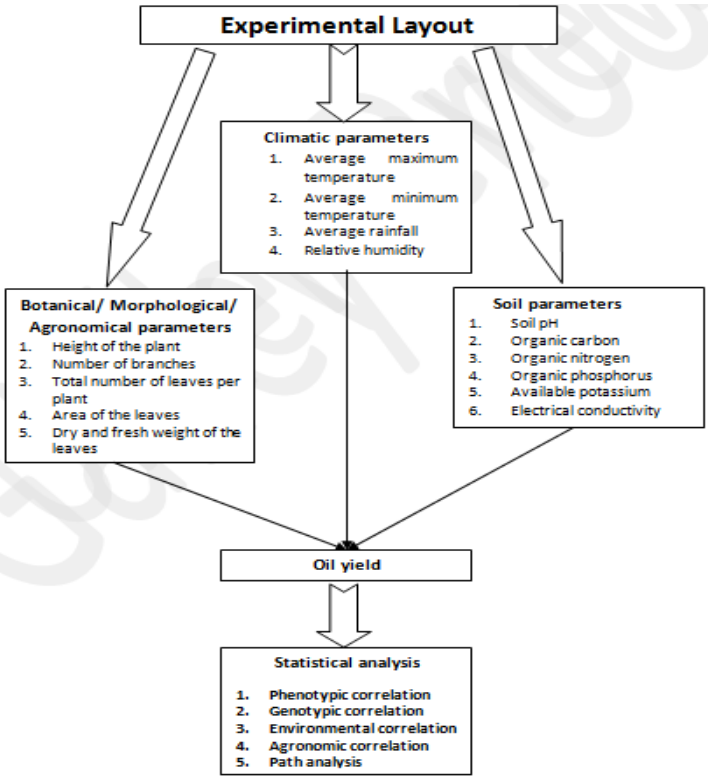

Figure 2
Experimental layout of the study

RESULTS AND DISCUSSIONS

The GC analyses of oil samples of different species of *Ocimum* in view of taking quantitative measure of eugenol content reveal that *Ocimum gratissimum* is the most potent eugenol producer. Though other species contains the same compound[20] the quantity of eugenol in the oil of this species is calculated 47.45% as formulated in our experiment (Fig 3&4), which is significantly higher than other constituents (Fig 5).Our finding corroborates with the findings of others who studied the organ specific variation of the essential oil of this species[21].The survey of the germplasm of this species of *Ocimum* from eight different districts reveals that there is a great quantitative variation of the agro-morphological characters (Table:1&2). Six agronomic parameters show significant variation among the districts (Table 3&4). The plant height in Coochbehar is significantly higher than Nadia. Similarly, the number of branches per plant in the same district is appreciably higher than that of Nadia and Midnapore (East). Highest value for the leaf area has been obtained from South 24-parganas. Leaf fresh weight and dry weight is highest in Bankura and Midnapore (East) respectively. The oil yielding competence of this species growing in Bankura district is significantly higher than that of all other districts studied. The fresh weight of leaves shows most significant correlation in a positive sagacity with the percentage of oil content. This morphological character is a nonflexible one, because it ascertains correlation with oil content at genetic level (Table 5&6). Area of the leaves could be considered as an important agronomic trait that has highest positive direct effect on oil production of leaves. The other traits in this species such as dry weight of leaves, total number of leaves and total number of branches per plant also have positive direct leading role on oil production but comparatively to a lesser extent. The statistical path analysis reveals that the characters like plant height, area of leaves and their fresh weight have positive indirect effect on oil production. Only two genotypic characters such as height of the plant and the dry weight of their leaves have maximum positive direct effect on oil bearing (Table7). The direct positive effect of dry weight of the leaves shows highest potentiality on the oil yield. Genotypic characters such as height of the plants, area of the leaves and the fresh weight of the leaves have indirect positive effect on oil manufacture. No agronomic traits are found significantly correlated with oil content in this species except the total number of leaves which acts positively in a direct way on oil development. The role of potassium content in the soil as macronutrients has highest direct effect in a positive sense on the oil yield in comparison to other macronutrients. All the three textural parameters are found to influence greatly the oil content in this species. The average minimum temperature of the site of collection have highest positive direct role on oil production than other climatic parameters studied (Table 8). To conclude it could be stated that the plants cultivated in Bankura district shows maximum oil production and the same site may be considered as suitable provider of microclimatic parameters for paramount oil production. The area of the leaves phenotypically and dry weight of the leaves genotypically are mainly important in oil production. So emphasis should be given on these two traits to augment productivity of oil and eugenol as well. The available potassium in the soil is also crucial factor which manipulates better oil production. Thus potassium based fertilizer application to the soil may be rewarding for oil production to assist the level of economic benefits at the best for the cultivators as well as entrepreneurs who extract oil from the plants to sustain uninterrupted supply to the industry.

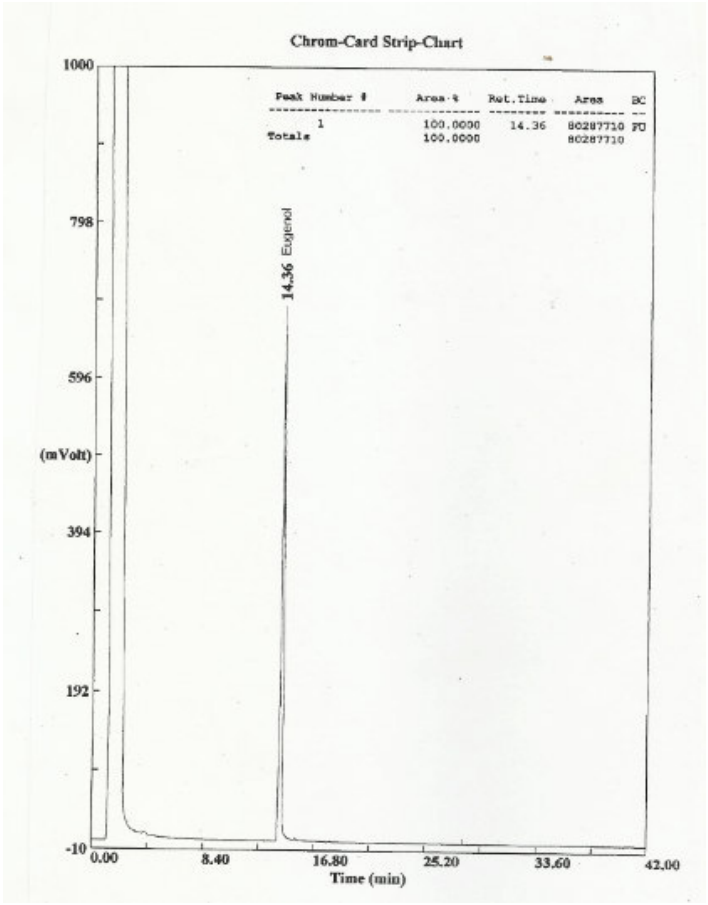

Figure 3
Gas Liquid Chromatogram of the authentic sample of Eugenol

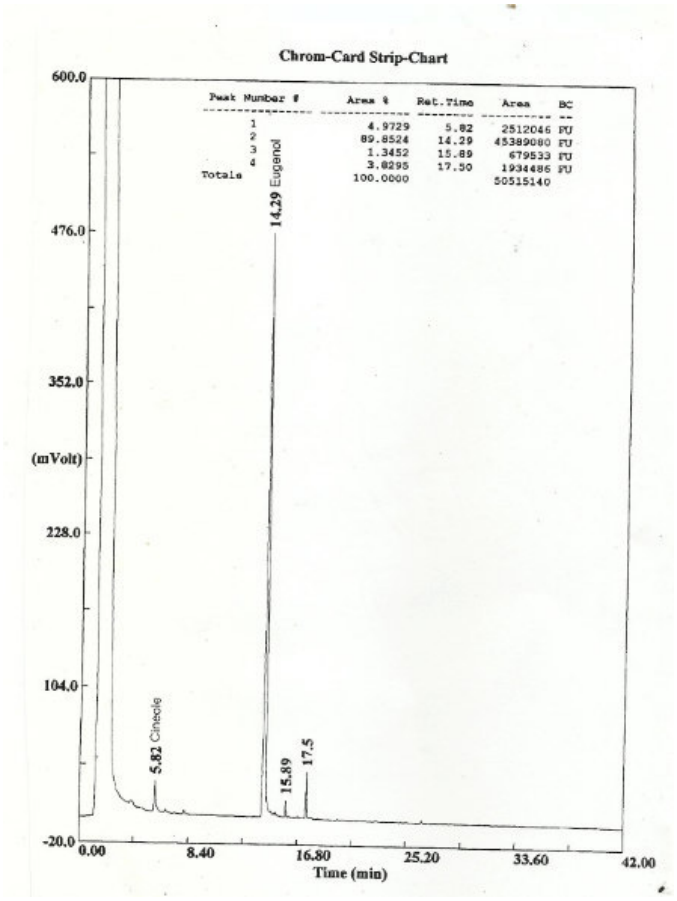

Figure 4
Gas Liquid Chromatogram of the essential oil obtained from *O. gratissimum*.

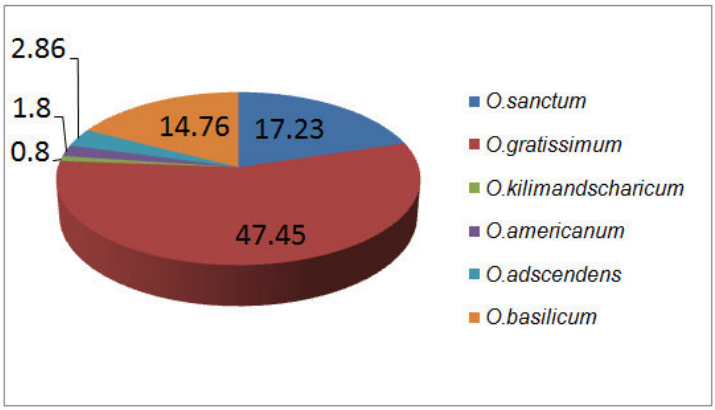

Figure 5
%age of eugenol content in the oil of different species of Ocimum. The eugenol content in the oil in O.gratissimum is significantly higher than other species as estimated through GC analysis of oil sample.

Table 1
Variation of the morphological parameters and oil content of *Ocimum gratissimum* Linn. collected from different districts of West Bangal

Name of the species.	The site of collection	Habit	Age of plant (month)	Height of the plant (cm)	Total no. of leaves	Total no of branches/ plant	Area of leaves (cm^2)	Fresh weight of 100 leaves	Dry weight of 100 leaves	% of oil content
Ocimum gratissimum	24-Pargana (north)	Perennial	12	120.63	1022	88	16.82	35.42	3.218	0.27
	24-Pargana (south)		12	124.64	1125	84	17.63	38.486	3.498	0.36
	Howrah		15	130.42	1228	89	18.02	42.068	3.824	0.28
	Bankura		12	122.84	1088	82	19.41	43.162	3.923	0.32
	Coochbehar		12	124.38	1132	86	19.31	43.085	3.833	0.24
	Nadia		15	132.63	1306	92	17.52	39.163	3.387	0.23
	Midnapore (East)		16	134.64	1386	96	16.95	36.192	3.208	0.27
	Malda		12	123.65	1092	86	17.02	37.821	3.392	0.22

Table 2
Estimation of genetic parameters

Characters	Height of the plant. (cm)	Total number of leaves	Total number of branches/ plant	Area of leaves (cm^2)	Fresh weight of 100 leaves	Dry weight of 100 leaves	% of oil content
H^2	0.145	0.000	0.141	0.334	0.410	0.350	0.357
GA	1.74	0.00	1.48	0.77	2.62	0.23	0.04
GCV	1.73	0.00	2.18	3.65	5.03	5.02	11.94
PCV	4.55	10.87	5.81	6.31	7.86	8.15	19.98

PCV= Phenotype coefficient of variation

Table 3
ANOVA of different morphological parameters and oil content of 0f O.gratissimum Linn.

Source	Variable	SS	df	MSS	F	Sig
Repl	Plant height	0.59	2	0.29	0.01	0.99
	No. of leaves	16.75	2	8.38	0.00	1.00
	Branch per plant	1.82	2	0.91	0.04	0.96
	Leaf area	0.04	2	0.02	0.03	0.98
	Leaf fresh weight	0.21	2	0.11	0.02	0.98
	Leaf dry weight	0.00	2	0.00	0.02	0.98
	Oil content	0.00	2	0.00	0.15	0.86
Site	Plant height	305.49	7	43.64	1.51	0.24
	No. of leaves	95890.96	7	13698.71	0.84	0.57
	Branch per plant	243.53	7	34.79	1.64	0.20
	Leaf area	14.77	7	2.11	2.51	0.07
	Leaf fresh weight	122.39	7	17.49	3.08	0.04
	Leaf dry weight	1.02	7	0.15	2.78	0.049
	Oil content	0.04	7	0.01	2.66	0.06
Error	Plant height	405.12	14	28.94		
	No. of leaves	227425.92	14	16244.71		
	Branch per plant	296.68	14	21.19		
	Leaf area	11.79	14	0.84		
	Leaf fresh weight	79.39	14	5.67		
	Leaf dry weight	0.73	14	0.05		
	Oil content	0.03	14	0.00		
Total	Plant height	711.20	23			
	No. of leaves	323333.63	23			
	Branch per plant	542.03	23			
	Leaf area	26.60	23			
	Leaf fresh weight	202.00	23			
	Leaf dry weight	1.75	23			
	Oil content	0.06	23			

Table 4
Mean comparison by Duncan's test (at 5% level of significance)*

Site	Age	Plant height	No.Leaf leaves	Branches/ plant	Leaf area	Leaf fresh wt.	Leaf dry wt.	Oil content
24-Pargana (north)	12	125.23^{ab}	1125.00	87.00^{ab}	17.49^{ab}	38.66^{ab}	3.51^{abc}	0.30^{ab}
24-Pargana(south)	12	129.95^{ab}	1175.33	86.67^{ab}	18.75^{a}	41.80^{a}	3.71^{abc}	0.26^{b}
Howrah	15	126.57^{ab}	1168.67	91.00^{ab}	17.06^{ab}	36.55^{b}	3.28^{c}	0.27^{b}
Bankura	12	127.72^{ab}	1161.67	87.80^{ab}	18.69^{a}	42.18^{a}	3.76^{ab}	0.36^{a}
Coochbehar	12	135.56^{a}	1289.67	93.76^{a}	18.59^{a}	41.11^{ab}	3.53^{abc}	0.26^{b}
Nadia	15	122.54^{b}	1078.67	84.30^{b}	17.19^{ab}	37.02^{b}	3.37^{bc}	0.25^{b}
Midnapore(East)	16	127.34^{ab}	1133.33	83.37^{b}	18.29^{ab}	42.03^{a}	3.84^{a}	0.25^{b}
Malda	12	128.91^{ab}	1246.67	89.10^{ab}	16.63^{b}	37.04^{b}	3.27^{c}	0.23^{b}

Similar alphabet denotes homogeneous means

Table 5
Phenotypic, genotypic and environmental correlation coefficients of Ocimum gratissimum Linn.for different traits in arrayed form.

Agronomic characters	Height of the plant	Number of leaves	Total number of branches/ plant	Area of the leaves (cm^2)	Fresh wt. of 100 leaves	Dry weight of leaves	% of oil content
Height of plant	1.000						
	1.000						
	1.000						
Number of leaves	0.787*	1.000					
	845.785*	1.000					
	0.764*	1.000					
Total number of branches/ plant	0.657	0.761*	1.000				
	0.879*	553.754**	1.000				
	0.620	0.766*	1.000				
Area of the leaves (cm^2)	0.223	-1.146	-0.325	1.000			
	1.194**	–	0.675	1.000			
	-0.053	-0.368	-0.623	1.000			
Fresh wt. of 100 leaves	0.263	-0.077	-0.354	0.897**	1.000		
	0.656	420.277**	-0.035	0.971**	1.000		
	0.145	-0.188	-0.486	0.857**	1.000		
Dry weight of leaves	0.091	-0.214	-0.500	0.868**	0.965**	1.000	
	0.410	48.493**	-0.293	0.868**	0.979**	1.000	
	-0.007	-0.282	-0.593	0.869**	0.957**	1.000	
% of oil content	-0.214	-0.129	-0.145	0.286	0.173	0.233	1.000
	0.009	–	0.186	0.597	0.737**	0.664	1.000
	-0.291	-0.067	-0.251	0.122	-0.176	-0.019	1.000

Note : If correlation coefficient is found more than one, it is due to error in estimating genetic parameters.
Sig at 5%level, **Sig. at 1% level.

Table 6
Phenotypic path results due to different plant morphological characters on oil content of *Ocimum gratissimum* (oil content as effect variable).

	Ocimum gratissimum Linn.					
Agromorpho-logical traits	**Plant height**	**No. of leaves**	**Total number of branches / plant**	**Area of leaves**	**Fresh weight of 100 leaves**	**Dry weight of 100 leaves**
Plant height	**-0.8305**	0.3399	0.2622	0.1763	-0.2315	0.0695
No. of leaves	-0.6536	**0.4319**	0.3038	-0.1154	0.0678	-0.1634
Total number of branches / plant	-0.5456	0.3287	**0.3991**	-0.2569	0.3116	-0.3818
Area of leaves	-0.1852	-0.0631	-0.1297	**0.7906**	-0.7895	0.6629
Fresh weight of 100 leaves	-0.2184	-0.0333	-0.1413	0.7092	**-0.8801**	0.7369
Dry weight of 100 leaves	-0.0756	-0.0924	-0.1996	0.6862	-0.8493	**0.7637**

Table7
Genotypic path matrix showing the direct and indirect effect of agro-morphological characters on oil content in *Ocimum gratissimum* (oil content as effect variable)

Agromorpho-logical traits	Plant height	Total number of branches / plant	Area of leaves	Fresh weight of 100 leaves	Dry weight of 100 leaves
Plant height	**2.136**	-0.998	2.203	-6.318	2.986
Total number of branches / plant	1.876	**-1.136**	1.244	0.335	-2.133
Area of leaves	2.551	-0.767	**1.845**	-9.353	6.322
Fresh weight of 100 leaves	1.401	0.040	1.792	**-9.629**	7.133
Dry weight of 100 leaves	0.875	0.333	1.601	-9.429	**7.284**

Table 8

Agropath matrix showing the direct and indirect effect of different agronomic traits on oil content in *Ocimum gratissimum* Linn.(Direct effects are on the main diagonal).

Agro-Agromic Traits	Age	Plant height	Number of leaves	Branches per plant	Leaf area	Fresh wt. of leaves	Dry wt. of leaves	Soil pH	Electrical Conductivit ..	Orga-nic Carbon	Available (K)	Available (N)	Available (P)	Av. Max. temp (^{0}C)	Av. Min. Temp (^{0}C)	Av. Rain fall	Rela-tive humi-dity	% of Silt	% of Clay	% of Sand
Age	**1.53**	0.08	-0.57	0.35	-0.07	0.16	0.03	-0.46	-0.60	-0.03	0.32	-0.07	-0.04	-0.38	3.95	1.32	-0.04	-0.42	-0.26	0.54
Plant height	0.75	**-0.17**	0.96	-0.53	0.15	-0.35	-0.31	0.52	0.06	0.21	-.20	0.09	-001	2.31	-2.27	-1.32	-0.08	0.17	-0.21	0.06
Number of leaves	0.83	-0.15	**1.05**	-0.62	0.04	-0.05	0.26	0.23	0.20	-0.02	-0.32	0.156	-0.11	1.90	-3.07	-0.66	0.15	-0.08	-0.52	0.54
Branches per plant	0.70	-0.12	0.86	**-0.75**	0.00	0.21	0.67	0.22	0.39	-0.07	-0.70	0.12	-0.21	1.67	-2.98	-0.31	0.02	0.44	-0.69	0.50
Leaf area	0.37	-0.08	0.15	0.00	**0.29**	-0.85	-1.43	0.78	-0.14	0.10	-0.13	-0.02	0.15	1.23	0.36	-0.62	-0.37	0.42	0.75	-1.09
resh wt. of leaves	0.28	-0.06	0.05	0.17	0.27	**-0.91**	-1.60	0.54	-0.31	0.31	0.45	0.05	0.18	0.67	0.84	-0.24	-0.22	0.17	0.71	-0.91
Dry wt. of leaves	0.03	-0.03	-0.17	0.31	0.25	-0.89	**-1.65**	0.44	-0.41	0.30	0.56	0.05	0.19	0.38	1.69	-0.38	-0.25	0.18	0.66	-0.83
Soil pH	-0.70	0.09	-0.24	0.17	-0.22	0.48	0.72	**-.01**	0.36	-0.35	0.41	0.10	-0.19	-0.64	1.42	0.69	0.36	-0.49	-0.67	1.07
Electrical Conductivity	-1.02	0.01	-0.23	0.33	0.05	-0.32	-0.76	-.40	**-0.90**	0.21	0.90	0.03	0.14	-0.09	3.55	-1.60	-0.13	-0.20	-0.19	0.33
Organic Carbon	0.06	-0.04	-0.03	-0.06	0.14	-0.35	-0.60	0.43	-0.22	**0.82**	0.44	-0.37	0.38	0.20	0.62	-1.35	-0.64	0.73	1.05	-1.54

Agro-Nomic Traits	Age	Plant height	Number of leaves	Branches per plant	Leaf area	Fresh wt. of leaves	Dry wt. of leaves	Soil pH	Electrical Conductivity	Orga-nic Carbon	Available (K)	Available (N)	Available (P)	Av. Max. temp (^{0}C)	Av. Min. Temp (^{0}C)	Av. Rain fall	Rela-tive humi-dity	% of Silt	% of Clay	% of Sand
Available (K)	-0.44	0.03	-0.30	0.47	0.03	-.37	-0.82	-0.38	-0.73	0.32	**1.12**	-0.07	0.31	-1.05	2.40	-0.56	-0.13	-0.12	0.37	-0.30
Available (N)	-0.20	0.03	-0.31	0.17	0.01	0.08	0.15	0.20	0.04	0.61	0.15	**-0.50**	0.30	-0.91	0.49	-0.17	-0.40	0.21	1.42	-1.68
Available (P)	0.12	0.00	-0.26	0.35	0.09	-0.37	-0.74	0.20	-0.29	0.70	0.77	-0.34	**0.44**	-0.79	0.84	-0.59	-0.46	0.39	1.18	-1.50
Av. Max. Temp (^{0}C)	-0.20	0.13	-0.68	0.43	-0.11	0.21	0.21	-0.57	-0.03	-0.06	0.40	-0.16	0.12	**-2.93**	0.98	2.43	0.12	-0.14	0.77	-0.70
Av. Min. Temp (^{0}C)	-1.36	0.08	-0.72	0.50	0.02	-0.17	-0.63	-0.32	-0.72	0.12	0.60	-0.06	0.08	-0.64	**4.44**	-1.08	-0.19	-0.12	0.06	0.04
Av. Rain fall	-0.58	-0.06	0.20	-0.07	0.05	-0.06	-0.18	0.20	-0.41	0.32	0.18	-0.03	0.08	2.05	1.38	**-3.47**	-0.34	0.20	-0.67	0.61
Relative humidity	-0.08	-0.02	-0.21	0.02	0.14	-0.27	-0.56	0.48	-0.16	0.70	0.19	-0.28	0.27	0.47	1.15	-1.60	**-0.75**	1.18	0.52	-1.17
% of Silt	0.43	-0.02	-0.05	-0.22	0.08	-0.10	-0.20	0.32	0.12	0.39	-0.09	-0.07	0.12	0.26	-0.36	-0.45	-0.58	**1.52**	-0.11	-0.61
% of Clay	0.21	0.02	-0.29	0.28	0.11	-0.35	-0.58	0.36	0.09	0.46	0.22	-0.38	0.28	-1.20	0.13	1.25	-0.21	-0.09	**1.87**	-2.04
% of Sand	-0.38	-0.01	0.26	-0.17	0.14	0.38	0.63	-0.49	-0.13	-0.58	-0.16	0.39	-0.31	0.94	0.09	-0.97	0.40	-0.42	-1.76	**2.18**

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CONFLICT OF INTEREST

Conflict of interest declared none.

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