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Physico-chemical properties of essential oil in vetiver (*Vetiveria zizanioides* (L.) Nash) as influenced by different planting methods and nutrition

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Abstract
An experiment was conducted at Medicinal and Aromatic Plants Unit, Saidapur Farm, Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka to study the physico-chemical properties of essential oil in vetiver (*Vetiveria zizanioides* (L.) Nash) as influenced by different planting methods and nutrition during July 2015- July 2016. The experiment was laid out in split plot design with three main plot and six sub plot comprising of eighteen treatment combinations with two replications. The main plot consisted of three planting methods; P_1– Ridge and furrow method, P_2– Bed method and P_3– Bag method. The sub plot consisted of six fertilizer levels; F_1- 25:25:25 kg NPK/ha, F_2- 50:25:25 kg NPK/ha, F_3- 75:25:25 kg NPK/ha, F_4- 25:50:25 kg NPK/ha, F_5- 50:50:25 kg NPK/ha and F_6- 75:50:25 kg NPK/ha. For all the treatments, farm yard manure @ 10 tonnes per ha was applied. Among the planting methods, bag method (P_3) recorded significantly higher dry root yield (4085.78 kg/ha) and essential oil yield (85.34 kg/ha) compared to other planting methods. Among the different levels of fertilizers, the higher dose of fertilizer (F_6- 75:50:25 kg N, P_2O_5 and K_2O per ha recorded significantly higher dry root yield (3,453.19 kg/ha) and essential oil yield (73.32 kg/ha) compared to other fertilizer levels. The interaction effects were also found significant. The bag method (P_3) with fertilizer levels of 75:50:25 kg NPK/ha (F_6) recorded significantly higher dry root yield (4333.52 kg/ha) and essential oil yield (94.25 kg/ha) compared to all other interactions. The planting methods and fertilizer levels individually and also in combination did not result in any significant differences for essential oil content. The vetiver oil samples were analysed for their physico-chemical properties. The samples were dark colour, turbid, opaque in P_1F_6, medium in P_2F_6 and light brown colour, transparent, thin in P_3F_6 treatment combinations, whereas the samples were light brown colour, transparent, thin in P_1F_1, P_2F_1 and P_3F_1 combinations. The oil recorded maximum values in all parameters *viz*., specific gravity (1.032) in P_1F_6, refractive index (1.5225) in P_3F_6, acid value (48.09) in P_1F_6, ester value (121.19) in P_3F_6 and saponification value (159.34) in P_3F_6 combinations. In case of odour evaluation, the oil was woody, fresh, distinct rosy aroma in P_1F_6, typical vetiver aroma in P_2F_6 and P_3F_6 combinations, whereas P_1F_1 had camphoraceous, dilute aroma, P_2F_1 had rosy, characteristic, persistent woody aroma and P_3F_1 had rosy, typical vetiver aroma.

Keywords: Essential oil, Nutrition, Physico-chemical properties, planting methods, Vetiver

1. Introduction
Among horticulture crops, medicinal and aromatic plants forms one of the important groups which have a unique role in sustaining pharmaceutical, perfumery and cosmetic industries in India (Raviprasad Sajjan and Venugopal, 2017) [10]. Of the thousands of aromatic plants, a few have attained the status of commercial crops which are being cultivated on large scale. Vetiver is one such indigenous aromatic plant being cultivated in India.

Vetiver or Khus botanically known as *Vetiveria zizanioides* (L.) Nash Syn. *Chrysopogon zizanioides* (2n=20) is a densely tufted perennial important aromatic grass belonging to family Poaceae. Vetiver is having several vernacular names in India such as Usirah, Sugandhimulah in Sanskrit, Lavancha in Kannada, Vettiver in Tamil, Khus-khus in Hindi and Bengali, whereas, it is more commonly known as Vetiver in English (Archana Pareek and Ashwani Kumar, 2013) [1]. Vetiver is indigenous to India and widely cultivated in the tropical and sub-tropical regions of the world. In India, khus grass grows wild in Rajasthan, Haryana, Uttar Pradesh, Gujarat, Bihar, Orissa, Madhya Pradesh and throughout South India. It is systematically cultivated in the North Indian states of Rajasthan, Uttar Pradesh and in the South Indian states of Kerala, Tamil Nadu, Karnataka and Andhra Pradesh.

The commercially important part i.e. roots of vetiver on steam distillation yield an aromatic high grade natural essential oil which is mainly used as a fixative in perfumery and cosmetic industries and for the scenting soaps and extensively used in tobacco, pan masala and beverage industries. The oil has antifungal, antibacterial, anticancer, anti-inflammatory and antioxidant activities, thus having application in the pharmaceutical industries (Rashmi and Singh, 2008) [9]. Root is cooling, bitter, alexiteric, stomachic, carminative, astringent, stimulates immune system, menstruation, useful in headache, burning sensations, ulcers, rheumatism and diseases of blood.

The present annual world production of vetiver oil is approximately 250-300 tonnes, while the demand is nearly 400 tonnes. Haiti and Indonesia account for 80 per cent of total vetiver oil production in the world. In India, about 20-25 tonnes of oil is produced annually, which is far below the Indian demand level (100 tonnes) which is met by imports. The heavy demand of vetiver oil in Indian as well as international markets has motivated Indian farmers to take up commercial cultivation of vetiver (Raviprasad Sajjan and Venugopal, 2017) [10].

The cultural practices including planting methods and nutrition management are the important factors that play a vital role in growth and productivity of vetiver.

Despite its economic importance, growers are not in a position to produce good quality vetiver with high productivity due to difficulty in harvesting of roots, as the roots grow deep into soil, sometimes upto 2-3 feet. Boonklinkajorn and Visuttipitakul (2001) [3] observed that there was a great loss of root caused by harvesting procedure. Since the grass extends its fibrous roots in all directions, a certain portion of the roots was unavoidably cut off. The amount of roots left in the soil is believed to be considerable. Conventionally khus roots are harvested manually using traditional tools like spade, pickaxe and narrow spade etc., which causes significant damage and root losses (approx. 30-40%). Alternatively, a plough would be run to loosen the soil along the rows of plant to facilitate easy pulling out of clumps of vetiver. Besides, this method requires huge involvement of drudgeryful manual labour (345-350 man days/ha) (Tiwari, 2014) [11]. To overcome these problems and making the vetiver cultivation sustainable and profitable, there was a need to find out suitable planting methods so that harvesting of roots become easy.

Plant nutrition is also a major factor which influences the growth and development of crops that are grown commercially, under normal agro-climatic conditions. The deficiency of major nutrients namely nitrogen, phosphorus and potassium are common and they pose serious problems in commercial crop production. Inadequate supply of these nutrients even for a short period is detrimental to plant and has a negative effect on yield. Use of organic manures alone cannot fulfil the nutrients requirement. Therefore, it is imperative to make use of organic manures along with inorganic fertilizers to maintain soil fertility and to get sustainable yields (Harshavardhan *et al*., 2016) [4].

Therefore, a research study was conducted to find out the impact of different planting methods and nutrition on the physico-chemical properties of vetiver essential oil.

2. Materials and Methods

An investigation was conducted at Medicinal and Aromatic Plants Unit, Saidapur Farm, Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka to study the physico-chemical properties and principal components of essential oil in vetiver (*Vetiveria zizanioides* (L.) Nash) as influenced by different planting methods and nutrition during July 2015- July 2016. The soil of experimental plot was clay loam with soil pH 7.36, organic carbon content of 0.57%, available nitrogen of 147 kg/ha, available phosphorus of 39.30 kg/ha and available potassium 144 kg/ha. The experiment was laid out in split plot design with three main plot and six sub plot comprising of eighteen treatment combinations with two replications. The main plot consisted of three planting methods *viz*., P_1– Ridge and furrow method, P_2– Bed method and P_3– Bag method. The sub plot consisted of six fertilizer levels; F_1- 25:25:25 kg NPK/ha, F_2- 50:25:25 kg NPK/ha, F_3- 75:25:25 kg NPK/ha, F_4- 25:50:25 kg NPK/ha, F_5- 50:50:25 kg NPK/ha and F_6- 75:50:25 kg NPK/ha. For all the treatments, farm yard manure at the rate of 10 tonnes per ha was applied. The gross plot size of each treatment was 2.7m x 2.4m and net plot size was 1.8m x 1.8m, respectively. The individual plots were levelled and the layout was made as per the plan.

Planting

For planting purpose, healthy planting materials were obtained from the nursery of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP) Research Centre, Bengaluru. The healthy clumps were uprooted and individual tillers or root slips were separated from the mother plant. Shoots were cut at 25 cm height above the ground level, roots were trimmed and dry foliage removed before they were used for planting. During planting, ridges of 15 cm height were made at 45 cm apart in case of ridge and furrow method; and raised beds of 15 cm height (two rows/bed) of required size were prepared in case of bed method. The empty cement bags (50 kg capacity) were washed and filled (upto $3/4^{th}$ height) with soil and FYM mixture in case of bag method. In case of ridge and furrow method, the slips were planted singly on one side of the ridge at 30 cm spacing between each slip. In case of bed method, the slips were planted singly in rows of 45 cm apart at 30 cm spacing between each slip. In case of bag method, root slips were planted singly in each bag and bags were kept at requisite spacing.

The farm yard manure @ 10 tonnes per ha was applied to all the treatments one month before planting. Nitrogen, phosphorus and potassium were applied in the form of urea (46% N), single super phosphate (16% P_2O_5) and muriate of potash (60% K_2O), respectively as per the treatments. At the time of planting, half dose of N and full dose of P and K were applied as a basal dose. Remaining half of nitrogen was applied after four months of planting. The experimental field was regularly irrigated and weeded.

Harvesting

After 12 months of planting, the aerial (stem) portion was cut at a height of 20-25 cm in all the experimental plots. Later, the JCB was used for efficient uprooting of clumps upto 50 cm depth in case of ridge and furrow method and also in bed method. In case of bag method, the bags were cut with a knife and torn, then the clumps were separated. Care was taken not to damage the roots and avoid loss of roots in all the three planting methods. The clumps were washed gently in clean running water to remove the adhering soil taking care so that the finer roots are not lost. Then the roots were separated from the clump and were shade dried for 12 hours by spreading on a clean dry floor.

Distillation

Hydro distillation method was employed to extract the oil. Hundred grams of the shade dried roots under each treatment was cut into small pieces and distilled in Clevenger's apparatus (Plate 1) for both the replications. The period of distillation was kept constant for 36 hours.

Plate 1: Clevenger's apparatus for distillation of oil

Root yield per hectare

The dry root yield from each plot was recorded as root yield per plot. Then the dry root yield per plot was converted to per hectare (ha) on area basis and expressed in kilograms (kg).

Essential oil content

The shade dried roots were used for estimation of essential oil content (%) by using Clevenger's apparatus and expressed in percentage.

$$\text{Essential oil content (\%)} = \frac{\text{Quantity of essential oil collected (g)}}{\text{Weight of roots (100 g)}} \times 100$$

Essential oil yield

The essential oil yield (kg/ha) was computed by using the following formula and expressed in kg per ha.

Essential oil yield (kg/ha) = Dry root yield (q/ha) × Essential oil content (w/w) (%)

Physico-chemical properties of essential oil

The quality of the oil was judged based on their physico-chemical properties including odour evaluation. This analysis was carried out at Division of Horticulture, University of Agricultural Sciences, GKVK, Bengaluru- 65.

Physical parameters

1. Colour

The colour of the essential oil of vetiver samples was recorded through visual observations by using colour chart.

2. Specific gravity

Specific gravity of the oils was measured at room temperature as described by Beckett and Stenlake (2001) [2] by using the following formula

$$\text{Specific gravity} = \frac{\text{Weight of 10 ml sample}}{\text{Weight of 10 ml water}}$$

3. Refractive index

Refractive index was measured using a refractometer (Model: DG-NXT). A drop of water was placed on the lens and calibrated. Then the oil sample was placed on the lens and the reading was noted down at room temperature (25-27 ^{o}C) as outlined by Beckett and Stenlake (2001) [2].

Chemical properties

1. Acid value

In 100 ml conical flask, 0.2 g of oil was weighed and 10 ml of absolute ethanol was added to it and surface heated for 1 min. To this mixture 2-3 drops of phenolphthalein indicator was added and titrated against 0.1 N KOH until the colour turned to pink (Philips, 2002) [8].

$$\text{Acid value} = \frac{\text{Volume of KOH consumed} \times 56.11 \times 0.1}{\text{Weight of oil}}$$

2. Ester value

In 100ml conical flask, 0.7 g of oil was weighed and 25 ml of alcoholic KOH was added to it and refluxed for half an hour. When the mixture was still warm, it was added with 4 drops of phenolphthalein indicator and titrated against 0.5 N HCl until the end point turned to colourless from pink as outlined by Philips (2002) [8].

$$\text{Ester value} = \frac{\text{(Blank- Sample) Volume of KOH consumed} \times 56.11 \times 0.1}{\text{Weight of oil}}$$

3. Saponification value

The saponification value was computed as described by Philips (2002) [8] by using the following formula.

Saponification value = Acid value + Ester value

Odour evaluation of essential oil

Oil samples were evaluated for odour characteristics by a professional perfumer. All the three notes *viz*. top note, body (middle or heart) note and base (after or dry out or bottom) note were taken into account while assessing the odour characteristics of the oil. Oil was also checked for burnt, off odour or charred odour.

3. Results and Discussion

Dry root yield (kg/ha), Essential oil content (%) and Essential oil yield (kg/ha)

The data pertaining to dry root yield, essential oil content and essential oil yield as influenced by planting methods and fertilizer levels at harvest in vetiver is presented in Table 1.

The data clearly indicated that the planting methods and fertilizer levels individually and in their interaction significantly influenced the dry root yield (per ha) and essential oil yield (per ha) in vetiver.

Among the planting methods, bag method (P_3) recorded significantly higher dry root yield (4085.78 kg/ha) and essential oil yield (85.34 kg/ha) compared to other planting methods. The least dry root yield (2,406.29 kg/ha) and essential oil yield (47.58 kg/ha) was recorded in ridge and furrow method. The better growth and higher root yield in bag method may be due to better rhizospheric environment provided to the growing plants, easy penetration of roots into the soil and inturn easy growth of roots and optimum utilization of supplied nutrients and less leaching losses. These findings of the present investigation are in conformity with the findings of Boonklinkajorn and Visuttipitakul (2001) [3] and Tiwari (2014) [11].

Among the different levels of fertilizers, the higher dose of fertilizer (F_6- 75:50:25 kg N, P_2O_5 and K_2O per ha recorded significantly higher dry root yield (3,453.19 kg/ha) and essential oil yield (73.32 kg/ha) compared to other fertilizer levels. However, least dry root yield (2,780.49 kg/ha) and essential oil yield (57.07 kg/ha) were found with 25:25:25 kg NPK/ha (F_1). Higher values in growth, yield attributes and increased root yield under increased level of nutrients (F_6) could be attributed to better synthesis of metabolites due to application of higher levels of fertilizes in combination with farm yard manure (FYM). This has helped in enhancing the uptake of nutrients by the plants and accelerated the metabolic activates leading to greater accumulation of photosynthates inturn gave maximum dry matter production. The photosynthates which are synthesized in the leaves are translocated to the roots resulting in increased growth of yield components accounting for maximisation of final root yield. Increased root yield was also related to better vegetative growth of plants in terms of plant height, number of tillers and number of leaves which had positive and significant correlation with yield. Consistently least root yield was recorded in F_1 (25:25:25 kg NPK/ha) which could be attributed to least production of metabolites, less uptake of nutrients, poor vegetative growth and yield attributes. These findings are in conformity with the findings of Patra *et al*. (2004) [7] and Rashmi and Singh (2008) [9].

The interaction effects were also found significant. The bag method (P_3) with fertilizer levels of 75:50:25 kg NPK/ha (F_6) recorded significantly higher dry root yield (4333.52 kg/ha) and essential oil yield (94.25 kg/ha) compared to all other interactions. These findings are well corroborated with the findings of Man Singh *et al.* (2002) [5] and Patra *et al*. (2004) [7].

The planting methods and fertilizer levels individually and also in combination did not result in any significant differences for essential oil content.

Physico-chemical properties and odour evaluation of vetiver essential oil

The data on Physico-chemical properties and odour evaluation of vetiver essential oil as influenced by different planting methods at lowest and highest level of fertilizers are presented in Table 2.

The results were not analysed statistically. The results showed that the samples were dark in colour, turbid, opaque in P_1F_6, medium in P_2F_6 and light brown colour, transparent, thin in P_3F_6 treatment combinations, whereas the samples were light brown colour, transparent, thin in P_1F_1, P_2F_1 and P_3F_1 combinations.

The specific gravity of the oil was maximum (1.032) in P_1F_6 and least (1.013) in P_2F_6 combinations. The refractive index was maximum (1.5225) in P_3F_6, whereas it was least (1.5153) in P_1F_1 combination. The oil had maximum acid value (48.09) in P_1F_6 and minimum value (38.15) in P_3F_6 combination. The oil had maximum ester value (121.19) in P_3F_6 and least value (28.06) in P_3F_1 combination. The saponification value was maximum (159.34) in P_3F_6 and minimum value (72.95) in P_3F_1 combination.

The odour evaluation showed that the oil was woody, fresh, distinct rosy aroma in P_1F_6, typical vetiver aroma in P_2F_6 and P_3F_6 combinations, while P_1F_1 had camphoraceous, dilute aroma, P_2F_1 had rosy, characteristic, persistent woody aroma and P_3F_1 had rosy, typical vetiver aroma.

The typical vetiver aroma was found in P_3F_6, P_2F_6 and P_3F_1 which is in general, due to the presence of major ketones (α-Vetivone and β-Vetivone). The presence of alcohol (khusimol) has contributed typical vetiver odour and excellent fixative properties. It is desirable to store the oil for few months for aging, after which the odour becomes rich and long lasting.

Higher the specific gravity, heavier will be the oil and better will be the quality of oil. The specific gravity was higher in P_1F_6. Hence the oil was better in quality. The major constituent khusimol renders aroma to vetiver oil. Similar trends were also reported by Patra *et al*. (2004) [7] and Nidhi *et al.* (2011) [6] in vetiver.

Table 1: Dry root yield, essential oil content and essential oil yield as influenced by planting methods and fertilizer levels at harvest in vetiver.

Sub plot - Fertilizer levels (F)	Dry root yield (kg/ha)				Essential oil content (%)				Essential oil yield (kg/ha)			
	Main plot - Planting methods (P)											
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
F_1: 25:25:25 kg NPK/ha	2,076.05	2,492.78	3,772.65	**2,780.49**	1.992	2.093	2.059	**2.05**	41.35	52.17	77.68	**57.07**
F_2: 50:25:25 kg NPK/ha	2,176.23	2,592.16	3,981.79	**2,916.73**	1.976	1.906	2.163	**2.02**	43.00	49.41	86.13	**59.51**
F_3: 75:25:25 kg NPK/ha	2,274.14	2,450.49	4,034.07	**2,919.57**	2.055	2.184	1.988	**2.08**	46.73	53.52	80.20	**60.15**
F_4: 25:50:25 kg NPK/ha	2,479.38	2,902.41	4,154.20	**3,178.66**	1.933	1.906	2.174	**2.00**	47.93	55.32	90.31	**64.52**
F_5: 50:50:25 kg NPK/ha	2,603.83	3,065.74	4,238.46	**3,302.68**	1.926	1.976	1.969	**1.96**	50.15	60.58	83.46	**64.73**
F_6: 75:50:25 kg NPK/ha	2,828.09	3,197.96	4,333.52	**3,453.19**	1.991	2.170	2.175	**2.11**	56.31	69.40	94.25	**73.32**
Mean	**2,406.29**	**2,783.59**	**4,085.78**	**3,091.89**	**1.98**	**2.04**	**2.09**	**2.04**	**47.58**	**56.73**	**85.34**	**63.22**
	S. Em. ±		**C. D. @ 5 %**		**S. Em. ±**		**C. D. @ 5 %**		**S. Em. ±**		**C. D. @ 5 %**	
Main plot	135.06		421.55		0.002		NS		3.09		9.76	
Sub plot	44.70		140.36		0.001		NS		1.65		4.99	
Interaction	65.66		200.42		0.001		NS		3.01		9.55	

P_1: Ridge and furrow method
P_2: Bed method
P_3: Bag method

Table 2: Physico-chemical properties and odour evaluation of essential oil in vetiver as influenced by different planting methods and nutrition obtained in different treatments.

Sl. No.	Parameters	BIS Value	KSDL Value	F_6: 75: 50:25 kg NPK/ha (Highest fertilizer dose)			F_1: 25:25:25 kg NPK/ha (Lowest fertilizer dose)		
				P_1	P_2	P_3	P_1	P_2	P_3
Physical Parameters									
1.	Colour	Brown to	Brown to	Dark,	Medium	Light brown,	Light brown,	Light brown,	Light brown,

		reddish brown viscous liquid	reddish brown viscous liquid	Turbid, opaque		transparent, thin	transparent, thin	transparent, thin	transparent, thin
2.	Specific gravity	0.9926-1.0444	0.985-1.020	1.032	1.013	1.015	1.015	1.015	1.021
3.	Refractive Index @ 24.5 °C	1.588-1.5306	1.5132-1.5242	1.5213	1.5216	1.5225	1.5153	1.5154	1.5158
Chemical Parameters									
4.	Acid value	8.4 – 40.1	Max 35	48.09	38.84	38.15	39.27	39.27	44.89
5.	Ester value	5.6 – 24.6	20 to 64	94.26	112.22	121.19	44.09	56.11	28.06
6.	Saponification value	-------	---------	142.35	151.06	159.34	83.36	95.38	72.95
Odour evaluation									
7.	Odour evaluation	Rosy odour	---------	Woody, fresh and distinct rosy aroma	Typical vetiver aroma	Typical vetiver aroma	Camphoraceous and dilute aroma	Rosy aroma. characteristic and persistant woody aroma	Rosy aroma, typical vetiver aroma, cooling and refreshing

P_1: Ridge and furrow method
P_2: Bed method
P_3: Bag method

4. Conclusion
Thus, based on the study it can be concluded that for commercial production of vetiver the bag method of planting with the fertilizer level of 75:50:25 kg NPK/ha along with farm yard manure (FYM) at the rate of 10 tonnes per ha is found suitable for getting higher root yield and better quality of vetiver oil.

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