Indian Journal of Agricultural Sciences **86** (8): 1004–9, August 2016/Article
https://doi.org/10.56093/ijas.v86i8.60519

Variability in yield and phytochemicals content in mandukapanri (*Centella asiatica*) as influenced by nutrient management

R S JAT[1] and N A GAJBHIYE[2]

ICAR–Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat 387 310

Received: 3 April 2015; Accepted: 9 May 2016

ABSTRACT

A two years field experiment was conducted to study the influence of nutrient management on herbage yield, triterpenes content and their correlation with NPK, triterpenes yield, and NPK content and their use efficiencies in *Centella asiatica* (L.) Urban. Results indicated that application of FYM 15 tonnes/ha and NPK 60:50:60 at planting and NK 60:60 kg/ha at 5th harvest as basal along with N 20 kg/ha as split application at each harvest recorded highest dry herbage yield. The asiaticoside (AS) and madecassoside (MS) content increased with FYM application, whereas, decreased with NPK, however, asiatic acid (AA) and madecassic acid (MA) content markedly increased with FYM and NPK both. Highly strong relationships were exhibited between plant NPK content with AA and MA content. Triterpenes yield was recorded highest with the application of FYM 15 tonnes/ha and NPK 60:50:60 at planting and NK 60:60 kg/ha at 5th harvest as basal along with N 20 kg/ha as split application at each harvest. Application of FYM and NPK significantly influenced N and K content and their agronomic and use efficiencies, whereas, P content and it's agronomic and use efficiency were significant with NPK and non-significant with FYM. Thus, application of FYM 15 tonnes/ha and NPK 60:50:60 at planting and NK 60:60 kg/ha at 5th harvest as basal along with N 20 kg/ha as split application at each harvest found optimum to harvest maximum herbage and triterpenes yield and to produce quality raw drugs of *C. asiatica*.

Key words: Asiaticoside, Asiatic acid, *Centella asiatica,* Madecassoside, Madecassic acid, Nutrient management

Centella asiatica (L.) Urban, commonly known as mandukaparni and synonymous to European marsh pennywort (*Hydrocotyle vulgaris*), is a perennial, prostrate, faintly aromatic, stoloniferous tropical medicinal plant. The crop attains full vegetative growth in 3-4 months and the leaves with petiole or the whole plant including the roots are used for medicinal uses. *Centella asiatica* is rich source of pentacyclic triterpenes like; asiaticoside, centelloside, brahmoside, brahminoside, thankuniside, sceffoleoside, centellose and madecassoside, and asiatic, brahmic, centellic and madecassic acids (Dubery 2009). It was known for its nutritional and pharmaceutical values as remedy of several diseases in traditional as well as in modern medicines. Most commonly, it is used as wound healing agent and as constituent in brain tonics for developmentally disabled people (Mamedov 2005). It is among the top 10 drugs in the category of anti-ageing and central nervous system used worldwide (Bhavna and Jyoti 2011).

Medicinal plants produces secondary metabolites as principal bioactive compounds, however, their concentration and yield vary according to growing environmental conditions. Content of bioactive compounds of *C. asiatica* varies with location, climate and agronomic practices (Randriamampionona *et al*. 2007). The production of secondary metabolites in the plants is a result of diverse physiological, biochemical, metabolic and genetic regulations and can be manipulated by alteration in the growing conditions (Malik *et al*. 2011). Nutrient management is an important and critical aspect under good agricultural practices to produce the optimal yield in terms of both quality and quantity of any medicinal plants intended for health purposes. Integrating organic manures with inorganic fertilizers was reported to increase yield and chemical constituents in many medicinal plants (Siddiqui *et al*. 2011). Despite the several uses and potential demand as future crop, *Centella asiatica* being a new crop under cultivation, the scientific information for its cultivation particularly on nutrient management is not available. The study was conducted with the hypothesis that integrated use of organic and inorganic nutrient sources would increase yield and quality of *C. asiatica*, and have significant relationship among NPK and principal bioactive compounds

[1]Senior Scientist (e mail: rsdevgudha@yahoo.co.in), ICAR-Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur, Rajasthan. [2]Senior Scientist (e mail: gajbhiye_narendra@yahoo.com), ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat.

content in the plant.

MATERIALS AND METHODS

The experimental site was situated at the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat, India (22°3′ N latitude, 72°57A′ longitude and at an altitude of 43 m above mean sea level). It has a semiarid, subtropical climate with hot dry summers and mild winters. The mean maximum temperature during the hottest month of May was about 39.7°C, while the mean minimum temperature in winter month of January was as low as 10.0°C. The normal period of monsoon was from third week of June and extends up to end of September. The mean annual rainfall during period of experiment was 880 mm and 95% received from July–September. The soil was sandy clay loam (clay-25%, silt-22% and sand-53%), moderately calcareous, hyperthermic, vertic ustochrepts.

Experimental treatments were applied based on the approach of plant uptake and nutrient supplying capacity of soil to meet out the requirement of the crop under given agro-ecological conditions. Total 21 combinations of FYM as organic source and NPK fertilizers as inorganic sources and split application of nitrogen were evaluated. Three levels of FYM (0, 10 and 15 tonnes/ha) and seven levels of NPK (kg/ha) (N0:P0:K0, N40:P30:K40, N80:P40:K50, N120:P50:K60, N10:P30:K40+N10 as split application at each harvest, N30:P40:K50+N15 as split application at each harvest, and N60:P50:K60+N20 as split application at each harvest) were tested in split plot design and replicated thrice. On reviewing the nutrients balance in soil at 4th harvest, it was found that soil P content has built-up more than crop requirement and thus, applied only N and K as per treatments without P for further experimentation. Details of treatment applications are given in Table 1.

The field was ploughed, levelled and divided in to 4 m × 3.9 m size plots. FYM was applied and mixed with the soil 15 days before planting, whereas, NPK fertilizers were applied as per treatments. *C. asiatica* cultivar Vallabh Medha planted on 20 June 2011 at 30 cm row to row and 15 cm plant to plants distance. The crop is shade loving hence, the field was covered with agro shade net of 50% shade with the help of poles and iron wires. Irrigations were given at weekly interval to maintain the soil moisture level at field capacity after cessation of monsoon. The crop attained full crop growth after 5 months of planting and harvested in the month of December (1st harvest). Then subsequent harvests were taken at grand growth stage in the month of February (2nd harvest), April (3rd harvest) and June (4th harvest) in the first year. In the second year, after harvest in June the crop was fertilized as per soil nutrient balance and harvested in the month of October (5th harvest), February (6th harvest) and April (7th harvest).

The composite soil samples from surface soil (0-15 cm) at before start of experimentation and after completion of two years (7th harvests) were collected from three points randomly in each plot and mixed into one sample after removing the surface organic materials and fine roots. The soil was air-dried in shade, ground to pass through a 2-mm sieve and used for the estimation of pH, organic carbon (Walkley and Black 1934), and available nutrients N (Subbiah and Asija 1956), P (Olsen *et al*. 1954) and K (Hanway and Heidel 1952) content. Five plants from net plot area were collected randomly from each plot at each harvest and measured leaf number per node and leaf area using leaf area meter (LI-COR, LI-3100C model). Then washed and oven dried the leaves with petiole at 45°C for 48 hours. After drying, plant samples were ground to pass through a 0.5 mm mesh sieve and analysed for N, P and K content using standard methods (Jackson 1973). Calculated the plant NPK content (%), agronomic efficiency of N (AEN), agronomic efficiency of P (AEP) and agronomic efficiency of K (AEK), and nitrogen use efficiency (NUE), P use efficiency (PUE) and K use efficiency (KUE) as given below:

AEN = Herbage yield from N fertilized plot (kg/ha) - herbage yield from control plot (kg/ha)/amount of N applied (kg/ha)

AEP = Herbage yield from P fertilized plot (kg/ha) - herbage yield from control plot (kg/ha)/amount of P applied (kg/ha)

AEK = Herbage yield from K fertilized plot (kg/ha) - herbage yield from control plot (kg/ha)/amount of K applied (kg/ha)

Table 1 Rates of application of NPK and FYM, and total NPK applied during the study.

Treatment		Total NPK applied (kg/ha)		
		N	P	K
FYM (t/ha)				
F_1	0	0	0	0
F_2	10	60	21	70
F_3	15	90	31.5	105
NPK (kg/ha)				
T_1	NPK 0:0:0	0	0	0
T_2	NPK 40:30:40 at planting and NK 40:40 at 5th harvest as basal	80	30	80
T_3	NPK 80:40:50 at planting and NK 80:50 at 5th harvest as basal	160	40	100
T_4	NPK 120:50:60 at planting and NK 120:60 at 5th harvest as basal	240	50	120
T_5	NPK 10:30:40 at planting and NK at 10:40 at 5th harvest as basal and N 10 as split application at each harvest	70	30	80
T_6	NPK 30:40:50 at planting and NK after 30:50 at 5th harvest as basal and N 15 as split application at each harvest	135	40	100
T_7	NPK 60:50:60 at planting and NK after 60:60 at 5th harvest as basal and N 20 as split application at each harvest	220	50	120

NUE = N uptake from fertilized plot (kg/ha) - N uptake from control plot (kg/ha)/amount of N applied (kg/ha) × 100

PUE = P uptake from fertilized plot (kg/ha) - P uptake from control plot (kg/ha)/amount of P applied (kg/ha) × 100

KUE = K uptake from fertilized plot (kg/ha) - K uptake from control plot (kg/ha)/amount of K applied (kg/ha) × 100

A modular HPLC system was used for estimation of all four secondary metabolites (asiaticoside, madecassoside, asiatic acid and madecassic acid) as per procedure described by Rafamantanana *et al*. (2009). Established a linear gradient calibration curves over the range 0.334 -3.34 µg/ml for MS (r^2=0.9995) and AS (r^2=0.9994), and 0.166-1.66 µg/ml for AA (r^2=0.9993) and MA (r^2=0.9995). Extracted one gram of sample in 90% methanol, and filtered and dried and dissolved in HPLC grade methanol and loaded 20 µl sample in HPLC at a flow rate of 1 ml/min and measured the concentration of secondary metabolites in solution. The values of content were calculated on dry weight basis (µg/g) = (Analyte concentration µg/ml) × (sample volume in ml/weight of sample in g). The total content of triterpenes was estimated by summing the quantity of all four triterpenes. Similarly, the total triterpenes production was estimated by multiplying the triterpenes content with herbage production on unit area basis.

The data obtained were subjected to statistical analysis using Microsoft Excel. Analysis of variance (ANOVA) was done as per the procedure outlined by Gomez and Gomez (1984). The significant differences between treatments were compared with the least significance (LSD) at 5% level of probability (P=0.05). Pearson's correlations between N, P, K, AS, MS, AA and MA were calculated using SAS software and the relationships showed at P=0.01 and 0.05 level of significance in the matrix.

RESULTS AND DISCUSSION

Plant growth and yield

Significant increment in number of leaves per node was recorded with FYM and NPK applications (Table 2). FYM 15 tonnes/ha applied at planting produced maximum number of leaves per node (10.3) which was significant over control (9.8), however, non-significant with FYM 10 tonnes/ha (10.1). The number of leaves per node with FYM 15 tonnes/ha increased by 5.1% over control. NPK fertilizers markedly increased number of leaves per node and produced maximum with NPK 60:50:60 at planting and NK 60:60 kg/ha at 5^{th} harvest as basal along with N 20 kg/ha as split application at each harvest (10.5) and recorded 9.4% increment over control.

FYM or NPK application did not bring any significant increment in leaf area (cm^2/leaf), however, recorded maximum with FYM 15 tonnes/ha and NPK 60:50:60 at planting and NK 60:60 kg/ha at 5^{th} harvest as basal and N 20 kg/ha as split application at each harvest (Table 2).

Table 2 Effect of FYM and NPK on number of leaves/node and leaf area (mean of seven harvest) and herbage yield (total of seven harvest)

Treatment	Number of leaves/node	Leaf area (cm^2/leaf)	Herbage yield (tonnes/ha)
F_1	9.8	11.3	5.9
F_2	10.1	11.8	6.5
F_3	10.3	11.9	7.1
CD (P=0.05)	0.3	NS	0.77
T_1	9.6	10.8	5.3
T_2	9.8	11.5	6.1
T_3	10.1	11.5	6.5
T_4	10.1	11.9	6.9
T_5	9.8	11.5	6.3
T_6	10.4	12.3	7.0
T_7	10.5	12.3	7.4
CD (P=0.05)	0.3	NS	0.50

See Table 1 for treatment details.

Application of FYM and NPK fertilizers as well as split application of N markedly affected herbage yield (total of seven harvests) (Table 2). FYM 15 tonnes/ha applied at planting recorded significantly highest herbage yield (7.1 tonnes/ha) over control (5.9 tonnes/ha). Herbage yield increased by 20.3% with FYM 15 tonnes/ha over control. Application of NPK 60:50:60 at planting and NK 60:60 kg/ha at 5^{th} harvest as basal and N 20 kg/ha as split application at each harvest recorded significantly highest herbage yield (7.4 tonnes/ha) which was higher by 39.6% over control.

The study revealed that application of FYM and NPK with split application of N gave significantly higher herbage yield than no application and basal application of N. It might be due to better synchrony of nutrient availability to the crop through combined use of organic and inorganic nutrient sources. The literature showed the synergistic effect of organic and inorganic nutrients on herbage yield might be attributed due to the supply of essential micro and macro nutrients from organic sources (Banik *et al*. 2006), and additional supply of major nutrients from the inorganic fertilizer. Favourable effects of FYM were also attributed due to improvement in soil properties which might have provided better environment for plant growth and yield. Split application of N recorded more pronounced effect over basal application in the study since, *C. asiatica* is a perennial crop and nutrient supply throughout the growth period is critical. Similar results were also reported by Zhu *et al*. (2009) in many medicinal plant.

Triterpenes content and correlation studies

Triterpenes; asiaticoside, madecassoside, asiatic acid and madecassic acid were analyzed by validated HPLC method and studied the effect of FYM and NPK on their content in plant and total yield on unit area basis (Table 3). The asiaticoside content increased with FYM application and decreased with NPK application. Highest AS content

Table 3 Effect of FYM and NPK on AS, MS, AA and MA content

Treatment	AS (%)	MS (%)	AA (%)	MA (%)
F_1	0.31	0.83	0.19	0.15
F_2	0.37	0.92	0.21	0.16
F_3	0.39	0.87	0.25	0.19
CD (P=0.05)	0.03	0.08	0.01	0.02
T_1	0.39	0.88	0.19	0.13
T_2	0.39	0.93	0.22	0.14
T_3	0.38	0.91	0.22	0.18
T_4	0.34	0.86	0.23	0.18
T_5	0.36	0.87	0.19	0.15
T_6	0.32	0.88	0.21	0.18
T_7	0.32	0.80	0.24	0.20
CD (P=0.05)	0.05	0.09	0.02	0.02

See Table 1 for treament details.

(0.39%) was recorded with FYM 15 tonnes/ha and no NPK application. Application of FYM 10 tonnes/ha and NPK 40:30:40 at planting and NK 40:40 kg/ha at 5th harvest as basal recorded maximum MS content (0.92 and 0.93%), respectively, and decreased with further increase in FYM and NPK levels.

Contrary to AS and MS, AA and MA content increased markedly with application of FYM and NPK both. Application of FYM 15 tonnes/ha and NPK 60:50:60 at planting and NK 60:60 kg/ha at 5th harvest as basal along with N 20 kg/ha as split application at each harvest recorded highest AA content (0.25 and 0.24%) and MA content (0.19 and 0.20%), respectively.

The Pearson's correlation matrix of plant nutrients (N, P and K) and triterpenes (AS, MS, AA and MA) content showed varied degree of correlation among each other (Table 4). N content significantly and positively correlated with P (r=0.86896**) and K (r=0.74005**) content and similarly, correlated with AA (r=0.59552**) and MA (r=0.85796**) content while, not correlated with AS and MS content.

P content significantly correlated with N and K content and AA (r = 0.59153**) and MA (r = 0.78437**) content while, not correlated with AS and MS content. K content also significantly correlated with N and P content and AA (r = 0.75173**) and MA (r = 0.76572**) content while, not correlated with AS and MS content. Among triterpenes content, significant and positive correlation was observed in AS and MS (r = 0.75291**) content, and AA and MA (r = 0.57186**) content while, AS and MS were not correlated with either AA or MA and vice versa.

The asiaticoside, madecassoside, asiatic acid and madecassic acid content showed varied degree of correlation with plant NPK content. The availability of key macronutrients during the growth increased their uptake and concentration in plant have significant potential to affect bioactive compounds. These results were also supported with Pearson's correlation which revealed that NPK content have significant and positive correlation with triterpenes content in plants. The synergistic effect on the production of bioactive compounds of the plant is also attributed due to the combined effect of beneficial microbes, micronutrients and chemical metabolites from FYM, and major nutrients from the inorganic fertilizer. The present findings are in close proximity to the findings of Siddiqui *et al*. (2011). The meagre response to asiaticoside and madecassoside concentration showed that all bioactive compounds were not affected significantly with mineral nutrition. Similar results were also reported by Mogren *et al*. (2006) on little or no effect of mineral nutrition on polyphenol production in onion.

Triterpenes yield

Total triterpenes (AS, MS, AA and MA) yield were also calculated for more commercial interpretation of the results on per unit area basis and recorded highest with FYM and NPK and split application of N (Fig 2). Application of FYM 15 tonnes/ha with NPK 60:50:60 at planting and NK 60:60 kg/ha at 5th harvest as basal along with N 20 kg/ha as split application at each harvest recorded highest total triterpenes yield (139 kg/ha) followed by FYM 15 tonnes/ha and NPK 30:40:50 at planting and NK 30:50 kg/ha at 5th harvest as basal along with N 15 kg/ha as split application at each harvest (130 kg/ha). FYM 15 tonnes/ha with NPK 60:50:60 at planting and NK 60:60 kg/ha at 5th harvest as basal along with N 20 kg/ha as split application at each harvest increased total triterpenes yield by 95.8% over control.

The conjunctive use of FYM and NPK resulted into ample supply of macro and micronutrients and also improved soil physical and chemical properties which might have increased herbage yield and triterpenes content as revealed from the Pearson's correlation study, and thereby lead to

Table 4 Pearson correlation matrix between N, P, K and AS, MS, AA and MA content

Parameters	N	P	K	AS	MS	AA
P	0.86896**					
K	0.74005**	0.66944**				
AS	-0.12933	-0.05629	0.34630			
MS	-0.26699	-0.17615	0.04441	0.75291**		
AA	0.59552**	0.59153**	0.75173**	0.32106	-0.04155	
MA	0.85796**	0.78437**	0.76572**	0.02552	-0.19628	0.57186**

*Correlation is significant at the 0.05 level (1-tailed). **Correlation is significant at the 0.01 level (1-tailed).

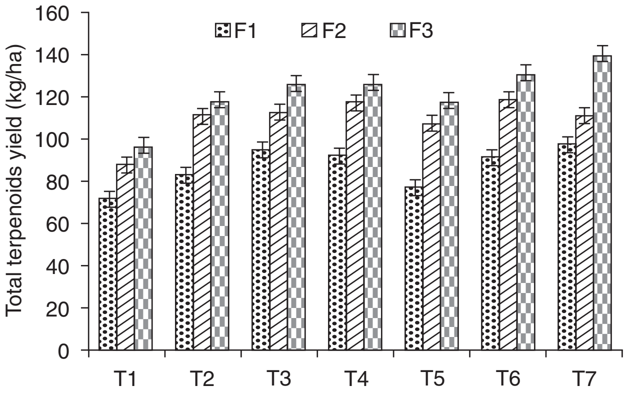

Fig 1 Effect of conjunctive use of FYM and NPK on total triterpenes yield. Error bars represent the standard error of the mean.

increased total triterpenes yield. The results of studies on a number of medicinal plants also confirm that the yield and production of bioactive compounds in medicinal plants were infiuenced by the ample supply of nutrients (Dethier *et al*. 1997). Even then, the content of pharmacologically active components in medicinal species basically determined by the genetic composition of the plant and cannot exceed speciûc thresholds (Samuelsson 1992).

NPK content and use efficiencies

Application of FYM and NPK influenced plant NPK content and agronomic and use efficiencies of NPK (Table 5). The highest plant N content (2.8%), AEN (13%) and NUE (51%) were recorded with FYM 15 tonnes/ha. Application of NPK 60:50:60 at planting and NK 60:60 kg/ha at 5th harvest as basal along with N 20 kg/ha as split application at each harvest recorded maximum N content (3.3%), AEN (15%), whereas maximum NUE (60%) was recorded with NPK 30:40:50 at planting and NK 30:50 at 5th harvest as basal along with N 15 kg/ha as split application at each harvest.

Plant P content and agronomic and use efficiency of P influenced non-significantly with organic manure while significantly with inorganic fertilizers (Table 5). Application of NPK 30:40:50 at planting and NK 30:50 at 5th harvest as basal along with N 15 kg/ha as split application at each harvest recorded highest plant P content (0.28%) and AEP (38%), whereas, PUE (14%) was maximum with NPK 60:50:60 kg/ha at planting and NK 60:60 at 5th harvest as basal and N 20 kg/ha as split application at each harvest.

Application of FYM 15 tonnes/ha and NPK 60:50:60 at planting and NK 60:60 at 5th harvest as basal and N 20 kg/ha as split application at each harvest recorded highest plant K content (2.6 and 2.5%), AEK (13 and 16%) and KUE (47 and 49%), respectively (Table 5).

Considering the effect of the treatments on plant nutrient composition, a significant increase was observed in N, P and K content in plants with the use of FYM and NPK fertilizers. This might be due to combined application of FYM and chemical fertilizer which has increased availability of native nutrients to the plants resulting in a higher yield (Bhandari *et al*. 2002) and increased nutrients use per unit of produce of the crops (Narwal and Chaudhary 2006). Improvement in the efficiency of nutrients when used in conjunction with manure, also might be due to the enhanced inherent nutrient supplying capacity of the soil coupled with improved soil physical properties (Hati *et al*. 2006), better rooting and higher nutrient and water absorption by the crops (Zhang *et al*. 1998). The results are in close proximity to the findings of Siddiqui *et al*. (2011) considering the effect of the treatments on plant chemical composition, and reported a small but significant increase in N, P and K content in *C. asiatica* treated with the combined application of compost tea and NPK.

These fndings revealed that application of FYM 15 tonnes/ha along with NPK 60:50:60 at planting and NK 60:60 kg/ha at 5th harvest as basal and N 20 kg/ha as split application at each harvest recorded increased herbage yield as well as concentration of principal bioactive compounds and their yield per unit area, and simultaneously fulfil the

Table 5 Effect of FYM and NPK on NPK content and agronomic efficiency and use efficiency of NPK.

Treatment	N			P			K		
	Content (%)	AEN (%)	NUE (%)	Content (%)	AEP (%)	PUE (%)	Content (%)	AEK (%)	KUE (%)
F_1	2.5	9	36	0.26	27	10	2.1	10	32
F_2	2.7	11	45	0.26	33	12	2.4	12	41
F_3	2.8	13	51	0.27	37	12	2.6	13	47
CD (P=0.05)	0.18	1.6	3.7	NS	NS	NS	0.15	2.6	2.2
T_1	2.2	6	25	0.24	21	7	2.2	6	23
T_2	2.4	9	39	0.26	30	10	2.3	10	35
T_3	2.7	11	41	0.27	33	12	2.4	12	39
T_4	2.9	10	45	0.27	33	12	2.5	12	46
T_5	2.4	12	44	0.26	34	11	2.3	11	44
T_6	2.9	13	60	0.28	38	13	2.4	14	46
T_7	3.3	15	55	0.28	38	14	2.5	16	49
CD (P=0.05)	0.09	2.0	3.1	0.01	3.6	1.1	0.13	2.9	4.8

See Table 1 for treatment details.

guidelines of good agricultural practices of WHO for the production of quality raw drugs. From a practical point of view, these results will have a positive impact on the commercial cultivation of *C. asiatica* and supply of high quality raw material for pharmaceutical use of this crop.

REFERENCES

Banik P, Ghosal P K, Sasmal T K, Bhattacharya S, Sarkar B K and Bagchi D K. 2006. Effect of organic and inorganic nutrients for soil quality conservation and yield of rainfed low land rice in subtropical plateau region. *Journal of Agronomy Crop Science* **192**: 331–43.

Bhandari A L, Ladha J K, Pathak H, Padre A T, Dawe D and Gupta R K. 2002. Yield and soil nutrient changes in a long-term rice–wheat rotation in India. *Soil Science Society of America Journal* **66**: 162–70.

Bhavna D and Jyoti K. 2011. *Centella asiatica*: the elixir of life. *International Journal in Ayurveda and Pharmacy* **2**(2): 431–8.

Dethier M, Sakubu S, Ciza A, Cordier Y, Menut C and Lamaty G. 1997. Aromatic plants of tropical Central Africa. XXVIII. Inûuence of cultural treatment and harvest time on vetiver oil quality in Burundi. *Journal of Essentialp Oil Research* **9**: 447–51.

Gomez K A and Gomez A A. 1984. *Statistical Procedures for Agricultural Research*. Wiley Inter science, New York.

Hanway J J and Heidel H. 1952. Soil analysis methods as used in Iowa State College Soil Testing Laboratory. *Iowa Agriculture* **57**: 1–31.

Hati K M, Swarup A, Singh D, Mishra A K and Ghosh P K. 2006. Long-term continuous cropping, fertilization and manuring effects on physical properties and organic carbon content of a sandy loam soil. *Austratian Journal of Soil Research* **44**: 487–95.

Jackson M L. 1973. *Soil Chemical Analysis*. Prentice Hall of India Pvt Ltd, New Delhi.

James J T and Dubery I. 2009. Pentacyclic tritepenoids from the medicinal herb *C. asiatica* (L.) Urban. *Molecules* **14**: 3 922–41.

Malik A A, Suryapani S and Ahmad J. 2011. Chemaical vs organic cultivation of medicinal and aromatic plants: the choice is clear. *International Journal of Medicinal and Aromatic Plants* **1**(1): 5–13.

Mamedov N. 2005. Adaptogenic, geriatric, stimulant and antidepressant plants of Russian far east. *Journal of Molecular and Cellular Biol*ogy **4**: 71–5.

Mogren L M, Olsson M E and Gertsson U E. 2006. Quercetin content in ûeld-cured onions (*Allium cepa* L.): effects of cultivar, lifting time, and nitrogen fertilizer level. *Journal of Agriculture and Food Chemistry* **54**: 6 185–91.

Narwal R P and Chaudhary M. 2006. Effect of long-term application of FYM and fertilizer N on available P, K and S content of soil. (*In*) *18th World Congress of Soil Science in Philadelphia*, Pennsylvania, USA, July 9–15, 2006.

Olsen S R, Cole C V, Watanabe F S and Dean L A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *US Deptartment of Agriculture Circular*, p, 939.

Randriamampionona D, Diallo B, Rakotoniriana F, Rabemanantsoa C, Cheuk K, Corbisier A M, Mahillon J, Ratsimamanga S and El Jaziri M. 2007. Comparative analysis of active constituents in *Centella asiatica* samples from Madagascar: application for ex situ conservation and clonal propagation. *Fitoterapia* **78**: 482–9.

Samuelsson G. 1992. *Drugs of Natural Origin. A Textbook of Pharmacognosy*, pp 17–26. Swedish Pharmaceutical Press, Stockholm.

Siddiqui Y, Islam T M, Naidu Y and Meon S. 2011. The conjunctive use of compost tea and inorganic fertiliser on the growth, yield and terpenoid content of *Centella asiatica* (L.) urban. *Scientia Horticulturae* **130**: 289–95.

Subbiah B V and Asija G L. 1956. A rapid method for the estimation of nitrogen in soils. *Current Science* **25**: 259–60.

Walkley A and Black I A. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modifcation of the chromic acid titration method. *Soil Science* **37**: 29–38.

Zhang H, Oweis T Y, Garabet Y and Pala M. 1998. Water use efûciency and transpiration efûciency of wheat under rainfed condition and supplementing irrigation in a Mediterranean type environment. *Plant Soil* **201**: 295–305.

Zhu Z, Liang Z, Han R and Wang X. 2009. Impact of fertilization on drought response in the medicinal herb *Bupleurum chinense* DC: growth and saikosaponin production. *Indian Crops Productivity* **29**: 629–33.