



Herbal biomass, secondary metabolites, water use and economic efficiencies of *Centella asiatica* influenced with irrigation water regimes

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ABSTRACT

Experiment was conducted on *Centella asiatica* (L.), Urban an important hydrophytic perennial medicinal plant to study the effect of water regimes on biomass production, secondary metabolites content and production, irrigation water use efficiency (IWUE), water economic efficiency (WEE) and economic returns in upland ecosystem. Maximum leaf area (22.1 cm²/leaf) and number of leaves (11.4/node) were recorded at 1.4 and 0.4 IW/CPE water regime, respectively. However, irrigation at 0.8 IW/CPE ratio produced optimum leaf area and number of leaves per node. Water application at medium regimes at 0.8 IW/CPE ratio produced significantly highest dry herbage yield (1301 kg/ha), triterpenes content (1.7%) and their yield (22.3 kg/ha), and gross return (182.1×10³ ₹/ha), net return (60.3×10³ ₹/ha) and benefits cost ratio (1.5). Significantly higher IWUE (6.9 kg/ha cm) and WEE (295 ₹/ha cm) were recorded at 0.4 IW/CPE ratio with minimum water use and longer application intervals. Thus, water application at medium regimes (0.8 IW/CPE ratio) found optimum to produce quality herbal biomass under commercial cultivation in uplands ecosystem.

Key words: Biomass, *Centella asiatica*, Irrigation water, Secondary metabolites, Water use efficiency

Centella asiatica (L.) Urban, popularly known as India Pennywort and synonymous to European marsh pennywort (*Hydrocotyle vulgaris*), is among the top 10 drugs in the category of anti-ageing and central nervous system (CNS) drugs used worldwide (Bhavna and Jyoti 2011). It is a rich source of pentacyclic triterpenes like; asiaticoside, centelloside, brahmoside, brahminoside, thankuniside, sceffoleoside, centellose and madecassoside, and asiatic, brahmic, centellic and madecassic acids (James and Dubery 2009). It is popular rasayana drug and is used as medhya rasayana in the CNS disorders like epilepsy, schizophrenia and cognitive dysfunction. It is also used as wound healing agent and as constituent in brain tonics for developmentally disabled people (Shrestha and Dhillion 2003, Zainol *et al.* 2003, Mamedov 2005).

The conditions of water scarcity or abundance both creates adverse impact on plant biochemical process related to photosynthesis directly and the entrance of carbon dioxide into stomata indirectly. Deficit irrigation, is the most significant factor restricting plant growth and crop productivity in the majority of agricultural fields of the world (Abedi and Pakniyat 2010) and alter the biochemical

properties of plants (Marchese and Figueira 2005, Zobayed *et al.* 2005). Limited water supply generally has negative effect on plant growth and development, whereas positive effect on biosynthesis of secondary metabolites, enzyme activities and solute accumulation (Sangwan *et al.* 2001). Recent advances in the understanding of secondary metabolism in plants, lead to attempts to increase the production of bioactive compounds desired for their medicinal, aromatic and culinary uses (Marchese *et al.* 2009). Plants under stress accumulate secondary metabolites very quickly (Kuc 1995), whereas under prolonged stress could lead to a reduction in content (Marchese *et al.* 2010). However, moderate water stress lead to increase the biosynthesis of secondary metabolites in many medicinal and aromatic plants and increase quality (Jaafar *et al.* 2012, Khalil and Kader 2011, Aradooei *et al.* 2013). These aspects need sufficient attention to develop the cost effective and efficient water management techniques suiting to particular soil-crop-climate conditions.

Thus, the present investigation was undertaken to optimise the regimes of irrigation water for the production of quality herbal biomass of *C. asiatica* with maximum efficiency of water use.

MATERIALS AND METHODS

The experiment was conducted at ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat (India) (22°35' N latitude, 72°55' E longitude and at an altitude of 45.1 m above mean sea level). The region (Anand, Gujarat) is characterized by

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semi-arid, sub-tropical climate with hot dry summers and mild winters. Mean monthly rainfall, temperature, relative humidity and evaporation were recorded throughout the duration of the experiment at the site. The annual rainfall received during the period of experiments was 946 mm during the year 2012-13 and 1142 mm during 2013-14, of which 95% received from June–September. The minimum temperature was recorded in January (10 °C in 2013 and 12 °C in 2014), whereas maximum temperatures was in May (40 °C) in both the years. Evaporation was also recorded higher in May (9.95 mm in 2013 and 10.5 mm in 2014). The relative humidity was minimum in the month of March (25%), whereas maximum in September (95%). The soil of the experimental field was sandy clay loam (clay-25%, silt-22% and sand-53%), moderately calcareous, hyperthermic and vertic ustochrepts.

The field was kept fallow during the winter and then ploughed and harrowed in the mid-May. Farmyard manure at the rate of 15 tonnes/ha and NPK 60:50:60 kg/ha was applied and mixed thoroughly in the soil with cultivator and planked. The test crop was planted at 30 cm row to row and 15 cm plant to plant spacing under 50% green shade net in July on the onset of monsoon. The water regimes were scheduled as per IW/CPE ratio given by Dastane (1972). The water depth was kept constant at 5 cm and the cumulative pan evaporation (cm/day) was measured by class-A pan evaporimeter. Total six water regimes at 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 IW/CPE ratios were applied in randomized block design and replicated thrice. The treatments were imposed after 100% ground cover of the crop. The crop was managed as per recommended practices and applied N 20 kg/ha at each harvest, and harvested at full leaf developmental stage. Total 7 harvests were taken during the study period, number of irrigations, water applied during the harvests are presented in the Table 1.

The number of leaves/plant were measured from ten plants on net plot area. After counting, the leaves were separated from the individual plants and used to measure leaf area (cm²) using leaf area meter. The plants from net plot area were harvested, washed and weighed. Then taken one kg plant sample and dried in shade for two days

Table 1 Number of irrigations and total quantity of water applied at different water regimes (IW/CPE ratio) (Total of 7 harvests)

IW/CPE ratio	Number of irrigations	Total water applied (ha cm)
0.4	34	170
0.6	42	210
0.8	54	270
1	64	320
1.2	71	355
1.4	81	405

IW: Irrigation water, CPE: Cumulative pan evaporation, ha: Hectare, cm: Centimetre

and then in oven at 45 °C for 48 hr and calculated the moisture percentage. Based on moisture content the dry biomass production was calculated on per ha basis and used for statistical analysis. The leaf samples collected were dried, powdered and prepared for high performance liquid chromatography (HPLC) extract using 90% methanol in water (3×75 ml, 1.0 hr) under reflux of a thermostatically controlled water bath maintained at 90 °C temperature.

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 ($r^2=0.9995$) as showed in the standard and extract's chromatogram. Extracted one gram of sample in 90% methanol, and filtered and dried. Then the extracted samples were 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 ($\mu\text{g/g}=(\text{Analyte concentration } \mu\text{g/mL})\times(\text{sample volume in mL/weight of sample in g})$). 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 lead significant differences (LSD) at 5% level of probability ($P=0.05$). Also calculated the per cent coefficient of variation (CV%).

RESULTS AND DISCUSSION

Growth and biomass production

Application of various water regimes markedly influenced leaf number and area, and recorded a reverse trend (Fig 1). The increase in water regimes from 0.4-1.4 IW/CPE ratio linearly increased leaf area ($R^2=0.94$) and recorded highest (22.1 cm²/leaf) at 1.4 IW/CPE ratio. Application of water at 1.4 IW/CPE ratio recorded 12.8% increment in leaf area over 0.4 IW/CPE ratio. However, increased water regimes decreased leaf numbers ($R^2=0.97$) and found highest (11.4/node) at lowest water regime at 0.4 IW/CPE ratio. Water application at 1.4 IW/CPE ratio decreased leaf number by 6.1% over 0.4 IW/CPE ratio. The optimum level of leaf area and number per node was found at medium water regime of 0.8 IW/CPE ratio. Increase in IW/CPE ratio from 0.4-1.4 increased leaf area most probably due to increased availability of water for photosynthetic energy processes, cell enlargement and cell division. Whereas, number of leaves decreased with frequent irrigations might be due to partial submerged conditions in the field which have favoured ethylene production and

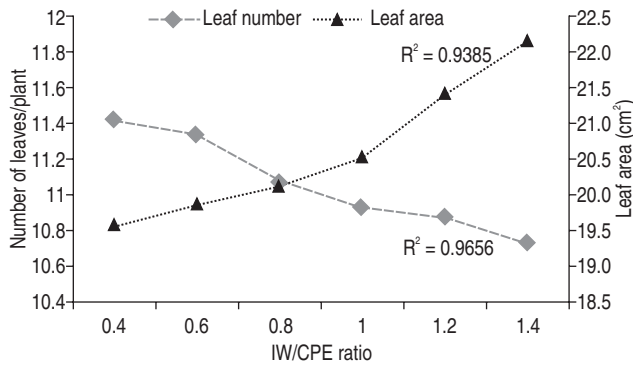


Fig 1 Leaf area and leaf number of *C. asiatica*

caused petiole abscission and leaf fall. Literature reviewed showed that water availability affect the growth of many medicinal plants like *Hypericum brasiliense* (Nacif and Mazzafera 2005), *Bupleurum chinense* (Chung *et al.* 2006, Zhu *et al.* 2009) and *Mentha piperita* (Khorasaninejad *et al.* 2011). Considering the facts, application of water at 0.8 IW/CPE ratio found optimum to produce desired leaf area and number, and optimum growth.

Harvest-wise the dry herbage yield was recorded and observed significant variation with water regimes up to 4th harvest (Table 2) and then found no response from 5th to 7th harvest might be due to build-up of soil moisture in the soil layers with continuous irrigation. However, the mean herbage yield of all the harvests significantly influenced with water regimes. The herbage yield increased with the increase in water regimes from 0.4-0.8 IW/CPE ratio and started to decrease with further increase in water regimes from 1.0-1.4 IW/CPE ratio. Irrigation at medium water regime at 0.8 IW/CPE ratio recorded maximum herbage yield across all the harvests as well as the mean herbage yield (1301 kg/ha). Water application at 0.8 IW/CPE ratio increased mean herbage yield by 10.3% and 7.8% over 0.4 IW/CPE ratio (1180 kg/ha) and 0.6 IW/CPE ratio (1207 kg/ha), respectively. The lowest mean herbage yield (1080 kg/ha) was recorded at 1.4 IW/CPE ratio. Irrigations at higher water regimes at 1.0, 1.2 and 1.4 IW/CPE ratio decreased herbage yield by 5.15, 13.69 and 17.0% over 0.8 IW/CPE

ratio, respectively.

Water availability either excess or deficit affects various plant physiological functions like cell enlargement, cell division, leaf area and leaf number and various biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Hendawy and Khalil 2005, Faroog *et al.* 2008, Jaleel *et al.* 2008a, Jaleel *et al.* 2008b). The maximum dry herbage production obtained at medium water regimes (0.8 IW/CPE ratio) might be due to optimum water availability for plant's physico-biochemical activities. Further, it might be due to optimum phenology (leaf area and number) at medium water regimes which has contributed to the final biomass production. Literatures reviewed also showed that moderate irrigation levels improved all growth and yield attributes in *Hibiscus sabdariffa* (Khalil and Kader 2011). And irrigation at deficit levels significantly reduced growth parameters and yield in rosemary (Hassan *et al.* 2013) and *Bunium persicum* (Saeidnejad 2013).

Secondary metabolites content and production

Irrigation at various water regimes markedly influenced total triterpenes content and their production in *C. asiatica* (Fig 2). The triterpenes content and yield increased with increasing water regimes from 0.4-0.8 IW/CPE ratio and decreased with further increase in water regimes from 1.0-1.4 IW/CPE ratio. The application of irrigation at medium water regimes at 0.8 IW/CPE ratio recorded highest total triterpenes content (1.71%) and increased by 2.0% over 0.4 IW/CPE ratio. Further, increase in water regimes above 0.8 IW/CPE ratio decreased the triterpenes content and recorded lowest (1.5%) at 1.4 IW/CPE ratio. The water regimes at 1.0, 1.2 and 1.4 IW/CPE ratio decreased triterpenes content by 4.7, 7.0 and 9.9% over 0.8 IW/CPE ratio, respectively. Similar trend was also recorded for total triterpenes yield and recorded highest (22.3 kg/ha) at 0.8 IW/CPE ratio which is 12.6% and 7.7% higher over 0.4 and 0.6 IW/CPE ratio, respectively. The water regimes at 1.0, 1.2 and 1.4 IW/CPE ratio decreased triterpenes yield by 9.4, 19.7 and 25.1% over 0.8 IW/CPE ratio, respectively.

Irrigation at various water regimes might have created

Table 2 Effect of water regimes on harvest-wise and mean dry herbal biomass production (Two years)

IW/CPE ratio	Dry herbage yield (kg/ha)							Mean
	1 st harvest	2 nd harvest	3 rd harvest	4 th harvest	5 th harvest	6 th harvest	7 th harvest	
0.4	722	1205	1943	515	1405	1287	1179	1180
0.6	790	1225	1946	518	1439	1303	1229	1207
0.8	895	1320	2116	525	1568	1334	1346	1301
1.0	825	1262	2014	490	1417	1291	1343	1234
1.2	716	1093	1752	419	1392	1275	1210	1123
1.4	648	1072	1707	418	1386	1198	1130	1080
LSD (P=0.05)	150.4	143.3	266.6	75.6	NS	NS	NS	61.7
CV (%)	10.8	6.6	7.7	8.6	8.8	7.3	7.93	2.9

IW: Irrigation water, CPE: Cumulative pan evaporation, CD: Critical difference, CV: Coefficient of variation, NS: Non-significant

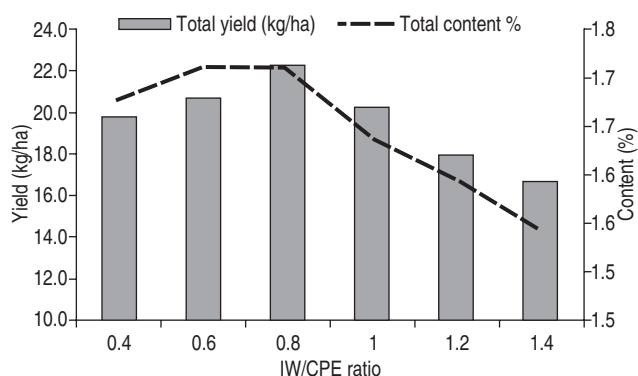


Fig 2 Effect of water regimes on triterpenes content and yield

various levels of water stress in the plant and affected the biosynthesis of secondary metabolites. In the present investigation, total triterpenes content recorded maximum when irrigated at medium water regimes (0.8 IW/CPE ratio) which might have created moderate water stress and changed the plant functions of the biosynthesis of secondary metabolites and thus, increased the total triterpenes content and production. The results were supported by the reports that under moderate water stress the photo assimilates redirected towards secondary metabolism (Marchese and Figueira 2005) and increase the accumulation of biologically active compounds in many medicinal and aromatic plants (Aradooei *et al.* 2013, Hossein *et al.* 2013, Marchese *et al.* 2010). The triterpenes content and production recorded less at lower as well as at higher water regimes might be due to severe water stress either due to deficit or excess water availability cause negative effect on the synthesis of secondary metabolites. The higher water regimes decreased the triterpenes content due to oxygen deficiency which might have reduced the plant biochemical process. The lesser triterpenes content and production were also reported by Hansen and Seufert (1999) and Duhl *et al.* (2007).

Water use and economic efficiency

The total water applied is the function of application rate and its intervals. In all the regimes, the application rate was kept constant at 5 cm, whereas intervals were dependent on water regimes. By virtue of the method of water application (IW/CPE ratio), the water applied at frequent intervals at high water regimes and *vice versa*. The application intervals decreased from 16 days at 0.4 IW/CPE ratio to 7 days at 1.4 IW/CPE ratio (Fig 3). Thus, it has increased the number of applications from 34 at 0.4 IW/CPE ratio to 81 at 1.4 IW/CPE ratio (Table 1). The total water applied for all the seven harvests were increased from 170 ha cm at 0.4 IW/CPE ratio to 405 ha cm at 1.4 IW/CPE ratio (Fig 3). The quantum of water applied at 1.4 IW/CPE ratio increased by 138% over 0.4 IW/CPE ratio.

The total water applied at various water regimes during all the harvests affected the efficiency of water use (herbage yield per unit of water use) and its economics (net income per unit of water use). Significantly highest IWUE (6.9 kg/ha cm) was recorded at water regime 0.4 IW/CPE ratio and

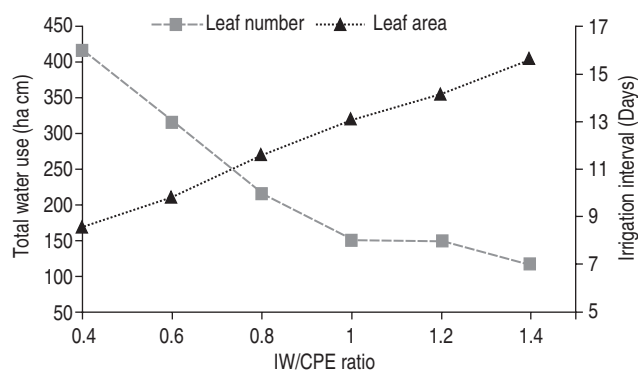


Fig 3 Water use and application intervals in *C. asiatica*

decreased drastically with further increase in water regimes and recorded 60.9% decrement at 1.4 IW/CPE ratio (2.7 kg/ha cm) (Table 3). The WEE was recorded markedly higher (₹295/ha cm) at 0.4 IW/CPE ratio and decreased with increasing water regimes up to 1.4 IW/CPE ratio (₹50/ha cm). Water economic efficiency decreased by 83.0% at 1.4 IW/CPE ratio over 0.4 IW/CPE ratio. The higher IWUE and WEE were recorded at low water regimes might be due to less water applied at longer intervals and efficient utilization of water for plant functions during the crop growing period. Results were also supported by Hassan and Ali (2014) in coriander with minimum water use.

Economic returns

The various irrigation regimes significantly influenced gross and net return as well as benefit cost ratio (B:C ratio) of production (Table 4). The water regime at 0.8 IW/CPE ratio fetched highest gross return (182.1 × 10³ ₹/ha), net return (60.3 × 10³ ₹/ha) and B:C ratio (1.5), however, decreased with further increase in water regimes (>0.8 IW/CPE ratio). Irrigation at 0.8 IW/CPE ratio increased gross return, net return and B:C ratio by 10.3, 20.4 and 7.1% over 0.4 IW/CPE ratio, respectively. Irrigation at 0.8 IW/CPE ratio recorded highest gross and net returns and benefit cost ratio of production might be due to optimum utilization of water for higher herbage production and resulted into realization

Table 3 Effect of water regimes on water use and economic efficiency (Mean of 7 harvests).

IW/CPE ratio	Irrigation water use efficiency (kg/ha cm)	Water economic efficiency (₹/ha cm)
0.4	6.9	295
0.6	5.7	244
0.8	4.8	223
1	3.9	149
1.2	3.2	83
1.4	2.7	50
LSD(P=0.05)	0.3	36
CV (%)	3.1	11

IW: Irrigation water, CPE: Cumulative pan evaporation, CD: Critical difference, CV: Coefficient of variation

Table 4 Effect of water regimes on economic return and benefit cost ratio (Mean of 7 harvests)

IW/CPE ratio	Gross return ($\times 10^3$ ₹/ha)	Net return ($\times 10^3$ ₹/ha)	B:C ratio
0.4	165.1	50.1	1.4
0.6	169.0	51.3	1.4
0.8	182.1	60.3	1.5
1	172.8	47.7	1.4
1.2	157.2	29.6	1.2
1.4	151.2	20.3	1.2
LSD(P=0.05)	8.6	8.6	0.1
CV (%)	3	11	2.9

IW: Irrigation water, CPE: Cumulative pan evaporation, CD: Critical difference, CV: Coefficient of variation. Sale price of herbage: ₹30/kg.

of maximum economic returns.

It can be concluded that optimization of water regimes is essential to enhance the herbal biomass and secondary metabolites production in medicinal plants even in *C. asiatica* which is considered as a hydrophytic plant by nature. Irrigation at medium water regimes at 0.8 IW/CPE ratio were found optimum to produce maximum herbage yield, triterpenes content and their yield in *C. asiatica* and to fetch the maximum economic returns. These results will have a positive impact on the commercial cultivation of *C. asiatica* in upland ecosystem and to supply the high quality raw drug for the traditional medicinal uses as well as for the pharmaceutical industries.

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REFERENCES

- Abedi T and Pakniyat H. 2010. Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). *Czech Journal of Genetics and Plant Breeding* **46**(1): 27–34.
- Aradooei S, Zakerin A R and Aboutalebi A. 2013. Effect of different levels of irrigation period and foliar application of methanol on essence of sweet basil (*Ocimum basilicum* L.). *Annals of Biological Research* **4**(6): 220–3.
- Bhavna D and Jyoti K. 2011. *Centella asiatica*: the elixir of life. *International Journal in Ayurveda and Pharmacy* **2**(2): 431–8.
- Chung I M, Kim J J, Lim J D, Yu C Y, Kim S H and Hahn S J. 2006. Comparison of resveratrol SOD activity, phenolic compounds and free amino acids in *Rehmannia glutinosa* under temperature and water stress. *Environmental and Experimental Botany* **56**: 44–53.
- Dastane N G. 1972. *A practical manual for water use research*. Navbharat Publication Mandir, Pune, India.
- Duhl T R, Helmig D and Guenther A. 2007. Sesquiterpene emissions from vegetation: a review. *Biogeosciences Discuss*

4: 3987–4023.

- Farooq M, Basra S M A, Wahid A, Cheema Z A, Cheema M A and Khaliq A. 2008. Physiological role of exogenously applied glycinebetaine in improving drought tolerance of fine grain aromatic rice (*Oryza sativa* L.). *Journal of Agronomy and Crop Science* **194**: 325–33.
- Gomez K A and Gomez A A. 1984. *Statistical Procedures for Agricultural Research*. Wiley Inter Science, New York.
- Hansen U and Seufert G. 1999. Terpenoid emission from *Citrus sinensis* (L.) under drought stress. *Physics and Chemistry of the Earth (B)* **24**: 681–7.
- Hassan F A S, Bazaid S and Ali E F. 2013. Effect of deficit irrigation on growth, yield and volatile oil content in *Rosmarinus officinalis* L. plant. *Journal of Medicinal Plants Studies* **1**.
- Hassan F A S and Ali E F. 2014. Impact of different water regimes based on class-A pan on growth, yield and oil content of *Coriandrum sativum* L. plant. *Journal of the Saudi Society of Agricultural Sciences* **13**: 155–61.
- Hendawy S F and Khalid K A. 2005. Response of sage (*Salvia officinalis* L.) plants to zinc application under different salinity levels. *Journal of Applied Science Research* **1**: 147–55.
- Hossein V, Hamid R V, Reza B and Mohammad J S. 2013. Effect of irrigation and nitrogen fertilizer on grain yield and essential oil percentage of medicinal plant Ajowan. *International Journal of Agronomy and Plant Production* **4** (5): 1 013–22.
- Jaafar H Z E, Ibrahim M H and Fakri N F M. 2012. Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL), malondialdehyde (MDA) and photosynthetic responses of Malaysian Kacip Fatimah (*Labisia pumila* Benth). *Molecules* **17**: 7 305–22.
- Jaleel C, Sankar B, Murali P V, Gomathinayagam M, Lakshmanan G M A and Panneerselvam R. 2008a. Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation. *Colloids and Surfaces B: Biointerfaces* **62**: 105–11.
- Jaleel C, Gopi R, Sankar M, Gomathinayagam M and Panneerselvam R. 2008b. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. *Comptes rendus Biologies* **331**: 42–7.
- James J T and Dubery I. 2009. Pentacyclic triterpenoids from the medicinal herb *C. asiatica* (L.) Urban. *Molecules* **14**: 3922–41.
- Khalil S E and Kader A A S A. 2011. The influence of soil moisture stress on growth, water relation and fruit quality of *Hibiscus sabdariffa* L. grown within different soil types. *Nature and Science* **9** (4): 263–78.
- Khorasaninejad S, Mousavi A, Soltanloo H, Hemmati K H and Khalighi A. 2011. The effect of drought stress on growth parameters, essential oil yield and constituent of peppermint (*Mentha piperita* L.). *Journal of Medicinal Plants Research* **5**: 5 360–5.
- Kuc J. 1995. Phytoalexins, stress metabolism and disease resistance in plants. *Annual Review of Phytopathology* **33**: 275–97.
- Mamedov N. 2005. Adaptogenic, geriatric, stimulant and antidepressant plants of Russian far east. *Molecular and Cellular Biology* **4**: 71–5.
- Marchese J A and Figueira G M. 2005. The use of pre-and post-harvest technologies and good agricultural practices in the production of medicinal and aromatic plants. *Brazilian Journal of Medical and Biological Research* **7**: 86–96.
- Marchese J A, Ming L C, Franceschi L, Camoचना R C, Gomes G D R, Paladini M V, Capelin D and Marchese C F. 2009. Medicinal plants used by “Passo da Ilha” rural community in

- the city of Pato Branco, southern. *Brazil. Anais da Academia Brasileira de Ciências* **8**: 691–700.
- Marchese J A, Ferreira J F S, Rehder V L G and Rodrigues O. 2010. Water deficit effect on the accumulation of biomass and artemisinin in annual wormwood (*Artemisia annua* L., Asteraceae). *Brazilian Journal of Plant Physiology* **22**(1): 1–9.
- Nacif de Abreu I and Mazzafera P. 2005. Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. *Plant Physiology and Biochemistry* **43**: 241–8.
- Rafamantanana M H, Rozet E, Raelison G E, Cheuk K, Ratsimamanga S U, Hubert P H and Quetin-Leclercq J. 2009. An improved HPLC-UV method for the simultaneous quantification of triterpenic glycosides and aglycones in leaves of *Centella asiatica* (L.) Urb (Apiaceae). *Journal of Chromatography B* **877**: 2396–2402.
- Saeidnejad A H, Mohammad K A F I, Hamid R K and Mohammad P. 2013. Effects of drought stress on quantitative and qualitative yield and antioxidative activity of *Bunium persicum*. *Turkish Journal of Botany* **37**: 930–9.
- Sangwan S N, Farooqi A H A, Shabih F and Sangwan R S. 2001. Regulation of essential oil production in plants. *Plant Growth Regulators* **34**: 3–21.
- Shrestha P M and Dhillon S S. 2003. Medicinal plant diversity and use in the highlands of Dolakha district. *Nepal Journal of Ethnopharmacology* **86**: 81–96.
- Zainol M K, Hamid A A and Yusof S. 2003. Antioxidant activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) Urban. *Food Chemistry* **67**: 456–66.
- Zhu Z, Liang Z and Han R. 2009. Saikosaponin accumulation and antioxidative protection in drought-stressed *Bupleurum chinense* DC plants. *Environmental and Experimental Botany* **66**: 326–33.
- Zobayed S M A, Afreen F and Kozai T. 2005. Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. *Plant Physiology and Biochemistry* **43**: 977–84.