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Effect of integrated manuring on growth and yield of *Centella asiatica* (L.) Urb.

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Abstract: *Centella asiatica* is an important ethnomedicinal plant. Effect of integrated manuring on growth of *Centella asiatica* was investigated using vegetative clones from Kirtipur, Kathmandu. The plantlets were grown in earthen pots containing soil, with integrated manuring [Urea(%) : FYM(%), 75:25; 50:50; 25:75], individual manuring (100 % Urea, 100 % FYM) and control conditions (no manure). The experimental design was completely randomized and each treatment had forty plants. We examined number of leaves per ramet, petiole length, specific leaf area, number of primary branches, number of flowers per ramet and plant biomass. The number of leaves per ramet, leaf area and number of flowers per ramet were significantly higher in integrated manuring than other treatments. Biomass production in integrated manuring (50 % Urea and 50 % FYM) was seven times higher than in control; it was five times higher than in complete organic manuring (100 % FYM) and 1.5 times higher than in inorganic manuring (100 % Urea).

Resumen: *Centella asiatica* es una planta etnomedicinal importante. Se investigó el efecto de la fertilización con abonos integrados en el crecimiento de esta especieusando clones vegetativos procedentes de Kirtipur, Katmandú. Las plántulas fueron cultivadas en macetas de barro consuelo al que incorporó abono integrado [urea (%) : FYM(%), 75:25; 50:50; 25:75], abono individual (100 % de urea, 100 % FYM) y condiciones control (sin abono). El diseño experimental fue completamente al azar y cada tratamiento tuvo cuarenta plantas. Se examinó el número de hojas por ramet, la longitud del pecíolo, el área foliar específica, el número de ramas primarias, el número de flores por ramet y la biomasa de la planta. El número de hojas por ramet, el área foliar y el número de flores por ramet fueron significativamente mayores con el uso de abonos integrados que con otros tratamientos. La producción de biomasa con el abono integrado (50 % de urea y 50 % de FYM) fue siete veces mayor que en el control;fue cinco veces mayor que con el abono orgánico total (100 % FYM) y 1.5 veces mayor que con abono inorgánico (100 % de urea).

Resumo: O A *Centella asiatica* é uma planta etnomedicinal importante. Através do uso de clones vegetativos provenientes de Kirtipur, Kathmandu, estudou-se o efeito da adubação integrada sobre o crescimento de *Centella asiatica*. As plântulas foram cultivadas em potes de barro contendo solo, com adubação integrada [Ureia (%) : FYM (%), 75:25; 50:50; 25:75], de adubação individual (100 % de ureia, 100 FYM %) e controlo (sem adubo). O delineamento experimental foi inteiramente casualizado e cada tratamento tinha quarenta plantas. Examinámos o número de folhas por rameto, o comprimento do pecíolo, a área foliar específica, o número de ramos primários, o número de flores por rameto e a biomassa da planta. O número de folhas por rameto, área foliar e número de flores por rameto foram significativamente maiores sob regime de adubação integrada do que com os outros tratamentos. A produção de biomassa com adubação integral (Ureia 50 % e FYM 50 %) foi sete vezes maior do que a do controlo; foi cinco vezes maior do que com adubação orgânica completa (100 FYM %) e 1,5 vezes maior do que com adubação inorgânica (100 Ureia %).

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Key words: Farm yard manure, growth, integrated manuring, urea, yield.

Introduction

Centella asiatica (L.) Urb. (Family Apiaceae) is widely distributed in tropical and subtropical regions of Nepal and is being collected by the local people for medicinal uses. Aerial parts are traditionally used against fever, high uric acid levels, high blood pressure, and also as a memory enhancer (Devkota & Jha 2008). The species is widely used in pharmaceutical companies and Ayurvedic formulations. Although over exploitation of the species is now widespread due to high market demand, no serious effort has been made for its planned cultivation in Nepal. Invasive plant species such as *Parthenium hysterophorus* (Linn.) has also been threatening the populations of *C. asiatica* (Karki 2009). Due to these reasons, appropriate agrotechnology needs to be developed for wider cultivation of the species, which will help conserving this species in the wild.

Proper fertilizer management for a medicinal plant species is important for increasing its yield and maintaining the quality of active principles. Nitrogen is recognized as one of the most limiting nutrients for crop growth and can be supplemented with organic materials (e.g. animal manures, crop residues, green manures etc.) or from inorganic fertilizers (urea, ammonia, nitrate etc). Application of organic materials like crop residue increases mineralization and mineral N pools in soil (Pandey & Rai 2007). Nitrogen amendment in soil increases 26 - 41 % of crop yields (Maier *et al.* 1994) and is one of the major nutrients of plant that involved in chlorophyll synthesis, and influences stomatal conductance and photosynthetic efficiency (Bidwell 1974). Farmers have traditionally utilized various kinds of organic materials to maintain or improve fertility and productivity of their agricultural soils. To increase the crop quality especially of medicinal and aromatic plants, organic fertilization is more acceptable than chemical fertilizers (Griffe *et al.* 2003). Beneficial effects of application of organic fertilizers on growth and yield of millet and wheat were demonstrated by Radwan & Hissein (1996), Mekki *et al.* (1999) and El-Kholy & Gomaa (2000). Addition of compost markedly improved the productivity of *Mentha spicata*, *M. pulegium* and *M. longifolia*, where an increase in composting level from 3.5 to 7.5 Mg ha^{-1} resulted in a significant increase in all the growth parameters and chemical constituents studied (Khalil & El-Sherbeny 2003). A significant increase in the yield of *Curculigo orchioides* Gaertn. was also reported with the addition of poultry manure (Joy *et al.* 2005) . Effect of organic and inorganic fertilizers on growth and yield of various medicinal plants have been studied by several workers (Das *et al.* 2007; Hasanuzzaman *et al.* 2008).

Complete substitution of inorganic fertilizers by organic fertilizers like vermicompost, or FYM, however, is not advisable because the nutrient concentrations of organic manures are generally low compared to inorganic fertilizers. This necessitates application of very large quantities of organic fertilizer to meet the nutrient requirements of the crops. Further, nutrients in organic manures are released gradually (Kumaraswamy 2002), which may be advantageous only to certain crops.

To meet the nutrient requirements of short duration crops such as *C. asiatica*, a judicious mix of organic and inorganic sources may be appropriate. Therefore, the present study was undertaken to evaluate the effect of organic amendments enriched with chemical fertilizers on yield and some selected growth traits of *C. asiatica*.

Materials and methods

Treatments

A pot culture experiment in a completely randomized block design was established in the garden of Central Department of Botany (CDB), Tribhuvan University (TU), Kirtipur, Kathmandu, (85° 17.32' E Long; 27° 40.20' N Lat, 1350 m asl), Nepal during October, 2007 and May, 2009. Total amount of N used was 20 kg ha^{-1} as recommended in Anonymous (2006). The recommended dose of N was met from two different sources. i.e. FYM (cattle manure) and urea mixed in different proportions with soil. FYM used for the study was 5 - 8 months old. Soil for the experiment was collected from the garden of Central Department of Botany. The soil of the experimental site was clay loam. Five replicate samples of soil and FYM were analyzed for pH, nitrogen and organic carbon. The proportion of urea and FYM (farmyard manure) varied by thoroughly mixing them with soil, thus six treatments reflecting a gradient of decreasing Urea: FYM content (%), 100:0 (T_1), 75: 25(T_2), 50: 50

(T_3), 25:75 (T_4), 0:100 (T_5) and control (soil without manure; T_6) were prepared. Treatments; T_2 (Urea: FYM, 75: 25), T_3 (Urea : FYM, 50: 50) and T_4 (Urea : FYM, 25:75) were considered as integrated manuring; treatments T_1 (Urea : FYM, 100:0) and T_5 (0: 100) were considered as individual manuring and treatment T_6 (no manuring) as control.

Plant material

In order to reduce the probability of genetic differences among the plantlets, all the plantlets were collected from a 5 m x 5 m plot located in a less disturbed site of the garden of Central Department of Botany. The cuttings of plantlets were more or less of uniform size with four leaves in each. A single plantlet was planted in each pot having a diameter of 20 cm. The pots were uniformly irrigated once in every two day. Altogether 240 plants, forty plants for each treatment were planted. Planting was done in October, 2007. All pots and treatments were rotated each week to counter any positional effects of pots within treatments. The experiment was replicated twice during succeeding season and the data were pooled.

Growth measurement

Plant growth traits and biomass were recorded in May, 2008 as the plants were fully mature and were in peak flowering condition. Ninety mature leaves per treatment were measured for petiole length (PL) and specific leaf area (SLA). Length and width of each leaf were measured. Length and width of each leaf were multiplied by a conversion factor following Zobel *et al*. (1987) to obtain leaf area. Then the leaves were oven dried at 60 ºC for 48 h and the biomass of each leaf was determined using an electronic balance (0.001g; Model- CA 153-10AACAAA). Specific leaf area was obtained as the ratio of area and biomass of each leaf. Leaf nitrogen (N) content was determined by modified microKjeldahl method following the procedure described by Horneck & Miller (1988). For leaf N analysis, twenty samples from each treatment were taken. Five leaf samples from each treatment were taken for determining chlorophyll-a, chlorophyll-b and total chlorophyll content. Chlorophyll content was determined following the method of Arnon (1949) by using spectrophotometer.

Number of nodes (NNB) occurring in each primary branch was noted. Internodal lengths (IND) were also measured on primary branches arising from mature rosettes. The numbers of leaves (NLN) and primary branches (NBN) arising from rosette were also counted. Total number of flowers per mature rosette was also noted. Dry mass of individual plant was also measured by drying the plant in hot air oven at 60 °C for 48 h.

Statistical analyses

The significance of the difference between the means of measured attributes among the treatments was analyzed by one way analysis of variance (ANOVA), followed by multiple range test (Duncan test). The amount of variation in the parameters in response to the treatments was assessed by calculating the coefficient of variation (CV), computed as the standard deviation expressed as the percentage of mean. Statistical Package for Social Science (SPSS, version, 11.5, 2002) was used for all statistical analyses.

Results

Nutrient content of soil and FYM

Nutrient contents of FYM had soil nitrogen (1.21 %),OC (20.12 %) and pH (8.2); while soil had soil nitrogen (0.14 %), OC (1.32 %) and pH (5.83).

Morphological traits

Different combinations of manuring significantly affected the leaf characteristics of *Centella asiatica*. Among the leaf traits, the extent of variation was the highest in specific leaf area (CV = 79, Table 1) and lowest in leaf width (CV = 5). The highest number of leaves per ramet was observed in treatment T_3 (50:50; Urea : FYM). Longest petiole length and relatively higher SLA were observed in treatment T_3 (50:50; Urea : FYM) followed by T_2 (75 : 25; Urea : FYM) and T_4 (25:75; Urea : FYM). The highest number of flowers per ramet (16.82) was also obtained at T_3 (50:50; Urea: FYM) (Table 2). There was significant difference in number of primary branches ($P < 0.001$), stolon length ($P < 0.001$) and number of stolons ($P < 0.001$) among the treatments (Table 2). But these traits did not vary significantly with the variation of urea and FYM in the integrated manuring treatments (T_2-T_4).

Leaf nutrient and chlorophyll content

Leaf N content ranged from 1.12 (T_6) to 2.2 % (T_3), and it varied significantly with treatments ($P < 0.001$; Table 1). There was no significant difference in leaf N between T_1 (Urea: FYM, 100 %

Table 1. Leaf characters of *Centella asiatica* (L.) Urb. in different combinations of urea and farm yard manure. For each parameter significant difference between means among different treatments are indicated by different letters (Duncan test, α = 0.05). *F* and *P* values were obtained by one way analysis of variance (ANOVA). (Treatments; T_1: 100 % Urea; T_2:75 % Urea, 25 % FYM; T_3: 50 % Urea, 50 % FYM; T_4: 25 % Urea, 75 % FYM; T_5 : 100 % FYM; T_6 : control (no manure).

Treatments	No. of leaves per- ramet	Petiole length (cm)	Leaf length (cm)	Leaf width (cm)	Dry mass of individual leaf (g)	SLA (cm^2 g^{-1})	Leaf N (%)	Chl a (mg g^1)	Chl b (mg g^1)	Total Chl (mg g^1)
T_1	5.84^a	5. 42^b	2.15^b	3.6^b	0.037^b	203^a	1.6^b	5.17^b	2.08^b	7.25^b
T_2	6.84^{ab}	6.68^{bc}	2.52^c	3.83^{bc}	0.027^b	345^b	1.88^c	8.35^d	3.06^c	11.41^d
T_3	7.2 ^{b}	8.23^c	2.92^e	4.45^d	0.023^a	406^c	2.2^c	9.01^e	3.02^c	12.03^d
T_4	6.46 ^{b}	6.86^{bc}	2.80^d	4.03^c	0.025^a	330^{ab}	1.9^c	8.28^d	3.22^c	11.5^d
T_5	5.43 ^{a}	5.65 ^{b}	1.74^a	3.05^a	0.03^b	204^a	1.37^b	6.06^c	2.26^b	8.33^c
T_6	4.41^a	2.22^a	1.82^a	3.11^a	0.038^b	197^a	1.12^a	2.25^a	1.81^a	4.6^a
CV	23	16	12	5	27	79	19	30	11	28
F (x, y)*	20 (5, 234)	47 (5, 534)	59.12 (5, 534)	16.98 (5, 534)	54.56 (5,534)	3.23 (5, 534)	54 (5,114)	25 (5,43)	71 (5, 43)	29 (5,43)
P value	0.013	0.002	< 0.001	< 0.001	0.001	0.036	< 0.001	< 0.001	< 0.001	< 0.001

*x= df_1 & y= df_2.

Table 2. Growth traits and yield of *Centella asiatica* (L.) in different combinations of urea and farm yard manure. For each parameter, significant difference between means among different treatments are indicated by different letters (Duncan test, α = 0.05). *F* and *P* value were obtained by one way analysis of variance (ANOVA). (Treatments; T_1: 100 % Urea; T_2:75 % Urea, 25 % FYM; T_3: 50 % Urea, 50 % FYM; T_4: 25 % Urea, 75 % FYM; T_5: 100 % FYM; T_6: control, no manure). Sample size for all attributes was 40.

Attributes	No. of primary branch	No. of stolon	Diameter of ramet (cm)	Stolon length (cm)	Root length (cm)	No.of flowers/ramet	Dry mass of individual plant (g)
T_1	9.47^b	4.86^{ab}	8.06^{bc}	7.39^c	4.14^a	10.38^b	5.74^b
T_2	8.68^b	6.0 ^{c}	9.52 ^{d}	8.39^d	5.04^{ab}	16.52^d	8.62^c
T_3	9.33 ^{c}	6.2^c	9.7^d	8.98^d	5.87^a	16.82^d	8.68^c
T_4	8.16 ^{b}	5.59$^{b\ c}$	9.42^d	8.27^d	6.68^b	14.33^c	7.97^c
T_5	5.75 ^{a}	4.56^a	7.87^b	6.71^b	5.73^a	12.37^{ab}	1.70^a
T_6	4.35 ^{a}	7.46^d	4.01^a	5.71^a	7.2 3^b	8.82^a	1.23^a
CV	17	28	18	17	19	17	18
F (x,y)*	15.5 (5,234)	23 (5,234)	20 (5,234)	43 (5,234)	21 (5,234)	8 (5,234)	34.53(5,234)
P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

x = df_1, y = df_2.

Urea) and T_2 (Urea: FYM, 75:25). Total chlorophyll content ranged from 4.6 (T_6) to 12.03 mg g^{-1} (T_3). There was significant difference in total chlorophyll content among the treatments (P < 0.001) (Table 1).

Biomass production

Biomass production of *Centella asiatica* was affected significantly by manuring condition (Table 2). Dry mass of individual plant ranged from 1.23 g (T_6) to 8.68 g (T_3). Dry mass of individuals was significantly higher in integrated manuring than in individual application and in control. There was no significant difference in plant biomass production among the integrated manuring conditions (T_2-T_4; Table 2). The dry mass of individual plant was around seven times higher in integrated manuring (T_3; 50 : 50; Urea : FYM) than in control; it was 1.5 and 5 times higher than in application of 100 % urea and 100 % FYM, respectively.

Discussion

All measured traits of *Centella asiatica* were significantly higher in integrated manuring than in individual application of organic and inorganic manures and in control. The traits like petiole length, leaf length and leaf width in T_3 (50:50; Urea: FYM) treatment were higher than in other integrated manuring and individual application. In earlier studies the number of runners and flowers on strawberries in plots grown with food waste and paper waste vermicompost treatments was reported higher ($P \leq 0.05$) than on those grown with inorganic fertilizers only (Arancon *et al.* 2004). Application of organic manures significantly increased levels of organic C and N and the formation of water-stable aggregates, as compared with application of chemical fertilizers (Adrien 2006). Integrated manuring increases availability of macro- and micronutrients to plant which leads to high vegetative growth and absorption of nitrogen (Abd El-Lattief 2011; Bilal *et al.* 2000). In the present work it is likely that N uptake increased when the FYM was mixed with the mineral N fertilizer as compared to the 100 % FYM or 100 % N mineral fertilizer. It can be due either to the effect of FYM and mineral N fertilizer on improving soil physical properties, or to a higher mineralization of FYM which was due to mineral N inputs. Role of organics in increasing yield of *C. asiatica* could be attributed to supply of all essential nutrients due to continuous mineralization of organic manures. Phytohormones extracted from FYM help the plant to grow more luxuriously even with reduced doses of chemical fertilizers (Saraf & Tiwari 2004). This might be the reason for higher value of vegetative and reproductive traits of *C. asiatica* plant in integrated manuring system than in individual application.

Significantly higher values of leaf nitrogen and chlorophyll content were observed in integrated manuring. This may be due to the availability of higher amount of nitrogen to the plant in the integrated manuring (Das *et al.* 2007). Nitrogen supply affects leaf growth because it increases the leaf area of plants thus influencing photosynthesis. Photosynthetic proteins represent a large proportion to total leaf N (Evans 1989). Chlorophyll content is approximately proportional to leaf nitrogen content (Evans 1983). That was the reason for having higher value of leaf N and chlorophyll content in integrated manuring (T_2-T_4) than in other treatments (Table 1). In soyabean, chlorophyll content of leaf in combined application of 40 Mg ha^{-1} MWS (municipal solid waste), VC (vermicompost), and SS (sewage sludge) were also higher compared to chemical fertilizer and other organic fertilizer treatments (Pirdashti *et al.* 2010). It was reported that leaf chlorophyll content in 20/80 (vermicompost/feedstock) treatments were significantly higher than pure vermicompost (Ali *et al.* 2007). Higher value of chlorophyll concentration in leaf of maize and sorghum in integrated manuring of inorganic fertilizer with poultry manure than in individual application of manure was also reported by Amujoyegbe *et al.* (2007).

Integrated manuring had significant positive effect on dry mass of *Centella asiatica*. Crop biomass was positively correlated with per capita N consumption (Wani *et al.* 2010). Dry mass of individual did not vary significantly with the variation of urea and FYM in integrated manuring (T_2-T_4). To increase the crop quality organic fertilization is more acceptable than chemical fertilizers (Griffe *et al.* 2003). Application of FYM in soil not only increase the rate of supply and pool size of available N but also sustained the enhanced N pool throughout the annual cycle and maintains the fertility of soil (Ghoshal 2002). Therefore, application of FYM (organic) supplemented with small amount of inorganic fertilizer (Urea) was the best manuring condition for *C. asiatica* growth.

Higher plant biomass in integrated manuring could be due to the existence of favorable nutritional environment under the influence of FYM and inorganic fertilizers on vegetative and reproductive growth, which ultimately led to realization of higher yield. It was reported that the soyabean yield in application of 40 Mg ha^{-1} SS (sewage sludge) enriched with half chemical fertilizer was higher as compared to other fertilizer treatments (Pirdashti *et al.* 2010). The potato yield in the compost treatment was significantly higher than in the control, and comparable to that produced with 200 kg N ha^{-1} mineral fertilizer

(Fragstein & Schmidt 1999). Enhanced yield of *Abelmoschus esculentus* (okra) by application of a combination of cowdung and NPK was also reported by Okwuagwu *et al.* (2003).

In conclusion, application of organic manure supplemented by a small amount of inorganic fertilizer was the best manuring condition for cultivation of *Centella asiatica*. This information is helpful for planning cultivation practices of *C. asiatica*.

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