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The conjunctive use of compost tea and inorganic fertiliser on the growth, yield and terpenoid content of *Centella asiatica* (L.) urban

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ABSTRACT

The conjunctive use of a compost tea (CT) and an inorganic fertiliser (NPK) on the growth, yield and terpenoid content of *Centella asiatica* (L.) urban was evaluated. CT and NPK applied at half the recommended concentration resulted in the significant enhancement of vegetative growth, yield and antioxidant content. The synergistic effect of CT_{50} and NPK_{50} was highly pronounced on the bioactive components Asiatic acid, madecassoside and asiaticoside. The distribution of the total antioxidants was highest in roots (80%), followed by leaves (66%) and petioles (54%). There was a strong positive correlation ($r = 0.990$) between fertility and total antioxidant content, suggesting an enhanced synthesis of bioactive components resulting from the treatment. This response by *C. asiatica* to the integrated experimental treatment suggests a viable option for the commercial cultivation of the herb. Augmenting the vegetative biomass production and bioactive components will increase the availability of this medicinal herb for the treatment of various human ailments.

1. Introduction

Centella asiatica (L.) urban is a slender, stoloniferous perennial weed with a weak aroma. It is native to Sri Lanka, northern Australia and Malaysia, and flourishes well in humid areas in several tropical countries. Locally known as pegaga, *C. asiatica* is a tropical ethnomedicinal herb with a long history of therapeutic uses. It has been known for its nutritional and pharmaceutical value as a functional food and as remedy for many diseases in folk medicine. Most commonly, it is used as wound healing agent and as constituent in brain tonics for developmentally disabled people (Mamedov, 2005; Shrestha and Dhillion, 2003; Zainol et al., 2003).

The active principle components in *C. asiatica* are terpenoids, including asiatic, brahmic, and centellic acid, asiaticoside and madecassoside (Dutta and Basu, 1968; Singh and Rastogi, 1968). *C. asiatica* is one of the 25 top-selling herbs in the United States, while in Malaysia, the herbal industry is still at its infancy. Reviews on the phytochemistry and pharmacological activity of *C. asiatica* indicate significant differences in active constituent content among samples from different locations depending on the climate and agronomic practices adopted (Das and Mallick, 1991; Rouillard-Guellec et al., 1997; Jamil et al., 2007; Randriamampionona et al., 2007).

Inorganic fertilisers have significant effects on world crop production and are essential components of today's agriculture. Estimates show that agricultural production is raised by 50% as a result of chemical fertilisers, and 60% of the population owes its nutritional survival to nitrogen (N) fertilisers (Fixon and West, 2002). However, of the total applied N, less than 50% is recovered in the soil–plant system, while the remainder is lost to the environment (Abbasi et al., 2003). Hence, growing concerns about the negative impact of inorganic fertilisers on the environment and their future cost make it expedient to integrate a greater use of organic materials in cultivation practices to enhance crop yields.

There are intensive efforts worldwide to use organic manures to provide the same amount of food with less fossil fuel-based inorganic fertiliser. Integrating nutrient management with organic manures and inorganic fertilisers has been reported to increase yields and chemical constituents in *Plantago arenaria* (Kolodziej, 2006). The conjunctive use of organic nutrient sources with inorganic fertilisers was shown to increase the potential of organic fertiliser and to improve the efficiency of inorganic fertiliser so that the use of these fertilisers could be reduced up to certain levels. The use of microbe-enriched compost tea for nutrient mobilisation is becoming popular, and new systems are being developed to meet the requirements of different crops and cropping systems. Several studies have reported benefits from the use of compost and compost teas as organic substrate additives in plant cultivation and in the suppression of soil-borne diseases. It has been reported

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that compost teas obtained from agro-wastes were able to enhance the growth and yield of okra when sprayed weekly at full strength (Siddiqui et al., 2008, 2009).

To our knowledge, in spite of its growing importance, no serious efforts have been devoted to planning and organising the commercial propagation and cultivation of *C. asiatica* in Malaysia. The spontaneous collection of the plant from natural sources and over-exploitation are now widespread in response to high market demand. Hence, the present study was undertaken to determine the efficacy of compost tea in conjunction with the minimal use of inorganic fertilisers for enhancing yield and the production of active terpenoid components, with the ultimate aim of promoting the viable commercial cultivation of *C. asiatica*.

2. Materials and methods

2.1. Plant materials and cultivation

The study was conducted under glasshouse conditions at the University Agriculture Park, Universiti Putra Malaysia, from September 2008 to January 2009. Stem cuttings of *C. asiatica* plants were obtained from the local fresh herb market. A herbarium voucher specimen was prepared and deposited at the faculty herbarium. The daily temperature in the glasshouse ranged from 25–33 °C with 85% relative humidity (RH). Planting trays (54 cm × 38 cm × 12 cm) with drainage holes were used to grow the herb. Each tray was filled with 5 kg of soil mixture (topsoil, peat, sand at 2:2:3 v/v), and ten uniform 4-leaf cuttings were planted 12 cm apart to a depth of 2 cm. Water was supplied as required to maintain the field capacity.

2.2. Experimental treatments

There were a total of six treatment combinations of compost tea (CT) and inorganic fertiliser (NPK) arranged in a randomised complete block design, *viz.*: (T1) control (no fertiliser or compost tea); (T2) CT 1 L (100%); (T3) CT 1 L (50%); (T4) the manufacturer's recommended rate of nitrogen based on the sand, clay and loam in the soil was applied at a rate of 100 kg N ha^{-1} N using ammonium nitrate (33.5%); phosphorus was applied at a rate of 50 kg P ha^{-1} using calcium super phosphate (15.5%); and potassium was applied at a rate of 50 kg K ha^{-1} using potassium sulphate (48% K_2O) (100:50:50 kg ha^{-1}); (T5) NPK at half of the recommended rate (50:25:25 kg ha^{-1}); and (T6) CT 50% (1 L) + N P K (50:25:25 kg ha^{-1}). CT was prepared according to Naidu et al. (2010) by brewing commercial compost (empty fruit bunch and chicken manure) and water in a ratio of 1:5 (w/v, compost:water) supplemented with microbial starter. The physicochemical and microbial properties of the CT were determined and are presented in Tables 1 and 2. Application of CT (T2, T3 and T6) was performed every two weeks as described in Ref. Siddiqui et al. (2008), whereas the mineral NPK fertiliser treatments (T4, T5 and T6) were applied every four weeks at the recommended dosage.

2.3. Nutrient analyses

The determination of the macro- and micronutrients (N, P, K, Ca, Mg, Zn and Fe^+) in the soil mixture was performed according to the micro-Kjeldahl method (Ma and Zuazaga, 1942; Jackson, 1973). Nitrogen and phosphorus were analysed using an AutoAnalyzer, whereas potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), and iron (Fe), were analysed using the Atomic Absorption Spectrophotometer (PerkinElmer Model 310).

Table 1
The physiochemical properties of microbe-enriched compost tea and soil mix.

Nutrients/heavy metals	Microbe-enriched compost tea	Soil mix (initial)
pH	8.6	4.6
EC (dS m^{-1})	4.3	4.63
Bulk density (pB) (g cm^{-3})	–	0.21
Clay (μm)	–	28.800
Silt (μm)	–	5.390
Sand (μm)	–	68.55
Texture	Watery	Sandy clay loam
Organic carbon (%)	–	0.94
Nitrogen (%)	8.46	0.06
Phosphorus (%)	3.99	0.25
Potassium (%)	2.85	0.056
Calcium (%)	4.16	0.17
Magnesium (%)	1.22	0.32
Zinc (ppm)	0.27	ND
Iron (ppm)	0.29	ND

2.4. Growth measurements

Data on morphological traits were recorded at two-week intervals. Twenty plants per treatment were selected randomly for the measurement of quantitative traits. The ten quantitative traits recorded on fresh samples were as follows: number of leaves, leaf length, leaf width, petiole length, leaf area, specific leaf area (SLA), petiole length, length and width of leaves, and rosette diameter. Leaves and roots were oven dried at 60 °C for 48 h, and their dry weights were determined. Leaf area was determined from length and width measurements using the conversion factor described by Zobel et al. (1987). SLA was calculated as the ratio of leaf area and dry mass. The number of nodes occurring along each primary branch was noted. At harvest, ten plants per treatment were used to determine the fresh and dry herb yields. Plant samples were analysed according to the recommended procedure (Piper, 1926). At the termination of the experiment, 20 plants per treatment were washed in running tap water and separated into leaves, petioles and roots for extraction of bioactive compounds.

2.5. Extraction and analysis of antioxidative compounds

The plant samples were dried in a convection oven at 45 °C for 48 h until they reached a constant weight (Zin et al., 2002). The dried plant parts were ground, sealed in polyethylene bags, and stored in a refrigerator at 4 °C until they were ready for extraction.

Ten grams each of the dried, powdered samples was extracted with 100 ml of methanol in an incubator shaker at 25 ± 2 °C for 24 h. The extracts were then filtered, and the filtrates were rotary-evaporated at 40 °C for 30 min. The crude extracts were stored in liquid nitrogen.

2.5.1. Antioxidant activity: diene conjugation method

The antioxidant activity of extracts was determined using the diene conjugation method based on the linoleic acid model

Table 2
Microbial populations in microbe-enriched compost tea used in this study after three days of brewing.

Microorganisms	Colony forming unit (CFU ml^{-1})
Pseudomonas spp.	7.5×10^7
Lactic acid bacteria	8.9×10^7
Other bacteria	8.2×10^7
Actinomycetes	3.9×10^7
Yeasts	2.3×10^4
Trichoderma spp.	3.5×10^5
Other fungi	1.8×10^4