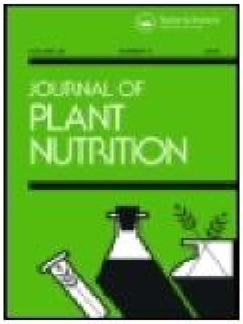

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Urea–hydroxyapatite nanohybrid as an efficient nutrient source in *Camellia sinensis* (L.) Kuntze (tea)

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ABSTRACT

Slow-release fertilizers based on nanomaterials have recently proven promise in increasing nitrogen use efficiency by plants and reduction in environmental hazards. We have previously reported the synthesis of an efficient and novel plant nutrient formulation based on urea–hydroxyapatite (HA) nanohybrid and its efficacy at small scale field trials conducted using rice as the model crop. This work focuses on a study of the effect of urea–HA nanohybrid fertilizer on the yield and quality of tea in farmer's fields selected from three climatic zones (Low Country, Mid Country, and UVA regions) in Sri Lanka for a period of three years. Experiments were carried out using treatments with half and full amounts of nitrogen recommendations to the tea plant, supplied through both conventional urea and nanohybrids at two and four splits per annum. Annual N requirement supplied through nanohybrids in Low Country and Uva showed a yield increment of 10–17% and 14–16%, respectively, compared with conventional recommendation. Nanohybrid fertilizer also allowed to reduce the number of fertilizer applications and the amount used by 50% resulting in matching yields to the conventional fertilizer applications. However, only a minute yield increase of 2–3% was observed in mid Country. Yield component analysis carried out in the Uva region was evident for increased tea yield obtained by application of slow-release nanofertilizer. Moreover, application of slow-release fertilizer significantly increased soil P, leaf N, and P concentration in Low Country tea yields. A positive effect of HA–urea nanohybrids was more pronounced during unfavorable climatic conditions.

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hydroxyapatite; hydroxyapatite–urea nanohybrids; mature tea; nitrogen use efficiency; slow-release; soil and plant nutrient status; yield

Introduction

Tea [(*Camellia sinensis*) (L.) O. Kuntze] is a perennial leafy crop which demands more nitrogen (N) than any other crops since the harvest is the young leaf of the plant (Han et al. 2008). It has been proven that the application of nitrogen in the form of chemical fertilizer promisingly increases the yield and as a result, farmers indiscriminately dump large quantities of nitrogen fertilizer in their tea fields. For example, most of the tea producing countries recommend using

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Table 1. Composition and nutrient content of fertilizer recommendations.

Mixture	Parts in the mixture			Total parts	Concentration in the mixture (%)		
	Urea	ERP	MOP		N	P_2O_5	K_2O
VPLC/880	587	126	167	880	30.7	4.1	11.4
VPUM/910	587	123	200	910	29.7	3.9	13.2
VPUVA/945	587	125	233	945	28.6	3.8	14.8

N, P_2O_5, and K_2O concentration in urea, ERP, and MOP are 46%, 28.5%, and 60%, respectively.

nitrogen fertilizer ranging from 580 to 2830 kg $ha^{-1}yr^{-1}$ (Tachibana, Yoshikawa, and Ikeda 1995). However, as recommended by the Tea Research Institute of Sri Lanka, the nitrogen application rate is less than 400 kg $ha^{-1} yr^{-1}$.

Long-term continuous excessive application of nitrogen fertilizer deteriorates the productivity of the tea bush (Sedaghathoor et al. 2009). High nitrogen rates aggravate the accumulation of high Theanine levels in tea roots owing to severe damages to feeder roots and deplete root starch reserves. Nevertheless, increasing the rates of nitrogen levels improve green tea quality and diminish black tea quality (Owuor 2001; Hamid et al. 2014; Cloughley, Grice, and Ellis 1983).

Current chemical fertilizers are highly water soluble and as a result they are only available for approximately a month for plant uptake (Han et al. 2008). Therefore, fertilizer use efficiency in tea fields becomes low with continuous application rates. As a result, only a small proportion of N ultimately is taken up by the tea crop. Extremely high application rates of nitrogen fertilizers aggravate N losses through volatilization, denitrification, and leaching, ultimately resulting in low nitrogen recovery efficiency which causes serious environmental pollution (Cassman, Dobermann, and Walters 2002). Application of conventional fertilizer results in 50–70% losses from the soil due to leaching and low N use efficiency by plants especially in tropical regions because of high temperature and precipitation (Gunaratne et al. 2016). A recent study carried out by Tea Research Institute in the low country region of Sri Lanka revealed that the loss of applied conventional fertilizer formulation of NPK, VPLC/880 mixture (Table 1) through leaching and volatilization were 28% and 22%, respectively (Liyanage 2016).

There are effective ways to increase the N use efficiency such as balanced fertilization, the timing of application, improving application techniques, and use of slow-release fertilizer (Han et al. 2008). Among the many methods, recent attention has been focused on introducing slow-release fertilizer which is capable of supplying its nutrients gradually over a specific period of time. Advantages of such slow-release fertilizers are enhancement in plant nutrient uptake efficiency, less frequent application, reduction in capital and labor outlay, the flexibility of release periods (40–90 days), and improved storage and handling properties.

Different types of slow and controlled release fertilizers such as coated controlled release fertilizers (CRF), uncoated CRFs, and bio inhibitors have been introduced to address the fertilizer-related challenges. However, none of them have shown promising results to date, globally (Gunaratne et al. 2016; DeRosa et al. 2010). Production of new and innovative fertilizers using nanotechnology based approaches is expected to resolve the issues of nutrient losses because of their nanoscale size and high surface to volume ratio (Gunaratne et al. 2016; DeRosa et al. 2010; Kottegoda et al. 2011; Dimkpa and Bindraban 2018; Pulimi and Subramanian 2016; Guo et al. 2018; de Silva et al. 2020). In this context, our group has introduced several types of NPK slow-release fertilizers. Urea-coated hydroxyapatite nanohybrids (HA–urea) have been used as a platform technology in order to derive different types of fertilizers. We have reported the efficacy of urea–HA nanohybrid with a N content of 40%, which is closer to that of commonly used urea, in rice cultivation (Kottegoda et al. 2017; Kottegoda, Priyadharshana, et al. 2014; Kottegoda, Siriwardhana, et al. 2014). The results suggested that up to 40% reduction in urea fertilizer with a 5–10% increase in the crop yields at farmer's level field trials. Further attempts have been made to encapsulate HA–urea nanohybrid into the cavities present in a softwood, *Gliricidia sepium*, in which the ultimate release characterizes are dominated by the rate of biodegradability of the plant material (Kottegoda et al. 2011; Kottegoda, Munaweera, Samaranayake, et al. 2013). In another innovation, HA–urea nanohybrid has been intercalated into layered nanomaterials, montmorillonite, and layered double hydroxides (Kottegoda, Munaweera, Madusanka, et al. 2013; Madusanka et al. 2017). The efficacy of these plant nutrient formulations have been studied using Ryegrass (Gunaratne et al. 2016). Furthermore, HA–citrate P slow-release formulation has been tested for corn (Samavini et al. 2018) and K intercalated montmorillonite nanocomposite has been tested at field level for rice (Sirisena et al. 2013). In addition, other groups have made attempts to incorporate urea into other nano-matrixes such as zeolite, clays, graphene, and layered materials (Manikandan and Subramanian 2017; Preetha and Balakrishnan 2017).

Reduction in fertilizer usage and number of applications are expected to have direct impact on the quality and the flavor of tea. It is noteworthy that Ceylon Tea is at high demand in world market due to its unique flavor. Therefore, development of such new fertilizer formulations are of high relevance and a timely need. This study focuses on the effect of HA–urea nanohybrids on tea yields in large scale fields in different climatic zones of Sri Lanka. Slow-release fertilizer has been applied at different frequencies and the soil and plant nutrient status, yield, and quality of tea have been investigated.

Materials and methods

Study location

Tea cultivating areas in Sri Lanka can be divided into four regions namely, Up Country (UC), Mid Country (MC), Low Country (LC), and Uva region. This experiment was carried out in three tea growing regions excluding UC. Nutrient losses in the UC is minimal compared to other tea growing regions due to low temperature, well-distributed rainfall, higher organic carbon content, and good bush cover. Each estate representing LC, MC, and Uva regions were selected for the study. Climatic, soil, and site information are given in Table 2. Soil and plant nutrient status before the commencement of trials is shown in Table 3.

Experimental design

The experimental design consisted of three replicates of seven treatments arranged in a randomized complete block design considering slope as a variant. Plots were surrounded by a guard row of tea bushes to prevent treatment effects in any adjacent plots. Each individual plot size was 33 m^2 and contained 40 bushes. Treatment details are given in Table 4. Four rounds of fertilizer treatments were applied per year.

HA–urea nanohybrid, (40% N and 6% P_2O_5) with a particle size <100 nm (Figure 1), produced by Sri Lanka Institute of Nanotechnology (Kottegoda et al. 2017) was applied as ground fertilizer (N and P source) along with MOP (K source) and compared with present TRI, Sri Lanka recommendation given to each region (Table 1).

Table 2. Experimental site information.

Site	LC	MC	Uva
Estate	Don Pedro	Kalabokke	Telbedde
Cultivar	TRI 2026	TRI 2023	TRI 2023, DN
Potential yield	4000 kg ha^{-1} yr^{-1}	3000 kg ha^{-1} yr^{-1}	3000 kg ha^{-1} yr^{-1}
Agro ecological region	WL1	WM3	IM3
Soil group[a]	Red Yellow-Podzolic Soil	Reddish Brown-Latasolic Soil	Red Yellow-Podzolic Soil
Soil series[b]	Dodangoda	Ukuwella	Badulla

[a]Moorman and Panabokke (1961).
[b]Mapa, Somasiri, and Nagarajah (1999).

Table 3. Soil and plant nutrient status of experimental sites before trial.

Location		Don Pedro (LC)	Kalebokka (MC)	Telbedde (Uva)
Soil chemical parameters	pH	4.68	5.66	4.80
	Organic C (%)	1.72	1.87	1.46
	Total N (g kg^{-1})	1.35	1.62	0.6
	P (ppm)	13	31	45
	K (ppm)	75	158	108
	Mg (ppm)	38	51	62
Leaf nutrient status (%)	N	2.68	2.78	3.03
	P	0.10	0.32	0.23
	K	1.08	1.03	1.49
	Mg	0.15	0.41	0.13
Fertilizer recommendation		VPLC/880	VPUM/910	VPUVA/945

Table 4. Treatment details of slow-release fertilizer trial.

Treatment	Description
T1	50% NPK through conventional fertilizers (4 Splits)
T2	50% NPK through conventional fertilizer (2 Splits)
T3	100 % N through (HA-urea nanohybrid) + 100% K through MOP (2 Splits)
T4	100 % N through (HA-urea nanohybrid) + 100% K through MOP (4 Splits)
T5	50% N through (HA-urea nanohybrid) + 100% K through MOP (4 Splits)
T6	50% N through (HA-urea nanohybrid) + 100% K through MOP (2 Splits)
T7	100% NPK through conventional fertilizer (4 Splits)

(T1, T2-VPLC/880-440 kg ha^{-1} yr^{-1} or VPUM/910-460 kg ha^{-1} yr^{-1} or VPUVA/945-472 kg ha^{-1} yr^{-1}; T3, T4-HA-urea nanohybrid-675 kg ha^{-1} yr^{-1}, MOP-167kg ha^{-1} yr^{-1}; T5, T6–HA-urea nanohybrid-338 kg ha^{-1} yr^{-1}, MOP-167 kg ha^{-1} yr^{-1}; T7-VPLC/880-880 kg ha^{-1} yr^{-1} or VPUM/910-920 kg ha^{-1} yr^{-1} or VPUVA/945-kg ha^{-1} yr^{-1}).

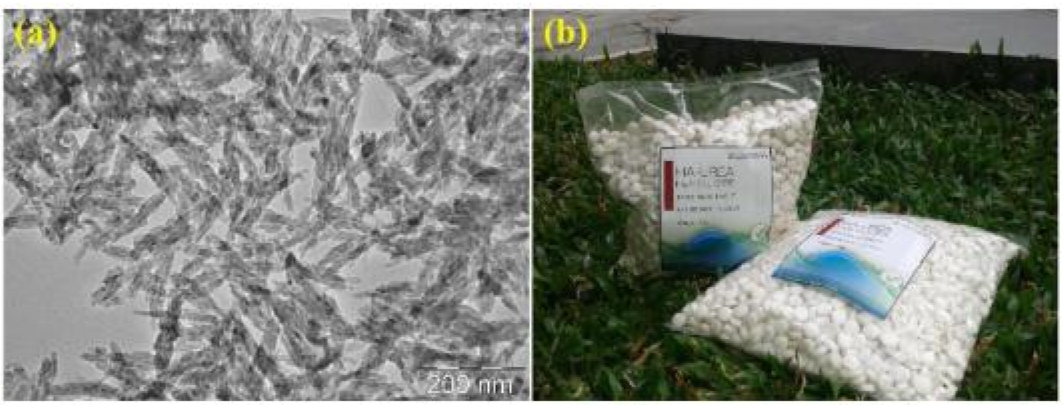

Figure 1. HA–urea nanohybrids (a) transmission electron microscopy (TEM) images at 200 nm scale; (b) pilot scale pelletized product developed at Sri Lanka Institute of Nanotechnology.

Soil sampling

Soil sampling was done at the end of each year. Soil samples at 0–15 cm depth were collected from three randomly selected locations within the plot. Samples were air-dried and sieved through 2 mm sieve prior to chemical analysis.

Plant sampling

Leaf sampling was done at the end of each year. Two mother leaves were collected from each bush in the plot to provide a composite sample. The leaves were oven dried overnight at 80 °C.

Dried samples were ground by using a micro hammer-cutter mill (1 mm) prior to chemical analysis.

Yield recording

Yield was recorded at 7–10 days interval throughout the experimental period. Harvest from each plot was weighed separately using a top loading balance. Tea yield was calculated by multiplying the fresh weight of harvest by 0.22.

Shoot density and shoot dry weight

Shoot density was determined by counting the mean number of harvestable shoots captured within a 0.09 m^2 grid randomly thrown thrice within each plot. Shoots falling within the grid were harvested, counted, and weighed. Shoots were oven dried overnight at 80 °C. Shoot dry weight was measured by dividing the dry weight by the number of shoots.

Weather conditions

Total precipitation during the LC trial period was 4859 mm and the mean temperature ranged from 30° to 35 °C. Total rainfall during the period is more than 25% higher than mean total rainfall of 3833 mm during the period of 2003–2013 (Meteorological observation, TRI, Ratnapura). Vast monthly variation in rainfall was observed during the trial (Figure 2) period. In Uva region, total rainfall during the trial period was 2373 mm and the mean temperature ranged from 23° to 28 °C.

Analytical procedures

Soil pH, available macronutrient concentrations in soil and collected leaf samples were measured. Soil pH was determined using a pH meter with the ratio of 1:2.5 (soil:water). Soil organic carbon was determined by Walkey and Black method (Nelson and Sommers 1996). Soil available phosphorous was determined by Borax extraction method (Beater 1949). Extraction of exchangeable K was done by using 1 M ammonium chloride (pH-7 adjusted) and determined using a flame photometer (Sherwood, Model 410). Total N in mature tea leaves was measured after Kjeldahl digestion. Mature leaves for P and K analysis were digested in a microwave oven (Anton Paar, Model Multiwave Go) using 50% aqua reagent and measured in spectrophotometer and flame photometer, respectively.

Tea shoots consisting of two leaves and an active bud were harvested from 40 bushes and converted to black tea, using miniaturized manufacturing techniques. Total color, brightness, theaflavin, and thearubigin content were measured using a spectrophotometric method as described by Roberts and Smith (1963). Total polyphenol was determined as gallic acid equivalent by the Folin–Ciocalteu colorimetric assay (ISO 14502-1, 2005). The total amino acid was determined using Ninhydrin method (Yemm, Cocking, and Ricketts 1955).

Statistical analysis

The collected data were statistically analyzed as completely randomized block design. Contrast analysis was performed with PROC ANOVA procedure using the Statistical Analysis System (SAS) Version 9.1. Mean separation was done using Least Significant Difference Test.

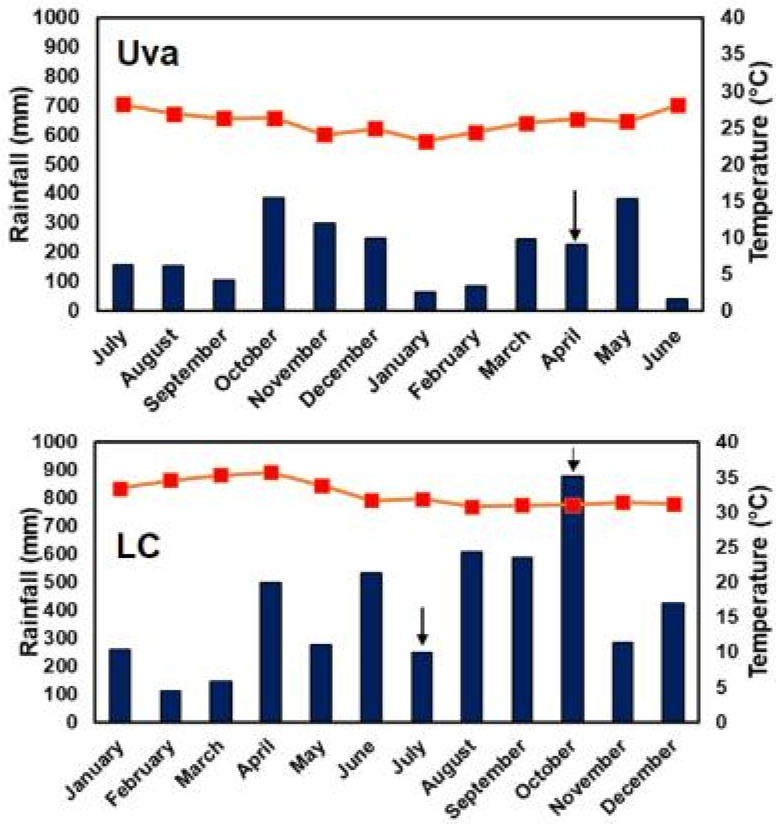

Figure 2. Monthly precipitation (bars) and monthly average temperature (line) during the trial period. Arrows show fertilizer application period.

Results and discussion

Effect of HA–urea nanohybrid fertilizer on mature tea yield

Results of slow-release fertilizer trials showed a significant yield difference among treatments in Low country and Uva tea growing regions (Figure 3). Yield increment due to application of HA–urea nanohybrids was observed in all the trials.

In LC and Uva regions, yields of pluckable shoots after application of slow-release fertilizer increased from 11% to 17% and 15% to 16%, respectively, compared with the yields obtained based on present fertilizer recommendations to those regions (T7). Yield increment observed in this experiment was similar to a slow-release fertilizer experiment carried out in China where they observed a 14% yield increase compared with uncoated urea (Han et al. 2008).

Yields from the fields where 50% of the recommended nitrogen amounts supplied through nano HA–urea in both 2 and 4 splits (T5 and T6) application were comparable to those obtained from the fields that followed the current recommendation (T7). The 1st year yields of T1 and T2 (50% N supplied via conventional urea) were comparable to those based on the present recommendation (T7) in the low country. However, a reduction in yields was noticed in trials carried out subsequently. Promisingly, in Uva region, T1 and T2 treatments showed a yield reduction with an average of 9%. During this trial, vast variation in temperature and heavy showers were experienced in these two regions. Therefore, the response of this slow-release fertilizer could not be clearly noticed. In these field trials, higher fertilizer use efficiency was achieved in T3 and T4

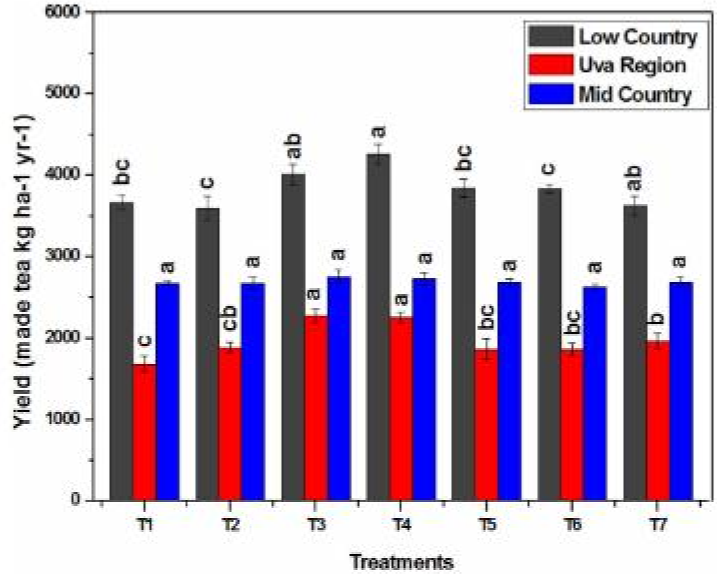

Figure 3. Effect of slow-release fertilizer on mature tea yield (kg $ha^{-1} yr^{-1}$). Different letters within a column show significant difference ($p \leq 0.05$).

compared with those from the present recommendations. In T3, where 100% N supplied through nano HA–urea in two splits saves the annual labor cost by half.

In the mid country trial, a significant difference in yield due to the application of nano HA–urea was not observed among treatments. A minute yield increment of 3% was observed in T3. Climatic conditions during the trial period were favorable for tea growth. During the trial period, average temperature ranged from 22° to 27 °C and 3450 mm of rainfall was received. Therefore, the positive effect of HA–urea nanohybrids was much less pronounced in this field experiment than the other regions.

Effect of nano-fertilizer on tea yield

Shoot density and shoot dry weight measurements were taken at Uva trial to find out its contribution to yield. Both shoot density and shoot dry weight showed a significant difference among treatments (Figure 4). HA–urea nanohybrids applied in four splits (T4) showed a significant difference in shoot density and shoot dry weight compared with present recommended conventional fertilizer (T7). However, HA–urea nanohybrids applied in two splits (T3) did not show any significant differences. Tea yield obtained in T4 was proven by the yield component analysis. Though T3 showed significant yield difference compared to T7 it was not reflected in yield component analysis.

Effect on quality indicators of black tea

Black tea quality was measured for the yield obtained from Uva trial. The application of slow-release fertilizer did not show any significant difference in tea quality parameters (Figure 5). Total color and brightness of tea infusions ranged from 3.67% to 4.58% and 20.3% to 24.6%, respectively. Good quality tea usually has higher values for brightness and theaflavin (TF) which contributes to brightness (Kottawa-Arachchi et al. 2011). Total polyphenol and total amino acids ranged from 20.3% to 24.6% and 1.26% to 1.48%. Good quality tea usually has a TR/TF ratio

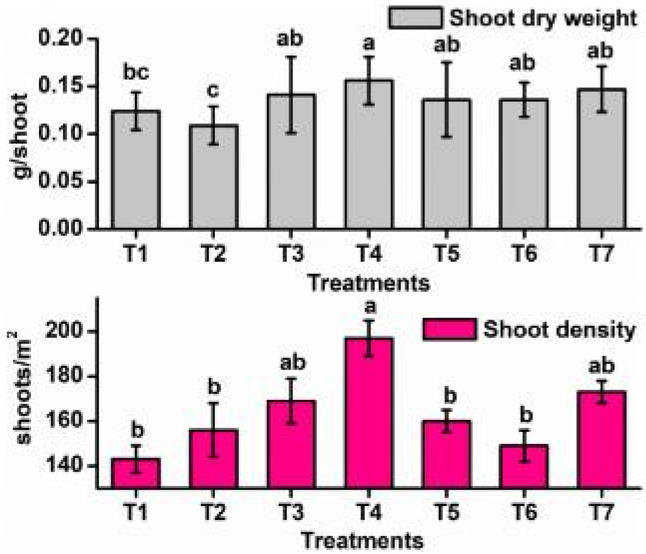

Figure 4. Effect of slow-release fertilizer on tea yield components. Different letters within a column show significant difference ($p \leq 0.05$).

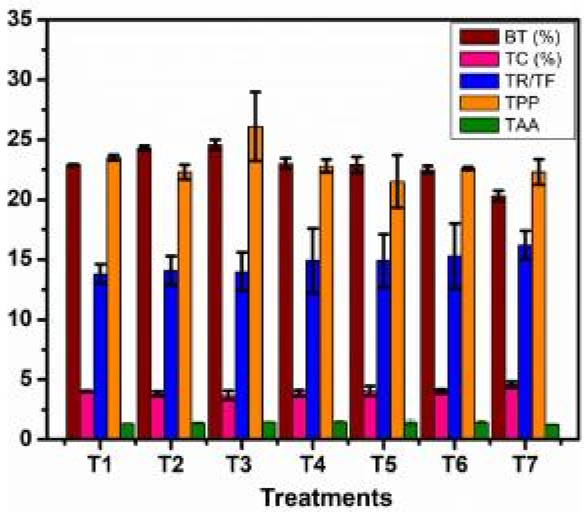

Figure 5. Effect of slow-release fertilizer on quality of black tea. BT, brightness of liquor; TC, total color of liquor; TR, thearubigin; TF, theaflavin; TPP, total polyphenol content; TAA, total amino acid content.

within the range of 10–12 (Roberts and Smith 1963). Nano-fertilizer applied treatments had TR/TF ratio within the range of 14–15.3 while the present recommended treatment had 16.2. Kottawa-Arachchi et al. reported that total color, brightness, total polyphenol, total amino acid, and TR/TF ratio in cultivar TRI 2023 during wet season were 4.06%, 19.41%, 21.8%, 1.18%, and 14.37, respectively (Kottawa-Arachchi et al. 2014). Application of nanohybrids comparatively increased the values of quality parameters such as brightness, total polyphenols, and total amino acids.

Soil nutrient status

In the trial carried out in LC, pH and organic carbon content of the study area did not significantly vary among treatments and ranged from 5.03% to 5.93% and 1.41% to 1.93%, respectively.

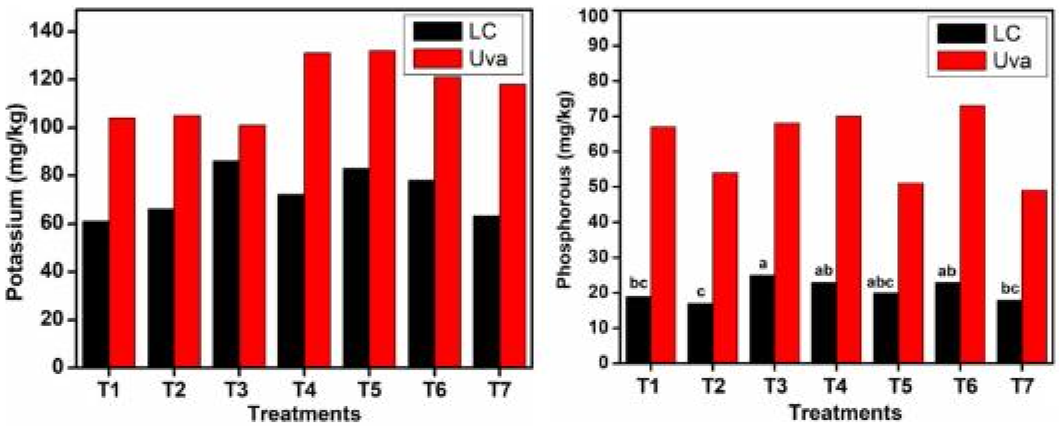

Figure 6. Soil nutrient status after the trial. Sufficiency level $p \geq 20\,mg\,kg^{-1}$, K 100–300 mg kg^{-1} (Hettiarachchi, Jayakody, and Gunaratne 2004).

The organic carbon content of Dodangoda soil series is 1–2% (Moorman and Panabokke 1961). Optimum pH range for better tea growth is 4.5–5.5. Soil phosphorous has significantly differed among the treatments. The higher values were observed in HA–urea nanofertilizer applied fields. HA–urea nanohybrid applied treatments had more P than the critical level while conventional fertilizer applied treatments had lower than the critical level (Figure 6). It may be due to higher P content in HA–urea nanohybrids (6.0% P_2O_5) than the present recommendation (4.10% P_2O_5). Soil potassium was not significantly different among the treatments. Soil potassium levels in all treatment applied plots were below the critical level despite the treatment. This could be due to the losses of applied K because of lower potassium specific sites and lower nutrient holding capacity of Dodangoda soil series.

In the Uva trial also pH and organic carbon content were not significantly different and ranged from 4.46% to 5.06% and 1.32% to 1.58%, respectively. Low organic carbon content of these soils may have resulted due to rapid degradation and mineralization of organic matter in the soil due to the prevailing high temperature in this region. All the treatments had more P than the sufficiency level and K was within the sufficiency level (Figure 6).

Effect on leaf macro nutrient concentration

Macro nutrient concentration of mother leaf samples is given in Figure 7. In the LC experiment, nitrogen and phosphorus concentration in the mother leaves significantly differed among the treatments, although all treatments were within the optimum range. K levels, except in one treatment, were found to be within the optimum level. At the Uva experiment, macronutrient concentrations were not significantly different among treatments and all treatments had concentrations within the optimum level.

Conclusion

Application of HA–urea nanohybrids results in enhancement of nitrogen use efficiency and reduces environmental impacts. Using the new fertilizer formulation up to 50% reduction of urea that is applied as the N source, was observed with up to 10–17% increment in the yields of tea. The urea–HA nanohybrids comparatively increased the quality parameters such as brightness, total polyphenols, and total amino acids in the resulting tea leaves. Finally, it can be concluded that use of HA–urea nanohybrid fertilizer is a potential alternative to reduce the high usage of urea fertilizer in tea cultivations, thus minimizing the amount of fertilizer needed in addition to reducing the number of applications.

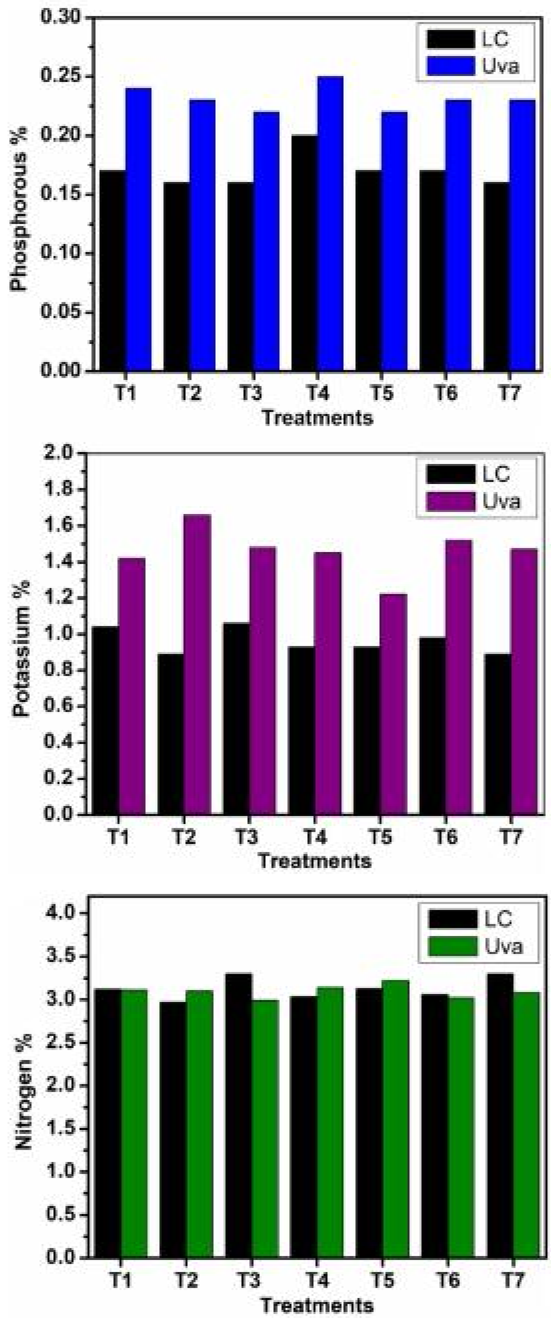

Figure 7. Macro nutrient concentration (%) in the mother leaves after the trial. Optimum ranges for leaf nutrients N (2.78–3.39%), P (0.12–0.15%), K (0.91–1.24%) (Kottawa-Arachchi et al. 2011).

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