ORGANIC VERSUS CONVENTIONAL FARMING OF TEA PLANTATION

Fui Seung Chin[1], Teck Yung Ho[2], Khim Phin Chong[1], Mohamadu Boyie Jalloh[1], & Nyet Kui Wong[2*]

[1]School of Sustainable Agriculture
Universiti Malaysia Sabah, Sandakan Campus, Mile 10 Jalan Sungai Batang, 90000 Sandakan, Sabah, Malaysia.
[2]School of Science and Technology
Universiti Malaysia Sabah, Locked Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia.

ABSTRACT. *A comparative study of organic and conventional farming system at two planting fields was conducted in Sabah Tea Plantation to determine the effect of management practices on soil nitrogen and pH, as well as leaf size and major polyphenol and caffeine content. Soil and leaves samples were from two selected fields; B29 (conventional field) and NO3 (organic field) in Sabah Tea Plantation, and were analyzed using UV spectrophotometer and high performance liquid chromatography methods. Organic farming system resulted in significantly higher soil pH (4.14), leaf length (15.14 cm), leaf width (7.33 cm) as well as major polyphenol content in tea shoot (172.42 mg/g) as compared to conventional farming system with soil pH (3.38), leaf length (13.19 cm), leaf width (5.58 cm) and major polyphenol content in tea shoot (107.03 mg/g). On the other hand, conventional farming system produced higher levels of ammonium (332.4 kg/ha) and nitrate (39.0 kg/ha) content in soil as compared to organic farming system (45.2 and 19.2 kg/ha, respectively). No significant difference in caffeine, epicatechin, epicatechin gallate and epigallocatechin content in leaves was observed between the two farming systems. The study provides some basic knowledge on soil nitrogen and pH level as well as tea major polyphenol and caffeine content in tea shoot of organic and conventional farming in Sabah Tea Plantation.*

KEYWORDS. Caffeine, Conventional, Nitrogen, Organic, Polyphenol

INTRODUCTION

Organic farming system (OFS) is the farming system that works in harmony with the nature without harming the natural environment or people who live or work in it. It creates a healthy balance between nature and farming, where crops and animals can grow, pests and weeds are to be controlled to an acceptable level for high yield benefits. Organic Farming system (OFS) is replacing conventional farming system (CFS) gradually due to increasing demands for organic food and growing environmental concerns. Studies reported that organic farming is able to increase the level of total nitrogen in soil and preventing nutrients leaching (Melero *et al*., 2006). On the other hand, conventional farming often gives many negative impacts such as soil erosion, nutrient runoff, loss of organic matter, impairment of environmental quality and pollution of natural water by agricultural chemicals (Diepeningan *et al*., 2006). Pests and diseases become more difficult to control as they become resistant to chemical fertilizers. In addition, the prolonged use of chemical fertilizers can stay in the soil for a long time and enter the food chain where they build up in the bodies of animals and humans, causing health problems.

Sabah Tea Plantation (STP) is in the lush tropical wilderness of Malaysia’s first ever World Heritage Site, Mount Kinabalu, sits on a 6,200-acre land at 2,272 feet above sea level.

It is surrounded by the world's oldest rainforest of about 130 million years. It is the only tea plantation in the state of Sabah, where it is also the third largest tea plantation after BOH tea and Bharat Tea in Cameron Highland, Malaysia. Sabah Tea Plantation is the largest single commercial tea plantation in Borneo with approximated area of 1,000 acres endowed with an interesting plant and agriculture resources, Camellia Sinensis. It is also one of the very few tea plantations in the world that certified by SKAL International (a professional organization of tourism leaders around the world based in Netherlands) to produce organic tea (Vanar, 2005). The organic farms in Sabah Tea Plantation are explored and developed from the deep tropical forests as shown in Figure 1.

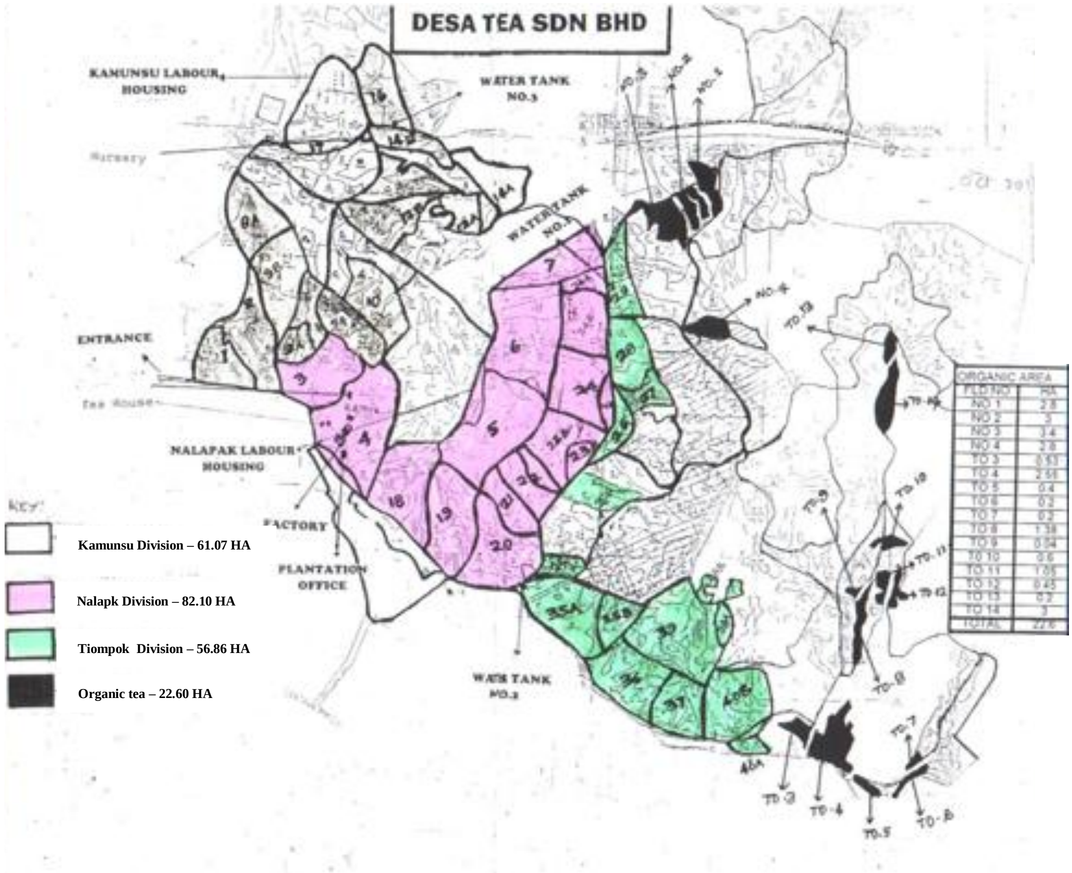

Figure 1. Organic Farms in Sabah Tea Plantation that Explored and Developed

Very little research was done on tea crop in Sabah regarding its soil nutrients under organic and conventional farming system and their potential effect on tea leaf growth as well as polyphenol and caffeine content in tea shoots. Polyphenols are of low molecular weight, secondary plant metabolites that consisted of a flavan nucleus as the main chemical backbone (Heim *et al*., 2002). They are usually water-soluble and are frequently stored in cell vacuole in combination with a sugar as glycosides (Dufresne and Farnworth, 2001). The four major catechins in tea, namely (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin-3-gallate (EGCG), together with gallic acid (GA) constitute approximately two-third of the total polyphenolic content in tea shoots (Zhen, 2002). Caffeine (1,3,7-trimethylxanthine) is a N-containing aromatic organic compound, where it is also the major alkaloid in tea plant (Ashihara *et al*., 2008).

This study was done to compare effect of different farming system (organic or conventional) on soil nitrogen (ammonium NH_4^+ and nitrate NO_3^-), pH content, tea leaf growth as well as major polyphenols and caffeine content in tea shoots. This study provides a basis understanding of the impact of differential agricultural practices on soil nutrient contents as well as plant biochemical contents.

MATERIALS AND METHODS

Study site

Sabah Tea Plantation (STP) is located at Kampung Nalapak (5° 55' 58.53" North, 116° 46' 22.44" East) of Ranau, Sabah with approximately 2,978 km^2 tea plantation area. The mean temperature of the study site ranges from 25 to 32 °C and the annual rainfall is approximately 2,000 mm $year^{-1}$. Soil used in tea plantation mainly consists of sandy loam texture with pH range from 4.0 to 4.5. two fields were selected for this study; Field B29 (conventional farmed field) and Field NO3 (organic farmed field), which share the same characteristics such size of area, altitude, date of tea planting and type of tea planted.

Samples

A total number of 18 soil samples (each soil sample consists of five sub-soil samples which were sampled at depth of 0 to 15 cm); nine were collected from Field B29 and another nine were from Field NO3, together with 108 middle tea leaves were randomly sampled from the two field in STP. For tea major polyphenol and caffeine content analysis, 25 tea shrubs were randomly selected from each Field B29 and Field NO3, where three newly formed tea shoots (consisting of the first two leaves and a bud) were plucked from each of the tea shrubs. All samples were air-dried at 30 to 35 °C and ground and sieved through a two mm mesh before chemical analysis was done.

Physical measurement and Chemical analyses

Soil pH was determined using pH meter with soil-water ration, 1:2.5 (Anderson and Ingram, 1993). Leaf size (length and width) was measured across the centre section of the leaf manually using ruler. Soil available nitrogen (N) in the form of ammonium (NH_4^+) and nitrate (NO_3^-) were determined at 636 nm and 410 nm respectively, following the method as described by Page *et al*. (1982), and Anderson and Ingram (1993) with the use of Varian 50 Win UV Spectrophotometer. Briefly, 2.5 g of soil was extracted with 25 mL of 2M KCl solution for an hour under continuous mechanical shaking and filtered. Then, 1 mL of EDTA reagent was added to 1 mL of the 2M KCl filtrate in 25 mL volumetric flask, and allowed to stand for one minute before 2 mL of phenol-nitroprusside reagent was subsequently added, followed by 4 mL of buffered hypochlorite solution. The flask was topped-up to 25 mL with distilled water and placed in water bath at 40 °C for 30 minutes. The absorbance of the colored complex was determined at wavelength of 636 nm against a blank solution for NH_4^+ content. For NO_3^- analysis, series of standard solution of NO_3^- were prepared, then 1.0 ml of 5% (w/v) salicyclic acid reagent (prepare by dissolving 5.0 g salicylic acid in 95 mL of concentrated sulphuric acid) was added into 1.0 ml of each standard solution as well as samples. All samples and standard with salicyclic acid reagent were vortex-mixed and left for 30 min, followed by 10.0 ml of sodium hydroxide reagent and left for an hour for colour development. Each standard and sample was then read at 410 nm. The final concentration of nutrient-N in soil filtrate (µg/mL) obtained from spectrophotometer was converted into kg ha^{-1} by multiplying with 2.0×10^6 (assume that one hectare of soil at the depth of 15 cm is equivalent to 2.0×10^6 kg).

Major polyphenol and caffeine content analysis

Major polyphenol and caffeine content in tea shoot were extracted using direct extraction method of Chin et al. (2009), which carried out with three series of subsequent extraction using 20% (v/v) aqueous acetonitrile in the sample weight (g) to solvent volume (mL) ratio of 1:10 (w/v). Quantification of major polyphenol (EC, ECG, EGC, EGCG and GA) and caffeine content in tea shoot was done by external standards of epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), gallic acid (GA) and caffeine (Caf) purchased from Sigma Chemical Co. (St. Louis, MO) using high performance liquid chromatography (HPLC) technique reported by Chin et al. (2010)

Quantification analysis

An Agilent Technologies 1200 series High Performance Liquid Chromatography (HPLC) system comprising vacuum degasser, quaternary pump, auto-sampler, thermostatted column compartment, and VWD detector was used for non-volatile components identification. The column used was an Eclipse XDB-C18 reversed phase (5 µm, 250 x 4.6 mm) with a C18 (5 µm, 30 x 4.6 mm) guard column. All solvents were filtered through a 0.45 µm millipore membrane filter disk and degassed before injection into a HPLC system. A gradient elution was carried out using the following solvent systems: Mobile phase A (0.1% (v/v) ortho-phosphoric acid), Mobile phase B (100% acetonitrile). Then, a linear gradient to 80% Mobile phase A (0.1% (v/v) ortho-phosphoric acid) and 20% mobile phase B (100% acetonitrile) was achieved in 30 min, following by a post-run of 3 min before next analysis cycle. Column temperature was set at 35^{o}C and the effluent was monitored at 280 nm with a flow rate of $1mL.min^{-1}$. The injection volume was set at 20 µL for each sample.

Statistical analysis

Differences among means were detected by one way ANOVA followed by LSD Post Hoc multiple comparison test. Results were considered significant at $p < 0.05$

RESULTS AND DISCUSSION

Effect of farming systems

The overall comparison between all of the parameters studied is shown in Figure 2. It shows that farming systems only significantly affecting soil ammonium ($p = 0.010$), soil nitrate ($p = 0.041$), soil pH ($p = 0.000$), leaf length ($p = 0.000$), leaf width ($p = 0.000$), epigallocatechin gallate ($p = 0.001$) and gallic acid ($p = 0.024$). Caffeine ($p = 0.530$), epicatechin ($p = 0.051$), epicatechin gallate ($p = 0.071$) and epigallocatechin ($p = 0.868$) are not significantly affected by the two farming systems.

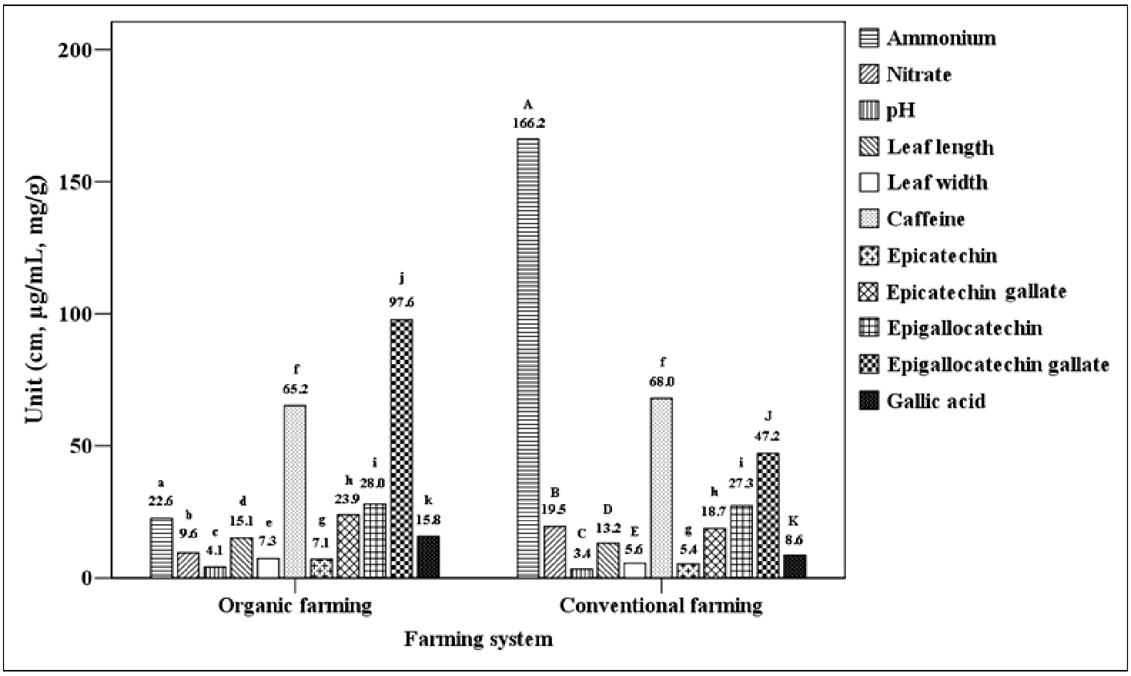

Figure 2. Mean values for eleven parameters studied under two different farming systems.

Bars with the same letter in lower case indicate no significant differences ($p < 0.05$).
Note: Unit of cm is for leaf length and leaf width, µg/mL is for soil ammonium and nitrate, and mg/g is for caffeine, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate and gallic acid.

Effect of farming system on soil ammonium
NH_4^+ content in CFS is 332.4 kg/ha whilst in OFS is 45.2 kg/ha of NH_4^+. Higher concentration of soil NH_4^+ in CFS (332.4 kg/ha) may due to excessive application of nitrogenous fertilizers. In tea plantations, excessive amounts of fertilizers are usually applied to ensure N is available for tea crop use. It is explained by the fact there are frequent harvests of tender young shoots containing high N content and in addition, regular pruning, removing large amounts of biomass (leaves, twigs) is carried out to maintain a vigorous vegetative growth (Vorgelegt, 2005). Whereas in OFS, organic fertilizer nutrient availability to plants depend on many environmental factors such as aeration, moisture, pH and temperature of soil, as well as the C: N ratio of the organic materials before decomposition and mineralization can take place (Zech *et al*., 1997). In other words, organic fertilizers require more time before nutrients become available for plant use as compared to chemical fertilizers (Tisdale and Nelson, 1975).

Effect of farming system on soil nitrate
Soil NO_3^- content in CFS and OFS are 39.0 kg/ha and 19.2 kg/ha respectively. Higher concentration of soil NO_3^- in CFS (39.0 kg/ha) than in OFS (19.2 kg/ha) can be explained by the same reason of fertilizers use. Low content of NO_3^- content in both CFS and OFS may due to leaching and denitrification as NO_3^- is known to be very mobile in soil and moves largely with the soil water. Under conditions of excessive rain, it will leach out from the upper horizons of the soil into deeper layers (Tisdale and Nelson, 1975). Diepeningen *et al*. (2006) also reported similar results in their study comparing the effects of organic and conventional management on chemical and biological parameters in agricultural soil. They suggest that soil type (clay or sandy soil) has a stronger effect on the soil characteristics rather than management type. Vorgelegt (2005) reported tea plants were adapted to NH_4^+ as the sole N

source due to their large capacity for NH_4^+ assimilation and growth as well as caffeine and polyphenol content does not response positively with increasing NO_3^- supply. Hence, the different in total NO_3^- content in both fields is of minor importance in tea growing soil.

Effect of farming system on soil pH

The soil pH measurement of CFS and OFS is pH 3.38 and pH 4.14 respectively. CFS is more acidic as compared OFS. Studies showed that nitrogenous fertilizers used in conventional tea cultivation such as ammonia sulphate, ammonium nitrate, ammonium sulphate nitrate, urea, calcium ammonium nitrate and ammonium chloride leading to acidity (Tee *et al.*, 1987; Ma *et al.*, 1990). In addition, continuous use of ammonium sulphate without the addition of lime (calcium carbonate) will reduce the soil pH (below 4.0) to level that is unsuitable for economic production of crops (Tisdale and Nelson, 1975).

Effect of farming system on tea leaf growth

Increased amount of N in the soil correlates positively with the tea leaf growth. However, when excessive amount of N present in soil or overdose, tea leaf growth will become toxic and retard the plant growth. This could be the reason for the lower tea leaf size observed in the CFS that had abundant NH_4^+ in soil. Wilson and Clifford (1992) reported, for unshaded tea, the increase in crop yield is directly proportional to the amount of N applied. Salisbury and Ross (1992) reported that excessive supply of N in soil leads to N-toxicity in plant. Plant toxicity can retard the growth and development of tea leaf and might consequently resulted in lower leaves size.

Effect of farming system on tea shoots major polyphenol and caffeine content

Concentrations of EGC, ECG and EC decreased slightly ($p > 0.05$) in the CFS. Large NH_4^+ supply in CFS soil significantly resulted into lower total major polyphenol content ($p = 0.002$) in young tea shoots, which agrees with previous findings in tea (Vorgelegt, 2005). The high N content in the CFS reduced EGCG and GA concentrations by 48% and 54%, respectively. Concentration of caffeine in tea shoots remained insignificantly different in both farming system ($p = 0.530$), despite high N supply in CFS. This observation suggests that biosynthesis of polyphenols is indirectly dependent upon N status of plants. Structurally flavonoids are carbon-based metabolites. It is assumed that their production is determined by availability of carbohydrates and increasing N supply often leads to decreasing of carbohydrate status in plants (Bryant *et al.*, 1983). Vorgelegt (2005) reported that concentrations of major catechins EGC, ECG and EC in young shoots decreased significantly with a concomitant reduction of glucose and fructose, but not sucrose concentrations when large N is present. This suggested that production of these catechins and caffeine were possibly linked to the availability of sugars. Apart from that, decreased of major polyphenols and caffeine synthesis in young shoots under ample N supply could be a result of low carbohydrate availability due to reduced photosynthate import from the photosynthetically active matured leaves. As the supply of NH_4^+ increased, C demand by roots would be raised so the NH_4^+ could be assimilated under abundant N supplied in soil. Thus, roots could in turn strongly compete for carbon (C) with young shoots leading to decrease substrates available for formation of polyphenols and caffeine in the young shoots.

Table 1. Caffeine and major polyphenol concentrations (mg/g) in young shoots of tea plants grown under different farming system.

Parameter	Mean ± standard deviation		ANOVA
	Organic Field (NO3)	Conventional Field (B29)	(*P*-value)
Caffeine (mg/g)	65.23 ± 10.37	67.99 ± 7.70	0.530
Epicatechin (mg/g)	7.13 ± 1.87	5.35 ± 1.71	0.051
Epicatechin gallate (mg/g)	23.86 ± 4.15	18.69 ± 6.87	0.710
Epigallocatechin (mg/g)	27.98 ± 8.44	27.26 ± 9.67	0.868
Epigallocatechin gallate (mg/g)	97.64 ± 31.60	47.18 ± 21.18	0.001
Gallic acid (mg/g)	15.80 ± 8.17	8.62 ± 2.81	0.024
Total Major Polyphenols	172.42 ± 44.56	107.03 ± 27.10	0.002

A *p*-value less than 0.05 indicate significant difference by LSD (ANOVA). Total major polyphenols is the sum of five parameters above except caffeine.

CONCLUSION

This study provides some basic knowledge about soil nitrogen content, pH as well as tea shoots caffeine and major polyphenol content of Sabah Tea Plantation. It has shown that the OFS has a higher polyphenol contents than CFS. Additionally, types of farming systems do have significant effect on soil pH, NH_4^+ and NO_3^- content, leaf length, leaf width, and total major polyphenol content which are main active constituents in drinking tea. Considering the importance of caffeine and polyphenols for tea quality and beneficial functions to human health, it is feasible that farming systems may be manipulated to produce tea that is rich in these compounds. Sabah Tea Plantation can adopt this data to manage their fertilizer addition to monitor the quality of Sabah Tea.

ACKNOWLEDGMENTS

This work is supported by the Fundamental grant Malaysia.

REFERENCES

Anderson, J. M. and Ingram, J. S. I. (Eds.). 1993. *Tropical Soil Biology and Fertility – A Handbook of Methods*. Second edition. United Kingdom: CAB International.

Ashihara, H., Sano, H. & Crozier, A. 2008. Review. Caffeine & related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. *Phytochemistry*. **69**:841-856.

Bryant, J. P., Chapin III, F. S. & Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* **40**:357-368.

Chin, F. S., Chong, K. P., Markus, A. & Wong, N. K. 2009. The impact of different extraction methods on yield of tea polyphenols and alkaloids. *8th Annual Seminar of Science and Technology 2009*.

Chin, F.S., Chong, K. P., Lum, M. S., Hong, C. Y., Markus, A. & Wong, N. K. 2010. A simple HPLC method for simultaneous analysis of tea polyphenols & alkaloids. *PEREKA 2010*. Universiti Malaysia Sabah, April 05-06.

Diepeningen, A. D., Vos, O. J., Korthals, G. W. & Bruggen, A. H. C. 2006. Effects of organic versus conventional management on chemical and biological parameters in agricultural soils. *Applied Soil Ecology*. **31**(2): 120-135.

Dufresne, C. J. & Farnworth, E. R. 2001. A review of latest research findings on the health promotion properties of tea. *Journal of Nutritional Biochemistry*. **12**:404-421.

Heim, K. E., Tagliaferro, A. R. & Bobilya, D. J. 2002. Flavonoid antioxidants: chemistry, metabolism and structural-activity relationships. *Journal of Nutritional Biochemistry*. **13**:572-584.

Ma, W. Cl., Brussaard, L. & Ridder, J. A. 1990. Long-term effects of nitrogenous fertilizers on grassland earthworms (Oligochaeta: Lumbricidae): Their relation to soil acidification. *Agriculture Ecosystem and Environment*. **30**(2): 71-80.

Melero, S., Porras, J. C. R., Herencia, J. F. & Madejon, E. 2006. Chemical and biochemical properties in a silty loam soil under conventional and organic management. *Soil & Tillage Research*. **90**(2): 162-170.

Page, A. L., Miller, R. H. & Keeney, D. R. 1982. *Ammonium by Colorimetric Method – Indophenol Blue Method. Method of Soil Analysis Part II – Chemical and Microbiological Properties.* American Society of Agronomy. Pp: 672-676.

Tee, B. G., Dudas, J. M., Pawluk, S. and Harapiak, J. T. 1987. Physical, chemical and micromorphological effects of nitrogen fertilizers on Chernozemic soils. *Geoderma*. **40**(2): 177-192.

Tisdale, S. L. & Nelson, W. L. 1975. *Soil Fertility and Fertilizers*. Second edition. New York: Macmillan Publishing Co.

Vanar, M. 2005. Sabah Tea acquires organic certification. *The Star* (December 10), p 10.

Vorgelegt, V. 2005. *Quality-related Constituents in tea (Camellia sinensis* (L.) O. Kuntze) as affected by the form and concentration of nitrogen and the supply of chloride. Ph.D. Thesis. Christian-Albrechts-Universität zu Kiel (University of Kiel).

Willson, K. C. & Clifford, M. N. 1992. *Tea – Cultivation to Consumption*. First edition. United Kingdom: Chapman and Hall.

Zech, W., Senesi, N., Guggenberger, G., Kaiser, K., Lehmann, J., Miano, M. T., Miltner, A. & Schroth, G. 1997. Factors controlling humification and mineralization of soil organic matter in the tropics. *Geoderma*. **79**: 117-161.

Zhen, Y. S. 2002. *Tea Bioactivity and Therapeutic Potential*. London and New York: Taylor & Francis.