

## Effect of bio-extracts on soil properties and tea-oil camellia (*Camellia oleifera*) yield grown in a mountainous area of northern Thailand

Kulapat Yimpak<sup>1, 2</sup>, Nattaporn Prakongkep<sup>2\*</sup> and Worachart Wisawapipat<sup>1\*</sup>

<sup>1</sup> Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

<sup>2</sup> Office of Science for Land Development, Land Development Department, Bangkok 10900, Thailand

**ABSTRACT:** Mountainous soils are prone to a gradual decline in soil health and crop productivity, affected by unsuitable agricultural measures. To sustain hilly natural resources, simple and practical measures to improve soil physical and chemical quality, plant production, and hill tribe revenues are prerequisites. Herein, we examined three measures: 1) banana shoot bio-extract, 2) chili bio-extract, and 3) mixed banana shoot-chili bio-extracts compared to water use as a control treatment for changes in soil health, plant yield, and economic return in a remote mountainous area of northern Thailand. A 0.6 L of the bio-extracts or water was supplied weekly to the surface soil underneath each tea-oil camellia plant, a perennial plant grown for soil conservation, for two consecutive years. The results revealed that the bio-extracts were rich in N, K, and Ca, with different micronutrients (Fe, Cu, Mn, and Zn). The chili bio-extract significantly ( $P < 0.05$ ) enhanced the formation of mesoaggregate fraction (0.25-2 mm, 54% of the total mass fraction) compared to the control (46% of the total mass fraction). The mixed bio-extract increased the soil pH, available P, and exchangeable Ca and Mg. The mixed bio-extract significantly promoted a higher tea-oil fresh fruit yield (3,594 kg/ha) than the control (2,662 kg/ha). The average annual revenue of the hill tribe from the mixed bio-extract application after two years was 2,478 USD/ha that is higher than the control of 1,262 USD/ha. The estimated revenue from using mixed bio-extract across the total area of the tea-oil production (589 ha) in Thailand was 1.46 million USD/year, gaining a higher profit margin of about 0.72 million USD/year than the control. This study highlights the simple and practical use of mixed plant-based extracts to improve soil physical and chemical properties and enhance tea-oil yield in the hill area.

**Keywords:** highland; economic benefit; microbial activator; soil management; soil nutrient

### Introduction

Mountainous areas are vital resources for agriculture, environments, and humans. These mountainous areas are the primary habitats of hill tribes whose lives have been involved in maize-shifting cultivation for several decades (Bruun et al., 2017; Charoenratana et al., 2021). However, shifting cultivation is an improper soil conservation measure, deteriorating soil physical, chemical, and biological properties and causing poor soil health, low crop productivity, and unsustainability of the well-being of the local hill tribes (Arunrat et al., 2023a; Arunrat et al., 2023b; Rahman et al., 2012). Currently, the accessibility of chemical and organic fertilizers to such remote and hilly areas remains costly and highly challenging. Therefore, conservation measures in such remotely highland areas should be simple, and soil amendment targeting to be applied should be locally found and easily affordable in the areas.

\* Corresponding authors: [asoil@hotmail.com](mailto:asoil@hotmail.com) (N.P.), and [worachart.w@ku.th](mailto:worachart.w@ku.th) (W.W.)

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However, limited evidence-based research focused on locally obtainable soil amendments with simple measures is poorly understood to date.

To solve the complexes of soil degradation and the well-being of local hill tribes, single and simple procedures need to be adopted progressively. One key measure expected to impact the area was ceasing maize shifting cultivations and introducing perennial plants for reforestation, decreasing soil erosion, improving soil health, and supporting hill tribe revenue sustainability. A potential plant for such soil conservation and human economics is the camellia oil tree (*Camellia oleifera* Abel.), which originated and has been cultivated in China for over 1,000 years (Deng and Zhang, 2020; Hu and Yang, 2018). Such tea-oil plant provides bio-oil with abundances of polyunsaturated fatty acids (i.e., linoleic and linolenic) and monounsaturated fatty acids (i.e., oleic) without trans-fat presentation, resembling olive oil (Distnaree, 2018). Its nutritional compositions have increased demands for commercial uses in high-value markets, such as supplementary healthy food, pharmaceutical, and cosmetics industries (Luan et al., 2020).

The *C. oleifera* was introduced into highland areas of Thailand in 2011 by Her Royal Highness Princess Maha Chakri Sirindhorn. The oil tree has been extensively cultivated at Pang Mahan village, Mae Fah Luang district, Chiang Rai province of Thailand. However, fruit yield gained in this area remains low and unstable, which may be induced by a lack of effective nutrient management guidelines. The area of Pang Mahan village is located in a highland (800 m above mean sea level, MSL) and hilly and mountainous regions (slope >35%) (HRDI, 2019), where shifting cultivation of maize was typically agricultural practice causing bald mountains (HRDI, 2017). Highly degraded red soils with major soil physical restrictions of high erosion and drought in summer are typically found in the area (He et al., 2001). Moreover, such soil types inherit many limitations of soil chemical properties, including soil acidity, Al toxicity, poor organic matter and different plant nutrients (e.g., nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg)), and lowering crop growth and production (Borhannuddin Bhuyan et al., 2019; He et al., 2011; Muindi et al., 2015; Wang and Zhang, 2016; Zhang et al., 2009). Such highly degraded soils in sloping areas are seriously needed for soil-water conservation practices and nutrient management technology to sustain plant production.

Microbial inoculants are considered a nutrient management strategy for soil restoration. (Chaudhuri et al., 2017). Microbial activator Super LDD2, a product from the Land Development Department used for soil improvement, increases plant nutrients and plant growth hormones. It comprises a consortium of microorganisms, including *Pichia membranifaciens*, *Lactobacillus fermentum*, *Bacillus megaterium*, *B. subtilis*, and *Burkholderia unamae*. Microbial activator Super LDD7, another product for pest control, consists of *Saccharomyces cerevisiae*, *Gluconobacter oxydans*, and *L. fermentum*. Various materials, such as fresh and succulent fruits, vegetables, fish, and snails, are used as substrates to multiply the Microbial activator Super LDD2. This bio-extract usually contains plant growth hormones (auxin, gibberellin, cytokinin), amino acids, humic acid, organic acids, vitamins, and minerals. Meanwhile, herbs such as tobacco, long pepper, derris, asiatic bitter yam, and chili are used as substrates for Microbial Activator Super LDD7, stimulating the production of alcohol and organic acids to extract active ingredients (Land Development Department, 2019). They have a positive impact on soil, plants, and ecosystems by enhancing plant productivity and improving soil health. They mobilize soil nutrients (phosphorus, sulfur, zinc, potassium, and iron), add atmospheric nitrogen to the soil, and improve soil structure (Mofokeng et al., 2021). Additionally, they secrete substances that attach to soil particles and promote soil aggregation stability. Bacteria produce exopolysaccharides, proteins, and other metabolites, while

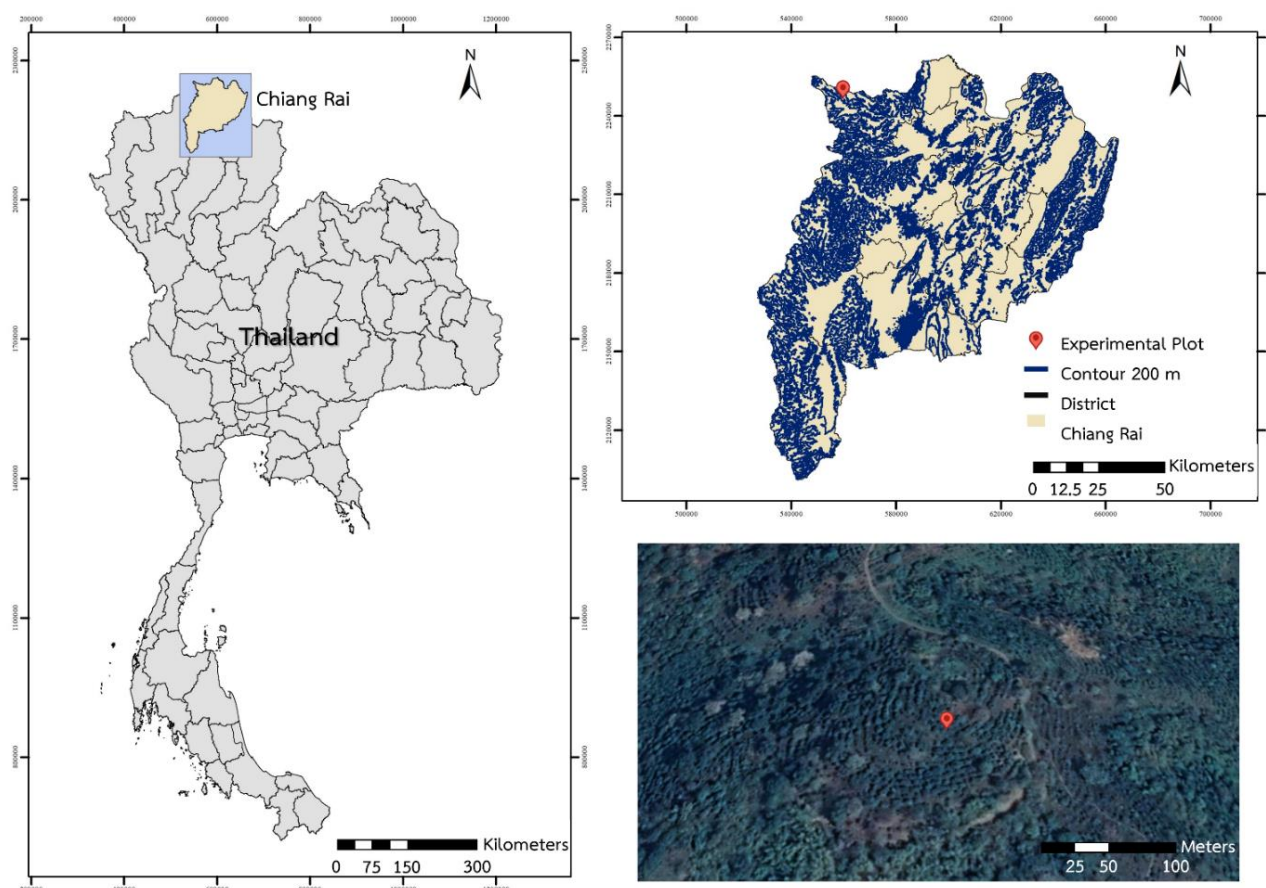
fungi form a hyphal network and exude proteins, organic acids, and polysaccharides to trap soil particles (Rashid et al., 2016).

This study aims to find and offer a simple and practical procedure to rehabilitate degraded highland soils to enhance the fruit yield of the tea-oil camellia. Here, we tested the effect of a weekly supply of bio-extracts derived from banana and chili, local plant residues, fermented with microbial inoculants on changes in soil physical and chemical properties and yield of the tea-oil for two consecutive years. The income and profit of these bio-extract applications were also assessed to obtain economic feasibility. The results obtained from this study will provide a simple and practical procedure for the sustainability of soil and human resources in mountain areas.

## Materials and Methods

### Study Site

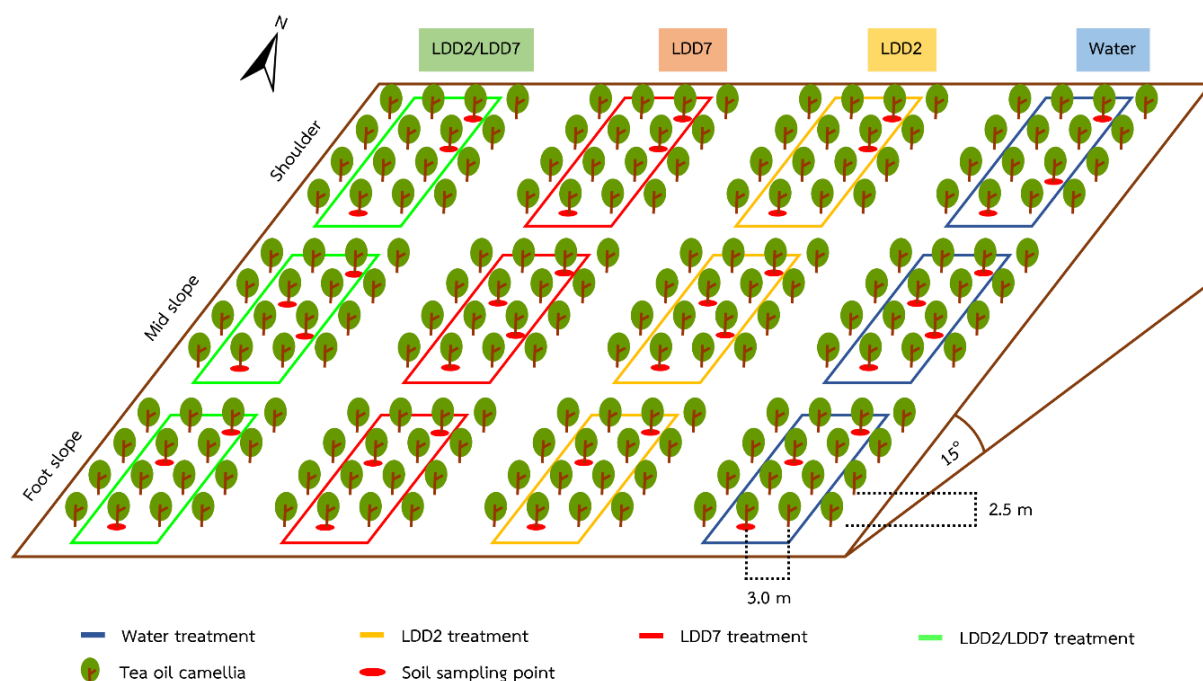
The studied mountainous area used for tea-oil camellia plantation is in Pang Mahan village, Mae Fah Luang district, Chiang Rai province, Thailand (20°18'48.6"N 99°34'22.1"E) (**Figure 1**). The field experiment was conducted from March 2019 to October 2021. The studied site is under a tropical climate, with a mean annual rainfall of 1,314 mm and a mean temperature of 26.4 °C (min-max = 20.6-32.2 °C). The area comprises hilly terrain located 930 m above MSL, with a slope in the degree of 15° (27% slope). The soil was locally named the Doi Pui soil series (Kandic Palehumults). Based on the profile description, the soil had a medium to slightly acid reaction, well-drained conditions, a slow to rapid runoff, and moderated soil permeability. Topsoil colors ranged from very dark brown to dark reddish brown, whereas subsoil colors ranged from reddish brown to red. The soil texture was loamy for topsoils and sandy loam to loam for subsoils. Soil profile data were collected before conducting the experiments. Three replicates of soils were taken from each topography: shoulder, mid-slope, and foot slope. The collected soil samples were air-dried, ground, and sieved through a 2 mm sieve. This process was conducted to observe any potential physiochemical variations in the soil and ensure homogenous testing for soil chemical properties. The finely ground soil materials, with particle sizes less than 0.5 mm, were prepared for analyzing organic matter (OM).



**Figure 1** The studied area of tea-oil camellia plantation in Chiang Rai, Thailand.

### Experimental Design

The experimental design was arranged in a Randomized Complete Block Design with four treatments and three blocks of different slope areas (i.e., shoulder, mid-slope, and foot slope) (**Figure 2**). Each experimental plot was sized 10 m in length and 6 m in width, containing eight tea-oil plants per plot. The plants, approximately 16-17 years old, were initially planted in 2006 as 3-4 year seedlings organized in rectangular plantation systems measuring 2.5 m by 3 m. The plant height and canopy looked resemble within the experimental area. The four treatments included 1) water as control, 2) banana shoot bio-extract (LDD2), 3) chili bio-extract (LDD7), and 4) mixed LDD2 and LDD7 bio-extract (LDD2/LDD7). The bio-extracts were diluted at 1:500 (bio-extract to water) with a total volume of 0.6 L of water, or the bio-extracts were supplied weekly for each plant. The bio-extracts or water were poured directly onto the soil surface within the canopy of the trees. All water or bio-extract applications were continued for two years, from March 2019 to October 2021, without the addition of chemical fertilizers or additional water.



**Figure 2** A layout of experimental plots for sampling soil and tea-oil camellia samples. The color of the plot line shows four treatments: the blue is sole water (Water), the yellow LDD2 bio extract (LDD2), the red LDD7 bio extract (LDD7), and the green LDD2/LDD7 bio extract (LDD2/LDD7).

The bio-extracts (LDD2 and LDD7) were prepared by mixing Microbial Activator products of the Land Development Department (LDD) with local plant residues (banana shoot or chili). The LDD2 bio-extract was fermented from 40 kg of chopped banana shoot, with 25 g (one pack) of Microbial Activator Super LDD2, 10 L of water, and 30 kg of molasses. The mixture was fermented for 7 days before application. The LDD7 bio-extract was prepared using 3 kg of chopped chili, 25 g (one pack) of Microbial Activator Super LDD7, 10 L of water, and 10 kg of molasses. This mixture was fermented at least 21 days before use.

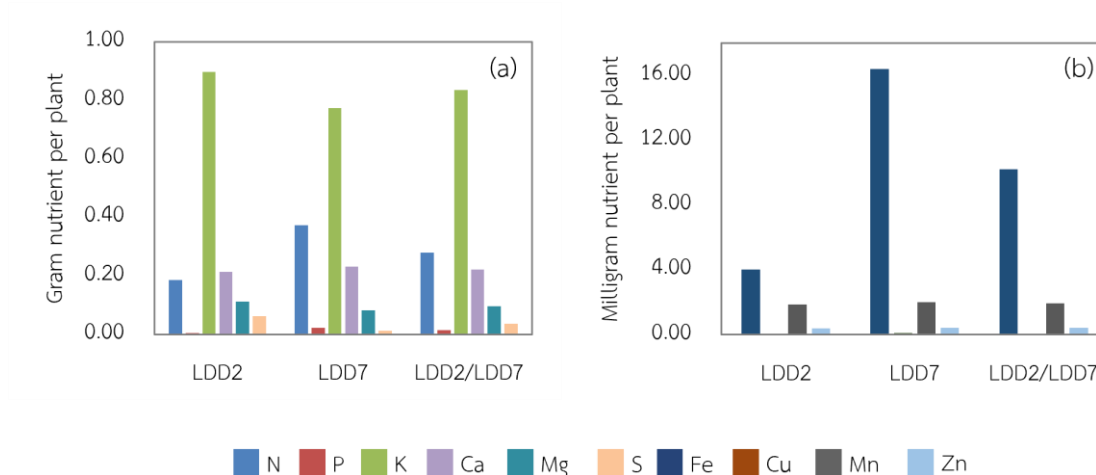
The chemical properties and elemental composition of LDD2 and LDD7 bio-extracts are given in **Table 1**. Both bio-extracts had a C/N ratio of about 17, with pH values of 4.50 and 3.66, EC values of 19.44 and 13.46 dS/m, and HA content of 25.85 and 25.85 g/L for the LDD2 and LDD7, respectively. The  $TN_b$ ,  $TP_b$ , and  $TK_b$  concentrations were 1.5-3.0, 0.04-0.17, and 6.2-7.22 g/L, respectively. The C/N ratio, pH, EC, HA, and  $TK_b$  values for both bio-extracts satisfactorily met some criteria standards for liquid organic fertilizers in Thailand, having a C/N ratio value <20, HA >10 g/L (Land Development Department, 2013), pH value <5.0, EC value <20 dS/m (Ministry of Agriculture and Cooperatives, 2005), and  $TK_b$  >4 g/L (Department of Agriculture, 2012). However, the  $TN_b$  and  $TP_b$  concentrations in the bio-extracts failed the requirements specified in the organic liquid fertilizer standard ( $TN_b$  >5 g/L and  $TP_b$  > g/L). In addition, the bio-extracts contained some total secondary macronutrients ( $TCa_b$ ,  $TMg_b$ , and  $TS_b$ ), accounting for 1.71-1.86, 0.66-0.90, and 0.1-0.5 g/L. The total of micronutrients, particularly  $TFe_b$ ,  $TMn_b$ , and  $TZn_b$ , were also observed in the bio-extract. The LDD2 extract had higher  $TK_b$ ,  $TMg_b$ , and  $TS_b$  than the LDD7 extract, whereas the LDD7 extract had higher  $TN_b$ ,  $TP_b$ ,  $TCa_b$ ,  $TFe_b$ ,  $TCu_b$ ,  $TMn_b$ , and  $TZn_b$  than the LDD2 extract. These variations in elemental profiling concentrations could be due to differences in the compositions of the fermented plant residues

which are banana shoots and chili used to produce the bio-extracts. Considering a weekly bio-extract supply for an experimental year (**Figure 3**), the data showed that each plant received  $TK_b$ ,  $TN_b$ , and  $TCa_b$  up to 0.90, 0.37, and 0.23 g nutrients/plant/year.

Soil profile data were collected before conducting the experiments. Three replicates of soils were taken from each topography: shoulder, mid-slope, and foot slope. The collected soil samples were air-dried, ground, and sieved through a 2 mm sieve. This process was conducted to observe any potential physiochemical variations in the soil and ensure homogenous testing for soil chemical properties. The finely ground soil materials, with particle sizes less than 0.5 mm, were prepared for analyzing organic matter (OM).

**Table 1** Chemical properties and composition of banana shoot bio-extract from Microbial Activator Super LDD2 (LDD2) and chili bio-extract from Microbial Activator Super LDD7 (LDD7)

Parameters	Bio-extract	
	LDD2	LDD7
C/N ratio	16.53 ± 0.47	17.30 ± 0.25
pH	4.50 ± 0.14	3.66 ± 0.03
EC (dS/m)	19.44 ± 0.26	13.46 ± 0.04
HA (g/L)	25.85 ± 0.07	25.85 ± 0.07
OM <sub>b</sub> (g/L)	42.80 ± 1.41	89.50 ± 0.85
TC <sub>b</sub> (g/L)	24.80 ± 0.07	51.90 ± 1.70
TN <sub>b</sub> (g/L)	1.50 ± 0.00	3.00 ± 0.14
TP <sub>b</sub> (g/L)	0.04 ± 0.00	0.17 ± 0.06
TK <sub>b</sub> (g/L)	7.22 ± 0.59	6.22 ± 0.23
TCa <sub>b</sub> (g/L)	1.71 ± 0.02	1.86 ± 0.10
TMg <sub>b</sub> (g/L)	0.90 ± 0.01	0.66 ± 0.08
TS <sub>b</sub> (g/L)	0.50 ± 0.00	0.10 ± 0.00
TFe <sub>b</sub> (mg/L)	32.40 ± 0.03	132.00 ± 4.24
TCu <sub>b</sub> (mg/L)	<0.001	0.85 ± 0.06
TMn <sub>b</sub> (mg/L)	15.00 ± 0.17	16.15 ± 0.39
TZn <sub>b</sub> (mg/L)	3.30 ± 0.01	3.55 ± 0.08



**Figure 3** Annual contents of (a) macronutrients and (b) micronutrients added to each plant.

### Data Collection and Analysis of Soil and Plant

After starting the experiment, ten soil samples were collected from the top layer of soil, at a depth of about 0-20 cm, beneath the shade of the ten specified plants for each treatment (**Figure 2**). The collected soil was divided into two portions for physical and chemical analyses. The physical analyses, including soil water aggregation stability (WAS), aggregate size distribution, and mean weight diameters ( $MWD_{wet}$ ), were undertaken using standard procedures (Kemper and Rosenau, 1986; Zheng et al., 2021). The soil samples were air-dried, ground, and sieved through 0.5 mm for organic matter (OM) analyses and 2.0 mm for chemical analyses. The analyzed standard chemical properties of soils were pH (1:1 soil-to-water ratio), electrical conductivity ( $EC_{1:5}$ , 1:5 soil-to-water ratio), available phosphorus (Avail. P; extracted with Bray-II extractable), exchangeable bases (Exch. Na, K, Ca, and Mg; extracted with 1M  $NH_4OAc$  at pH 7.0), cation exchange capacity (CEC), extractable sulfur (Extr. S; extracted with  $NH_4OAc$  at pH 5.0), extractable iron, manganese, copper, and zinc (Extr. Fe, Mn, Cu, and Zn; extracted with DTPA), and pseudo-total nutrient contents, obtained through pre-digestion in a nitric-perchloric mixture (1:2 v/v), at room temperature overnight, heated at 200 °C until the sample was uniformly white (Amacher, 1996; Hesse, 1971), followed by elemental analysis (Ca, Mg, Fe, Mn, Cu, and Zn) using an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Optima 8000; PerkinElmer; Massachusetts; USA), total inorganic phosphorus ( $TP_s$ ) using UV-Vis Spectrophotometer (Lambda 35, PerkinElmer, Massachusetts, USA) and total potassium ( $TK_s$ ) using a Flame photometer (Model 420, Sherwood, Cambridge, United Kingdom). The fine soil materials (<0.5 mm) were used for total carbon and total nitrogen ( $TC_s$  and  $TN_s$ ) using a CN Analyzer, modified from (International Organization for Standardization, 1995). (Flash 2000, Thermo Fisher Scientific, Massachusetts, USA). For quality control, blank and quality control samples were performed parallelly in each batch.

The chemical properties of bio-extracts were analyzed using standard procedures: pH, electrical conductivity (OSD, 2004), humic acid (HA; extracted with 0.5 M NaOH) (Chutibut and Phraankhan, 2008), Organic matter ( $OM_b$ ) and total C ( $TC_b$ ) were determined using wet oxidation modified from (Walkley and Black, 1934), and total nitrogen ( $TN_b$ ) by the Kjeldahl method (Jackson, 1967). The total phosphorus ( $TP_b$ ) was analyzed using the vanadomolybdate method (Barton, 1948). Total sulfate ( $TS_b$ ) was analyzed using the turbidimetric method using a

UV-Vis Spectrophotometer at 420 nm (Bardsley and Lancaster, 1965). Total potassium ( $TK_b$ ) was measured using a flame photometer, and concentrations of elements (Ca, Mg, Fe, Cu, Mn, and Zn) were analyzed using the ICP-OES after the nitric-perchloric digestion (2:1 v/v) and heated at 220 °C until the aliquot cleared (Hesse, 1971).

For plant data collection, the fruit yield was harvested and with the numbers and the weight of fruits per plant were collected to calculate the fruit weight per hectare from 24 specified plants within each treatment (**Figure 2**). Plant elemental concentrations were obtained from the index leaf, the youngest fully expanded leaves, collected from approximately 100 healthy leaves without any signs of disease or pest damage (October 2021). In each treatment, leaves were randomly sampled from ten specifically designated plants around their shrub (**Figure 2**). The plant leaves were kept in an ice box and quickly transferred to the lab. The collected leaves were gently wiped with a moistened cloth to remove dust and impurities. The leave samples were dried in an oven (60 °C), finely and homogeneously ground, and sieved through a 0.25 mm sieve to analyze the total contents of plant nutrients. The plant samples were digested using nitric-perchloric digestion (2:1 v/v) and heated at 220 °C until the aliquot cleared (Hesse, 1971). The total concentrations of nutrients ( $TCa_p$ ,  $TMg_p$ ,  $TFe_p$ ,  $TMn_p$ ,  $TCu_p$ , and  $TZn_p$ ) in the digested samples were measured using the ICP-OES. The total phosphorus ( $TP_p$ ) was analyzed by the vanadomolybdate method modified from (Barton, 1948), total sulfate ( $TS_p$ ) was analyzed by the turbidimetric method (Bardsley and Lancaster, 1965), and total potassium ( $TK_p$ ) was measured using a flame photometer. The concentrations of total carbon ( $TC_p$ ) and total nitrogen ( $TN_p$ ) contents were determined using a CN Analyzer as described above.

### Statistical Analysis

The normality of the datasets was checked using the Shapiro-Wilk test. Most datasets showed non-normal distribution. Therefore, the median value was reported instead of the mean value to show the central tendency of the data. The Mann-Whitney U test was also used to determine median differences between the two sample groups. All statistical tests were conducted at a significant level of  $\alpha = 0.05$ .

## Results and Discussion

### Soil Properties before the Experiment

#### Physical Properties

Soil before the experiment showed homogeneity of chemical and some physical properties both downward along the slope (**Table 2**) and across the experimental area (**Table 3**). Collectively, the topsoil's thickness was variable between 10 cm and 18 cm (**Table 4**). The soil depth at the shoulder and mid-slope was deeper than 80 cm, whereas the foot slope was much shallower, only 30 cm thick. Loam was the most dominant soil texture, with some samples having sandy loam or sandy clay loam texture. These soil characteristics could be considered highly suitable for tea-oil camellia cultivation as this plant can thrive in a wide range of soil textures, from sandy to clayey, with a minimum depth of 50 cm (Chaicharoenpong, 2020; Hajiboland, 2017).

#### Chemical Properties

The chemical analysis of the studied highly developed soils (**Table 5**) indicated that the soil pH ranged from 5.58 to 6.49, moderately to slightly acid, with a median value of 5.89 (Soil Survey Division Staff, 1993). This pH range



suits tea-oil production (pH 4.0-6.5) (Pantong, 2012). The  $EC_{1.5}$  value was from 0.04 to 0.20 dS/m, with the median value = 0.08 dS/m, indicating non-saline conditions (FAO, 1988). The concentrations of OM were 37.33 to 93.18 g/kg, median = 60.13 g/kg, and Avail. P (126-903 mg/kg, median = 204 mg/kg) were extraordinarily high. This was unexpected for highly developed soils that should have low OM and Avail. P concentrations (OSD, 2004). The high OM content could be due to a low organic matter decomposition rate, as revealed by our micromorphological analysis (**data not show**), which could have been influenced by the relatively lower temperature at higher altitude compared to lower ones (Chimdessa, 2023; Swetnam et al., 2017; Tashi et al., 2016). The high available P concentration could also be mainly derived from heavy P fertilization of local hill tribes as the P is not an element derived from parent rocks (Anirut et al., 2003). Conversely, Exch. Na, Ca, Mg, and CEC were very low, low, high, and medium, respectively (OSD, 2004). The high Exch. Mg could be related to either Mg inherent from their schist/mica schist soil parent rocks that may contain Mg-rich minerals such as magnesiochloritoid ( $(Fe/Mg)_2Al_2Si_4O_{23}(OH)$ ) (Anirut et al., 2003; Soil Science Department, 2005). The extractable Al (0.03 - 0.72 cmol<sub>c</sub>/kg, median = 0.20 cmol<sub>c</sub>/kg) was below the toxic threshold of <1 cmol<sub>c</sub>/kg (Moir and Morton, 2018). Overall, the studied soils before the experiment were not considered infertile but were high in OM and P with low soil acidity.

Although the soils had favorable soil chemical and physical properties as described above, vegetative growth and yield of the studied tea-oil camellia remained poor (**Figure 4**), with a low average fresh fruit yield of as low as 0.24 kg/plant/year from 2015-2018 (**Table 6**). The obtained fruit yield was considerably low (less than 1.5 kg/plant/year) (Zhao et al., 2017) and below the levels observed in tea-oil plantations in red soil regions of China, where plants yielded an average of 4.04 kg of fresh fruit/plant/year (Chen et al., 2023). In 2018, 89% of the tea-oil fruit yield in the studied site was categorized as Grade C and D (Grade A: >100 fruits/plant, Grade B: 50-100 fruits/plant, Grade C: <50 fruits/plant, and Grade D: no fruits) (**Table 7**). Overall, the tea-oil production in the area studied before the experiment was considered unproductive, requiring simple and practical measures to improve its yield, fruit grade, and even nutrition.



**Figure 4** A tea-oil camellia plant with poor vegetative growth.

**Table 2** Homogeneity testing of soil chemical properties of Ap soil samples before treatments downward to the slope of the experimental area

Soil properties <sup>1/</sup>	Topography						U-test
	Shoulder		Mid slope		Foot slope		
	Range	$\tilde{X} \pm SD$	Range	$\tilde{X} \pm SD$	Range	$\tilde{X} \pm SD$	
pH	5.58 - 6.49	5.82 ± 0.47	5.82 - 5.94	5.89 ± 0.06	5.68 - 6.09	5.97 ± 0.21	ns
EC <sub>1:5</sub> (dS/m)	0.08 - 0.20	0.12 ± 0.06	0.04 - 0.06	0.06 ± 0.01	0.05 - 0.12	0.08 ± 0.04	ns
OM (g/kg)	59.88 - 93.18	65.24 ± 17.88	60.13 - 76.82	64.81 ± 8.61	37.33 - 58.10	48.98 ± 10.41	ns
Avail. P (mg/kg)	126 - 903	154 ± 441	158 - 442	204 ± 152	148 - 375	370 ± 130	ns
Exch. Na (cmol <sub>c</sub> /kg)	0.01 - 1.19	0.04 ± 0.67	0.01 - 0.01	0.01 ± 0.00	0.01 - 0.05	0.01 ± 0.02	ns
Exch. K (cmol <sub>c</sub> /kg)	0.33 - 1.19	0.76 ± 0.43	0.35 - 0.71	0.65 ± 0.19	0.71 - 1.26	0.86 ± 0.28	ns
Exch. Ca (cmol <sub>c</sub> /kg)	4.44 - 15.37	6.41 ± 5.83	4.81 - 5.81	5.15 ± 0.51	1.99 - 4.96	4.69 ± 1.64	ns
Exch. Mg (cmol <sub>c</sub> /kg)	2.51 - 4.40	3.09 ± 0.97	2.57 - 3.84	3.49 ± 0.66	3.21 - 4.51	4.10 ± 0.66	ns
CEC (cmol <sub>c</sub> /kg)	13.64 - 17.55	15.05 ± 1.98	14.47 - 16.18	14.81 ± 0.91	11.89 - 14.59	13.58 ± 1.36	ns
Extr. Al (cmol <sub>c</sub> /kg)	0.03 - 0.72	0.14 ± 0.37	0.20 - 0.62	0.56 ± 0.23	0.16 - 0.48	0.17 ± 0.18	ns

<sup>1/</sup>pH; measurement using soil: water ratio of 1:1, EC<sub>1:5</sub>; measurement using soil: water ratio of 1:5; OM; Organic matter, Avail. P; Bray-II extractable P; Exch. Na, K, Ca, and Mg; NH<sub>4</sub>OAc extractable Ca, Mg, K, and Na, CEC; NH<sub>4</sub>OAc pH 7, Extr. Al; KCl extractable Al. \* and different letters above median values indicate statistical differences based on the Mann-Whitney U test (U-test) at  $\alpha = 0.05$ , whereas ns indicates non-statistical differences.

**Table 3** Homogeneity testing of soil chemical properties of Ap soil samples before treatments across the slope of the experimental area

Soil properties <sup>1/</sup>	Zone						U-test
	Left		Middle		Right		
	Range	$\tilde{X} \pm SD$	Range	$\tilde{X} \pm SD$	Range	$\tilde{X} \pm SD$	
pH	5.89 - 6.49	5.97 ± 0.33	5.68 - 5.82	5.82 ± 0.08	5.58 - 6.09	5.94 ± 0.26	ns
EC <sub>1:5</sub> (dS/m)	0.06 - 0.20	0.08 ± 0.08	0.04 - 0.08	0.05 ± 0.02	0.06 - 0.12	0.12 ± 0.03	ns
OM (g/kg)	58.10 - 93.18	76.82 ± 17.55	37.33 - 65.24	64.81±15.99	48.98 - 60.13	59.88 ± 6.37	ns
Avail. P (mg/kg)	370 - 903	442 ± 289	148 - 204	154 ± 31	126 - 375	158 ± 135	ns
Exch. Na (cmol <sub>c</sub> /kg)	0.01 - 1.19	0.05 ± 0.67	0.01 - 0.04	0.00 ± 0.02	0.01 - 0.01	0.01 ± 0.00	ns
Exch. K (cmol <sub>c</sub> /kg)	0.65 - 0.86	0.76 ± 0.11	0.33 - 0.71	0.35 ± 0.21	0.71 - 1.26	1.19 ± 0.30	ns
Exch. Ca (cmol <sub>c</sub> /kg)	4.69 - 15.37	5.81 ± 5.87	1.99 - 6.41	4.81 ± 2.24	4.44 - 5.15	4.96 ± 0.37	ns
Exch. Mg (cmol <sub>c</sub> /kg)	3.49 - 4.51	4.40 ± 0.56	2.51 - 3.21	2.57 ± 0.39	3.09 - 4.10	3.84 ± 0.53	ns
CEC (cmol <sub>c</sub> /kg)	14.59 - 17.55	16.18 ± 1.48	11.89 - 14.81	16.64 ± 1.47	13.58 - 15.05	14.47 - 0.74	ns
Extr. Al (cmol <sub>c</sub> /kg)	0.20 - 0.03	0.16 ± 0.09	0.72 - 0.48	0.56 ± 0.12	0.62 - 0.14	0.17 ± 0.27	ns

<sup>1/</sup>pH; measurement using soil: water ratio of 1:1, EC<sub>1:5</sub>; measurement using soil: water ratio of 1:5; OM; Organic matter, Avail. P; Bray-II extractable P; Exch. Na, K, Ca, and Mg; NH<sub>4</sub>OAc extractable Ca, Mg, K, and Na, CEC; NH<sub>4</sub>OAc pH 7, Extr. Al; KCl extractable Al. \* and different letters above median values indicate statistical differences based on the Mann-Whitney U test (U-test) at  $\alpha = 0.05$ , whereas ns indicates non-statistical differences.

**Table 4** Soil physical properties before treatments downward to the slope of the experimental areas

Topography	Replication	Horizon	Depth (cm)	Soil Color	Sand	Silt	Clay	Texture
					(%)			
Shoulder	1	Ap	0-10	7.5YR2.5/3 very dark brown	38.1	41.6	20.3	L
		Bt1	10-30	2.5YR4/4 reddish brown	41.8	35.4	22.8	L
		Bt2	30-50	5YR4/4 reddish brown	47.9	32.5	19.6	L
		Bt3	50-70	5YR4/6 yellowish red	53.2	31.0	15.8	SL
		BC	70-80	2.5YR5/8 red	59.0	29.8	11.2	SL
	2	Ap	0-15	5YR3/4 dark reddish brown	44.2	34.6	21.2	L
		Bt1	15-35	2.5YR4/8 red	44.7	33.7	21.6	L
		Bt2	35-70	2.5YR4/8 red	50.6	34.6	14.8	L
		Bt3	70-100	2.5YR5/6 red	52.8	32.3	14.9	SL
	3	Ap	0-15	5YR3/4 dark reddish brown	43.2	32.3	24.5	L
		AB	15-35	5YR3/3 dark reddish brown	43.8	34.5	21.7	L
		Bt	35-55	5YR4/6 yellowish red	47.3	31.2	21.5	L
		BC	55-80	2.5YR5/8 red	44.9	30.2	24.9	L
Mid slope	1	Ap	0-18	5YR3/2 dark reddish brown	43.2	34.8	22.0	L
		AB	18-30	2.5YR3/2 dusky red	54.2	24.4	21.4	SCL
		Bt	30-60	2.5YR5/6 red	49.8	32.6	17.6	L
		BC	60-80	2.5YR5/6 red	51.7	31.5	16.8	L
	2	Ap	0-10	7.5YR3/3 dark brown	46.4	30.0	23.6	L
		AB	10-30	7.5YR3/3 dark brown	44.1	34.8	21.1	L
		BC1	30-70	7.5YR3/4 dark brown	52.3	30.7	17.0	SL
		BC2	70-100	5YR5/6 yellowish red	54.7	30.0	15.3	SL
	3	Ap	0-15	7.5YR2.5/3 very dark brown	42.8	32.9	24.3	L
		AB	15-30	7.5YR3/3 dark brown	42.6	33.9	23.5	L
		BC	30-40	7.5YR3/3 dark brown	44.9	33.3	21.8	L
	Foot slope	1	Ap	0-10	2.5YR3/2 dusky red	41.3	34.7	24.0
BC			10-30	5YR3/3 dark reddish brown	43.6	34.0	22.4	L
2		Ap	0-10	2.5YR2.5/2 very dusky red	40.1	36.0	23.9	L
		BC	10-30	2.5YR3/3 dark reddish brown	38.5	34.7	26.8	L
3		Ap	0-10	2.5YR3/3 dark reddish brown	43.3	35.5	21.2	L
		BC	10-30	5YR3/3 dark reddish brown	46.8	31.8	21.4	L

**Table 5** Soil chemical properties range (minimum-maximum) and median values ( $\tilde{x} \pm SD$ ) of Ap soil samples before treatments of the experimental area

Soil properties <sup>1/</sup>	All samples		Interpretation	References
	Range	$\tilde{x} \pm SD$		
pH	5.58 - 6.49	5.89 $\pm$ 0.26	Moderately acid	Soil Survey Division Staff (1993)
EC <sub>1:5</sub> (dS/m)	0.04 - 0.20	0.08 $\pm$ 0.05	Non saline	FAO (1988)
OM (g/kg)	37.33 - 93.18	60.13 $\pm$ 15.84	Very high	OSD (2004)
Avail. P (mg/kg)	126 - 903	204 $\pm$ 249	Very high	OSD (2004)
Exch. Na (cmol <sub>c</sub> /kg)	0.04 - 1.19	0.01 $\pm$ 0.39	Very low	OSD (2004)
Exch. K (cmol <sub>c</sub> /kg)	0.33 - 1.26	0.71 $\pm$ 0.32	Very high	OSD (2004)
Exch. Ca (cmol <sub>c</sub> /kg)	1.99 - 15.37	4.96 $\pm$ 3.73	Low	OSD (2004)
Exch. Mg (cmol <sub>c</sub> /kg)	2.51 - 4.51	3.49 $\pm$ 0.74	High	OSD (2004)
CEC (cmol <sub>c</sub> /kg)	11.89 - 17.55	14.59 $\pm$ 1.61	Medium	OSD (2004)
Extr. Al (cmol <sub>c</sub> /kg)	0.03 - 0.72	0.20 $\pm$ 0.25	Nontoxic	Moir and Morton (2018)

<sup>1/</sup>pH; measurement using soil: water ratio of 1:1, EC<sub>1:5</sub>; measurement using soil: water ratio of 1:5; OM; Organic matter, Avail. P; Bray-II extractable P; Exch. Na, K, Ca, and Mg; NH<sub>4</sub>OAc extractable Ca, Mg, K, and Na, CEC; NH<sub>4</sub>OAc pH 7, Extr. Al; KCl extractable Al.

**Table 6** Data of fruit weight per individual tea-oil camellia plant before the experiment during the 2015-2018 crop years in the studied area

Crop years	Fruit weight (kg/plant/year)
2015	0.10
2016	0.20
2017	0.37
2018	0.27
Average	0.24

**Table 7** Grade of tea-oil camellia before treatment in 2018 in the plantation plot

Grade	Tea-oil camellia number (plant)	Percent (%)
A (>100 fruits/plant)	28	4
B (50-100 fruits/plant)	49	7
C (<50 fruits/plant)	355	50
D (no fruits)	277	39
Total	710	100

In the plantation area, termites, major insect pests responsible for damaging the roots and stems of Camellia plants, were observed (Getachew et al., 2020; LiHong et al., 2016). Additionally, despite the excessive use of chemical

N-P-K fertilizers, more than 1 kg of 15-15-15 per plant per year, as indicated by the chemical properties of the soil before treatment, the plants still exhibited low fruit productivity. Since microbial inoculants are widely recognized as a valuable nutrient management strategy for soil restoration, these procedures are considered simple, cost-effective, environmentally friendly, and possess multiple abilities, including enhancing nutrient availability and restoring soil microbiological degradation (Chaudhuri et al., 2017; Lal, 2015; Singh et al., 2016). Microbial Activator Super LDD2 and LDD7 were selected to address these issues. Due to their abundance in the area, Banana shoots and chili were chosen as substrates for multiplying the microbial activators LDD2 and LDD7, respectively. Banana pseudostem can be an alternative source of macro and micronutrients for producing bio-fertilizers (Padam et al., 2014). Research has shown that the nutritional composition of banana pseudostem sap varies in elements such as N, P, K, S, Ca, Mg, Mn, Fe, Zn, and Cu (Pathak and Patil, 2014). Genovia and Gapo (2016) reported that a 50% extract of chili pepper fruit combined with 50% distilled water demonstrated positive effects as an insecticide against termites (Genovia et al., 2016).

### Changes in Soil Properties Induced by Bio-extract Application

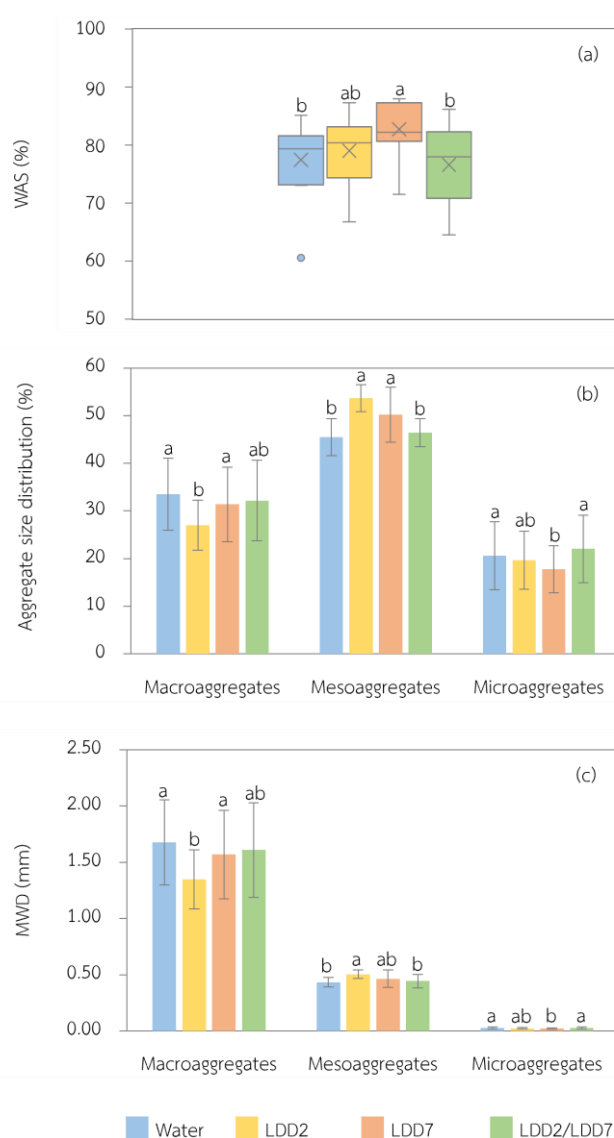
#### Physical Properties

The effects of bio-extraction on soil properties were compared with the control (water supply). The water-stable aggregates (WAS) percentage decreased in the following order: LDD7 (82.21%) > LDD2 (80.34%) > water (79.38%) > LDD2/LDD7 (77.95%) (**Figure 5a**). The LDD7 significantly promoted WAS compared to the control, LDD2, and LDD2/LDD7. There was no statistical difference in WAS between LDD2 and LDD7 treatments. The explanation for LDD7 to improve WAS could be due to its continuously providing the highest total C content for two years, and organic carbon could act effectively as the main binding agent promoting soil aggregate through clay-Fe-OM complexes (Sun et al., 2023). This data is consistent with the data from Yilmaz and Sönmez (2017) and shows that utilizing organic and bio-fertilizers improved the stability of macroaggregates and microaggregates through the cation bridge of clay with organic matter accompanied with humic acid in bio-extract, which can enhance soil aggregation (Norambuena et al., 2014).

For soil aggregates size distribution, mesoaggregate (0.25-2 mm) was the main aggregate size fraction, accounting for (46-54%, median =48%). The macroaggregate (>2 mm, 27-32%, median =32%) and the microaggregate class (<0.25 mm, 18-22%, median =20%) were present at smaller fractions and were different across all treatments (**Figure 5b**). Compared to the control treatment, single utilization of LDD2 or LDD7 bio-extract significantly increased mesoaggregate fraction but tended to decrease micro and macroaggregate fractions. This suggests the breakdown of macroaggregate into mesoaggregate and the formation of mesoaggregate from microaggregate. It is possible that some chemical compounds in the bio-extracts can act as binding agents forming the mesoaggregate fraction. For the decrease of macroaggregates, we hypothesized that compounds derived from the bio-extracts might also promote oil-tea root development, which is consistent with previous study showing that plant roots can breakdown macroaggregate (Nakamoto et al. 2001). However, this hypothesis needs further studies to indicate the effect of bio-extracts on oil-tea root architecture and soil aggregate formation. In addition, among the four treatments, the LDD2 bio-extract treatment had significantly higher mean weight diameter (MWD) values in the mesoaggregate class than others but significantly lower MWD values in the macroaggregate class (**Figure 5c**).

### Chemical Properties

After two years of utilizing the bio-extracts (LDD2 and LDD7), the soil pH values were significantly higher than those of the control treatment (**Table 8**). All bio-extracts significantly increase in Avail. P levels when compared to the control treatment. Moreover, the bio-extracts significantly enhanced Exch. Ca and Exch. Mg. Also, CEC, TN<sub>s</sub>, TP<sub>s</sub>, and TCa<sub>s</sub> contents tend to increase compared to the control treatment. Conversely, the water treatment significantly had higher TK<sub>s</sub> and TFe<sub>s</sub> contents than the bio-extract treatments. However, the combined LDD2/LDD7 bio-extract treatment increased EC<sub>1:5</sub> and Extr. Cu values compared to the other treatments. The increasing available P content could be related to an increase in soil pH that can promote the P availability for acidic soils, by decreasing P fixation by Fe and Al oxides under acidic conditions. Also, the addition of other nutrients in both extractable and total forms could be simply and directly related to the inherent of nutrients from the applied bio-extracts.



**Figure 5** Water aggregation stability (WAS) (a), aggregate size distribution (b), and mean weight diameters (MWD) (c) of soil under four treatments shown by box colors. Different letters indicate significant differences among treatments based on the Mann-Whitney U test (U-test) at  $\alpha = 0.05$ .

**Table 8** Median values ( $\bar{x} \pm SD$ ) of soil chemical properties of each treatment in 2<sup>nd</sup> year

Soil properties <sup>1/</sup>	2 <sup>nd</sup> year				U-test
	Water	LDD2	LDD7	LDD2/LDD7	
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
pH	5.92 $\pm$ 0.28 <sup>b</sup>	6.18 $\pm$ 0.21 <sup>a</sup>	6.19 $\pm$ 0.32 <sup>a</sup>	6.44 $\pm$ 0.23 <sup>a</sup>	*
EC <sub>1:5</sub> (dS/m)	0.08 $\pm$ 0.02 <sup>b</sup>	0.07 $\pm$ 0.02 <sup>b</sup>	0.08 $\pm$ 0.02 <sup>b</sup>	0.11 $\pm$ 0.03 <sup>a</sup>	*
OM (g/kg)	58.40 $\pm$ 10.98	69.35 $\pm$ 12.18	66.20 $\pm$ 10.45	67.85 $\pm$ 14.71	ns
Avail. P (mg/kg)	99 $\pm$ 52 <sup>c</sup>	505 $\pm$ 104 <sup>a</sup>	243 $\pm$ 217 <sup>b</sup>	228 $\pm$ 89 <sup>b</sup>	*
Exch. Na (cmol <sub>c</sub> /kg)	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	0.05 $\pm$ 0.05	0.01 $\pm$ 0.02	ns
Exch. K (cmol <sub>c</sub> /kg)	0.17 $\pm$ 0.07	0.22 $\pm$ 0.05	0.19 $\pm$ 0.08	0.17 $\pm$ 0.11	ns
Exch. Ca (cmol <sub>c</sub> /kg)	7.79 $\pm$ 1.59 <sup>b</sup>	9.80 $\pm$ 1.84 <sup>a</sup>	9.99 $\pm$ 2.66 <sup>ab</sup>	10.71 $\pm$ 1.61 <sup>a</sup>	*
Exch. Mg (cmol <sub>c</sub> /kg)	3.87 $\pm$ 0.64 <sup>c</sup>	5.00 $\pm$ 0.79 <sup>ab</sup>	3.73 $\pm$ 1.10 <sup>bc</sup>	5.04 $\pm$ 1.47 <sup>a</sup>	*
CEC (cmol <sub>c</sub> /kg)	19.30 $\pm$ 2.95 <sup>b</sup>	23.25 $\pm$ 1.53 <sup>a</sup>	21.66 $\pm$ 2.33 <sup>ab</sup>	19.59 $\pm$ 3.21 <sup>b</sup>	*
Extr. K (mg/kg)	319 $\pm$ 132	381 $\pm$ 96	328 $\pm$ 137	316 $\pm$ 191	ns
Extr. Ca (mg/kg)	1258 $\pm$ 297	1513 $\pm$ 309	1519 $\pm$ 448	1704 $\pm$ 303	ns
Extr. Mg (mg/kg)	351 $\pm$ 66 <sup>b</sup>	432 $\pm$ 60 <sup>a</sup>	344 $\pm$ 88 <sup>b</sup>	441 $\pm$ 99 <sup>a</sup>	*
Extr. S (mg/kg)	8.76 $\pm$ 1.84 <sup>a</sup>	7.29 $\pm$ 3.61 <sup>ab</sup>	4.77 $\pm$ 1.57 <sup>b</sup>	6.71 $\pm$ 4.06 <sup>ab</sup>	*
Extr. Fe (mg/kg)	49.14 $\pm$ 12.45	54.18 $\pm$ 9.44	46.28 $\pm$ 8.87	43.02 $\pm$ 9.73	ns
Extr. Cu (mg/kg)	1.07 $\pm$ 0.28 <sup>b</sup>	1.09 $\pm$ 0.30 <sup>b</sup>	0.98 $\pm$ 0.26 <sup>b</sup>	1.65 $\pm$ 0.62 <sup>a</sup>	*
Extr. Mn (mg/kg)	12.62 $\pm$ 6.01 <sup>ab</sup>	8.94 $\pm$ 3.08 <sup>b</sup>	8.55 $\pm$ 6.4 <sup>b</sup>	12.09 $\pm$ 6.90 <sup>a</sup>	*
Extr. Zn (mg/kg)	1.43 $\pm$ 0.68	2.48 $\pm$ 0.97	1.62 $\pm$ 0.55	1.73 $\pm$ 1.22	ns
TC <sub>s</sub> (g/kg)	41.83 $\pm$ 7.37	48.72 $\pm$ 3.03	43.42 $\pm$ 7.57	44.29 $\pm$ 07.90	ns
TN <sub>s</sub> (g/kg)	2.97 $\pm$ 0.41 <sup>b</sup>	3.35 $\pm$ 0.25 <sup>a</sup>	3.33 $\pm$ 0.39 <sup>ab</sup>	3.59 $\pm$ 0.59 <sup>a</sup>	*
TP <sub>s</sub> (g/kg)	1.67 $\pm$ 0.67 <sup>b</sup>	2.71 $\pm$ 0.45 <sup>a</sup>	1.96 $\pm$ 0.76 <sup>ab</sup>	2.14 $\pm$ 0.56 <sup>ab</sup>	*
TK <sub>s</sub> (g/kg)	14.73 $\pm$ 2.99 <sup>a</sup>	10.65 $\pm$ 1.17 <sup>b</sup>	11.73 $\pm$ 2.14 <sup>b</sup>	9.43 $\pm$ 2.02 <sup>b</sup>	*
TCa <sub>s</sub> (g/kg)	3.00 $\pm$ 0.88 <sup>b</sup>	4.18 $\pm$ 1.25 <sup>a</sup>	4.08 $\pm$ 1.16 <sup>ab</sup>	4.16 $\pm$ 0.78 <sup>a</sup>	*
TMg <sub>s</sub> (g/kg)	6.79 $\pm$ 1.80 <sup>a</sup>	5.94 $\pm$ 0.58 <sup>ab</sup>	4.66 $\pm$ 1.09 <sup>b</sup>	5.14 $\pm$ 0.84 <sup>ab</sup>	*
TFe <sub>s</sub> (mg/kg)	1596 $\pm$ 109 <sup>a</sup>	1372 $\pm$ 109 <sup>b</sup>	1332 $\pm$ 108 <sup>b</sup>	1300 $\pm$ 134 <sup>b</sup>	*
TCu <sub>s</sub> (mg/kg)	18.09 $\pm$ 3.17 <sup>ab</sup>	17.92 $\pm$ 1.87 <sup>ab</sup>	16.53 $\pm$ 1.39 <sup>b</sup>	19.19 $\pm$ 2.09 <sup>a</sup>	*
TMn <sub>s</sub> (mg/kg)	415.24 $\pm$ 73.99	410.91 $\pm$ 104.06	430.04 $\pm$ 77.53	495.78 $\pm$ 57.19	ns
TZn <sub>s</sub> (mg/kg)	33.71 $\pm$ 9.03 <sup>ab</sup>	40.31 $\pm$ 4.98 <sup>a</sup>	34.68 $\pm$ 6.67 <sup>ab</sup>	32.37 $\pm$ 5.15 <sup>b</sup>	*

<sup>1/</sup>pH; measurement using soil: water ratio of 1:1, EC<sub>1:5</sub>; measurement using soil: water ratio of 1:5; OM; Organic matter, Avail. P; Bray-II extractable P; Exch. Na, K, Ca, and Mg; NH<sub>4</sub>OAc extractable Ca, Mg, K, and Na, CEC; NH<sub>4</sub>OAc pH 7, Extr. K, Ca, Mg; NH<sub>4</sub>OAc pH 7 extractable Ca, Mg, and K, Extr. S; NH<sub>4</sub>OAc pH 5 extractable S, Extr. Fe, Cu, Mn, and Zn; DTPA extractable Fe, Cu, Mn, and Zn, TC<sub>s</sub>, TN<sub>s</sub>; determined using CN Analyzer; TP<sub>s</sub>; determined using UV-vis Spectrophotometer, TK<sub>s</sub>; determined using Flame Photometer, TCa<sub>s</sub>, TMg<sub>s</sub>, TFe<sub>s</sub>, TCu<sub>s</sub>, TMn<sub>s</sub>, and TZn<sub>s</sub>; determined using Atomic Absorption Spectrophotometer. \* and different letters above median values indicate statistical differences based on the Mann-Whitney U test (U-test) at  $\alpha = 0.05$ , whereas ns indicates non-statistical differences.

### Nutrient Concentrations in Tea-oil Leaves

There was a significant difference in the TN<sub>p</sub> content of tea-oil camellia leaves of LDD7 treatment with LDD2/LDD7 treatment and the TC<sub>p</sub> content of LDD2 treatment with other treatments (**Table 9**). However, there

were no significant differences among all treatments in the  $TP_p$ ,  $TK_p$ , secondary macronutrients ( $TCa_p$ ,  $TMg_p$ ,  $TS_p$ ), and micronutrients ( $TFe_p$ ,  $TCu_p$ ,  $TMn_p$ ,  $TZn_p$ ) in tea-oil camellia leaves.

**Table 9** Median values ( $\bar{x} \pm SD$ ) of element content in tea-oil camellia leaves of each treatment in 2<sup>nd</sup> year

Element content <sup>1/</sup>	2 <sup>nd</sup> year				U-test
	Water	LDD2	LDD7	LDD2/LDD7	
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
$TC_p$ (g/kg)	475.12 $\pm$ 7.26 <sup>b</sup>	484.37 $\pm$ 8.34 <sup>a</sup>	476.13 $\pm$ 16.21 <sup>ab</sup>	472.92 $\pm$ 8.87 <sup>b</sup>	*
$TN_p$ (g/kg)	18.73 $\pm$ 1.00 <sup>b</sup>	19.91 $\pm$ 2.50 <sup>ab</sup>	20.62 $\pm$ 1.72 <sup>a</sup>	21.04 $\pm$ 2.79 <sup>a</sup>	*
$TP_p$ (g/kg)	0.51 $\pm$ 0.06	0.47 $\pm$ 0.09	0.56 $\pm$ 0.11	0.53 $\pm$ 0.11	ns
$TK_p$ (g/kg)	5.83 $\pm$ 1.05	5.33 $\pm$ 1.40	5.92 $\pm$ 0.86	5.67 $\pm$ 1.03	ns
$TCa_p$ (g/kg)	28.53 $\pm$ 8.01	31.79 $\pm$ 4.97	25.15 $\pm$ 5.29	26.77 $\pm$ 2.92	ns
$TMg_p$ (g/kg)	61.13 $\pm$ 15.21	61.54 $\pm$ 11.29	69.24 $\pm$ 19.27	52.59 $\pm$ 10.16	ns
$TS_p$ (g/kg)	0.34 $\pm$ 0.08	0.39 $\pm$ 0.14	0.39 $\pm$ 0.09	0.38 $\pm$ 0.10	ns
$TFe_p$ (mg/kg)	37.51 $\pm$ 10.25	39.24 $\pm$ 4.66	35.47 $\pm$ 6.83	31.50 $\pm$ 8.53	ns
$TCu_p$ (mg/kg)	3.53 $\pm$ 0.61	3.13 $\pm$ 0.48	3.91 $\pm$ 0.79	3.40 $\pm$ 0.61	ns
$TMn_p$ (mg/kg)	898 $\pm$ 503	894 $\pm$ 334	995 $\pm$ 293	718 $\pm$ 411	ns
$TZn_p$ (mg/kg)	6.69 $\pm$ 1.89	7.51 $\pm$ 2.16	8.20 $\pm$ 2.12	6.47 $\pm$ 2.25	ns

<sup>1/</sup>  $TC_p$ ,  $TN_p$ ; determined using CN Analyzer;  $TP_p$ ; determined using UV-vis Spectrophotometer,  $TK_p$ ; determined using Flame Photometer,  $TCa_p$ ,  $TMg_p$ ,  $TFe_p$ ,  $TCu_p$ ,  $TMn_p$ , and  $TZn_p$ ; determined using Atomic Absorption Spectrophotometer. \* and different letters above median values indicate statistical differences based on the Mann-Whitney U test (U-test) at  $\alpha = 0.05$ , whereas ns indicates non-statistical differences.

The concentrations of N and C in the leaf in the LDD2 treatment may be attributed to the activity of certain microorganisms present in the microbial product, such as *Bacillus subtilis*. This microorganism has been shown to enhance the photosynthetic capacity of plants by influencing stomatal conductance (Li et al., 2016). Studies have also demonstrated that dipping pre-germinated sugarcane seedlings with *Bacillus subtilis* can improve the photosynthetic rate and water use efficiency (Fonseca et al., 2022). Similarly, co-inoculation treatments involving seed coating with *Bacillus* strains have enhanced nitrogen concentration and increased macro and micronutrients in maize root and shoot (Mumtaz et al., 2018). The application of LDD7 bio-extract serves as a biocontrol measure and may also enhance N and C content through the action of *Gluconobacter oxydans*. This bacterium exhibits several beneficial effects on plants, including nitrogen fixation, phosphate solubilization, and zinc solubilization (Dwivedi, 2020). Furthermore, foliar application of yeast (*Saccharomyces cerevisiae*) has been shown to improve photosynthetic attributes and yield in rice (Gao et al., 2014).

#### Tea-oil Camellia Fruit Yield

The combined application of LDD2/LDD7 in the first year resulted in higher and statistically significant differences in fruit number per plant, fruit weight per plant, and fruit weight per hectare than other treatments. These values were 3.0 times, 2.17 times, and 2.17 times higher than the control. In the second year, LDD2/LDD7 treatment provided 1.53, 1.35, and 1.35 times fruit number per plant, fruit weight per plant, and fruit weight per hectare higher than the control (**Table 10**). It should be noted that using single either LDD2 or LDD7 for two years



had the same fruit yield as the control treatment in the second year. Our work indicated that the combined use of LDD2 and LDD7 could provide the highest yield of the tea oil than the sole use of LDD2 or LDD7, or water. Compared with other studies, the fruit weight per plant in the LDD2/LDD7 treatment was comparable to the high fruit yield obtained by tea-oil camellia plants cultivated in China (Chen et al., 2023). This data suggests that the diverse nutrients (C, N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn) contained in the LDD2/LDD7 bio-extract mixtures could additionally

**Table 10** Data of fruit number (FN) and fruit weight (FW) per individual tea-oil camellia plant and fruit weight per hectare (FWh) in 1<sup>st</sup> year and 2<sup>nd</sup> year

Treatments	1 <sup>st</sup> year			2 <sup>nd</sup> year		
	FN	FW	FWh	FN	FW	FWh
	(fruits/plant)	(kg/plant)	(kg/ha)	(fruits/plant)	(kg/plant)	(kg/ha)
Water	20 <sup>b</sup>	1.2 <sup>b</sup>	1,597 <sup>b</sup>	30 <sup>b</sup>	2.0 <sup>b</sup>	2,662 <sup>b</sup>
LDD2	27a <sup>b</sup>	1.9 <sup>ab</sup>	2,529 <sup>ab</sup>	35 <sup>ab</sup>	1.7 <sup>b</sup>	2,263 <sup>b</sup>
LDD7	30a <sup>b</sup>	1.8 <sup>ab</sup>	2,396 <sup>ab</sup>	30 <sup>b</sup>	1.8 <sup>b</sup>	2,396 <sup>b</sup>
LDD2/LDD7	60 <sup>a</sup>	2.6 <sup>a</sup>	3,461 <sup>a</sup>	46 <sup>a</sup>	2.7 <sup>a</sup>	3,594 <sup>a</sup>
U-test	*	*	*	*	*	*

\* and different letters in the same column indicate statistical differences based on the Mann-Whitney U test (U-test) at  $\alpha = 0.05$ , whereas ns indicates non-statistical differences.

supply plant nutrients to soils, providing available nutrients particularly P, Ca, Mg, and Cu (**Table 8**). Based on the active microbial inoculants proved by the Land Development Department, the Microbial Activator Super LDD2 contained some microorganisms (i.e., *Pichia membranifaciens*, *Lactobacillus fermentum*, *Bacillus megaterium*, *Bacillus subtilis*, and *Burkholderia unamae*) that could have activated fermentation and digestion of plant residues, whereas the Microbial Activator Super LDD7 contained mainly contains some bacteria (i.e., *Gluconobacter oxydans* and *Lactobacillus fermentum*) that could triggered acetic and lactic production (Land Development Department, 2019). We postulate that active microorganisms in LDD2 inoculants might have triggered the decomposition of original organic matter in soils to become more available for plant uptake. This assumption is consistent with a recent study, showing that supplying 0.6 L of liquid *Bacillus subtilis* as a biofertilizer through an integrated water and fertilizer irrigation system, in addition to conventional fertilization, enhanced fruit yield, juice yield, edible rate, and improved fibrous root density of Tarocco blood orange trees (Qiu et al., 2021). Moreover, the microorganisms in LDD7 inoculants could provide acetic and lactic acids, which could form complexes with some cation nutrients and prolong the nutrient availability in soil systems. Moreover, the LDD2/LDD7 treatment could increase the pH and reach circumneutral conditions (i.e., pH 6.44). Increasing soil pH (pH 6.5) is the best pH condition for maximizing soil nutrient availability and mitigating soil metal toxicity, particularly Al, that typically occurs in acidic soil environments.

### Economic Benefits

In the first year, the application of LDD2/LDD7 increased by 1.4 kg in fruit weight per tea-oil camellia plant. Considering that the price of tea-oil camellia fruit is 0.70 USD/kg, according to a farmer interview, this increase translated to an additional income of 0.98 USD/plant. Given that there are 1,331 plants/ha, the income per hectare

was calculated and presented in **Table 11**. The data demonstrated that the income per hectare from the LDD2/LDD7 application was 1,309 USD higher than that from the control water application. Similarly, in the second year, the LDD2/LDD7 application yielded an additional income of 655 USD/ha compared to the control. On average, over the two years, the utilization of the mixed LDD2/LDD7 approach could earn an estimated additional profit of 1,216 USD/ha/year compared to the control. The current estimated plantation area in Thailand is 589 hectares (Pantong, 2012). We presume that the LDD2/LDD7 are applied to the tea-oil plantation area; the country's overall income

**Table 11** Income in 1<sup>st</sup> year, 2<sup>nd</sup> year, and averaged 2 years with different treatments

Treatments	Income per hectare (USD/ha)		
	1 <sup>st</sup> year	2 <sup>nd</sup> year	Averaged 2 year
Water	1,122 <sup>b</sup>	1,870 <sup>b</sup>	1,262 <sup>b</sup>
LDD2	1,777 <sup>ab</sup>	1,590 <sup>b</sup>	1,636 <sup>b</sup>
LDD7	1,683 <sup>ab</sup>	1,683 <sup>b</sup>	1,683 <sup>ab</sup>
LDD2/LDD7	2,431 <sup>a</sup>	2,525 <sup>a</sup>	2,478 <sup>a</sup>
U-test	*	*	*

\* and different letters in the same column indicate statistical differences based on the Mann-Whitney U test (U-test) at  $\alpha = 0.05$ , whereas ns indicates non-statistical differences.

from the tea-oil production can reach 1.46 million USD annually with an annual profit margin of about 0.72 million USD compared to the control water supplement. The production and labor costs associated with the application of bio-extract were minimal. This low cost is attributed to the receipt of free Microbial Activator products from the Land Development Department as well as the local abundance of banana shoot and chili, which enabled farmers to produce and apply the bio-extract themselves. Consequently, the costs related to these inputs were excluded from the economic benefit evaluation.

## Conclusions

Bio-extract application increased the fruit yield of tea-oil camellia cultivated in a mountainous area compared with the water supply. The combination of LDD2 and LDD7 bio-extract gained the highest tea-oil camellia fruit yield. Applying all bio-extracts tended to promote the mean weight diameter of the soil compared to the water control treatment. The single utilization of LDD2 or LDD7 bio-extract significantly increased mesoaggregate fraction but tended to decrease micro and macroaggregate fractions. Bio-extract application enhanced higher pH, Avail. P, Exch. Ca, and Exch. Mg, CEC, TN<sub>s</sub>, TP<sub>s</sub>, and TCa<sub>s</sub> contents. Furthermore, bio-extract increases the TN<sub>p</sub> content of tea-oil camellia leaves relative to control. Overall, this study suggests that a combination of LDD2 and LDD7 bio-extract should be applied in mountainous areas to improve both the physical and chemical properties of soils and tea-oil yield grown in mountainous regions.

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