CHAPTER 3

RESEARCH METHODS

The experiment was conducted in Banthuan village, Bantub Subdistrict, Mae Chaem District Chiang Mai Province , Northern Thailand, the experimental plot was located at altitude 1,238 m, latitude 18° 31' 04.81" N, and longitude 98° 17' 29.46" E. The average annual rainfall during the last 10 years was approximately 1,500 mm. Dominant soil is Acrisols according to The World Reference Base for Soil Resources, WRB (IUSS Working Group WRB, 2006) classification. Most conventional cultural practices in this area are contour planting based on intensive mono cropping, shifting or swidden cultivation, without any soil and water conservation strategy (Panomtoranichagul and Narubarn, 2008; Panomtoranichagul et al., 2009). This experimental plot had been used to study on "The improvement of anti-erosive and water harvesting practices in alley cropping to increase sustainable rainfed multiple crop production on sloping land", under The Uplands Program and National Research Council of Thailand (NRCT-DFG) for several years.

3.1 The Experimental Design

The experiment was designed as a Split-Split Plot in Completely Randomized Designed (Split-Split Plot in CRD) consisted of 3 replicates. Main plots were cultivated practices comprising conventional planting (CP) and furrow cultivation (CF). The 6 selected experimental plots with dimension of 5x12 m each were used as the Main-plot. Subplots were zinc applications by foliar spraying and no zinc

applications, whilst Sub-subplots were applications of lime, organic fertilizer, inorganic fertilizer and no lime and fertilizer application (Table 3.1 and Figure 3.1).

Table 3.1 *Experimental designed was a Split-Split Plot in Completely Randomized Designed (Split-Split Plot in CRD), Main plots were cultivated practices comprising conventional planting (CP) and furrow cultivation (CF), Subplots were zinc (Zn_1) and no zinc (Zn_0) applications, whilst Sub-subplots were lime (L), organic fertilizer (OF) and inorganic fertilizer (IF) applications.*

	Main-plot		Main-plot	
	Conventional Planting (CP)		**Furrow Cultivation (CF)**	
	Zinc application (Zn_1)	**None- Zinc application (Zn_0)**	**Zinc application (Zn_1)**	**None- Zinc application (Zn_0)**
Sub-subplot	**No liming and fertilization (CP)**	**No liming and fertilization (CP)**	**No liming and fertilization (CF)**	**No liming and fertilization (CF)**
Sub-subplot	**Lime application (L)**	**Lime application (L)**	**Lime application (L)**	**Lime application (L)**
Sub-subplot	**Organic-Fertilizer application (OF)**	**Organic-Fertilizer application (OF)**	**Organic-Fertilizer application (OF)**	**Organic-Fertilizer application (OF)**
Sub-subplot	**Inorganic-Fertilizer application (IF)**	**Inorganic-Fertilizer application (IF)**	**Inorganic-Fertilizer application (IF)**	**Inorganic-Fertilizer application (IF)**
	Subplot	Subplot	Subplot	Subplot

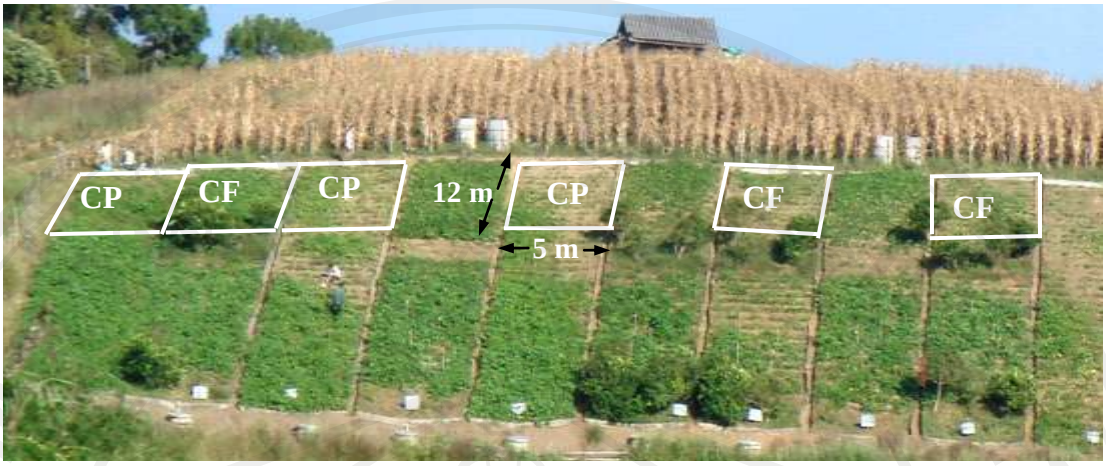

Figure 3.1 *The experimental plot consisted of 2 Main-plot, conventional planting (CP) and furrow cultivation (CF) and 4 Subplot of lime (L), fertilizers (organic, OF, and inorganic, IF) and foliar zinc (Zn) applications with 3 replicates of each treatment condition.*

3.2 Treatment Applications

Conventional planting (CP) was conducted by direct seeding along the contour line with minimum disturbing the soil surface. Furrow cultivation (CF) was done by constructing each furrow of 30 cm depth and 50 cm widths, with the ridge of 25 cm width and keeping topsoil in the furrow (Figure 3.2).

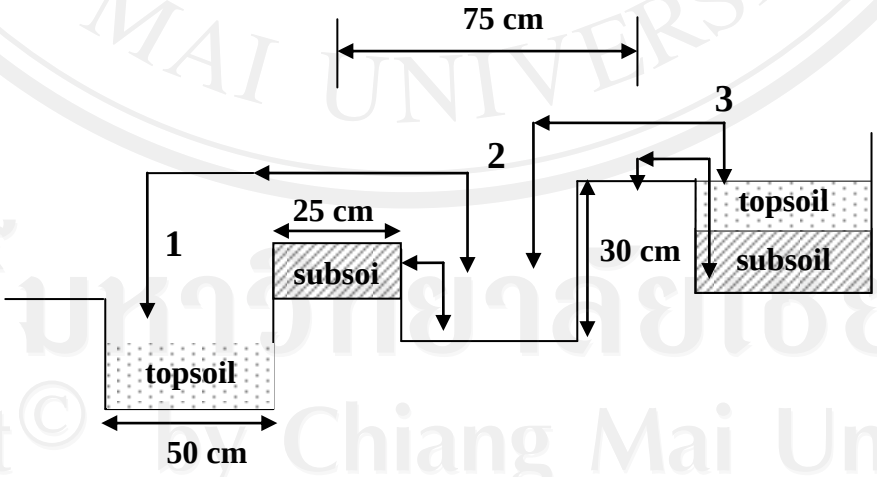

Figure 3.2 *Method of preparing furrow with 30 cm depth, 50 cm width and 25 cm width of the ridge and keeping topsoil in the furrow*

Lime (L, Calcium carbonate, $CaCO_3$) was applied as dry powder by banding along the contour planting rows or contour cultivated furrow at the rate 0.5 kg/row (2 t ha^{-1}) which was the rate of lime requirement, 2 weeks before crop sowing. Liming aimed to increase soil pH and phosphorus availability. Lime was applied on 22 August, 2009 and 17 September, 2010. The local commercial products of organic fertilizer (OF) has neutral reactions with pH 7.1, consisted of total N, P and K 1.30, 2.04 and 1.19 g/100g respectively, and inorganic fertilizer (IF, ammonium phosphate, 16-20-0), were applied as planting-row-banding at the rates of 130 and 65 g/row (333 and 167 kg ha^{-1} respectively). The fertilizers-applying rates were based on the normal farmer used rate. The comercial grade of zinc sulfate ($ZnSO_4.7\ H_2O$) was used as Zn-solution at 1 g l^{-1} concentration by foliar spraying with the rate of 1 l per 10 m^2. All fertilizers were applied at seedling stage, 2 weeks after germination, on 30 September, 2009 and 14 October, 2010 in the 1st and the 2nd experimental year respectively.

3.3 Cropping practices

Three different crop varieties were planted as multiple rotational relay cropping system. Sweet corn (*Zea mays saccharata*) was grown as the 1st crop during early-mid rainy season, followed by peanut *(Arachis hypogaea L.)* as the 2nd crop during the mid-late rainy season. Lablab bean *(Lablab perpureus Linn.)* was grown as the 3rd crop during late rainy – mid dry season. Each crop was sown at seeding rate of 3-4 seeds/pit with 40x75 cm spacing. Only one plant was left in the sowing pit after germination. This experiment was commenced in August, 2009 which was too late to collect all the data during growing sweet corn (the 1st crop). Therefore, data measurement was started during growing peanut (the 2nd crop), which was planted on

25 August, 2009. Lablab bean (the 3rd crop) was sown on 8 September, 2009. Only maize (*Zea mays* Linn.) and lablab bean were growing during the 2nd year-experiment, 2010 due to the late starting of rainy season. Maize (the 1st crop) was sown on 26 June, 2010 and lablab bean (the 2nd crop) was planted on 29 September, 2010.

3.4 Measurement of soil properties and nutrients contents.

Preliminary soil sampling (0-20 and 20-40 cm depth) and analysis was carryout for primary data before commencing the experiment on 22 August, 2009. The data was shown in Table 3.2.

Table 3.2 *Soil physical and chemical properties within 0-20 and 20-40 cm depth under conventional planting (CP) and furrow cultivation (CF) before commencing the experiment (22 August, 2009).*

Soil properties	**Conventional planting (CP)**		**Furrow Cultivation (CF)**	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm
Bulk density (BD,Mg m^{-3})	1.30	-	1.26	-
Particle density (PD, Mg m^{-3})	2.44	-	2.42	-
Total porosity (TP, m^3 m^{-3})	0.47	-	0.48	-
Field capacity (FC, m^3 m^{-3})	0.36	-	0.38	-
Aeration Porosity (AP, m^3 m^{-3})	0.11	-	0.10	-
Soil acidity (pH)	4.03	3.94	4.56	4.41
Organic matter (OM, g/100g)	3.41	1.89	3.34	1.84
Extractable phosphorus (P mg kg^{-1})	149	76	127	61
Extractable potassium (K, mg kg^{-1})	65	63	59	50
Extractable zinc (Zn, mg kg^{-1})	0.46	0.26	2.14	0.22
Cation exchange capacity (CEC, cmol(+)/kg)	10.32	8.58	10.24	8.23

During experimental period, soil sampling 0-20 cm depth were collected and measured for some soil physical properties , 3 times each year which were at seedling, flowering and harvesting stages of lablab bean. The studied soil physical properties were bulk density (BD), particle density (PD), total porosity (TP), field capacity (FC), and aeration porosity (AP). Monthly soils sampling (0-20 and 20-40 cm depth) were conducted for soil chemical and nutrients analysis which were soil acidity (pH), organic matter (OM), extractable phosphorus (Ext.P) and extractable zinc (Ext.Zn). Date of soil sampling and measurements for each soil properties are presented in Table 3.3. All soil properties and nutrient contents were measured and analyzed using standard methods presented in Table 3.4. The methods of analysis are described as follows.

(i) Bulk density (BD, Mg m^{-3}**)**

Soil cores (75x75 mm) were used to collect the representative undisturbed soil sample at 0-20 cm depth, 3 samples / plot. The volume of soil was equal to volume of soil core (Vt). The soil sample was dried at 105 ^{o}C for 24 hrs and weighed (Ms) (Blake, 1965). The BD is the oven-dry mass divided by the sample volume (equation 3.1)

$$BD = Ms / Vt \quad (3.1)$$

Table 3.3 *Date of soil sampling at different growing stages for analysis of the studied soil properties and available nutrients, which were bulk density (BD), particle density (PD), total porosity (TP), field capacity (FC), and aeration porosity (AP), soil acidity (pH), organic matter (OM), extractable phosphorus (Ext.P) and extractable zinc (Ext.Zn).*

Sampling date	**Soil sampling depth (cm)**	**Soil properties**	**Lablab bean growing stage**
22 August 2009	0-20	BD, PD, TP, FC, AP	Before lablab bean sowing
	0-20	pH, OM, Ext.P, Ext.Zn	
	20-40	pH, OM, Ext.P	
30 September 2009	0-20 and 20-40	pH, OM, Ext.P	Seedling
19 October 2009	0-20 and 20-40	pH, OM, Ext.P	Vegetative growth
15 November 2009	0-20	BD, PD, TP, FC, AP	Flowering
	0-20 and 20-40	pH, OM, Ext.P	
19 December 2009	0-20 and 20-40	pH, OM, Ext.P	Flowering - Producing seed yield
23 January 2010	0-20 and 20-40	pH, OM, Ext.P	Producing seed yield
20 February 2010	0-20	BD, PD, TP, FC, AP	Harvesting
	0-20 and 20-40	pH, OM, Ext.P	
17 September 2010	0-20	BD, PD, TP, FC, AP	Before lablab bean sowing
	0-20	pH, OM, Ext.P, Ext.Zn	
	20-40	pH, OM, Ext.P	
19 November 2010	0-20 and 20-40	pH, OM, Ext.P	Vegetative growth
18 December 2010	0-20 and 20-40	pH, OM, Ext.P	Flowering - Producing seed
14 January 2011	0-20	BD, PD, TP, FC, AP	Harvesting
	0-20	pH, OM, Ext.P, Ext.Zn	
	20-40	pH, OM, Ext.P	

Table 3.4 *Methods of soil analysis for the studied soil properties and available nutrients, which were bulk density (BD), particle density (PD), total porosity (TP), field capacity (FC), and aeration porosity (AP), soil acidity (pH), organic matter (OM), extractable phosphorus (Ext.P) and extractable zinc (Ext.Zn).*

Soil properties	Unit	Methods of measurement
(i) Bulk density (BD)	Mg m^{-3}	- Using core method modified from Blake (1965)
(ii) Particle density (PD)	Mg m^{-3}	- Using volumetric flask method
(iii) Total porosity (TP)	m^3 m^{-3}	- Calculation of TP as 1-BD/PD.
(iv) Field capacity (FC)	m^3 m^{-3}	- Using hanging column at 100 cm H_2O head or soil water suction at 100 mb and gravimetric method.
(v) Aeration Porosity (AP)	m^3 m^{-3}	- Calculation of AP as TP-FC
(vi) Soil reaction (pH)	pH unit	- 1:1 soil: water ratio suspension was measured by potentiometric method using a standardize pH meter (Kalra, 1995)
(vii) Organic matter (OM)	g/100g	- Wet oxidation by Walkley-Black procedure (Nelson and Sommers, 1982)
(viii) Extractable phosphorus (Ext.P)	mg kg^{-1}	- Bray II as extracting solution and measured by colorimetric method using spectrophotometer (Murphy and Riley, 1962).
(ix) Extractable zinc (Ext.Zn)	mg kg^{-1}	- Extracting by Diethylene triamine pentaacetic acid (DTPA) solution (Lindsay and Norvell, 1978) and measured by Atomic Absorption Spectrophotometer

(ii) Particle density (PD, Mg m^{-3})

Volumetric flask method was used to measure particle density (PD). Oven dry-soil core samples after BD measurement were ground and sieved through 2.00 mm opening sieve. Transfer ≤ 2.00 mm soil particles in to a volumetric flask of M_1 weigh and V volume, approximately 1/3 of the flask capacity. Weight the soil and flask (M_2) and add distilled water (ρ_w) to fulfill the flask volume. Remove entrapped air by gentle boiling of the water for several minutes with frequent gentle agitation of the contents. Cool the flask and its contents to room temperature and then add enough distilled water to fill the flask. The Flask and its content were weigh as M_3. The particle density was calculated as follows equation (Equation 3.2)

$$PD = (M_2 - M_1) / [V - (M_3 - M_2)/\rho_w] \quad (3.2)$$

(iii) Total porosity (TP, m^3 m^{-3})

Total porosity (TP) was determined after measurements of soil bulk density and particle density according to the procedures described in (i) and (ii). TP was calculated using Equation (3.3).

$$TP = 1\text{-}BD/PD \quad (3.3)$$

(iv) Field capacity (FC, m^3 m^{-3})

Field capacity (FC) was calculated as the volumetric soil water content at soil water suction of 100 cm H_2O column or 100 mb. The saturated soil core sample was

placed on a porous plate connected to hanging column at 100 cm height above free water level for 2-3 days to reach the equilibrium. The moist soil sample (Mt) was oven dried and weighted (Ms), soil water content at 100 mb or FC was calculated as volumetric water content, $m^3\ m^{-3}$) by equation 3.4 , when ℓw is water density.

$$FC = (Mt - Ms)/(Vt \times \ell w) \quad (3.4)$$

(v) Aeration Porosity (**AP**, $m^3\ m^{-3}$**)**

Aeration porosity (AP) was calculated as Equation 3.5

$$AP = TP - FC \quad (3.5)$$

(vi) Soil acidity (pH)

The air-dry soil (<2.00 mm) was weight 10 g and transferred into glass beaker, added 10 ml distilled water mix well with glass rod and allowed standing for 30 minutes. The suspension was stirred again before started measuring soil suspension-pH using potentiometric method by pH meter (Kalra, 1995).

(vii) Organic matter (**OM**, g/100g**)**

Soil organic matter (OM) was analyzed using wet oxidation method according to Walkley-Black procedure (Nelson and Sommers, 1982), reduction of potassium dichromate ($K_2Cr_2O_7$) by organic carbon in soil and subsequent determination of unreduced dichromate by titration with ferrous sulfate heptahydrate ($FeSO_4$-$7H_2O$).

Air-dry soil (0.5 mm) was weigh 1 g and transferred into Erlenmeyer flask and added 10 ml 1 N potassium dichromate and 20 ml concentrate sulfuric acid. Then allowed to stand for 30 minutes, add 100 ml distilled water. O-phenathroline ferrous sulfate indicator was used as 4-6 drop and titrated with 0.5 M ferrous sulfate heptahydrate.

(viii) Extractable phosphorus (**Ext.P**, mg kg^{-1}**)**

The air-dry soil (<2.00 mm) was weight 2.5 g and transferred into Erlenmeyer flask and added 25 ml Bray II solution (0.1 N HCl + 0.03 N NH_4F) as extractant. The sample was shaken for 1 minute and then it was passed through No.5 filter paper. The extracted phosphorus in solution was measured by colorimetric procedures, based on the reaction with Molybdenum Blue Color Reagent (Murphy and Riley, 1962). The color development reagent is made up by mixing Reagent A, ammonium molybdate $[(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_20]$ + concentrated sulfuric acid (H_2SO_4) + antimony potassium tartrate ($KSbOC_4H_4O_6$), with ascorbic acid. The soil phosphorus extract, 1- 2 ml, was transferred to 25 ml volumetric flask, add 4 ml Molybdenum Blue Color Reagent and add distilled water to fulfill the flask volume, and then stand for 30 minutes. The absorbance of the compound was measured at 882 nm in a spectrophotometer and was directly proportional to the amount of phosphorus extracted from the soil by compared with the standardized.

(ix) Extractable zinc (**Ext.Zn**, mg kg^{-1}**)**

Diethylene triamine pentaacetic acid (DTPA) was used as the extracting solution (Lindsay and Norvell, 1978). The air-dry soil (<2.00 mm) was weight (10 g) and transferred into an Erlenmeyer flask, with 20 ml extraction solution. The sample

was shaken for 2 hours and the suspension was passed through No.42 filter paper. Atomic Absorption Spectrophotometer was used to measure Zn in the clear solution and compared with the standard.

3.5 Measurement of Plant Growth, Plant Nutrient Uptake and Crop Yield

Only lablab bean growth during the late rainy-mid dry season were monitor as crop height (crop development) at different growing stages, and total dry matter including seed yield were measured at harvesting stages of lablab bean.

Six plants per replicate of each treatment had been monthly measured plant height for vegetative growth since germination to maturity stage. Crop biomass and yield productions were measured as dry weight (oven-dry at 65 ^{o}C for 24 hours) of total dry matter above ground level and seed yield per unit growing area.

Total plant phosphorus (total P) and total zinc (total Zn) in plant, were analyzed at harvesting stage (20 February, 2010 and 14 January, 2011) to measure plant uptake-phosphorus and zinc. The procedure of total phosphorus and zinc estimation are described as follows.

(i) Plant samples digestion

The 1 g of ground-plant sample was digested by 10 ml of di-acid mixture (HNO_3+ $HClO_4$). The plant sample content was transferred to 100 ml volumetric flask and fulfills with distilled water, and then filtered through No. 1 filter paper (FAO, 2008). This solution was used for total phosphorus (total P) and zinc (total Zn) estimation.

(ii) Total phosphorus (total P) in plant analysis

The 5 ml of digested solution was transferred to a 50 ml volumetric flask, and add 10 ml of vanadomolybdate reagent (ammonium molybdate – ammonium vanadate, $(NH_4)_6MO_7O_2.4H_2O$, in HNO_3), then make up the volume with distilled water. A yellow colour developed (30 minutes) and this yellow solution was measured by spectrophotometer at 410 nm wavelengths. Total P content determined from the standard curve (Ryan et al., 2001).

(iii) Total zinc (total Zn) in plant analysis

Total Zn in solution was measured by Atomic Absorption Spectrophotometer and compared with the standardized total zinc solution (Ryan et al., 2001).

(iv) Total phosphorus and zinc uptake by plant (total P and Zn-uptake)

The amount total P and total Zn uptake by plant was calculated as total P and total Zn concentration multiplied by total biomass production per unit growing area.

3.6 Data analysis

Inferential statistics were calculated to evaluate the effects of the studied treatments on soil properties, nutrient availability and crop yields. Least significant difference (lsd) with 95% probability or 0.05 significant level were calculated to determine the single and interaction effects of each studied treatment, cultivation practices (conventional planting and furrow cultivation), zinc, lime, organic and in organic fertilizers applications, on soil physical and chemical properties including seed yields of lablab bean, during the 2 experimental years.