



**EVALUATION OF BIO - FERMENTED LIQUID FERTILIZERS ON
GROWTH AND YIELD OF LETTUCE '*Lactuca sativa* var. *crispa*'
UNDER ORGANIC PRODUCTION**

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Title

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Title	Evaluation of Bio - fermented Liquid Fertilizers on Growth and Yield of Lettuce ' <i>Lactuca sativa</i> var. <i>crispa</i> ' Under Organic Production
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ABSTRACT

Concern over degrading agriculture environment and food safety issue along with high cost of agro-chemicals in conventional and yield limitations in organic farming, a new approach for organic fertilization is needed. In order to exploit the potential use of Bioferment (BF), this study examined water soluble NPK BF over its sourcing, nutrient and extraction timing thereby optimum rate for field application. The analysis on BF production showed that chicken manure (CM) extracted 1:1 with water yielded 2.5 fold NPK index than from cow dung (CD); however, P released 3 folds in CD BF. Among 3, 6, 9, 12 and 15 extraction days, the 15 and 9 days were the appropriate timings accumulating highest turnover in CM and CD BF respectively. The doses of best BF extract from CM and CD were drench tested and verified in two discrete organic Lettuce trials. The effect of 10 dose- levels was detected by applying compost as basal and fortnightly feeding of BF. N-based nutrient was supplied with $1220 \text{ mg/L}^{-1}/\text{m}^2$ from BF or $2600 \text{ mg/kg}^{-1}/\text{m}^2$ from Compost with and without reciprocations. Both field experiments sourcing CM BF and CD BF showed positive effects however, experiment 2 CD BF produced none significantly promoted value. Whereas, the experiment 1 CM BF revealed, among rates 100%,75%,50%,25% and nil ($200,150,100,50$ and nil ml/m^2) supplied with compost reciprocals nil, 25%,50%,75% and 100% ($\text{nil},250,500,750$ and 1000 gm/m^2), significant yield increased from BF 75:25 compost reciprocal. It gained (105%) highest yield 2237 g/m^2 , effective statue (39%), leaf number (49%) and leaf area (40%), followed by 50:50 reciprocals. This was confirmed by earlier growth vigor and high N depletion soil status. This study concluded without affecting conventional yield, CM BF could substitute 50-75 % organic mass hence potential use for alternative fertilization; in addition, contributing to synchronize crop timing, safety harvest and sustainable environment.

Key words: Bioferment, Extraction, Conventional/Organic farming, Compost, Loose-leaf Lettuce.

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TABLE OF CONTENTS

	Page
Abstract	(iii)
Acknowledgements	(iv)
Table of Contents	(v)
List of Tables	(viii)
List of Plates	(ix)
 Chapter 1 Introduction	
Background of the Study	1
Objectives	4
 Chapter 2 Literature Review	
Issues of Conventional Farming	5
Organic Farming and Sustainability	6
Organic vs. Conventional Development	7
Thai Organic Scenarios	10
Nutrient Balance and Organic manure	11
Solid Soil Manure vs. Bio-fermented Liquids	17
The Sourcing of Bio-fermented Fertilizer	18
Composting	21
IPM and Organic Farming	25
Organic Standards	27
Contamination and Microbiology	29
Organic Lettuce Growing	30
 Chapter 3 Materials and Methods	
Conceptual Framework	33
Materials and Procedures	34
Field Technique	40
Information Collection and Measurement	45
Analysis of Data	47

Chapter 4 Results and Discussions

Results

Bio-Ferment Property: Chicken Manure vs. Cow Dung	48
Production of water soluble NPK	49
Appropriate Timing for Extraction	49
Temperature and pH Fluctuations	51
Soil and Compost Property	53
Soil Properties before and after Harvesting	54
Compost Properties	
Experiment -1: Growth and Yield Parameters	
Leaf Numbers	56
Tallness	57
Leaf Area	58
Stem Diameter	58
Total Yield	59
Experiment -2: Growth and Yield Parameters	
Leaf Numbers	60
Tallness	61
Leaf Area	62
Stem Diameter	62
Total Yield	63

Discussions

Bioferment Extraction	64
Soil and Compost Property	65
Experiment -1: Chicken Manure BF vs. Compost	
Vegetative Growth	66
Total Yield	67

Experiment -2: Cow Dung BF vs. Compost

Vegetative Growth 68

Total Yield 69

Chapter 5 Conclusion and Recommendation

Conclusions 70

Recommendations 72

References 73**Appendices**

Appendix A The ANOVA table showing effect of reciprocal levels of
Chicken Manure BF Vs. Compost Application on Lettuce
Yield. 79

Appendix B Abstract of Paper Presentation. 80

Appendix C Additional Plates. 81

Appendix D Biography. 83

List of Tables

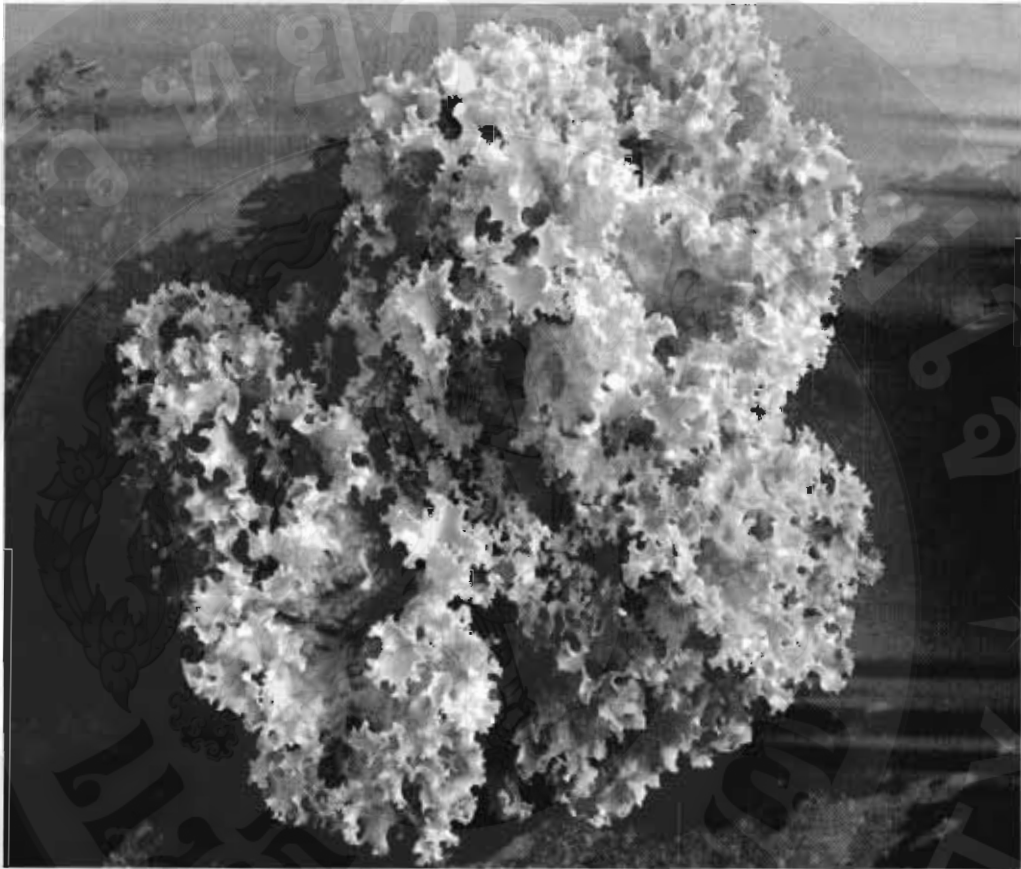
Table	Page
1 Nutrient Content by Sourcing and Type of Organic Fertilizer.	14
2 The amount of Nitrogen accumulated at weekly interval in 20 lbs Chicken Manure Tea.	20
3 Nutrients Assessment of the Requirements for Lettuce crop.	40
4.1 Treatment Structure: Experiment-1	41
4.2 Treatment Structure: Experiment-2	41
5 Types of Data Collection.	47
6 Analysis of Production of Water Soluble NPK Bioferments Score Sourcing Chicken Manure and Cow Dung over Time Period of Extraction.	48
7 Temperature and pH fluctuation during BF Extraction.	51
8 Determination of Soil Nutrient Contents.	53
9 Determination of Compost Nutrient Contents.	54
10 Effect of reciprocal level of Chicken Manure BF conc. vs. Compost on Growth and Yield of Lettuce Crop.	55
11 Weekly effect of Chicken Manure BF vs. Compost on Lettuce Leaf Number.	56
12 Weekly effect of Chicken Manure BF vs. Compost on Lettuce height.	57
13 Effect of reciprocal level of Cow Dung BF conc. vs. Compost percentage on Growth and Yield of Lettuce.	60
14 Weekly effect of reciprocal level of Cow Dung BF vs. Compost on Lettuce Leaf Number and Stem Diameter.	61

List of Plates

Plate	Page
1 Bioferment Scaling.	19
2 Generalized steps for Composting.	24
3 Extraction steps of Bioferment for both the Sourcing.	36
4 Field Design:-Experiment-1 X 2. RCBD.	42
5 Experiment Sampling Design.	42
6 Measurement of Lettuce Stem Diameter and Leaf fresh weight.	46
7.1 Production of BF from Chicken Manure and Cow Dung over time period.	48
7.2 Time period and Nutrient concentrations in BF Sourcing from Chicken Manure and Cow Dung.	50
7.3 Temperature and pH fluctuation during BF Extraction.	52
8 Cumulative effect of various parameters on Lettuce.	55
9 Effect of reciprocal level of Chicken BF vs. Compost on Lettuce Leaf number.	56
10 Effect of reciprocal level of Chicken BF vs. Compost on Lettuce Plant height.	57
11 Effect of Reciprocal level of Chicken Manure BF vs. Compost on Lettuce Leaf Area and Stem Diameter.	58
12 Effect of reciprocal level of Chicken Manure BF vs. Compost on Yield.	59
13 Effect of reciprocal level of Cow Dung BF vs. Compost on Lettuce Yield.	63

Chapter 1

Introduction



Loose Leaf Lettuce

'Lactuca sativa var. crispa'

Background of the Study

During the past four decades, particularly with the era of green revolution 1960, a large increase in the production of crop was achieved by the heavy reliance on chemical fertilizer and pesticide (FAO, 2003). This form of agriculture has been unable to build their productivity upon a cyclic and sustainable system, rather tend to unstable agro-ecosystems. Hazardous consequences have been realized. Alarms over degree of health hazards and degrading agric-environment urged to find alternatives over conventional agriculture. Organic farming is taken one of the strong alternatives to overcome these situations. However, one of the mostly announced issue is the yield lower than that of chemical farming so cause farmers often reluctant to enter into it. Such issue has been addressed as it is possible for organic farm to produce vegetable, herb and grasses with a high quality and a yield higher than or similar to that of chemical farm (Hussein and Hadid, 2003; Xu et al., 2003; Lynch et al., 2004; Hendawy, 2008). But, disinclination is found due to benefits also share to locality besides individual solely (Zanoli et al., 2007). Moreover, toward the quality and safety food matters have been explained and examples are laid as; doubled the ratio of vitamin "C" and NO_3 (Xu et al., 2003), chlorinated biocides and derivatives in mother's milk decreased (Vogtmann, 1984), carbohydrate and protein contains increased (Liu and Shijun, 2003) and vegetative growth expressed as plant height, leaf, root and stem weight increased (Hussein and Hadid, 2003) as compare to chemically produce. Similarly, improvements of animal physiological observations have been investigated through organic feeding system.

In organic vegetable production, during transitional period major plant nutrients (NPK) stress due to infertile soil and limited organic add-ons to crops is a serious agricultural problem. In large areas that affect plant growth and development leading toward a drastic reduction in quality and economic yield. Due to the replacement of chemical fertilizer, organic farming, however the consequences is seen even more distinctly and severely. Organic manures and bioferment are prerequisite for organic productions and it has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming (Splittstoesser, 1990). The bioferment sourcing from animal and poultry manures is known richen in NPK plus and capable of substituting chemical fertilizer (Hendawy, 2008; Zublena, 1997).

Lettuce crop (*Lactuca sativa* L.) is one of the most widely consumed salad crop, needs especial attention in terms of healthy produce. Preferentially under organic farming which avoids the nitrate and metallic accumulations and use of synthetic fertilizers. However, problems are laid by the lower yield during conversion. Biofermented liquids and or organic mass i.e. compost, manure are applied to overcome such problem and to meet the organic standards. Also some criticize such a way it can only be used for deficiency corrections but other suggests successful yield and quality achievements.

The primary thing to do is to find the best alternative from organic fertilizer. However, a lot of solid soil manures from domestics and urban waste as well are being investigated and applied. But, liquid bioferments which are known to be strong alternative and not yet properly investigated in terms of their potential concentrations and nutrient series over suitable extraction time and using different kinds of sourcing.

However, the benefits of bioferment owing to better nutrient value over solid manuring suggest various enhancements; as half of the soil applied N fertilizer i.e. residual soil N can be made accounted for plant (Papendick and Elliott, 1984). Higher efficiency of nutrient use and early nutrient availability are possible (Charlie, 2002). Heavy metal deposition is escape with cutting down of huge require amount of solids. Likewise the speed of absorption by drenching/foiar application is immediate and slower decomposition problem of the solid caused by interactions between the manure type and soil physics as interface from pH, temperature, moisture and proportion of cay particle can be reduced (Rochette, 2006). Therefore, to select, liquid bioferments applied can replace a much greater amount of solid nutrient mass that is soil-applied, and is immediately available to plant. It is seen importantly in intensive farming when the timing between previous and next cropping is limited and restricts the multiple cropping by slow compost decomposition up to 60 days (Zublena, 1997; Kuepper 2003) or shifting nutrient to next crop season.

Bioferment is the nutrient solution extracted and brewed aerobically from manure with microbial food source like molasses and grown up with beneficial microorganism (Grubinger,

2005). The process of aerobic fermentation or extraction 1:1, more than 7-14 days and judging by dark brown color or devoid of strong ammonia sting smell has been suggested (Stephens, 2003). After mineralized extract, the rate of concentration is assessed first so that the amount of soil organic mass requirement might be reduced could be verified. This organic tea can be built-in farm, safer, cheaply and in some case, content is even better than that of chemical fertilizer. But the direct fresh manure is detrimental to tender leaf like Lettuce (Miller, 1989).

Basically the organic matters sourcing from cow, and poultry manure contain low nutrient by its volume. High concentration of bioferments is obtained after a series of extraction process reducing the volume of organic materials. However, the concentration varies among sources as regional basis, animal feeds, breed and age with handling methods (Al-Kaisi et al., 2007). Therefore, to handle with the substitution of soil fertility materials on organic vegetable farm, it was required to verify whether the bio-fermented fertilizers are useful enough or not.

This study was mainly concern over development and verification of suitable concentrated bioferment liquid fertilizer, derived from Cattle and Poultry manures in northern Thailand's condition. Two experiments were performed in growth and yield of organically produced loose leaf Lettuce during three years of transitional phase field.

In this study, we examined and evaluated the best alternative organic fertilizer as bio-ferment, containing water soluble NPK derived from the fresh cow and poultry manure. Concentrated BF with reciprocated compost treatments were drench tested on RCBD designed organic Lettuce production plots. Technology of organic production was implemented according to ACT -1995 and ATTRA (2002) if not applicable from Thailand's Royal Project. Recommendations to the farmers were made base on result obtained from replicated trails.

The goals of this study as follows:

Objectives

Overall Objectives:

- Find out the viable alternative organic fertilizer for GAP through formulating environmental friendly, safer, low cost, potential alternative over chemical fertilizer as bio-fermented fertilizer.
- Cutting down the cost of production through reducing the large amount of soil solid organic mass management hazards and reducing the crop to crop time gap created by slow nutrient release of solid mass in organic farming.
- Extension of self relines technology of organic fertilization to produce healthy farm products under organic standard and export.

Specific Objectives:

1. To examine the sourcing of bioferment, potential concentration and the appropriate timing for bio-fermentation or extraction in order to produce best water soluble NPK formulation for plant application.
2. To evaluate the best bioferment as an alternative fertilizer for optimum rate of concentration reciprocal with compost fertilization.

Chapter 2

Literature Review

Issues of Conventional Farming

For years, industrialized forms of agriculture (also called conventional or modern agriculture) have been unable to build their productivity upon a sustainable system (Vogtmann, 1984), rather are dependent on large inputs of chemicals from industry. These farming systems considered the degrading agriculture environment as unimportant ecosystems and yields are inevitably associated with a risk due to soil fertility instability (Papendick and Elliott, 1984; splittstoesser, 1990). From the era of green revolution 1960, a considerable increase in the production of agriculture was achieved through the use of high yielding, hybrid varieties of crops and higher inputs of chemical fertilizer as well as plant protection chemicals (FAO, 2003). Heavy reliance on chemicals continues to rise with and tend to be degrading environment in the potential for crop yield. Besides failure to support and care for natural factors of inputs, however agricultural production continued to elevate but efficiency per unit area has started to decline (Pathak and Ram, 2003). The sustainability of the strategy adopted during green revolution era became debatable. It has been realized that the increase in the production was attained at the cost of soil health, environmental pollution, losses of indigenous crop diversity and poor health among rural people. These agricultural practices have caused irreparable damage to soil fertility, structure and water holding capacity (Papendick and Elliott, 1984; Jayamangkla, 2007). As a result disaster like soil desertification, loss of biodiversity, Climate change, food and water toxic residue hazards have been emerged.

The discriminate use of chemical fertilizer has not only resulted in various unwanted environment health hazards also with socio-economic problems. Highly proven the farmer who are caught in a difficult situation, have to continuously cope with a decreasing margin between costs and product prices; costs rises quicker than produce prices especially the cost of fertilizer (Panyakul, 2003). Many time the farmers have lack of money to purchase chemical fertilizer or even not available in time.

Organic Farming and Sustainability

The concerns over the issues of conventional farming particularly in environmental degradation and food safety matter, over all sustainability have prompted to search alternatives on top of the chemical farming. Considering the primary thing to find ecologically sound, viable and sustainable farming system for different soil and agro-climatic situations, organic farming system has been found one of the well-built alternatives. The organic farming systems have proved to be sustainable and it is not only the alternative to chemical farming but also became urgent for future generations (IFOAM, 2007; Pathak and Ram, 2003). This matter has also been suggested by different organic researchers, scientists and institutions (Vaheesan, 2003; Hiraoka, 2003; Scialabba and Wai, 2003; Hussein and Hadid, 2003; IHC Toronto, 2002; Vogtmann, 1984; Papendick and Elliott, 1984; FAO, 2007; Niwa et al., 2006 and Panyakul, 2003).

Evidences of positive role of organic farming have been indicated in both above and below ground biodiversity preservation and restorations. Contribution toward reducing chemical agriculture dominant pollutions such as 20 % of global anthropologic green house gas emission, 70 % and 40 % of threatened bird and plant species (Scialabba, 2003). A long term sustainability comparisons with chemical farming demonstrated (FAO, 2002) that organic farm deserves per hectare higher biological activity of 30-100 %, higher total mass of soil microorganism (30-40 %), reduction on nitrate leaching rate of 40-64 % and 30- 50 % of increase energy use efficiency.

Define: Organic farming has been defined and perceive according to focus as ecological farming, natural farming, perma-culture, sustainable farming and bio-dynamic farming. However, central concept is found to be similar. Broadly (IFOAM, 1995) defines as:

“Organic farming is an approach to agriculture where the aim is to create integrated, environmentally and economically sustainable agricultural production systems. Maximum reliance is placed on locally or farm-derived renewable resources and the management of self-regulating ecological and biological processes and interactions in order to provide acceptable levels of crop, livestock and human nutrition, protection from pests and diseases, and an appropriate return to the human and other resources employed”.

Reliance on external inputs, whether chemical or organic, is reduced. In many European countries, organic agriculture is known as ecological agriculture, reflecting this reliance on ecosystem management rather than external inputs. ATTRA (2002) announces the basic principles of organic farming are “Integrated Pest Management (IPM), Integrated Crop Management (ICM) and Integrated Farm Management (IFM)”.

Sustainability is considered chiefly due to plant growth and development that organic farming recognizes, depends on interaction between and among various organisms is important to maintain the ecosystem balance (Pilkaukas and Mehta, 2003).

Organic Vs. Conventional Development

In the past, organic farming was viewed through the eyes of inefficient, less productive and traditional practices of labor use but now it has been realized holistically by its broad responses toward sustainable locality and community, integrity with animal husbandry and sound ecosystems. In order to produce require inputs traditionally, farms have also combined several kinds of agricultural land use; developing country where the labor cost is low suggests the utility for organic production. In addition, the possibility of additional employment, increasing consumer health awareness, rising domestic as well as international market further promote the development toward organic farming.

There have been many studies on organic vegetable farming; however, one of the most state dilemma is the yield lower than that of chemical farming causing farmers' unwillingness to enter organic farming. This issue has been taken in hand by organic researchers. It is probable for organic farming to produce vegetables with a good quality and a good yield similarly to that of chemical farming (Hussien and Hadid, 2003; Xu et al., 2003) implementing good nutrient supply. However, reluctance is observed due to, unlike chemical farming benefits are also shared to community or locality or environment instead of individual only (Zanoli et al., 2007). This is also spelled out for the right of coming generations.

Toward yield, quality and safer food contrast

Chemical vs. organic production:

Regarding the yield obtained in organic vegetable farming reveals the growth at the early stage of leafy vegetable was lower in organic fertilizer than in chemical however, on later stage grew better and resulted in a final higher total yield (Xu et al., 2003). Also similarly (Hussein and Hadid, 2003) indicated in a nutrient research with higher yield found from cattle and chicken manure as compare with the others. Several other researchers have indicated contradictory results of lower yield too. However, many organic investigators believe in slightly lower yield during transitional period likely to be occurring due to soil fertility build up process and suggest it can be overcome afterward. Compensations are also indicated obtainable through price premium.

Furthermore, not only yield but also toward the quality and safer food matters are well explained and examples are shown. Like plants undergo changes under organic fertility produce different qualitative harvest in terms of essential nutrients and amount contents. Concentration of sugars, vitamin "C" significantly increased in leaf of Brassica grown under bioferments fertilizer and the ratio of vitamin "C" and NO_3 nearly doubled indicates the quality can be enhanced by organic as compare to inorganic feeding (Xu et al, 2003). This study is found important for nitrate accumulation problem like in leafy vegetable. A similar study by Liu and Shijun, (2003), reported that the yield and amount of carbohydrate and protein contain of Brassica in winter increases in organic nutrient media over inorganic, suggest the yield and quality obtained from organic nutrient is even better than the conventional farming. According to Aubert cit. Vogtmann (1984) the chlorinated biocides and derivatives in mother's milk decreases as the intake of organic produce increases, indicates organically produce are much safer than chemically produce. This is seen importantly for the health of infant as well as mother herself. It is found that food chemical changes on plant not only endanger the health but also leave harvest with toxic residues to consumer both human and animal (FAO, 2003) and emergence of fish disease in Thailand is the example (Panyakul, 2003).

Nutrient stress and relation to animal:

Chemical vs. organic production:

Much has been reported by researchers in relation to human kind however, the aspect of livestock has been little investigated. The influence of nutrient stress primarily under organic vs chemical farming has been established after 1970's health hazards reported from livestock researcher. The lower fertility in bulls receiving feeds produce under chemical fertilizer as compare to those feeds grown from FYM have shown earlier in 1975. Similarly, lowest death rate found in rabbits is the indication so far observed from feeding produce in organic farming can be taken as positive plant functional physiology that is created by organic production system, however the straight forth relations between these observation and feed from mineral nutrient is not been clear (Vogtmann, 1984).

Global Trends:

- Soil from many areas getting dead and economically cannot be re-cultivated,
- Organic is accepted for the process not the product,
- Bioferment is local, cheaper, safe and affordable by the farmer specially in developing country,
- The natural storage of chemical fertilizer (accept N nutrient) have been deployed,
- Farmer and consumers are now aware enough to behave according to consequences of adopting any of the product,
- Organic products fetch higher rate of return than the others,
- Many countries have already started to control over not to import the chemically produced product especially for vegetable,

As a result of world trend, it is reasonable to say the chemical grower can be put in mess to sell and use their product in future.

Thai Organic Scenarios

Local Thai farmers are continued to exercise traditional farming practices from its very beginning. Except shifting, although considered non competitive still deserves environment friendly. Beside the lots of intervention made by chemical farming, many of the farms still alive, such existence known to be the basis for organic farming in Thailand.

Furthermore, the northern mountain belt considered vulnerable due to shifting cultivation, where Royal project is devoted to uplift the living of people through organic farming. Retaining the ecology and benefits of the local people are the major goal of the projects. After 1980s movements the traditional farm started to transfer into chemical modern farming with heavy rely on excessive use of agro-chemicals. Consequences are started to expose as environmental as well as health hazards. Situation was worsening when many farmers reported to be indebted and forced out of their farmlands, fresh water fish disease emerged (Panyakul, 2003; Thailand's Royal Project, 2007).

This threat trend drew attention of policy maker and scientist toward formulating plan and policy for organic farming. As a result NGO based organization like AAN, GNENF together with government and 8th plan initiated the related program.

Predominantly rice, vegetable and fruit are the focal point of organic production. Among vegetables one of the most used world's salad crop, Loose Leaf Lettuce (*Lactuca sativa cv crispa* L) is popularly grown among the farmers affiliated in the Thai Royal Project. However, the lower yield has been observed during its transitional period. Nowadays, successful developments toward the organic production, harvesting, postharvest measures, packaging and transportation to exportation been achieved.

Nutrient Balance and Organic Manure

Soil Amendments:

All organic matter (mostly C: N < 30:1) incorporated in the soil and mulch, to all that is left or spread on it, and refers only microorganisms and microbiological products derived from plant and animal origin are known to be organic amendments (Splittstoesser, 1990). These are: compost, farm yard and other manures, green manure, crop residues, mulching materials, sawdust, dry grass, forest liter, husks and straws, urban waste, industrial or other organic by-product.

From very beginning of research a number of functions of soil amendments for soil fertility and crop production have been identified and explained consecutively by the researchers. Some of the major roles of soil amendments are as follows (Splittstoesser, 1990; ATTRA, 2002).

- Source of food for micro organism, earthworm increase,
- Water holding capacity increase by filling the space between coarse particles,
- Amount of usable water increase by a parting particles and draining of clay soil,
- De-cementing/de-crusting increase leading to easy root penetration,
- Better aeration leads to better root growth,
- Maintain temperature equilibrium,
- Release of package of nutrients rather certain amounts,
- Decay acids dissolve mineral nutrient from natural deposits,
- Widen Cat ion Exchange Capacity,
- Increase disease hindrance.

Organic farming entails development natural processes through holistically by time. The microorganism plays an important role to allow natural production of nutrient. Considering the boost up the soil thereby plant, it is referred as feed to the soil not to the plant. Addition of organic matter such as manure and compost increases the nutrient in soil however, the microorganism needed to withdraw such nutrient intern enhance the microbial population which again release the amount of nutrient, suppressing the harmful microorganism and releasing the important plant growth regulators.

Experiments on soil microbial activity, feeding on organic matter reveals suppressing harmful organism, and combining soil solarization with amendments enhanced protection from soil pathogens. Due to heating and generating of volatile compounds the soil borne disease and pest can be better managed. For example, the percentage of club-root disease incidence in 7 years organic vs. conventional farming found increase from 40 % to 90 % in conventional farming and nearly 0 % in organic farming (Niwa, 2006).

Amendment Principles:

- Feeding first to soil biomass recycle to soil solution and root to plant is a nature friendly phenomenon,
- Soil considered as a primary priority and long-term fertility is maintained,
- Mother breast feeding vs. bottle feeding to baby,
- Nutrient balance is achieved considering the amount of depleted nutrient through harvested product plus residue management,
- After Transitional Period no nutrient loss occurs to nutrient stress and depletion.
- Transitional Period depends on fundamental of soil contains at start, mostly chemical effect considered to be naturalized after 3 to 5 yrs of cutting down the supply, and during and after Transitional period soil empowered its parameter and considered to be strong enough to maintain the soil eco- system (Vogtmann, 1984).

In organic vegetable production, especially during the soil build up or conversion time from inorganic to organic system, experiences significant yield reduction. The reductions are seen according to degree of soil degradation before the system changed. This occurs because of the withdrawal of continuous fertilizer disrupt the established nutrient cycle and flow pattern in soil thereby plant then needs time to be re established (Papendick and Elliott, 1984). After the replacement of chemical fertilizer by organic in organic farming, the consequence is seen noticeably. This difference can be much greater where vegetable crops are involved and farm size is large tended. A better management effect of manure is likely to be occurred if the farm size holds not too big (ATTRA, 2002).

Organic manures are requirement for organic productions and it has been identified as an alternative to chemical fertilizer increasing soil fertility and crop yield in sustainable farming. Soil fertility is also amplified by improving water and nutrients holding capacity, increasing soil bio-life, releasing nutrients and widens CAC and better aeration and better soil structure (Vogtmann, 1984; Niwa, 2006). However, soil applied solid organic manure are still not devoid of agricultural problems in terms of heavy metal deposition, nitrate downward movement, slower decomposition, inefficient nutrient use and primarily, the large solid mass management hazards. The biological process of nutrient mineralization with soil microorganism varies and depends upon moisture, temperature, microorganism species and population size in the soil. So to access the nutrient availability often found unpredictable to nutrient use efficiency mostly as compare to liquid bioferments causing over application subsequently leading toward contamination and nitrate hazards (ATTRA 2002; Jayamangkala, 2007).

It has long been acknowledged that improper use of raw manure can adversely affect the quality of vegetables; as it breaks down in the soil; raw manure releases chemical compound i.e. Skatole and phenols (Grubinger, 2005). When absorbed by plant, these compounds can impart off flavors and odors to the vegetables. Acidification is also likely to be occurred due to releasing of different organic acids.

All the bioferments sourcing from cattle, chicken, pig, goat and horse contains nutrients in varying proportion but some of them are recognized to be ample in plant nutrient if concentrated could be able to substituting chemical kind of fertilizers (Matararino, 1994; Hendawy, 2008). In organic application, the evaluation containing NPK % from different sourcing suggests highest total yield can be obtained by chicken and cattle manure than from the others (Hussein and Hadid, 2003). Also, among manures analysis in terms of its nutrient contains, the poultry manure deserves highest total concentration above swine and dairy manure (Table 1).

Liquid manure vs. solid manure is investigated and higher contents of major nutrients have been demonstrated as follows:

Table 1 Nutrient Content by Sourcing and Type of Organic Fertilizer:

(lb/ton)

Materials	TKN	P ₂ O ₅	K ₂ O	Ca	Mg	S
Dairy Cattle:						
Fresh manure	10	5	8	4	2	1
Liquid	50.6	30.8	46.2	22	11	6.6
Poultry:						
Fresh manure	26	22	11	41	4	4
Liquid	136	130	81	77	15.4	17.6
Swine						
Fresh manure	12	9	9	8	2	2
Liquid	68.2	48.4	37.4	19.8	6.6	11
Horse						
Fresh manure	12	6	12	11	2	2
Sheep						
Fresh manure	21	10	20	14	4	3
Goat						
Fresh manure	22	12	18	-	-	-

(Adapted from: Zublena, 1997; Jayamangkala, 2007).

Major nutrients NPK management:

Since changed crop physiology under nutrient stress is found in organic production, crops constantly sense the changes in their environment. Internal changes of physio-chemical regulate the visible external symptom of expressions. Usually symptom exposes lateral stage for standard monitoring and often becomes difficult in recovery. However, the changes are equally observable no matter of organic and in-organic system of crop production; but in the organic system it is needed to be handled more vitally especially during transition soil build up process.

Nutrients such as nitrogen, potassium and phosphorus are important macronutrients for crops but are often deficient in the field. Little is known about how plants sense fluctuations into physiological and metabolic adaptations (Amtmann and Wareing, 2005). Nevertheless, their functional linkage are well explained, mostly the nutrient N is responsible as constituent of chlorophyll, proteins and nucleic acid, the P is for phospholipids, energy transfer and K is for regulatory functional activities.

In this regard, (Hermans et al., 2006) indicated when nutrients are scarce; plant often allocates a greater proportion of their biomass to the root system. This acclamatory response is a consequence of metabolic changes in the shoot and an adjustment of carbohydrate transport to the root. It has long been known that deficiencies of essential macronutrients (nitrogen, phosphorus, potassium and magnesium) result in an accumulation of carbohydrates in leaves and roots, and modify the shoot-to-root biomass ratio distinctly. Considering the urgent recovery on nutrient stress site, however, the better available of water can enhance the acquisition of elements overcoming the nutrient stress status of crop.

In organic growing the nutrients NPK sources are various organic materials from plant and animal origin. Animal or bird manures containing more than 30 C: N ratio undergoes decomposition and forming compost or bioferments are mostly used. The organic system of crop nutrition begins with proper care and nourishment of the organism responsible for the soil fertility process. This soil health is improved through recycling of organic manures with the combination of biological fixation, green manuring and biodiversity enhancement. Focus is drawn in the equal amount of replacement that is removed from the soil (Splittstoesser, 1990).

Nitrogen

N supply is most prominent that crop needs in the greatest amounts (Guerena, 2006). Usually after transition, the initial nitrogen available from animal manure, soil solid as well as bioferments is enough (ATTRA, 2006). However, the legume biological N fixation, cover crops and green manuring are required adequately during and after transition to ensure enhance balance

soil micro biology and fertility for soil hygiene and disease insect pest managements (Papendick and Elliott, 1984). But the proper fermented or decomposed products are necessary over its time and doses according to soil status and crop requirements. Once the soil builds up ensured, through cyclic manuring the N nutrients is not the limiting factors in organic vegetable production as it saves half of the leaching N from the rhizosphere. The mineralization of N and its availability to crop varies greatly, depending upon the N source, the temperature, humidity, texture and mainly microbial activity. If not enough, additional bio friendly fertilizers such as pelleted compost, fish emulsion, blood meal can be supplemented according to requirement of crops.

Phosphorus and Potash

P and K nutrients are second most required element known that influence quality and yield of crop. In most of the case the removal of P and K by crops relative to total amount of these nutrients in the rooting zone of soil are often small (Papendick and Elliott, 1984). So that the increase in the organic matter further increases the microbial activity and organic acids which in turn release and add to the levels of these natural elements. According to ATTRA (2006), manures and residues are enough to release natural and content phosphorus and potash requirement of the crops, in addition, rock phosphate and wood ash respectively if needed is an option and acceptable. However, the organic cultural practices play a vital role as the shallow plowing; intercultural operations help to prevent downward movement of element. Moreover, poultry manure is known by richen in K and the michorizal utilization are also suggested for phosphorus enhancement activity.

Micronutrients

Further need of micronutrient is not likely to be occurred where the biologically active soil with adequate organic matter is supplied and maintained (ATTRA, 2006). The soil food web which regulates the potensial activities of microorganism in turn benefits the plant by releasing various types of elements in to the soil solution. Nevertheless, the foliar feeding of bioferment is still likely to solve the micronutrient deficiency.

Solid Soil Manure vs. Bioferments Liquids

To find the best alternative derived from organic fertilizer, however, a lot of solid soil manures from cattle, poultry, pig, horse, goat, and urban as well are being investigated and field applied but, bio- ferment that is known to be strong substitute needs to be properly studied. The different sourcing in terms of their potential nutritional series requires verified.

Since there are no generally accepted recommendation or guidelines specifically for organic production with bioferment; however, the benefits of aerobic bio ferments due to increased nutrients concentration, increasing beneficial microorganism i.e. fertility building microbes 10^{11} per ml of liquid (Wikipedia, 2007) including lactic acid bacteria suggests various improvements.

Such as a tremendous downward movement of nitrate can be reduced (Ott et al., cit. Vogtmann, 1984). Similarly, half of the soil applied N fertilizer i.e. residual soil N can be made accounted for plant (Papendick and Elliott, 1984). Higher efficiency of nutrient use and early nutrient avail are possible. This is suggested due to increased microbes, increased nutrients movement at the rate of one foot per hour to all part of plant, providing 95 % use efficiency as compare to 10 % of soil solids (Charlie, 2002). Heavy metal deposition from solid mass is escaped. Likewise the speed of absorption and use by liquid application is immediate as it reserves the natural sugars. Further, slower decomposition problem of the solid caused by interactions between the manure type and soil physics such as proportion of clay and sand particle, temperature, pH, moisture and their interface can be reduced (Rochette, 2006). It plays important role in reducing the time gap between crop to crop plantations benefiting multiple and intensive cropping sum up 60 days (Kuepper 2003; Zublena, 1997). An interpretation of Virginia tech by Charlie (2002) shows liquid bioferment applied can replace a much greater amount of solid mass than that is soil –applied and immediately available to plant. Therefore, selecting bio-ferments, should replace a much solid soil mass management hazards and able to reduce the higher cost of production.

The Sourcing of Biofermented Fertilizer

Bioferment is defined by the nutrient solution extracted and brewed aerobically from manure with microbial food source like molasses and grown up with beneficial microorganism (Grubinger, 2005).

Due to the variation in nutrient concentrations and C:N ratio of sourcing, associated with different location, region, breed, feed, age and sanitation factors (Al-Kaisi et al., 2007) under domestics, the bio-fermented is prepared locally. Age and feed of livestock as cow, buffalo, goat, sheep, horse or poultry, piggery greatly affects the amount of nutrient found in bioferment. These variations are localized because of variations in their nutrients in feeds. However, this organic manure tea can be built-in farm, safer, cheaper and in some cases, contains are even better than that of chemical fertilizer (Gross, 2007; Martin and Nathan, 1984; Matarirano, 1994). It reserves wider range of multiple nutrients with substitution value to chemical fertilizer (Palm 1997; Hendawy, 2008).

While preparing biofermented fertilizers from different organic sources, it must be extracted and mineralized at a certain phase as time and quantity before they become plant-available (Crohn, 2006). The rate and concentrations of applications need to be assessed first in terms of liquid absorption and crop requirement so that the amount of reducible solid organic mass requirement which varies according to sources used, that can be quantified. These quantities are then collected to ferment or extract in the bioferment fertilizer.

Though variability among researches has been seen in bioferment extraction, timings, dilution and dose factors however, it is realized the liquid feeding (drenching as well as foliar) of such low-cost substrate containing not only specific plant nutrient, but also natural sugar that aids rapid entry and movement into and through plant, necessitates the further need of search and generalization.

Bioferment Scaling:

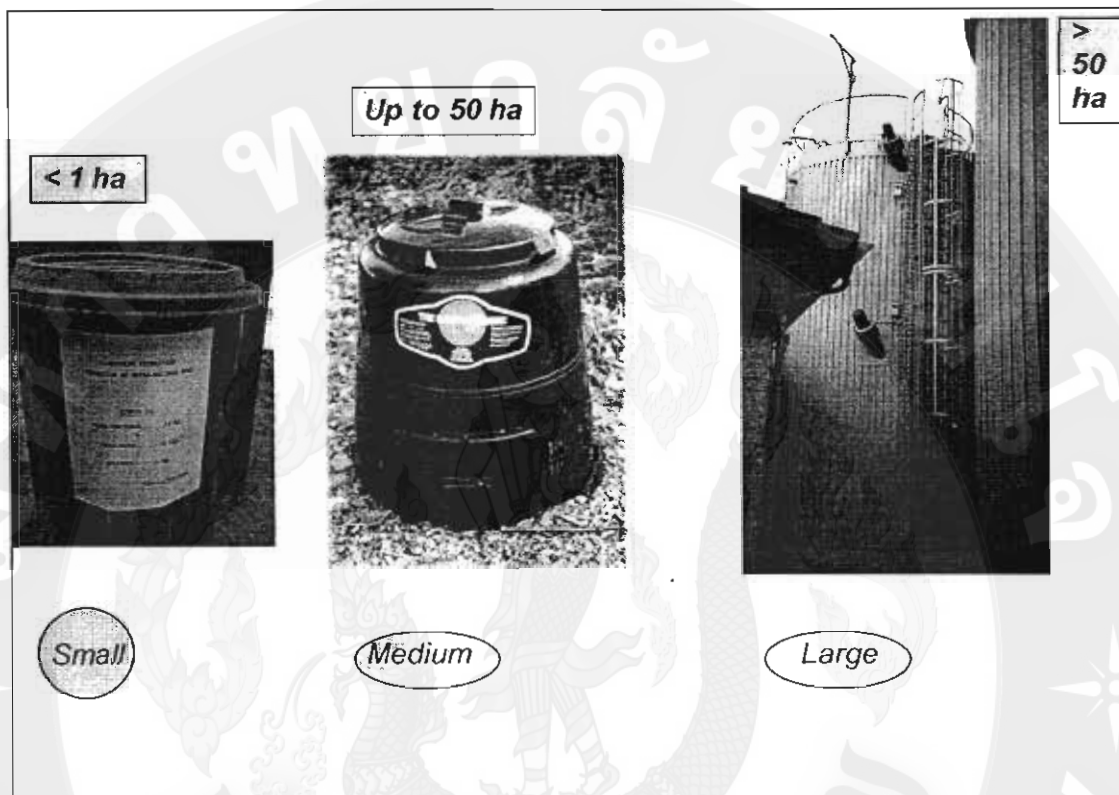


Plate 1 Bioferment Scaling collected from www.media.cce.cornell.edu; biopact.com; etc.

The amount of bioferment to be produce depends up on the nutrients concentration in it. However, as shown in the plate 1, biofermented liquid are prepared according to the crop cover area. It is suggested that the basal application of bioferment needs more amount as compared with foliar feeding (Matarirano, 1994).

Cow dung and Poultry manure based bio-ferments:

Fundamentally, the organic matters like cow dung and poultry manure contains low nutrients by its volume (Splittstoesser, 1990). High concentrations of bio-fermented are acquired after a series of extraction, decomposition and fermentation processes which in turn significantly reduces the volume of solid organic materials. In field study, the poultry manure containing narrow <15 C: N ratio than that of wide cow dung >15 indicates high production with delayed

mineralization (Palm, 1997). Putting forward the sources of bio-ferments and its doses to apply, (Matarirano, 2007) indicates fresh Cow dung and Poultry manure are good source for liquid fertilizer since they contain ample nitrogen, phosphorus, potash and other micronutrients. If concentrated it can provide nutrients just like chemical fertilizer, but the strong backup fresh poultry liquids are detrimental to tender leaf like Lettuce (Miller, 1989).

Table 2 The amount of N accumulated at weekly interval in 20 lbs Chicken Manure Tea

(ppm)

Nitrogen	1 week	2 week	3 week	4 week
NO ₃	102	32	42	18
NH ₄	655	732	832	860
Total	757	764	874	878

(Adopted from-Martin and Nathan, 1984)

A process of aerobic fermenting more than 7- 14 days and judging by dark brown water color or devoid of strong ammonia sting smell has been suggested (Grubinger, 2005), similarly 10-15 days are recommended by CIWMB (2008). The amount of rated manure to water while preparing bio- ferments controversially varies among researcher of different sources used, Stephens (2003), University of Florida stated preparing water: manure ratio with 1:1. However, rule of thumb (Richards, 2007) allow to extract in water at the rate of 2 pounds to 2 gallons, strain and use the liquid diluted 10-15 times have been practiced. Likewise (Liu and Shijun, 2003; Matarirano, 1994; Gross, 2006) suggested suitable result from the ratio of 1: 10 dry matter and water diluting it in 10 times has been suggested for liquid feeding. On an average, preparation of liquid manure takes 8-12 weeks time and one thousand liter of liquid dissolved in 4 thousand liter of water is used on plant as liquid spray (Pathak and Ram, 2003). In contrary, also found in terms of releasing days and mixing water as stated in table 2 (Martin and Nathan, 1984).

A method of compost extraction proposed by researchers at the Wood's Hole Laboratory uses an 8:1 water to compost dilution. Water is added to the compost and the mixture stirring for

about 10 minutes every day of the week long extraction period. Subsequently, the extract is filtered through several cheese cloths, and then store outside away from sunlight until use on crops. A double strength, less dilute solution is also prepared with a 4:1 water to compost ratio (Silvia, 2001). Over this contribution still little been evaluated the rate and time of applications under the nutrient series of bio-fermented materials.

The amounts of nutrients release from organic materials are a function of their physical/chemical composition, the amounts set and surrounding factors. In this regards, Nhamo et al. (1985) concluded there is a positive correlation between the initial %N, C: N and % C and net N mineralized after 7 days of incubation and a weak correlation with lignin. The characteristics of cattle manure showed that it is highly variable with N content that ranged between 0.10 and 2.76% and manure poses a C:N ratio narrow <15 (chicken manure) and broad >15 (cow dung). The %N, %C, lignin and the C: N ratio is the most reliable indices of manure nutrient release. However, the C: N ratio found to be negatively related to N mineralization (Chandwick et al., 2000; Palm, 1997).

In terms of bioferment doses to apply, apart from unequal contains among sourcing and crop requirements, there little have been explained in relation to nutrient concentration needed to apply through bioferments. However, many researchers have suggested similar doses to comply with i.e. foliar application as mass and water and dilution to 1:10:10 (Matarirano, 1994), or hydroponic solution to 200 ppm N (Martin and Nathan, 1984), or fertigation (Gross, 2007), or absolute 200 ml BF per liter of water (Hendawy, 2008) and absolute dry base to 200 gm per m² (Xu et al., 2003).

Composting

Composting is the natural process of 'rotting' or decomposition of organic matter by microorganisms under controlled conditions. Raw organic materials such as crop residues, animal wastes, food garbage, some municipal wastes and suitable industrial wastes, enhance their suitability for application to the soil as a fertilizing resource, after having undergone composting.

Compost is a rich source of organic matter. Soil organic matter plays an important role in sustaining soil fertility, and hence in sustainable agricultural production. In addition to being a source of plant nutrient, it improves the physio-chemical and biological properties of the soil. As a result of these improvements, the soil: (i) becomes more resistant to stresses such as drought, diseases and toxicity; (ii) helps the crop in improved uptake of plant nutrients; and (iii) possesses an active nutrient cycling capacity because of vigorous microbial activity. These advantages manifest themselves in reduced cropping risks, higher yields and lower outlays on inorganic fertilizers for farmers (Atta -Atia. 2005).

Types of composting:

In anaerobic composting, decomposition occurs where oxygen (O) is absent or in limited supply. Under this method, anaerobic micro-organisms dominate and develop intermediate compounds including methane, organic acids, hydrogen sulphide and other substances. In the absence of O, these compounds accumulate and are not metabolized further. Many of these compounds have strong odors and some present phytotoxicity. As anaerobic composting is a low-temperature process, it leaves weed seeds and pathogens intact. Moreover, the process usually takes longer than aerobic composting. These drawbacks often offset the merits of this process, i.e. little work involved and fewer nutrients lost during the process.

Aerobic composting takes place in the presence of ample O₂. In this process, aerobic microorganisms break down organic matter and produce carbon dioxide (CO₂), ammonia, water, heat and humus, the relatively stable organic end product. Although aerobic composting may produce intermediate compounds such as organic acids, aerobic micro-organisms decompose them further. The resultant compost, with its relatively unstable form of organic matter, has little risk of phytotoxicity. The heat generated accelerates the breakdown of proteins, fats and complex carbohydrates such as cellulose and hemi-cellulose. Hence, the processing time is shorter. Moreover, this process destroys many micro-organisms that are human or plant pathogens, as well as weed seeds, provided it undergoes sufficiently high temperature. Although more nutrients are lost from the materials by aerobic composting, it is considered more efficient and useful than

anaerobic composting for agricultural production. Composting objectives may also be achieved through the enzymatic degradation of organic materials as they pass through the digestive system of earthworms. This process is termed as vermicomposting.

The aerobic composting process:

The aerobic composting process starts with the formation of the pile. In many cases, the temperature rises rapidly to 70-80 °C within the first couple of days. First, mesophilic organisms (optimum growth temperature range 20-45 °C) multiply rapidly on the readily available sugars and amino acids. They generate heat by their own metabolism and raise the temperature to a point where their own activities become suppressed. Then a few thermophilic fungi and several thermophilic bacteria (optimum growth temperature range 50-70 °C or more) continue the process, raising the temperature of the material to 65 °C or higher. This peak heating phase is important for the quality of the compost as the heat kills pathogens and weed seeds.

The active composting stage is followed by a curing stage, and the pile temperature decreases gradually. The start of this phase is identified when turning no longer reheats the pile. At this stage, another group of thermophilic fungi starts to grow. These fungi bring about a major phase of decomposition of plant cell-wall materials such as cellulose and hemi-cellulose. Curing of the compost provides a safety net against the risks of using immature compost such as nitrogen (N) hunger, O deficiency, and toxic effects of organic acids on plants.

Eventually, the temperature declines to ambient temperature. By the time composting is completed, the pile becomes more uniform and less active biologically although mesophilic organisms re-colonized the compost. The material becomes dark brown to black in color. The particles reduce in size and become consistent and soil-like in texture. In the process, the amount of humus increases, the ratio of carbon to nitrogen (C: N) decreases, pH neutralizes, and the exchange capacity of the material increases.

Compost making steps:

Compost piling on a well-drained site would benefit from nutrients running off the pile. Pile can be built gradually in layers. The layers are distinctly fill according to the rules of thumb supplying organic materials amounting those are locally available.

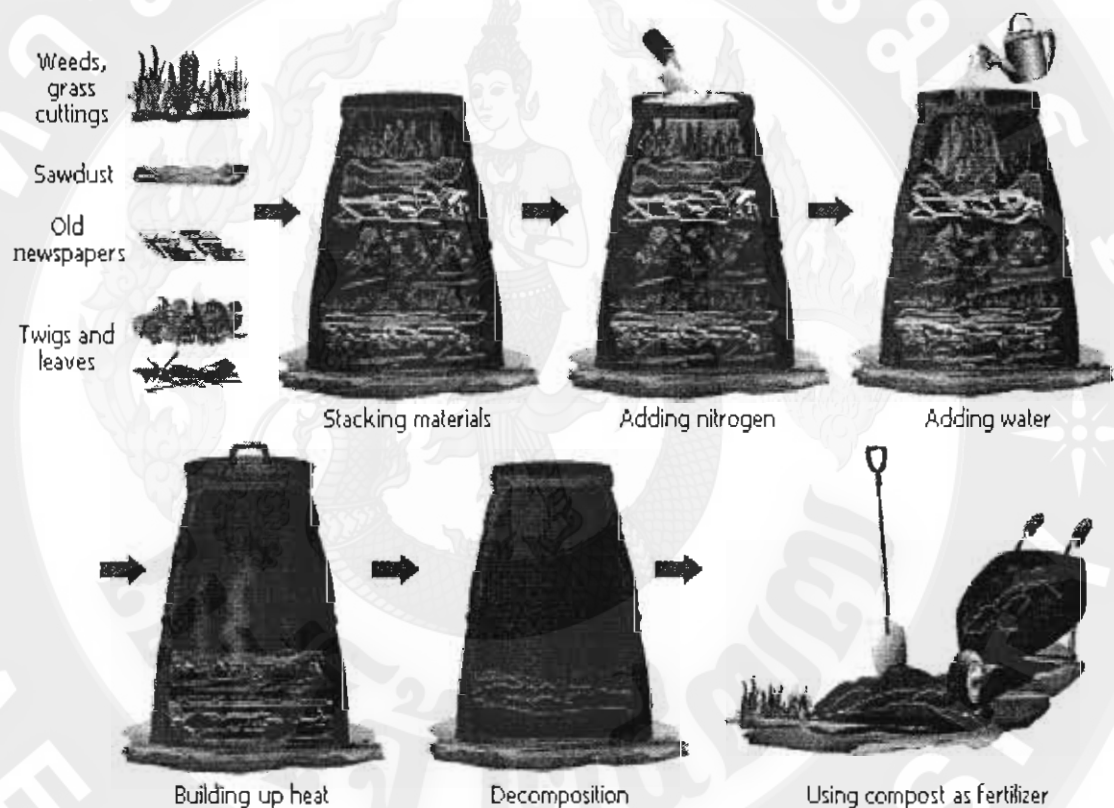


Plate 2 Generalized steps for Composting (picsearch.com, 2008)

As shown in the schematic diagram there are various steps involve while making the compost however, such steps are seen differentiated by type as well as quality of various material used in compost piling.

Common Steps

It can be mixed and blended at one time.

- To ensure good aeration and drainage, putting down a 3-inch layer of coarse plant material, such as small twigs or chopped corn stalks, or a wooden pallet.
- Adding about 8 to 10 inches of leaves or other dry organic wastes.
- Providing nitrogen for compost-promoting microorganisms by adding 2 to 3 inches of fresh grass clippings or fresh manure.
- If no soil is included in compost material, a sprinkling of soil or a compost starter to each layer to inoculate the pile with microorganisms.
- Moisten the pile as added leaves and other dry material.

Mixing the materials thoroughly, shaping the pile so its center is lower than its sides, to help water flow into the pile. Keeping the pile moist but not soaking wet. Within a few days, it should heat up. If not, it may lack nitrogen or moisture. If the pile emits an ammonia smell, it is too wet or too tightly packed for oxygen circulation; turning the heap and adding coarse material to increase air space. Once a month, turning the pile with a pitch fork and putting the outside materials on the inside and vice versa.

The plant materials should decompose into compost within five months in warm weather and longer under cool or dry conditions. The center of the pile should reach 160 F. to kill most weed seed, insects and eggs, and disease organisms. Composting may be completed in one or two months if the materials are shredded, keeping moist, and turning several times to provide good aeration (Adopted from- ATTRA, 2002).

IPM and Organic farming

Firstly, in organic system of production the plant protection measures are build in by the feeding and raising of soil microorganism mass which in turn suppress the harmful organism, secondly by enhanced soil physics and plant bust up (Gamliel, 2004), thirdly the botanical

pesticide products. Integrated Pest Management (IPM) is a broad ecological approach to pest management uses a variety of pest prevention or control techniques that target the entire pest complex of a crop ecosystem. Integrated management of pests ensures high-quality agricultural production in a sustainable, environmentally safe, and economically sound manner.

The built in soil health is based on soil biology, which is responsible for the cycling of nutrients. The complex interactions of this biological community are known as the soil food web. The soil ecosystem is composed of bacteria, fungi, protozoa, nematodes, algae, arthropods (insects and mites), and large soil-dwelling mammals like moles, ground squirrels, and gophers. The photo synthesizers or primary producers in this system use the sun's energy to convert atmospheric carbon into sugars. Other organisms feed off these primary producers. Dead organisms and their byproducts decompose, becoming the soil's organic matter that stores nutrients and energy. Plants use these nutrients, preventing them from accumulating in soil and water. The life cycle of all these organisms improves the condition of soils by enhancing structure, water-infiltration and water-holding capacity, and aeration. This results in healthy plants that are more productive and resistant to pests.

Chemical agricultural methods are not conducive to maintaining ecological equilibrium, because of constant tilling and synthetic inputs to the soil. The plants in the conventional system are nutritionally out-of-balance, receiving much inefficient nitrogen. The extra nitrogen formed free amino acids that are not tied up in proteins, stimulating the insect-pests to feed and deposit eggs. During organic evolution, plants obtained nutrients solely from the soil food web. It is the slow release of nitrogen in this system that ultimately causes no pest reproduction, in which the plant's immune system is stimulated to resist pest attack. Another plant protection phenomena attributed to soil microbial activity is induced and acquired systemic resistance. The concept of healthy soils being responsible for plant health has long been known to organic farmers.

IPM is based on the following components: pest identification, monitoring, mechanical and physical controls, cultural controls, biological controls, and mostly plant origin chemical controls. The biological and cultural insect pest controls for crops involve by the understanding

the ecology of agricultural systems. When there is a diverse farm scale involving many types of plants and animals, the likelihood of severe insect pest outbreaks diminishes considerably. That is created production methods that complement natural systems. The use of beneficial insect habitats along crop field borders increases the presence of beneficial insects. These habitats provide shelter, food (pollen and nectar), and act as refuges that attract pests' natural enemies to fields. When biologically beneficial insects are released, these field-edge habitats will encourage the beneficial to remain and continue their life cycle there, helping to reduce pest populations. Some pests may also inhabit the field-edge habitats; therefore, these habitats are monitored along with the crop (Adopted from- ATTRA, 2006).

Organic Standards

In recent years organic vegetable production has developed rapidly and this trend continues and became attractive export opportunity for most developing countries. It has attained an annual growth rate of 20 -30 % and at the same time a considerable premium price to grower (Hiraoka, 2003).

Consumers expect their food to be nutritious and safe, and trust in the organic label. This is also the key to success for growers. One of the essential elements distinguishing organic farming is the existence of organic logo of production standards and certifications. Since the organic production system avoids the uses of synthetic agricultural inputs, such as synthetic pesticide, herbicide, fertilizer, and veterinary drugs, preservative and additive, GMO, the potential hazards posed by synthetic residues are prevented. Also the microbial and mycotoxins contaminations are protected from hygienic and good agricultural practices. Organic farming with restrictions and provisions need to be agreed and verified with organic standard compliance if the product is to be exported (FAO, 2001; IFOAM, 2007).

“Organic logo” is a term that denotes products that have been produce accordance with organic standards throughout production to marketing stage. Therefore the organic is a process claim rather than a product claim. Registration is legal mechanism that permits a grower to sale

product as organic. This is accomplished after the use of list of permitted agricultural practices imposed with the organic standard. Similarly, certification is a process whereby an organic production, handling and marketing is certifies as good production and handling practices as organic. Such certification needs certifying agency to certify a product as produced through organic practice. Organic standards are developed by different country by its own domestic use purpose however; international standards are also developed for the exploitation of global markets.

“IFOAM standard” is the first basic international framework for organic standard production and processing (IBS) expressed in 1980 by International Federation of Organic Agricultural Movements. This reflects how organic products are grown, produced, processed and handled as basic standard and general principles. Whereas, “Codex Alimentarius” emerged in 1999 under the WHO/FAO of United Nations organization facilitate the production and trade of organic product worldwide; and Codex Commission approved its requirement basically in line with IFOAM and EU Regulation. This standard defines organic agriculture as “holistic system which enhances agro-ecosystems health, and emphasizes locally adapted systems as opposed using of synthetic materials” (FAO, 2001). The US National Organic Standard (NOS) is USDA seal initiated in 2002 needs to be accreditation if organic product is marketed in the United States. Likewise in the EC, EEC 1992 determines the requirement of organic product to be imported in EU countries. JAS explains the organic standard needed for production and trade with Japan, the established JAS is based on the Codex guidelines for organic farming institutionalized from 2001 (Jayamangkala, 2007).

The ACT standards as Organic Agriculture Certification Thailand was established in 1995 by Government and NGOs independently and basically in line with IFOAM basic standards. It guides the standards of production, handlings and processing of organic products and certifies the concerns in Thailand (Panyakul, 2003; Jayamangkla, 2007).

Contamination and Microbiology

Several chemical hazards are associated with food. General occurrence of atmospheric pollution, natural inbuilt i.e. zinc, solanin, histamines and/or industrial disposal or radioactive such as arsenic, cadmium, copper, lead, mercury and polychlorinated biphenyls-PCBs are equally found in conventional and organically produce. These and embedded pollution like chlorinated hydrocarbon, heavy metal depositions are difficult to remove only by organic practice. Chemical pollutants often entered into food by excess or improper fertilizer used along with misused of veterinary drugs and improper livestock managements with contaminated animal feedings. Sludge is prohibited in organic farming reducing heavy metal and toxic dioxin, PCBs (ACT-1995).

Whereas in the case of microorganism associates; the ready-to-eat vegetable Lettuce is been reported contamination with pathogen such as *Salmonella* and *Listeria*, due to agronomic system employed by the organic growers. The organic fertilizer and manure are taken in to consideration (Kakiomenou et al., 1998). However, not only the bacteria but also varieties of protozoa, parasites, viruses and fungi or their toxins deteriorate the food safety mostly from untreated and unmanaged organic mass. In this regards, exclusive research is lacking in terms of pathogen survival and time as well as method that effects the potential contamination. Soil inoculums are suppressed by boost up the beneficial microorganism. The organic system of production must prevent firstly contaminating from source and for secure the organic fertilizer are used after proper treatment or by well decomposition. The care for contamination of *Listeria*, *E.coli*, *Staphylococcus*, *Prion* and *Salmonella* from manure source; the *Aflatoxins* from inbuilt moulds are current concern of organic system of production (Jayamangkala, 2007).

Nitrate reduction:

Reducing Nitrate accumulation during periods of short daylight length, there is a health risk associated with nitrate accumulation in leafy greens. Nitrates are converted in the body to toxic nitrites which reduce the blood's capacity to carry oxygen. Additionally, nitrites can help form carcinogenic nitrosamines. In winter, leafy vegetables can easily contain the acceptable

daily intake level of nitrate for an adult in a mere couple of ounces of crop, unless special efforts have been made to reduce the nitrate levels. Spinach, mustard greens and collards contain about twice as much as lettuce; radishes, kale and beet roots often have 2 times lettuce levels. Turnip greens are especially high at 3 times lettuce levels. To reduce levels to the minimum possible during the high-nitrate-accumulating winter period, harvesting is done only after at least 4, and preferably 6, hours of bright sunlight avoiding harvesting on overcast days. Keeping the soil moisture adequate, with P, K, Mg, Mo, use of organic compost and the crops warm is practiced. Similarly avoiding over-matures crops and discarding the outer leaves. Once lettuce is harvested, nitrates will convert into nitrites as long as temperatures are merely cool, rather than cold. So for healthy lettuce, refrigeration is needed after harvesting (Dowling et al., 2006)

Organic Lettuce Growing

General Understanding:

Lettuce vegetable is found compatible in organic production systems. Its organic production in terms of sustainable growth and development relies on management techniques that replenish and maintain long-term soil fertility by optimizing the soil's biological activity. This is achieved through various organic cultural practices and use of organically accepted bio-rationales and fertilizers. However, the leaf nitrate accumulations, off-flavors and plant protection measures are important in this crop.

The Lettuce (*Lactuca sativa* L.) $2n = 2x = 18$, is the member of the Asteraceae family (previously known by compositae family) and the order of the campanulales, also known as the sunflower or thistle family. The name lettuce is derived both in Latin and English from the milky juice of the plant. The Latin root word is *Lac* (Lactuca) or mil. Lettuce was probably derived from the French *laitue* "milk", referring to its peculiar milky juice. Lactucarium is the Lettuce opium giving sleepy taste (Nonneche, 1922; Etafererahu, 1996).

The Lettuce is the world's most used salad or fresh crop. It's essential element contents and morphological as well as vividness drastically varies giving a lots of human choice. It is

extensively grown in cool season and best adapted to temperate locations classed as half hardy. While tropical climate proceeds the Lettuce tends to bolt rather than forming a head, better flavors tend to develop, and a disorder called tip burn becomes prevalent. A minimum of 4.4 degree to maximum of 29.4 degree centigrade is known favorable to Lettuce growing. Lettuce that grows best on well-drained muck soils. Due to high summer temperatures, lettuce is not grown to any extent in excessive hot weather or dryness can cause pithiness, bitterness and bolting. Once-over harvesting is essential for efficiency. Therefore, it is important that the crop matures uniformly. To ensure consistency, seed should possess a high germination percentage. Because of the high cost of seed and plant thinning, precision seeding should be used with coated seed. Lettuce seed should be dropped every 2.5-3.0 inches for 111000 seeding per hectare. Lettuce Crop seeding transplants can be started in greenhouses in late February or March for transplanting into fields in late March (OU, 2008).

The Lettuce crop is an annual plant of about 50 days which forms a rosette of leaves at the base and subsequently a tall flower stem from 30 to 100 cm high. It is favorably grown with spacing 25 - 30 cm apart and along the 30 cm row to row spacing raised or flat field depending upon irrigation and fertigation system (ATTR, 2006). Commercially, Lettuce is harvested before the formation of the flower stalk. Some cultivars pose from a definite head, where as others are merely a loosely formed rosette of leaves (Halfacre and Barden, 1979). Leaves often many and usually nearly sessile are spirally arranged in a dense rosette. Considerable diversity occurs in color, shape, texture and leaf margins between different forms. Leaf margin may be lobbed, smooth or finely divided and leaf color can vary from light to dark within various colors. Among the Lettuce crops grown, the loose leaf Lettuce (*Lactuca sativa* cv. *Crispa* L.) largely grown in northern Thailand.

Soil and Fertility:

The Lettuce crops are heavy feeders and it can grow on variety of soil as long as the soil provides adequate nutrient, moisture and are well drained. The challenge is to maintain good soil nutrient and hygiene. The organic materials like animal manures are the prerequisite to supply

adequate nutrient in soil. NPK elements are cycled through the animal manure and bio ferments. Based on soil contains, rule of thumb 14-20 ton of organic manure/compost material is applicable per year basis during transitional frame reducing 3-5 tons / ha later (ATTRA, 2007; Jayamangkala, 2007). A condition of Nitrogen, Phosphorus and Potash i.e. 56000, 7000 and 60000 ppm concentration respectively through plant analysis have been suggested for Lettuce crop (Roorda and Smilde, 1981).

However, the bioferments fertilizers are often combined with organic mass in order to reduce the cost of production as well as for enhancement of the nutrient uptakes. For balance nutrients acquisition, reduction in the down ward nutrient movement or leaching such as nitrate, shallow ploughing, proper irrigation and drainage are essentially managed, and weeding is timely done. The organic manure, after decomposition gives soil humus which in turn releases the nutrients available to crop. However, it depends upon the amount of biomass supply and parental nutrient circumstances of soil. The initial nutrient contents of soil are enough to convey lots of essential nutrients mostly after acquiring the transitional periods of soil (Guerena, 2006). If not enough, additional bio friendly fertilizers such as rook phosphate or ashes can be supplemented according to requirement of crops. Further needs of micronutrient are not likely to occur where the biologically active soil with adequate organic matter have been adequately augmented (ATTRA, 2006).

Chapter 3

Materials and Methods

Conceptual Framework

Bioferments, as stated before are prepared after extraction over time period and mineralized before they become plant available. Since cow or chicken manure holds ample NPK it can be drawn into water soluble solution and use as foliar and soil drench applied. However, the nutrients concentrations on bioferments depend upon source variation like locality, kinds of feed, breed and sanitations measure applied to cow and chicken so that bioferments fertilizers are prepared and evaluated first before plant applications. The Loose Leaf Lettuce cultivar poses a unique taste in associated food and extensively used worldwide for fresh table salad also found very popular in Thailand.

As far as extraction methodology is concerned, varies greatly among researcher no generalized recommendation is found. In this study the concentrations of water soluble NPK production in biofermented were obtained after a series of extraction and fermentation processes and getting hold of more than two weeks to accomplish, accompanied as stated in CIWMB (2008); US University of Florida (2003) and field applications as Nhamo et al. (1985); Palm (1997). Extraction was achieved through aerobically using anti-sting such as molasses. Molasses are the food source for beneficial microorganism as well as sting smell reducer so far as known. Anti sting was used for Lettuce crops because it is extensively consumed as fresh table salad food.

In present method, firstly, fresh cow and chicken manure with water and molasses were allowed to extract over various days time and each nutrient solution at fix interval was evaluated for its best NPK concentrations; and secondly, best formulated bioferment (title: 3.2.5) for the optimum rate of field concentration reciprocated with compost materials be verified. Two Lettuce crop trials with labeled treatments were conducted at Maejo University Chiang Mai supplying the NPK requirements of the crop through composted mass vs. bioferment drenched in organic crop production system, details as follows:

Materials and Procedures

1. Cultivar:

A composite leafy vegetable called Loose- leaf Lettuce (*Lactuca sativa* var. *crispa* L.) of Asteraceae family abundantly found in Thailand was grown in an organic farm at Maejo University. This crop deserved and thrived well under organic farming system. However, being used freshly, adequate care was taken while drench fertilization of bio-ferments in order to avoid stinging smell and microbial contaminations. For such case, at the time of extraction molasses were properly streamlined. Lettuce crop was transplanted in winter season after 21 days of nursing in organic media as peat moss from nearby market according to recommendation made by VDD of University. For experiment the organic seed was brought from the sponsors of Thailand's Royal Project.

Lettuce Nursery Management:

Organic seeds 45 grams were sown in 8 plastic trays containing each 105 holes seeding with 2 seeds per holes and were kept under plastic house for germination. The trays were filled first with 24 kgs peat moss moisture with tape water then seed were sown near evening. Germination was recorded at 91% after 5 day of sowing, showing ample viability. Irrigation solely with water was performed everyday at two batch morning and evening until before transplanting. Weak and deformed seedlings were thinned after 10 day keeping only one seedling per hole to allow enough strength of seedlings. From 5 day before transplanting, the seedlings were hardened by exposing it during morning and evening hours outside from the plastic house. No disease insect and weeds were seriously observed during nursery period. No any application of plant protection material and nutrient supply was made. Seedlings were found well developed, enough strong and green however, some seedlings experienced unequal upright growth (appendix: B). At the time of transplanting 630 seedlings were selected randomly and remaining 20 % seedlings were kept for application of gap filling.

2. Bioferment Extractions (Production of Water Soluble NPK):

Extraction method: Bucket Fermentation (ATTRA, 2002).

Extraction Materials:

Fresh Cow Dung and Poultry Manure were collected from Maejo University, Livestock and Poultry farm, molasses and other materials from nearby market.

- 1) Chicken manure (Fresh) – 10 kilogram.
- 2) Cow dung (Fresh) – 10 kilograms.
- 3) Fresh Water – amount of 10 liter for each kind.
- 4) Molasses - 1 kilogram for each batch.
- 5) Burlap Sacks (Bag) -4 numbers.
- 6) Plastic Drum – 20 Liter- 2 numbers.

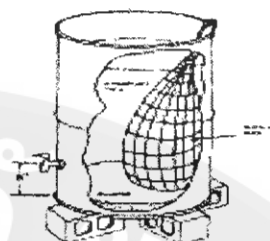
Extraction procedures and time period:

Continuous extraction of water soluble NPK solution and sampling of 2 samples in every 3 days intervals from each kind of sourcing comprising 10 NPK samples analysis were performed. Determined upon start of negative (lesser) concentrations from continuous NPK analysis in every 3 days sample, 15 days frequency limitation was confirmed. Extractions were carried out in 10:10 w/v (1:1) water and manure each for suitable extraction, as referred before by Stephens (2003); CIWMB (2008). To ensure proper release of nutrient into the water solution, addition of molasses for microbial feeding as well as manual stirring at each sampling was accomplished. The strong ammonia smell was also observed.

Activities performed two batches:

- Mixing of the 1 kilogram of molasses and 10 kilogram of sourcing of each kind.
- Putting the mixed manure in each bag and tying bag's neck.
- Keeping the filled Bags into the plastic drum with 10 liter of fresh water supply.
- Allowing it to extract and discharge for 3, 6, 9, 12, 15 days, stirring at samplings.

Each 50 ml sample-extractions were drawn into sample tube from each drum and sent to laboratory for analysis. Every time the remaining extract was kept as in the same Drum (plate-3).

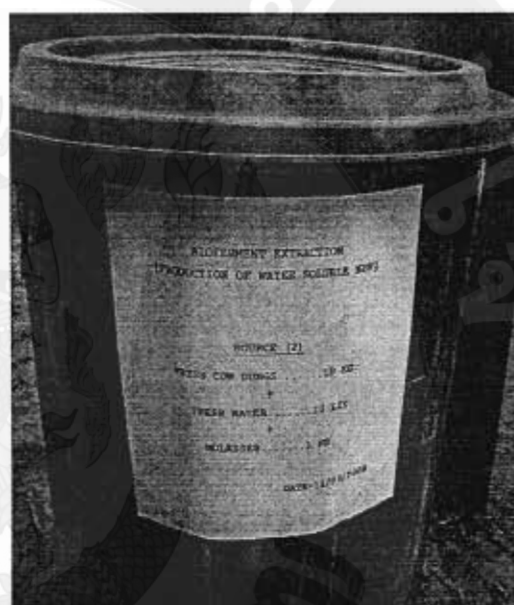


(1) Preparation

(2) Extraction: 3, 6, 9, 12, 15 days.



S1 -(Chicken Manure BF)



S2 -(Cow Dung BF)



(3) Filtration



(4) BF Field Application

Plate 3 Extraction steps of Bioferment for both the Sourcing.

Method of BF nutrient analysis:

Nutrient analysis was performed as adopted analysis at Maejo University soil laboratory guide.

- **For N analysis Kjeldahl (TKN) Method** (Nelson and Sommers, 1980; AOAC, 1985).

[N_{org} 2 h 12 ml H_2SO_4 -Salicyclic + 1.5 g $Na_2S_2O_3 \cdot 5H_2O$ + Kjeltab].

$$\text{Formula: \% N} = \frac{N_{HCL} \times f_{HCL} \times \text{ml}_{HCL} \times 0.014 \times 100}{W_s}$$

Wherein: N_{HCL} = Normality of HCL

f_{HCL} = Factor obtained by standardization

W_s = Weight sample.

- **For P analysis** (AOAC, 1980; Benton, 1988)

[30 min: 10 ml stock sol. + 10 ml NO_3 -vanadomolybdate + WL 430 nm; solution 1:10 water].

$$\text{Formula: ppm P} = (\text{ppm in solution} - \text{ppm blank}) \times 50 / 5 \times 100 / W_s$$

- **For K analysis FE Spectrophotometry Method** (AACC, 1976; AOAC, 1985).

[1.9066 KCl + 1 lit. H_2O + 10 ml stock sol. + 0.1N HCl; soil and solution 1:10; FES].

$$\text{Formula: ppm K} = (\text{ppm in solution} - \text{ppm blank}) \times 100 / W_s \times d_f = \text{dilution factor.}$$

The analyzed data are shown in table 3- 4.

3. Compost material preparation and analysis:

Compost material was prepared in vegetable farm, Maejo University. A mixture of animal and poultry manure, lawn and field vegetable clips, mushroom byproducts as well as EM starter were allowed to decompose aerobically up to 3 month. The turning up and material moistening were accomplished. Composted material was well decomposed and pliable. Prior to field application 3 compost samples analysis for nutrient NPK, Ca, Mg, S, and OM were performed at laboratory of Land Reform, Chiangmai, Thailand as in table 3.

4. Soil sample analysis: (before and after experiment)

Before transplanting, soil samples were taken from the organic Lettuce experiment plots at organic vegetable production field, VDD Maejo University. With the help of soil Auger 15 initial samples were taken representative sampling from every side of 120 sqm field and 10 cm below ground. All the samples were mixed and reduced to half kg by half to half discarding method. Soil sample analysis for its NPK Ca, Mg, S and OM was accomplished at the same laboratory as compost's analysis shown in table 3.

After harvesting, as stated above procedure 10 soil samples were taken for each treatment representing from 3 initial samples from each replication and reducing into one sample per treatment. Analysis was done at same laboratory as before. This analysis was used to confirm the nutrient loss or gain incurred during cropping period.

Based on bioferment, compost and soil nutrient analysis the field trials were further implemented.

5. Nutrient assessments: Calculating the treatment rates of concentration:

The major nutrients supply for Lettuce crop as NPK (table 3) (Roorda and Smilde, 1981) was considered matching up to total plant requirements, reserving available soil nutrient in soil profile. Whereas, depending upon analysis data from bioferment and compost, the concentrations or rates of bioferment as well as compost material applications were calculated according to total crop requirement. Thereby set for field applications. Though the 10:1 extraction or conversion index (CI) for source manure to water was required to produce BF as nutrient necessitate by lettuce, in this study, for the sake of suitable extraction with 1:1 manure to water and product of nutrient rate with CI was verified in field experiment. As described earlier, the general recommendations or research outcomes specifically for bioferment are lacking in terms of concentrated doses to apply and also differs greatly according to factors of sourcing. In this study, to see what accounts the yield variation, the treatment rates of concentrations (doses) were combine formulated as follows:

BF Treatments were structured 3 ways on N base application (table 4) in part by using the (1) unitary formula thereby extraction or conversion index (CI), and (2) in reference to the foliar feeding general practice (Matarirano, 1994), hydroponic conc. (Martin and Nathan, 1984), absolute quantity of BF per liter of water or per m^2 (Hendawy, 2008; Xu et al., 2003). Whereas, according to generally adopted practice in organic vegetable farming compost was applied. That the approx. rate of organic manuring (cattle - chicken, 28-10 ton/ha, Splittstoesser, 1990; ATTRA, 2007) to compost inclusion 10 tons per ha (a cost-benefit dose by AGRIS, 2006), resultant rate for its higher application value of 1000 gm per m^2 i.e. 2600 mg N per kg^{-1} was applied. The field was considered after transitional period. The compost applications were same for both the experiments differing the BF doses. Reciprocations of BF and compost material were executed comply with percentage supply through its maximum application value (table 4).

BF Calculations- Chicken Manure and cow dung: (N-nutrient based)

Preparation of BF requirement 100 liters drench per ha undiluted.

Total crop requirement equivalent 123000 ppm of NPK index or 56000 ppm of N.

Highest application value equivalent 200 ml of BF per m^2 , as referred below:-

Bioferment scaling: small

- 10 kg of chicken manure into 10 liter of water (1:1) yielding 17985 ppm of NPK index or 6100 ppm of N from BF extraction analysis.
- In 100 liter BF, it is needed, $123000 \times 100 / 17985 = 685$ kg of manure; but for N supply
i.e. $56000 \times 100 / 6100 = 918$ kg of chicken manure required.
- Conversion index (CI. w/v) rounded by 10:1, due to some volatile, other loss incurred.
- 100 liter undiluted BF per ha equivalent $100 \times 1000 / (\text{appx. } 2/3 \text{ ha drench}) \approx 20 \text{ ml} / m^2$.

But, in present study the conversion index was used as 1:1 (w/v) manure to water solution (also Stephens, 2003) for proper extraction. Therefore, maximum value for drench application as $20 \text{ ml} \times 10 \text{ (CI)} = 200 \text{ ml BF per } m^2$, i.e. 1220 mg for smooth application adding with 1 liter of water / m^2 liquid feeding was used. This is in reference with similar quantity used by Hendawy (2008), hydroponic nutrient value (Martin and Nathan, 1984) and 1:10:10 foliar feeding (Matarirano, 1994). Whereas, due to approx half of the nutrient found in cow dung, its bioferment dose was executed twice as much as of the chicken manure bioferment.

Nutrient assessment compilation:

Table 3 Nutrients Assessment of the Requirements for Lettuce crop:-

(ppm)

Nutrients	Crop* requirement	Soil available (pre-plant)	Nutrient supply	Nutrient Contents Analysis		
				Compost	Bioferments (table-5)	
					S1 (15d)	S2 (9d)
Nitrogen	56000	4700	56000	2600	6100	1090
Phosphorus	7000	256	7000	218	458	2500
Potash	60000	144	60000	297	11400	3940
Total index	123000	5100	123000	3115	17958	7530

[* Source: Roorda and Smilde, 1981]

- Information on soil, compost and bioferments was obtained after laboratory analysis.
- S1 and S2 indicate sourcing as chicken manure and cow dung respectively.
- Nutrient supply -Nitrogen (NH_3) 1220 mg N per liter of water per m^2 from BF or 2600 mg N per kg per m^2 from intact nutrient of Compost, considering 35 to 45 % of nutrient not available during cropping season.

Field Technique:

Two Loose-Leaf Lettuce field trials were conducted under organic farm at Maejo University. The crop management practices followed organic standard recommended by Organic Agriculture Certification Thailand (ACT-1995) and National Sustainable Agriculture Information Service (ATTRA) NCAT (2002), if not applicable from the Thai royal project.

A Randomized Complete Block Design (RCBD) was designed for each experiment with total of 10 labeled treatments of bioferment concentration rates reciprocated with compost materials. Each treatment was randomly assigned and observed under 3 replications block efficacy. Gradient variations also considered and 30 plots of (2 x 1) sqm together with 21 plants (25 x 25 cm^2 plant to plant and row to row) per plot were assigned.

Treatments structures:

Concentrations of BF supplied to Lettuce crop through reciprocal level of bioferment drenching vs. compost basal based on table 3 and calculation.

Table 4.1 Treatment Structure: Experiment-1. Reciprocal Chicken Manure BF vs. Compost Materials:-

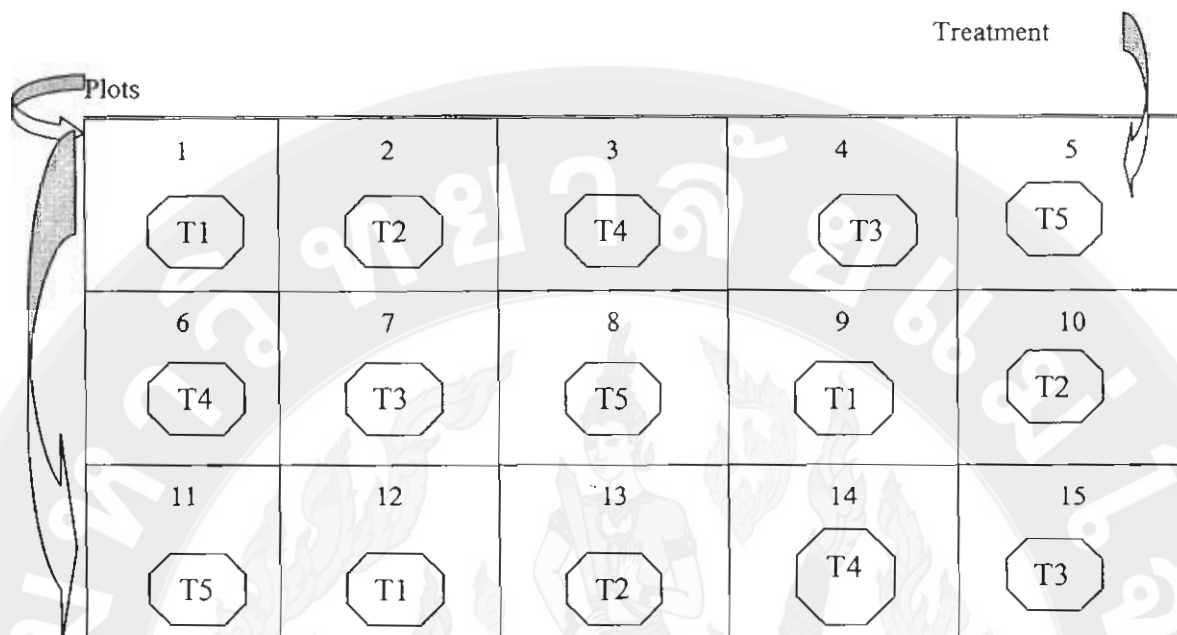
Treatments	T1	T2	T3	T4	T5
Drench BF vs. Compost (%)	100 : 00	75 : 25	50 : 50	25 : 75	00 : 100
Drench BF concentration	200 ml / sqm	150 ml / sqm	100 ml / sqm	50 ml / sqm	00 ml / sqm
Compost mass concentration	00 gm / sqm	250 gm / sqm	500 gm / sqm	750 gm / sqm	1000 gm / sqm

Table 4.2 Treatment Structure: Experiment-2. Reciprocal Cow Dung BF vs. Compost materials:-

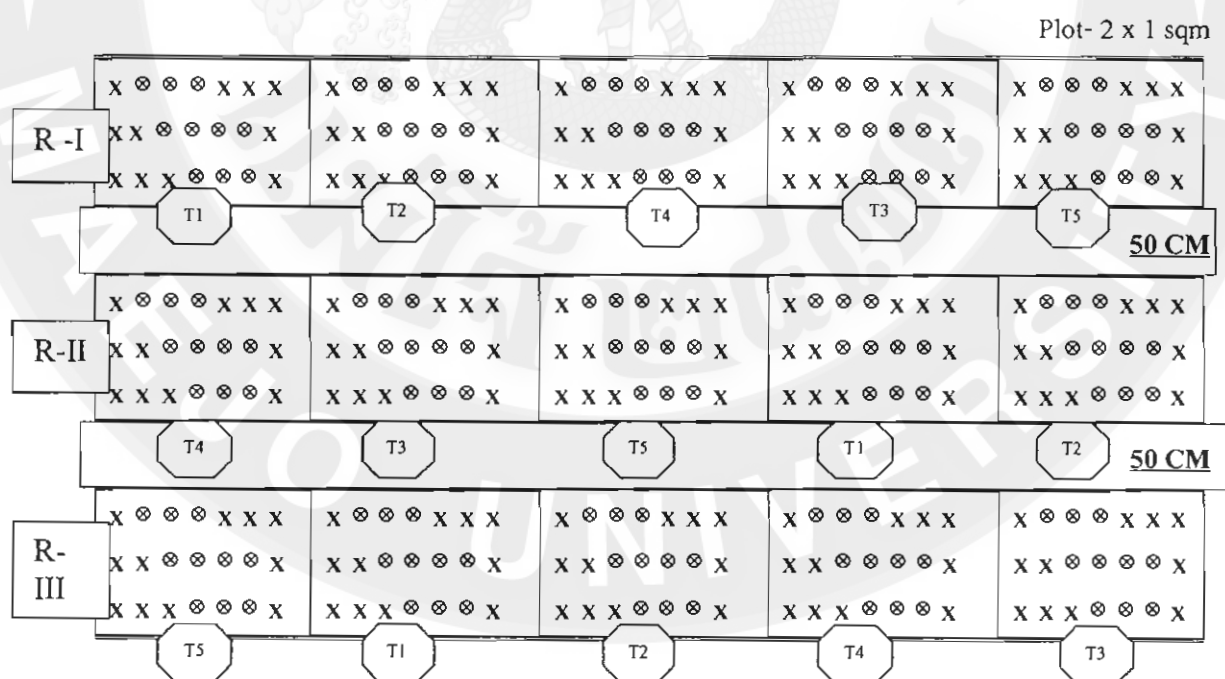
Treatments	T1	T2	T3	T4	T5
Drench BF vs. Compost (%)	100 : 00	75 : 25	50 : 50	25 : 75	00 : 100
Drench BF concentration	400 ml / sqm	300 ml / sqm	200 ml / sqm	100 ml / sqm	00 ml / sqm
Compost mass concentration	00 gm / sqm	250 gm / sqm	500 gm / sqm	750 gm / sqm	1000 gm / sqm

Mode of nutrient application:

- Compost mass as basal dose before seedling transplanting.
- Bioferment drench ratio (50 %, 25 % and 25 %) as 3 batches fortnightly at and after seedling transplanting. BF was supplied per liter of water drenching near base of each plant in corresponding plot to facilitate proper distribution.



Replication -3 (Blocks)

Plate 4 Field Design:-Experiment-1 X 2. RCBD**Plate 5** Experiment Sampling Design:

Arrangement of Lettuce Experiment plots total area (12 X 10) sqm.

⊗ ⊗ ⊗ as number of total sampling plant 10 per plot.

Field experiment materials used:

- Lettuce seedlings 630 - compost material 50 kg,
- Bioferments Chicken and Cow dung 10 liter
- Mulching plastic 100 sqm.- Needle stick
- Garden pipe - Spade and rake- Planter
- Measuring cylinder- Watering cane - Sprayer
- Spading fork- Cylindrical bucket - Organic pesticide
- Measuring tape - Bamboo stick 35 for Tagging
- Weighing machine - Vernier caliber
- Metal scale - Wheelbarrow -Board and string

Transplanting and experiment seating:

The organic field layout and soil preparation were performed during early winter after the soil sampling had made. Soil ploughing, planking and removal of weed accomplished with power tiller followed by manual work. The field with 120 sqm was divided into 30 plots having 2 x 1 sqm each and half meter passage between replication as well as treatment plots as shown in plate 04 and 05. For both the experiments, 5 treatments each designed in 3 replications as RCBD in which each treatment appeared in every replication. Replications were made perpendicular to thought- soil gradient, trust for reducing the experimental error raised if any by soil nutrient flow.

After the preparation of replication and treatment plots, compost material was supplied depending upon the treatment structures (table 4). With slightly raised leveled plots, black plastic mulch cover was spread covering the plots so that weed problem and moisture loss as well as nutrient erosion could be mitigated. Each plot was longitudinally divided into 3 rows having 25 cm spacing apart and each row into 7 holes equally with 25 cm spacing apart. That comprised 7 plants in each row accommodating 21 plants per plots. The 10 cm holes were made with the help of seedling planter or auger through punching the mulch plastic.

Organically grown 360 seedlings from nearby nursery were transported to field using wheelbarrow and set for planting. Single seedlings per hole planted in each hole manually in the evening. Depending upon the treatment structure (table 4), the first batch of bioferment (50 %) application was adopted after a short irrigation.

Management adopted during growing period:

Regular daily basis irrigation was provided except for the raining day until harvest with the help of water can approximately 1 liter per plant. The application of second and third (25 % each) bioferment were done at successive fortnight i.e. 15 and 30 day after transplanting. Though the plastic mulch worked very well, but continuous hand weeding from passage and planting hole at weekly interval also performed. Considering the seedling mortality during first week of transplanting, total 75 out of 630 seedlings (12 %) were gap filled. Death of seedlings occurred due to intense sunlight trapped by the black plastic mulch, however the heat is found useful to kill the notorious weeds. Interestingly no any kind of serious disease insect pest was observed during the growth period. Even liable aphid population not observed. However, a few symptom of tip burn (Ca deficiency) found during the second week after transplanting. No any kind of organic pesticide, insecticide or other nutrient application were materialized during whole growing period.

Rainfall of 160 mm and temperature between 23 to 31 deg centigrade received during the growing period (www.chaingmai.net/weather, 2008). No any pattern of natural disaster observed. Plants were found poor to vigorous according to treatment employed. Harvesting was done by one time basis at 38 day after transplanting (appendix B).

The sample plants in every plot were randomly selected as shown in plate 5 before first measurement was made and set for schedule measurements from the same plants. Measurement and information collection according to schedule was performed on different parameters of growth and development as stated (title 3.4, table 5).

Information Collection and Measurement

Information was collected and measurements were made on bioferment production analysis, compost and soil analysis as well as plant growth and development indicators. Particularly on:

- 1 Laboratory evaluation of bio-fermented liquid for water soluble NPK nutrient status. Time duration assessment for best extraction sourcing from chicken manure and cow dung. During bioferment extraction, the daily temperature fluctuations were recorded with thermometer. Similarly, the pH flux and influx measurements in every 3 days were performed with laboratory pH meter.
- 2 Laboratory analysis of soil properties for NPK plus content of soil both before transplanting and after harvesting were accomplished.
- 3 Laboratory analysis of sampled composted materials for NPK was performed.
- 4 Information collection about climate and disease pest aspects and
- 5 Measurements of crop yield, growth and development parameter carried out on (table 5) Germination, plant height, leaf number, leaf area (LA), stem diameter, harvest leaf weight and total yield individually in every instance from same sample identified plants.

The two measurements i.e. on plant height and leaf number during growth were taken at every week interval i.e. 5 times until harvesting to confirm the rate of increment. The data on plant height using metal scale was measured from base of stem to highest apex of leaf primordial. Similarly, the leaf number addition at every week was counted during the same period. In addition, at harvest, other yield parameters as stem diameter using vernier caliper and leaf area with its largest leaf length and breadth were measured. Finally, the fresh weight separately from treatment plots as well as replication wise accomplished from sampled plant and set for statistical analysis (plate 6).

Stem Diameter:



Fresh leaf weight:



Plate 6 Measurement of Lettuce Stem Diameter and Leaf fresh weight:

Table 5 Type of Data Collection:

Phenotypic parameter	Data collection type
<u>Water soluble NPK BF analysis</u>	-Every 3 days
Type of manure	Cow dung /Poultry manure
No. of days to extract	3, 6, 9, 12 and 15 days
pH and temperature data	During BF extraction period
Compost material analysis:	NPK Before Transplanting
Seed germination	Percentage
Plant height (cm)	Every week and at harvest
Leaf number (no.)	Every week and at harvest
Leaf area (LA- cm^2)	At harvest
Stem diameter (mm)	At harvest
Total wt. of leaf (gm)	At harvest
Total yield. (gm)	At harvest
Plant protection and bio pesticide record	Occurrences
Rainfall and Temperature record	During cropping period
Soil nutrient analysis: NPK	Before experiment, after harvesting

Analysis of Data:

All data under RCBD were statistically analyzed with Sirichai Statistics Version 6.00 an interactive statistics program for agricultural research. F-test and Least Significant Difference (LSD) were used for the comparison between means at 5% probability level. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were applied for the analysis of the result obtained from the measurement on growth and development and yield parameters. Block efficacy was assessed through replication error using every parameter obtained from the resulted measurements. The bioferment along with its measured parameters were analyzed through common mathematical interpretations.

Chapter 4

Results and Discussions: Results

Bioferment Extraction Properties: Chicken Manure vs. Cow Dung:

Table 6 Analysis of Production of Water Soluble NPK Bioferments (BF) Score Sourcing from Chicken Manure and Cow Dung over Time Period of Extraction:-

(ppm)

Source Materials	No. of Discharge Days				
	3 d	6 d	9 d	12 d	15 d
S₁: Chicken Manure:					
N	3050	4100	5300	5600	6100
P	695	218	840	635	458
K	10620	10710	10640	10690	11400
Total index	14365	15028	16780	16925	17958
S₂: Cow Dung:					
N	810	910	1090	1050	1130
P	145	1005	2500	325	318
K	4320	4320	3940	3810	4560
Total index	5275	6235	7530	5185	6008

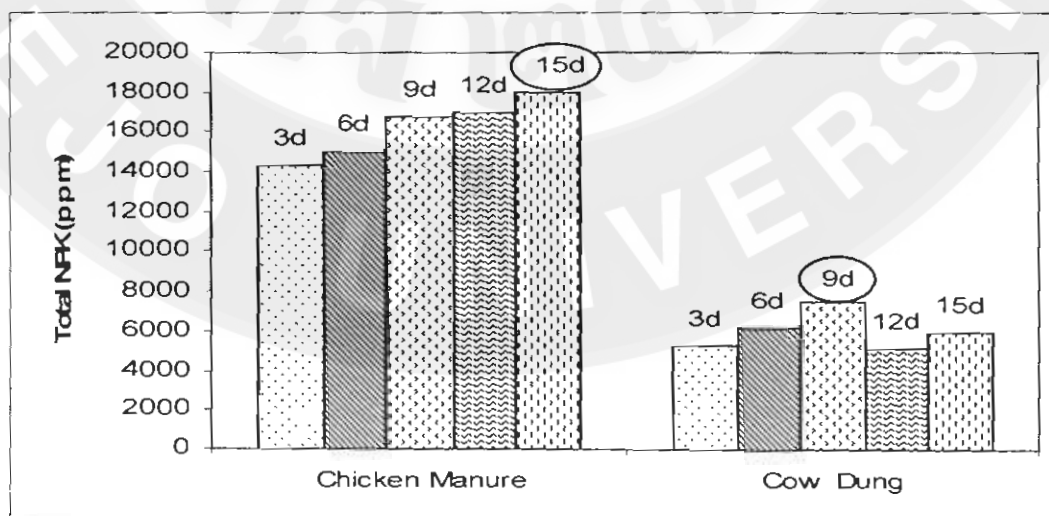


Plate 7.1 Production of BF from Chicken Manure and Cow Dung over time period:

Production of water soluble NPK:

The highest total concentration index with 17958 ppm of NPK nutrient in bioferment was obtained sourcing from chicken manure whereas, cow dung accumulated 7530 ppm highest score of same nutrients. This higher accumulation from chicken manure than cow dung was acquired with higher initial beginnings that assessed 14365 ppm and steady higher increment until peak cumulative upregulation. Afterward, assessment showed that the steady but slower increments amounting retardant rate of cumulative rise. However, the production of water soluble NPK sourcing from cow dung was acquired with lower initial nutrient build up (5275 ppm) releasing lower steady increment amount until peak assessments of downregulation. But, onwards fluctuated decline amount accumulations were observed in the evaluation periods. Nevertheless, P released higher of 2500 ppm in cow dung than that of chicken manure (458 ppm); finally, chicken manure resulting in the extractions, yielded two and half fold more of total nutrient as compared with cow dung. The accumulations were similarly found higher of $K > N > P$ sourcing both from chicken manure and cow dung (table 6, plate 7.1).

Appropriate Timing for Extraction:

The upper most resultant data from analysis of bioferment sourcing from chicken manure and cow dung extraction were observed in 15 and 9 days respectively that accumulated highest total nutrient concentration. Correspondingly, the similar patterns of nutrient accumulation over time period of extraction from both the sourcing obtained that the initial rise continued to release up to 9 days irrespectively the amount drawn. However, it is found that the 9 day's onward the accumulation from chicken manure increased by decreasing rate of nutrient release until 15 days of highest total assessment. Whereas from cow dung, nutrient accumulations were declined and negative until 15 days assessment though some fluctuated nutrient accumulations were observed. Besides some diminutive fluctuation, the nutrients accumulations for N and K showed similar series of constant and steady increment during peak releasing period from both the sourcing. However, the P released highest during 9 day's extraction causing uppermost total nutrient accumulation from cow dung (table 6, plate 7.2).

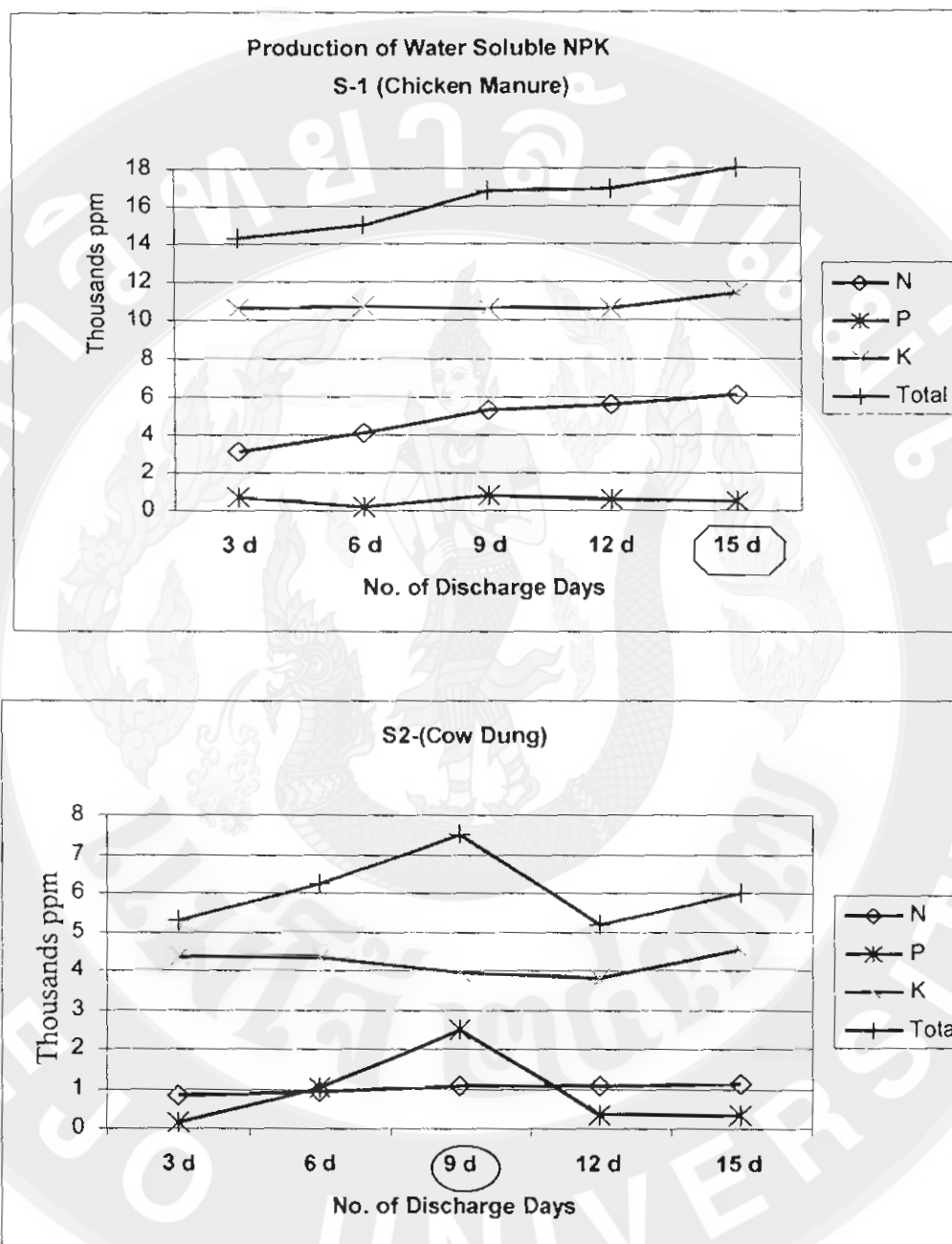


Plate 7.2 Time period and Nutrient concentration in BF sourcing from Chicken Manure and Cow Dung:

Temperature and pH fluctuations:

The daily recorded temperature during chicken manure BF extraction showed that the continuous rise from first day's 27 degree to 35 degree temperature, acquiring highest heat during 7-8 days extraction. Whereas, subsequently highest of 32 degree temperature recorded in releasing days of 6-7 for cow dung BF solution. However, in the peak nutrient releasing days of 9 and 15 (table 6) respectively for cow dung and chicken manure found to be little declined (31 and 30 degrees) at their peak temperatures. The little higher temperature of 35 degree was observed in chicken manure bioferment as compared with 32 degree in cow dung bioferment during whole extraction days of 15 and 9 days respectively (table 7, plate 7.3).

Table 7 Temperature and pH fluctuation during BF Extraction:

No. of Extraction Days	S1-Chicken Manure		S2-Cow dung	
	Temperature (°C)	pH	Temperature (°C)	pH
1 st =0 day	27	5.80	27	5.60
2	28	5.05	28	5.02
3	31	-	29	-
4	31	4.61	30	4.22
5	33	-	31	-
6	34	-	32	-
7	35	6.04	32	4.63
8	35	-	31	-
9	33	-	31	-
10= day 9	33	6.17	30	5.50
11	30	-		
12	29	-		
13	30	6.23		
14	30	-		
15	29	-		
16 = day 15	30	6.30		

Similarly, the pH value for chicken manure BF was observed as lower as 4.61 scales in 4 days extraction to its initial value of 5.8. Whereas, showing similar pH value of 4.22 in 4 days of extraction also found in cow dung BF. However, at peak accumulation days of 15 and 9 (table 6) , the pH values were observed upregulated by 6.30 and 5.50 scales respectively in chicken manure and cow dung BF apparent toward alkalization (table 7, plate 7.3).

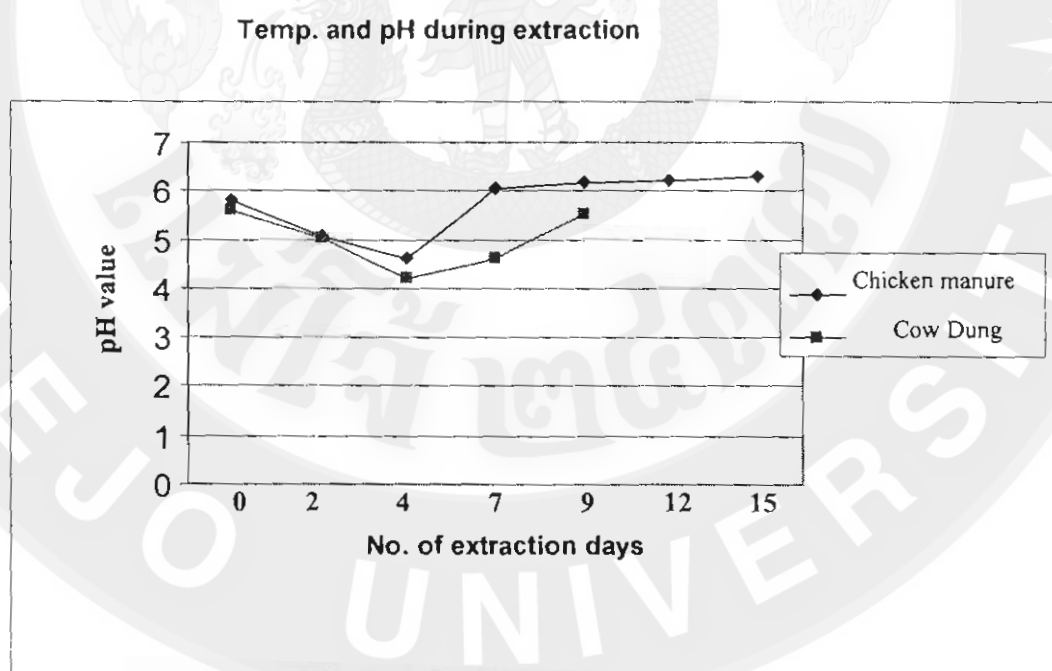
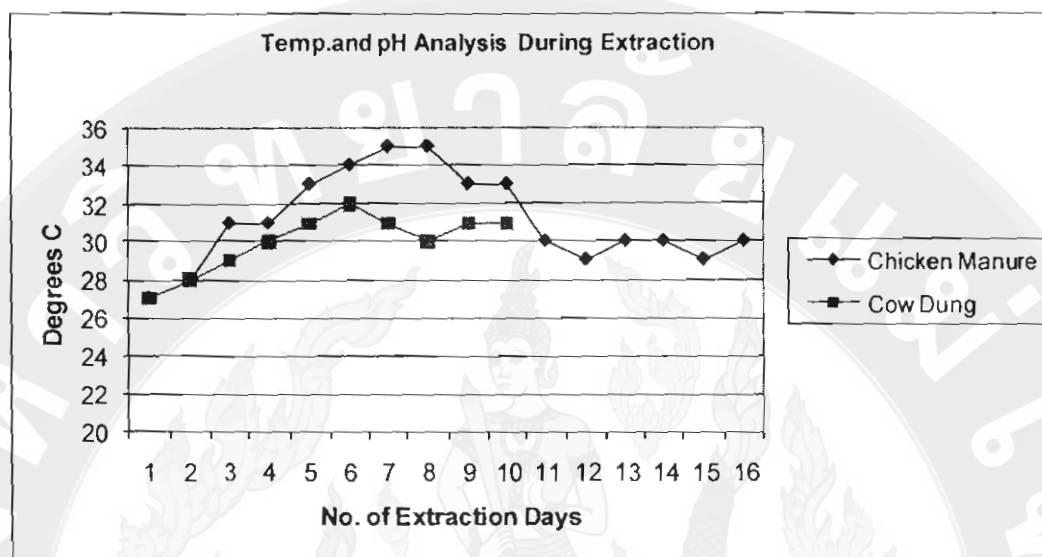


Plate 7.3 Temperature and pH fluctuation during BF Extraction:

Soil and Compost Property:

Soil Properties before and after harvesting

Table 8 Determination of Soil Nutrient Contents:

(ppm)

Soil analysis	N	P	K
1. Before Transplanting	4700	256	144
2. After Harvesting			
Experiment-1	Chicken Manure BF vs. Compost		
Treat-1 (100 % BF)	885	291	172
Treat-2 (75 % BF)	635	294	188
Treat-3 (50:50)	900	287	179
Treat-4 (75% Com)	930	286	164
Treat-5 (100% Com)	1010	291	170
Experiment-2	Cow Dung BF vs. Compost		
Treat-1 (100 % BF)	805	287	171
Treat-2 (75 % BF)	680	287	131
Treat-3 (50:50)	855	285	128
Treat-4 (75% Com)	870	288	137
Treat-5 (100% Com)	960	294	161

The data presented in table 8 showed that the soil nutrients contained as NPK before transplanting (4700, 256, 144 ppm of NPK respectively) was found deviated in their amount contained, tested after harvest in both the experiments. The decreased amounts of nitrogen (N) from all treatments' plots were in general similar however, the highest decreased amount of N (remaining 635 ppm of N) was found in the chicken manure experiment 1 as nutrient supplied with 75 % BF: 25 % compost (150 ml/m² BF combined 250 gm/ m² compost). The similar trend of decreased N amount in the treatment plots among the treatments level reciprocals was also observed from the cow dung BF experiment 2.

The highest left over nitrogen or lowest decreased amount to 1010 ppm and 960 ppm of N respectively from chicken manure BF and cow dung BF experiment were observed in treatment plots from nutrient supplied in sole through compost as 100 % (i.e. 1000 gm/ m² compost) for both experiments.

Whereas, the phosphorus and potash contents were noticed slightly up regulated in the soil of treatment plots from the both experiments that is the tested soil after experiment showed increased P and K amount to its respective initial contents among all the treatment reciprocals. From both the experiments of chicken manure BF and cow dung BF with its compost reciprocals, the increased amount of P and K trends were similarly observed among all the treatment levels.

Compost properties:

As shown in table 9, the nutrient contents analysis as Nitrogen, Phosphorus and Potash from compost was found total yielding of 3115 ppm of NPK index. The individual nutrient available observed highest of 2600 ppm for nitrogen, 218 ppm for phosphorus and 144 for potash. It suggested compost is richen in nitrogen.

Table 9 Determination of Compost Nutrient Contents:

Compost	Nitrogen	Phosphorus	Potash	Total index
ppm	2600	218	297	3115

Experiment -1: Growth and Yield Parameters

Table 10 Effect of Reciprocal Level of Chicken Manure BF conc. vs. Compost on Growth and Yield of Lettuce Crop:-

Treatment [BF : Compost / qm]	Leaf number	LA -lxb (cm ²)	StemDM (mm)	Plant ht (cm)	Yield (gm/m ²)
100 % BF [200 ml : 00 gm]	26.99 ^b	159.35 ^b	7.90	13.26 ^{bc}	1259.75 ^b
75 BF :25 Comp [150 ml : 250 gm]	40.32 ^a	222.75 ^a	9.96	17.09 ^a	2237.54 ^a
50:50 BF vs Comp[100 ml:500gm]	33.79 ^{ab}	224.68 ^a	8.37	15.18 ^{ab}	1832.14 ^{ab}
25 BF:75 Comp [50 ml : 750 gm]	29.42 ^b	164.87 ^b	7.55	12.29 ^c	1088.04 ^b
100 % Comp [00 ml : 1000 gm]	27.46 ^b	182.39 ^{ab}	7.83	12.92 ^c	1386.14 ^b
Confidence level at 5 %-F test	*	*	ns	**	*
LSD at .05	9.1	43.5	1.6	2.1	729
CV %	[15.4 %]	[12 %]	[10.3 %]	[7.7 %]	[24.8 %]

Means followed by different letters differ significantly at ($P < 0.05$). Block means significant on plant height. BF- Bioferment, Comp- Compost, LA-leaf area, DM- diameter, ht- height.

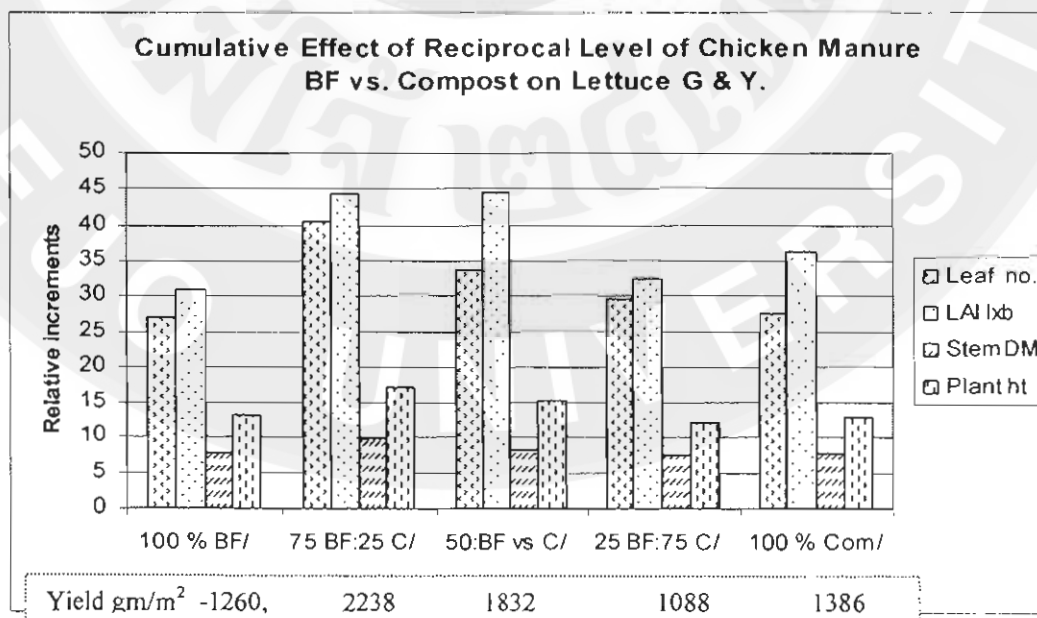


Plate 8 Cumulative effect of various parameters on Lettuce:-

Leaf Numbers:

The counted up highest mean leaf number of 40 at harvest with reciprocal treatment -2 i.e. nutrient supply 75 % through chicken manure BF (150 ml BF :250 gm compost /m²) differed significantly ($p < 0.05$) to its other reciprocals. It was followed by treat-3 (leaf # 33) supplied with 50 % BF and rest of the treatment showed similar results (table: 10 and plate: 8). Whereas, the weekly (7, 14, 21, 28 and 35 days after transplanting) growth to leaf number among the treats observed similar initial increase (week 1), significant rise for treat -1 and 2 (leaf # ~8 for both) than other treats in 2nd week however, shifting later on higher significant count was found in 4-5 week with treat-2 (leaf # 40) and followed by treat -3 (leaf # 33) at harvest.

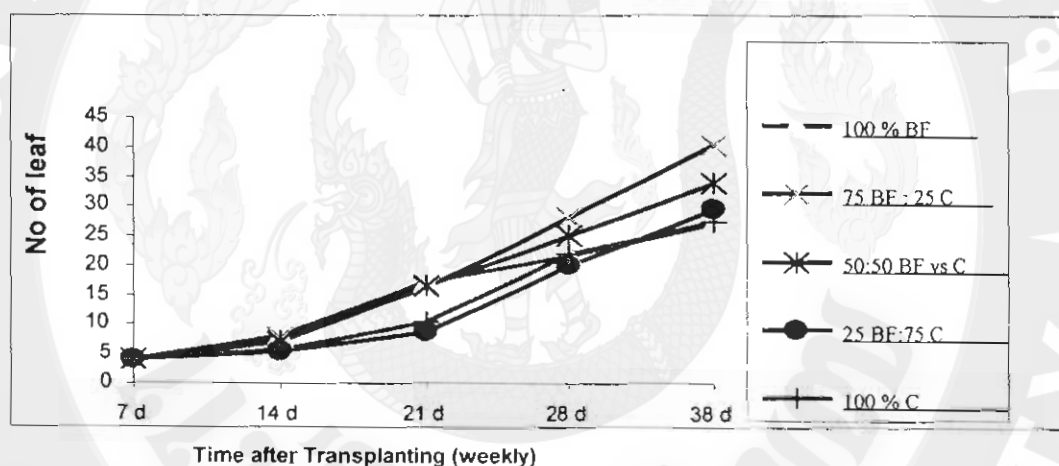


Plate 9 Effect of reciprocal level of Chicken BF vs. Compost on Lettuce

Table 11 Weekly effect of chicken manure BF vs. compost on Lettuce Leaf Number:-

Treatment	Time after Transplanting (weekly)				
	7 d	14 d	21 d	28 d	38 d
100 % BF	4.05	8.11 ^a	17.26	21.61	26.99 ^b
75 BF : 25 C	4.23	7.91 ^a	16.19	27.97	40.32 ^a
50:50 BF vs C	3.99	6.96 ^{ab}	16.66	25.18	33.79 ^{ab}
25 BF:75 C	4.00	5.56 ^b	8.89	20.06	29.42 ^b
100 % C	3.98	5.66 ^b	10.34	21.93	27.46 ^b
level at 5 %-F test	ns	*	ns	ns	*
LSD at .05	0.5	1.7	6.8	7.7	9.1

Tallness:

The study found that the plant height of Lettuce through application of treat 2 as nutrient supply 75 BF: 25 compost (150 ml: 250 gm/m²) yielded highest value (17 cm) differing significantly ($p < 0.05$) than other treatment reciprocals at 5 week harvest (table 10). Tallness pattern was followed by treats -2 (17 cm) > 3 (15 cm) 1 > (13 cm) > 5 and 4 (12 cm). However, the weekly ht exposure found highly significantly differed at every week except initials. Subsequent shifting significant results were obtained as at 2nd week with treat 1 (5.3 cm) followed by treat 2 (4.8 cm) and treat 3 (4.6 cm); modified at 3rd week highly significant with treat 2 (10.7 cm) followed by treat 1 and 3. Whereas, at 4th week the treatment effect was observed greatly varied with treat 2 (14.2 cm) followed by similar other reciprocals (table 12, plate 10).

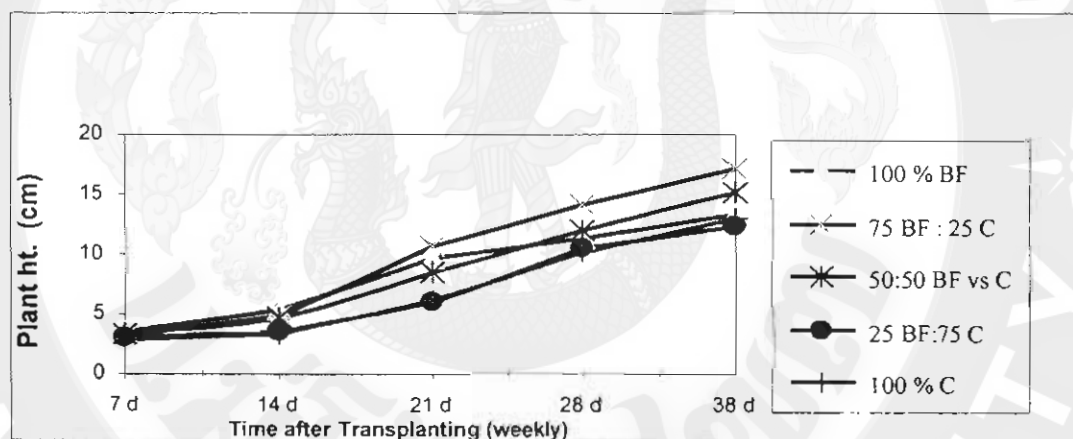


Plate 10 Effect of reciprocal level of Chicken BF vs.Compost on Lettuce plant height.

Table 12 Weekly effect of chicken manure BF vs. compost on Lettuce height.-

Treatment	Time after Transplanting (weekly)				
	7 d	14 d	21 d	28 d	38 d
100 % BF	3.42	5.36 ^a	9.64 ^{ab}	11.39 ^b	13.26 ^{bc}
75 BF : 25 C	3.19	4.81 ^{ab}	10.75 ^a	14.24 ^a	17.09 ^a
50:50 BF vs C	3.26	4.66 ^{ab}	8.58 ^b	11.95 ^b	15.18 ^{ab}
25 BF:75 C	3.06	3.50 ^{bc}	6.02 ^c	10.44 ^b	12.29 ^c
100 % C	2.87	3.30 ^c	6.23 ^c	10.15 ^b	12.92 ^c
Level at 5 %-F test	ns	*	**	**	**
LSD at .05	0.6	1.3	1.8	1.9	2.1

Leaf Area (LA):

Data presented in table 10 and plate 11 on Leaf Area (LA) assessed by largest leaf length and breadth at harvest showed significant difference among treatment means. Treat 3 (nutrient supplied with 50 BF:50 Compost) and treat 2 (nutrient supplied with 75 BF:25 Compost) yielded highest similar leaf expansion of 224 and 222 cm² respectively followed by treat 5 (182 cm²) with 100 % compost. However, treat 4 (164 cm²) and treat 1 (159 cm²) produced similar lowest size with its largest leaf.

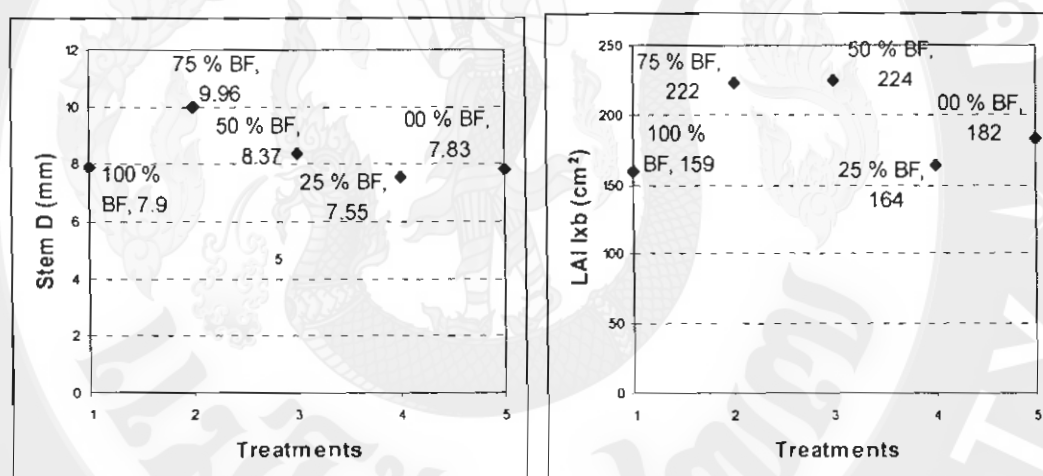
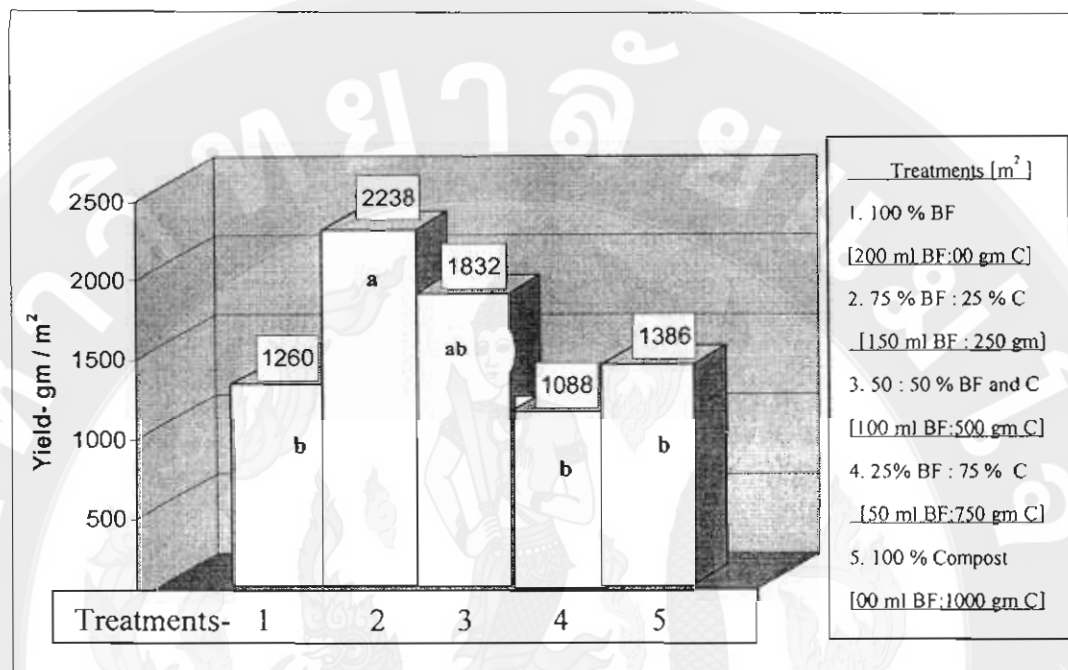


Plate 11 Effect of Reciprocal level of Chicken Manure BF vs. Compost on Lettuce Leaf Area and Stem Diameter:-

Stem Diameter:

The mean stem diameter valued by Vernier Caliper at harvest was found similar among the treatments showing non significant stem thickness. However, the highest enlargement was observed in treat 2 i.e. nutrient supplied through 75 % BF (150 ml BF: 250 gm Compost /sqm) at 9.9 mm ($p < 0.05$) subsequently by treat 3 (8.4 mm) > treat 1 (7.9 mm) > treat 5 (7.8 mm) and treat 4 (7.5 mm). The mean range showed thicker than 7.5 mm to 9.9 mm among all the treatments (table 10 and plate 11).

Total Yield:**Plate 12** Effect of reciprocal level of Chicken Manure BF vs. Compost on Lettuce Yield

The study found that the yield treatment means were significantly different ($p < 0.05$). The highest total fresh leaf weight of 2.2 kg per m² was obtained from treat 2 through supplying nutrient 75 % BF and 25 % compost (150 ml BF : 250 gm compost per m²). It was followed by treat 3 i.e. with 50:50 BF and compost (100 ml BF:500 gm compost per m²) yielding 1.8 kg per m² . However, the treatments 5 (1.3 kg), treat 1 (1.2 kg) and treat 4 (1.0 kg) produced similar leaf fresh mass (table: 10 and plate: 12). As shown by ANOVA outcome (appendix: A) the yield means among treatments ranged from 1.0 to 2.2 kg per m² and least differing value with 729 gm at 5 % level. The highest contribution of BF (T 2) supplied with 75 % nutrient (150 ml BF : 250 gm compost per m²) yielded at 105 % higher production than that of lowest contribution (T 4) from 25 % BF (50 ml BF:750 gm compost per m²). The CV value has shown 24.8 %. However, the block contribution ($F=0.35$, $p < 0.05$) found to be non significant for its replicated mean yields.

Experiment -2: Growth and Yield Parameters

Table 13 Effect of reciprocal level of Cow Dung BF vs. Compost % on Growth and Yield of Lettuce:-

Treatment	[BF : Compost / m ²]	Leaf number	LA- lxb (cm ²)	StemDM (mm)	Plant ht (cm)	Yield (gm/m ²)
100 % BF	[400 ml : 00 gm]	29.10	159.07	6.96	11.96	945.55
75 BF : 25 C	[300 ml : 250 gm]	32.47	161.16	7.16	12.15	1038.62
50:50 BF vs C	[200 ml : 500 gm]	31.86	150.07	7.18	12.32	995.12
25 BF:75 C	[100 ml : 750 gm]	31.27	170.74	7.45	11.70	914.16
100 % Compost	[00 ml : 1000 gm]	31.75	163.31	7.59	11.38	979.16
level at 5 %-F test		ns	ns	ns	ns	ns
LSD at .05		11.5	70	1.7	3.8	566
CV %		[19.5 %]	[23 %]	[12.6 %]	[17. %]	[30 %]

Means followed by different letters differ significantly at ($p < 0.05$).

BF- Bioferment, C- Compost, LA-leaf area, DM- diameter, ht- height.

Leaf Numbers:

The data presented in table 13 shows that the number of leaf counted at harvest obtained from the 5 level of reciprocals supplied through cow dung BF vs. compost found non significant. However, the nutrient supplied from 75 % BF (300 ml BF) to its reciprocal 25 % compost (250 gm/m²) observed highest leaf number (32) than others.

Similarly, (table 14) the weekly leaf treatment means showed not affected from any kind of reciprocals supplied with cow dung BF and compost to its number except 3rd week. But, the similar increments were observed regularly until the harvest. During 3rd week the treat 2 (75 % BF with 300 ml /m²) yielded significantly highest no of leaf (16 leaves) and followed by treat 1 (14 leaves) and treat 3 (12 leaves).

Table 14 Weekly effect of reciprocal level of Cow Dung BF vs. Compost on Lettuce Leaf
Number and Stem Diameter.

Leaf (No.)						Plant ht. (cm)				
Tr.	Time after Transplanting (weekly)					Time after Transplanting (weekly)				
	7 d	14 d	21 d	28 d	38 d	7 d	14 d	21 d	28 d	38 d
T1	3.55	7.28	13.92 ab	23.83	29.10	3.11	5.22	8.69	10.45	11.96
T2	3.71	6.74	16.88 a	25.14	32.47	2.99	3.93	8.37	10.92	12.15
T3	3.69	6.82	12.09 abc	21.27	31.86	2.63	3.50	6.68	9.71	12.32
T4	3.72	5.32	9.56 bc	23.03	31.27	2.87	3.99	6.99	10.10	11.70
T5	3.55	5.23	8.28 c	20.38	31.75	2.87	3.45	6.77	9.55	11.38
F	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
LSD	0.6	1.8	5.3	8.2	11.5	0.7	2	3	3.3	3.8
CV	[9%]	[15%]	[23%]	[19%]	[19%]	[13%]	[26%]	[22%]	[17%]	[17%]

Means followed by different letters differ significantly at ($p < 0.05$).

Tallness:

The result on plant height of Lettuce grown in applications of all reciprocal nutrient supplies through cow dung BF vs. compost showed subsequently similar rise pattern throughout the growing period and at harvest. Although the tallness's were not significantly affected by the treat applications the crop statue elongated highest in treat 3 and treat 2 i.e. nutrient supplied through 50:50 BF and compost ; 75 :25 BF and compost respectively (12 cm) followed by others at 11 cm long (table: 13 and 14).

Leaf Area (LA):

Data presented in table 13 on Leaf Area assessed by largest leaf length and breadth at harvest showed no significant enlargement among treatment means. The result on leaf size of Lettuce grown in applications of all reciprocal nutrient supplies through cow dung BF vs. compost showed similar expansion patterns of leaf at harvest. Although the leaf extensions were not significantly affected by the treat applications, the crop canopy expanded highest in treat 4 i.e. nutrient supplied 25 BF : 75 compost (170 cm^2) followed by treat 5 (163 cm^2) > treat 2 (161 cm^2) > treat 1 (159 cm^2) and least treat 3 (150 cm^2) (table 13).

Stem Diameter:

The mean stem diameter valued by Vernier Caliper at harvest was found similar among the treatments showing no significant stem thickness. Although the thicknesses were not significantly affected by the treat applications, however, the highest swelling was observed in treat 5 i.e. nutrient supplied through 100 % compost ($1000 \text{ gm compost /m}^2$) at 7.6 mm thick, subsequently by treat 4 (7.4 mm) and less others. The mean range showed thicker than 6.9 mm (T 1) to 7.6 mm (T 5) among all the treatments (table 13).

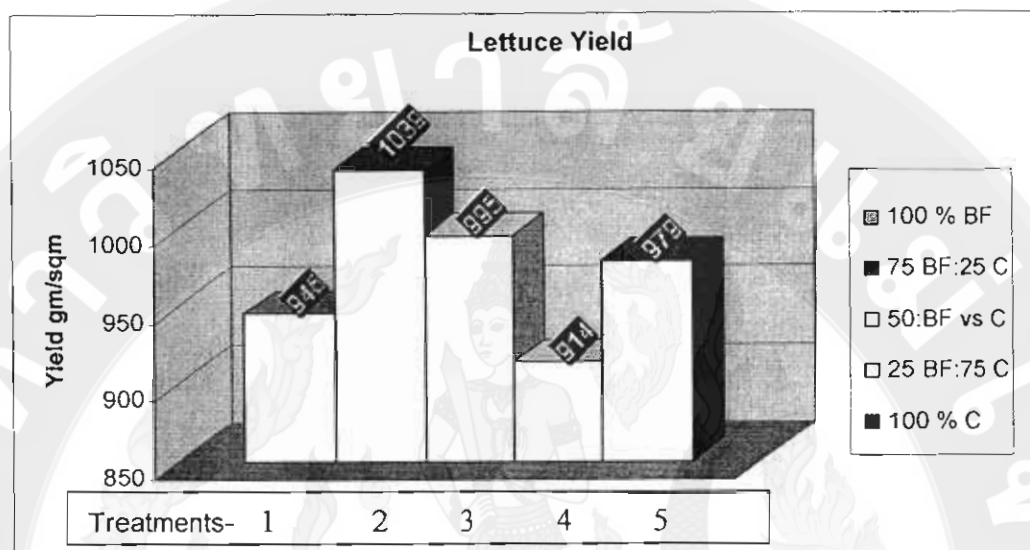
Total Yield:

Plate 13 Effect of reciprocal level of Cow Dung BF vs. Compost on Lettuce Yield:

The study found that the yield treatment means were not significantly difference ($p < 0.05$) in all the reciprocals applications of nutrient supplied through cow dung BF vs. compost. However, the highest total fresh leaf weight of 1.04 kg per m^2 was obtained from treat 2 through supplying nutrient 75 % BF : 25 % compost (300 ml BF : 250 gm compost per m^2). It was followed by treat 3 i.e. with 50:50 BF and compost (200 ml BF:500 gm compost per m^2) yielding 0.99 kg per m^2 , other treatments produced similar leaf fresh mass (table 13 and plate 13). As shown by ANOVA outcome the yield means among treatments ranged from 0.91 to 1.4 kg per m^2 . The highest contribution of BF (T 2) supplied with 75 % nutrient (300 ml BF: 250 gm compost per m^2) yielded 13 % higher production than that of lowest contribution (T 4) from 25 % BF (100 ml BF:750 gm compost per m^2). The CV value has shown 30 %. However, the block contribution found to be non significant for its replicated mean yields.

Discussions

Bioferment Extraction

The extraction score for best water soluble NPK bioferment revealed that the highest release of nutrients obtained during the 15 day (17958 ppm of NPK) and 9 day (7530 ppm of NPK) of withdrawal respectively sourcing from chicken manure and cow dung (table 6 and plate 7.1). The higher initial content of N and K nutrients in chicken manure source (Zublena, 1997; Atta - Atia, 2005) might have caused the higher released amount (2.5 fold) of these nutrients in solution as compared with cow dung that contents lower amount resulting less accumulations. Similarly, the narrow C: N ratio ($< 15:1$) of chicken manure (Palm, 1997) could have delayed the time to release of nutrients by lingering activities of aerobic microorganism due to less availability of carbon to them for the energy simulation. This is obviously seen by the longer time of 15 days needed to be best extracted in the solution sourcing from chicken manure than that of 9 days from cow dung. This is in similarity work done by Gross et al. (2007), Martin and Nathan (1984). However, unlike chicken manure BF the P release observed higher in cow dung BF indicates in the same ways as rich content of organically bond P in stipulated sourcing conditions to release into solution. Primarily, the production of water soluble NPK or extraction that might be associated with microorganism activities, pH and temperature involved as:

Since the suitable microbial feeding, molasses that was added in solution caused rapid growth of various complex microorganisms like lactic acid bacteria, yeast, actinomycetes, fungi and other aerobic bacteria (Diver, 2002) to act promptly utilizing the available C: N ratio for their growth and development. As shown by temperature rises for the organisms to involve readily under their various activities released and washed-out organic N continuously and steadily until the availability of bund and linear over the time period of 9 and 15 days for cow dung and chicken manure respectively. Also, the P releases might be due to involvements by microbial activities such as P mobilizing, P dissolving bacteria and hydrolysis of organic bond which is acquired by generation of profile pH acidifications (table 7 and plate 7.3). Steady and continuous extractions of P also observed over the peak time period, but the negative fluctuations found in

both the BF after peak releasing was possibly onward period pH alkalization as shown in plate 7.3. It has been shown that the proportion of organic P in the soil profile reaches a maximum and falls off, and adsorption appears by depressing phosphate availability with High pH thus, can be associated with dramatically opposite effect on P availability. The microbial activity depends on temperature and is highest in the range of 30 to 45 °C (Mengel and Kirkby, 1987) needs further verifications. The hydrolysis of organic K bond by microorganism acted and released was also continuous and steady during extractions. However, the small ups and downs of curve were observed due to possible dissimilarity during manual stirrings.

By this examination, it could be explained that this observed result of longer days followed by released of higher nutrient in chicken manure BF might be due to the higher intact nutrient contain and subsequently lower C:N ratio in source thereby lingering the microbial activities during extraction. Further, the richen P in Cow Dung BF is seen importantly for combine formulation with higher N and K from Chicken Manure BF. However, the later on solution alkalization and loss of phosphorus needs to be studied further.

Soil and Compost Property

The result shown in table 8 indicated that the N nutrient uptake by the crop was sharply increased with applied all different levels of reciprocals. It is confirmed due to the increased removal of N contains from soil profile and commensurate with highest leaf harvest (table: 10 and 13) in both the experiments. It also led to the N supplied by all levels of treatments was insufficient though nutrient supplied was formulated according to plant requirements. However, the effect of precipitation with 160 mm and other possible N losses during growth period needs further verification. As shown by the data the plot supplied with higher level chicken manure BF i.e. 150 ml/m² reciprocal with low level compost i.e. 250 gm/ m² removed highest level of N content had more favorable effect on growth parameters. The promoted effect of chicken manure BF combine compost might be attributed to the direct initial nutrient released by BF conducive to quick biological available form to plant and microbial uptake to steady mineralization of compost mass later on. This result is in close with Hendawy (2008); Xu (2003) i.e. plant growth may

benefit from rhizosphere microflora that enzymatically enhance the conversion of nutrient unavailable to available for growth. However, the soil P and K content after harvest shows treatment application rates were sufficient or even more than the crop requirement since their amount found to be not intensely changed after the reasonable yield.

Experiment -1: Chicken Manure BF vs. Compost

Vegetative Growth:

The data illustrated from this experiments in table 10 revealed the positive effect of chicken manure BF reciprocated with compost on vegetative growth i.e. plant height, leaves no, stem diameter and leaf size. Each supplying with equal nutrient array, the 5 levels of dose was verified. It is clear from the obtained data that applying chicken manure BF reciprocal at 75 % (150 ml per m^2) level with compost at 25 % (250 gm per m^2) found significant increments on plant height (39 %), leaf number (49 %), leaf area LA (40 %) but less effect on stem diameter. This higher value was closely followed by supplying nutrient at 50: 50 chicken manure BF and compost ($100 \text{ ml BF: } 500 \text{ gm compost per m}^2$). Whereas, applying nutrient alone with BF or compost the lowest values were obtained. It was confirmed that adding chicken manure BF combined compost to the soil at effective level (50-75 % BF : Compost 25-50%) had increased leaf number and plant height in early weeks which in turn, grew up steadily and reflected on highest value at harvesting. Also the highest N removal value coincided in the same treatment plot supported further. On the contrary, adding alone the BF 100 % (200 ml per m^2) though yielded early crop height and leaf number which later on could not supported regular increment resulting lower final value. Whereas, the early slower rise tallness and leaf number (although afterward weeks grew more rapidly) in 100 % compost (1000 gm per m^2) created by slow release of nutrient gained less value up to harvesting, as shown by early to later shifting increments in plate (9 and 10). The highest growth value achieved on applications of 75 and 50 % BF reciprocals with respective compost component could be due to quick availability of nutrient in BF initiated early plant vigor expressed as crop height and leaf number and subsequently supported by sustained nutrients from compost on later stage ($p < 0.05$). The obtained result is similar with those of Hussein and Hadid (2003); Xu et al. (2003) at field application of organic

mass showing early growth limitation following higher later growth and Hendawy (2008) working with compost BF causes herbs height increments.

Total Yield:

The best Lettuce yield applying reciprocal nutrient level through 75 % BF (150 ml BF: compost 250 gm/ m²) was sufficient to produce reasonable yield (22 ton/ha) in this study. This level of BF when coupled with lower level of compost 25% (250 gm / m²) led to significant accumulation of fresh leaf mass (105 %) than other substitutes. The data demonstrated in table (10) and plate 8 and 12 evident that the all applied treatments favorably promoted effect on the accumulation of the Lettuce leaf fresh weight. Mean while the nutrient supplied with two highs levels of chicken manure BF (75% and 50%) reciprocals with low level compost (25% and 50%) gave significant effect on fresh mass production over either the sole (100 %) application of BF (200 ml/ m²) or compost (1000 gm / m²).

It could be explained by the fact that the cumulative growth effect expressed as leaf number, plant height, and leaf expansion (Plate: 8) was confirmed reflected on Lettuce yield as obtained data in table (10). Furthermore, the higher substituted part of solid organic compost mass (75% to 50%) with BF levels (100 to 150 ml/ m²) produced more fresh leaf mass due to favorable combined effects. The beneficial effect of chicken manure BF on Lettuce yield might be due to its instant nutrition and/or its microbial functions available earlier thereby early promotion of various growth parameters. For its direct nutrition, the BF could provide nutrients for easy plant absorption as well as nutrients in biological available to both plant and microbial uptake. However, unlike growth in sole BF, the combine application of compost mass is needed for its steady growth afterwards. It was verified by the BF applied solely with 200 ml BF per m² and similarly, the compost solely at 1000 gm per m² yielded less effective production ($p < 0.05$) revealed as not complete fertilizer without combination. Unfortunately, the determined effect of BF on Lettuce species yet not reported from other organic researchers, though this study found similar work done by Liu and Shijun, 2003; Xu et al. (2003) on Brassicas and Hendawy (2008) working with compost BF caused herbs mucilage increments.

Experiment -2: Cow Dung BF vs. Compost

Vegetative Growth:

In this study with cow dung BF vs. compost mass reciprocated at 5 levels of concentrated dose, as shown in table 13 we found none significantly promoted growth value of various growth parameters expressed as leaf number, height elongation, stem thickness and leaf area. Similarly, (table 14) the weekly leaf no. and crop height increments found also not vigorously affected from any kind of reciprocals however, in general, the growth and development patterns were consistent over time periods showing similar increments until the harvest. However, as revealed pattern in chicken manure BF the shifting increments between 2nd and 3rd weeks were found on leaf number resulting from the nutrient level of (75 % BF with 300 ml /m²) that yielded efficient no of leaf counted on 21 day after transplanting. Although, the data demonstrated at harvest from this experiment in table 13 revealed the less effect on vegetative growth yet, each 5 levels of dose supplying with equal nutrient concentration were clearly verified. The all added levels to the soil applying cow dung BF reciprocal at 100 %, 75 %, 50 %, 25 % and nil (400, 300, 200, 100 and nil ml per m²) corresponding with compost at nil, 25 %, 50 %, 75 % and 100 % (nil, 250, 500, 750 and 1000 gm per m²) had similar increased value reflected at harvesting.

The obtained less satisfactory upper value (table 10 and 13) on growth parameters from cow dung BF reciprocals as compared with chicken manure BF were the consequences might be due to less availability of essential nutrient at early stage, leading less vigorous growth throughout the growing period. However, the compost was supplied at the same level as in chicken manure BF assessment. In this study, among the dose levels supplied, neither the combined reciprocals nor the individual levels (100 % BF and /or compost) showed effective increments further evoked the deficient availability of nutrient from each of the five levels. Unlike in chicken manure BF, in this assessment any of the levels could not initiated the earlier growth encouragement and later on to support by sustained nutrient from compost. It confirmed the experiment failure to detect the difference among the reciprocal levels applied with more predictably the out of available nutrient range. Since we did not find the similar study performed yet, further need of verifications was appreciated. However, Nhamo et al. (1985) indicated Polyphenol contents are low in cattle manure for the browning.

Total Yield:

The illustrated data on Lettuce fresh leaf total mass in table 13 and plate 13 revealed the yield differences were not detectable ($p < 0.05$) among all the reciprocals applications of nutrient supplied through cow dung BF vs. compost. However, the highest total fresh leaf weight of 1038 gm per m² was obtained through supplying nutrient 75 % BF : 25 % compost (300 ml BF : 250 gm compost per m²). It was followed with 50:50 BF and compost (200 ml BF:500 gm compost per m²) yielding 995 gm per m², other treatments produced similar results. Besides, the contribution alone either from BF and / or compost, the higher reciprocal of nutrient supply through BF (75 %) accumulated the upper most yield differed by 13 % to its lowest contributor from lower reciprocal of BF (25 %).

In this study, although there were no negative effects on yield among the doses applied with 5 levels of reciprocals, the less favorable combined effects on total leaf yield was observed as compared with chicken manure BF. It could be explained due to the less cumulative growth effect expressed as leaf number, plant height, and finally LA, validates reflected on lesser leaf mass as obtained parameters data in table 13. Furthermore, importantly comparable effect with nutrient supplied through 75 % and 50 % BF from both the experiment resulted from sourcing as chicken manure and cow dung BF with its same compost reciprocal. This clearly confirms the highest level of favorable combine effect of BF vs. compost on lettuce leaf mass conveying similar trends of substitution value.

Chapter 5

Conclusion and Recommendation

Conclusion

In this study, it could be concluded that the production marks for best water soluble NPK bioferment is obtainable during the 15 days and 9 days of extraction respectively sourcing from chicken manure (17958 ppm of NPK) and cow dung (7530 ppm of NPK). It suggested appropriate timing for peak nutrient release with highest nutrients accumulation thereby potential plant applications, as field verified. The higher intact nutrient contents of chicken manure however, lingering the time for extraction caused 2.5 fold more discharge of nutrient in BF than that from cow dung. Nevertheless, cow dung BF importantly revealed richen in P content (3 fold) than from chicken source. The average nutrient concentration hierarchy observed as $K > N > P$ in both the BF, along with considerable role of pH (4.22) and diminutive temperature rise (35°C) during extraction. However, the down regulated P concentration and pH alkalization after the peak releasing day in the cow dung BF solution was less understood.

The study has shown, BF extracted in 1:1 chicken manure to water over aforesaid time, adjusted to conversion index (CI) 10:1 and 100 liter of undiluted BF per ha as crop requirement yielded significant lettuce harvest. However, the BF sourcing from cow dung has revealed no effective increments.

By this examination, we formulated the BF rate as we found the effective lettuce yield from the fertilization through higher level of chicken manure BF (75% at 150 ml/m^2) combined with its reciprocal compost (25% at 250 gm/m^2). Due to the higher nutrient availability from chicken manure BF at early stage of growth thereby supported to steady increments created by compost reciprocals make possible of effective lettuce yield. Unlike BF, the longer duration needed for compost to initiate early growth competition was also reflect on the final yield. Moreover, the higher amounted nitrogen removal from such plot soil profile possibly returns the yield consequences.

Whereas, the contrary was found obtainable using cow dung BF besides the yield trend has similarly shown. Also, the higher P content analyzed in cow dung BF did not reflected on growth and yield parameters.

The presented findings suggest that the significant yield was combine effect of BF and compost rather than the application of them individually; indicating each of them alone is not a complete fertilizer. However, the obtained yield (22.2 ton/ha) was considered similar or more than that obtainable from chemical fertilizer. The existence of relationship between BF and compost has been importantly realized to its substitution value of 50 to 75 % as large organic mass management hazards. Moreover, in this study combined BF could perform as chemical fertilizer yields, though not as a complete fertilizer alone. In addition to the richen laboratory analysis of BF as an alternative fertilizer subjectively proved with field experiment.

From the forgoing data it can be summarized that use of safer, economical, locally available BF combine substitutes with compost (50 -75 %) has a significant potential for Lettuee crop improvement. Therefore, avoids and/or reduce cost of chemical and/ or organic solid fertilization as an effective non chemical way of crop improvement, in addition reducing crop to crop timing, creating healthy harvest and sustain agro environment.

Recommendation

By this study, we could recommend the following facts for related implications:-

- ✦ The BF extraction with 1:1 source manure to water thereby adjusted with extraction or conversion index (CI 10:1) should be convenient.
- ✦ The production of best water soluble BF that shows most efficient in terms of recovery of NPK nutrient is to soak fresh chicken manure and cow dung in Burlack bag placing in suitable container for appropriated timing of 15 and 9 days respectively.
- ✦ The chicken manure BF is the superior alternative drench fertilizer for organic Lettuce production, instead of using cow dung BF alone as well as synthetics fertilizers.
- ✦ According to foregoing data apply 150 ml of chicken manure BF combine compost mass at $250 \text{ gm} / \text{m}^2$ formulated to obtain highest production yield from lettuce crop.
- ✦ For the optimizing cow dung BF substituted dose, applying widen concentrated dose as well as the possibility of combine effect of chicken manure (with its richen N and K) and cow dung BF (with its higher P) should be explored further. Careful study on the down regulated P index and pH alkaline after peak releasing day in BF is importantly needed.
- ✦ Question to be answer next includes the other factors such as role of biotic abiotic factors involved should be explored to fully exploit a wider range of BF possibility.
- ✦ By this study result an integrated recommendation are made that it is possible for organic farming to produce Lettuce with higher yield than that of conventional farming if the nutrient supply through BF is well formulated, recommend both from environmental and economic standpoints.
- ✦ However, the cost: benefit and qualitative aspects for transmittable human disease in Lettuce should be considered for future study.

Reference

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Appendix – A

The ANOVA table

The ANOVA table showing effect of reciprocal levels of chicken manure BF vs. compost application on Lettuce yield:-

Treatment [BF : Compost per sqm]	Lettuce Yield, gm / sqm			Mean
	1	2	3	
100 % BF [200 ml : 00 gm]	1097.25	1275.01	1407.00	1259.75 ^b
75 BF : 25 Compost [150 ml : 250 gm]	2286.37	2018.10	2408.49	1237.65 ^a
50:50 BF vs Compost [100 ml : 500 gm]	1942.50	2123.31	1430.62	1832.14 ^{ab}
25 BF:75 Compost [50 ml : 750 gm]	1094.10	840.00	1330.03	1088.04 ^b
100 % Compost [00 ml : 1000 gm]	1824.38	1672.54	661.50	1386.14 ^b

Analysis of Variance:- SIRICHAJ STATISTICS VERSION 6.0

Source	df	SS	MS	F	0.05
Block	2	106101.37	53050.6888	0.35 ^{ns}	4.46
Treatment	4	2629175.2	657293.8055	4.38*	3.84
Ex. Error	8	1199462.2	149932.7835		
Total	14	3934738.8	281052.7763		
GRAND	MEAN	1560.7466,	CV	24.81%	
LSD	0.05	729.05782			

The logo of Maejo University is a large, circular emblem in the background. It features a central figure of a Thai deity or guardian spirit standing on a lotus, holding a sword and a mace, surrounded by flames. The figure is encircled by a ring of Thai script. The outermost ring of the logo contains the text "MAEJO UNIVERSITY" in English and Thai script.

Appendix - B

Abstract of the Paper Presentation in National Horticultural Congress, Thailand held at Maejo University, ChiangMai, during May 6-8, 2009.

Effect of Bioferment Fertilizer on Growth and Yield of Lettuce (*Lactuca sativa* var. *crispa*) Under Organic Production

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Abstract

In order to exploit the potential use of bioferment fertilizer (BF), the rates of concentrated BF sourcing from Chicken Manure (CM) and Cow Dung (CD) extracted with 1:1 water were drench tested and verified in two discrete organic Lettuce trials. The BF analysis showed that extracted from CM released 2.5 fold NPK index than from CD; further, among 3, 6, 9, 12 and 15 fermentation days, 15 and 9 days were appropriated timing, accumulated the highest (17,958 and 7530 ppm NPK) turnover respectively. The effects of 10 dose-levels on growth and yield were detected by supplying crop with reciprocals of nutrient through BF and compost. Both the field experiments as CM BF and CD BF with its compost reciprocals showed positive effects. However, CD BF yielded none significantly promoted value. Whereas, the experiment with CM BF found that among the concentrated rates at 100%, 75%, 50%, 25% and nil (200, 150, 100, 50 and nil ml/m²) supplied with compost at nil, 25%, 50%, 75% and 100% (nil, 250, 500, 750 and 1000 g/m²), the dose with BF 150 ml/m² (75 %) combine compost 250 g/m² (25 %) showed optimum. It significantly gained (105%) highest yield 2237 g/m², effective tallness (39%), leaf no (49%), leaf expansion (40%) and less effect on stem thickness. This was confirmed by earlier growth expressions reflected on yield; also complied higher N removal soil status. This study suggested existence of relationship (combine effect) with BF and compost; concludes without affecting the conventional yield, CM BF could substitute considerable (50-75%) amount of organic mass, hence a new approach for organic fertilization to reduce cost, synchronize crop-timing, safety harvest and sustain environment

Key words: Bioferment, Extraction/ Fermentation, Conventional/Organic farming, Drench, Compost, Loose-leaf Lettuce.



Appendix – C

Additional plates

1 Lettuce Nursery



2 Lettuce Transplanting



3 Lettuce field -4th week



4 Lettuce field at harvest



5 Lettuce growth at 2nd week day



6 Lettuce growth at harvest





Appendix - D
Biography

BIOGRAPHY

Name	Keshab Adhikari	
Date of Birth	May 28, 1962	
Educational Background	1984-1987	B. Sc. Ag (Plant Science) Tribhuvan University, Nepal
	2007-2009	M. Sc (Horticulture) Maejo University, Thailand
	1989 to date	Senior Horticulturist, Government of Nepal Ministry of Agriculture and Cooperative District Agriculture Development Offices, Fruit development Division
Past Affiliations	USAID project IRDP-Nepal JICA Horticulture Develop Project- Nepal SNV-Netherland-Nepal	
Proficiency	Organic Horticulture Extension	
Research Experience	Researcher	
	Socio-economic Research and Investigations	
International Training	December, 1999	Nutrition and Human Diet FAO, TCP/Nepal
	September, 2004	Intensive Vegetable Growing under Various Environment Condition, CINADCO, Israel
Publications	June, 1997	Off-Season Vegetable Production, Technical Notes on- Mango Cultivation Tech
	May, 2009	Effect of Bioferment Liquid fertilizers on Growth and Yield of Lettuce 8 th National Horticulture Congress, Maejo University, Thailand