

Full Length Research Paper

Production of liquid fertilizer by fermented livestock liquid and application on crops

Yong-Hong Lin^{1*}, Yi-Lang Wei², Juei-Yu Chiu², Tai-Yuan Chen³ and Chih-Hang Chang³

¹Department of Plant Industry, National Pingtung University of Science and Technology, Pingtung, Taiwan.

²Department of Environmental Science and Engineering, National Pingtung University of Science and Technology, Pingtung, Taiwan.

³Kaohsiung District Agricultural Research and Extension Station, Pingtung, Taiwan.

Received 3 January, 2020; Accepted 29 April, 2020

The livestock liquid commonly contains humic acid, amino acids and minerals; hence, it can be used for the fertilization of crops. These studies were conducted to examine the use of livestock fermented liquid in producing liquid fertilizers and irrigation application on two kinds of crops (tomato and wax apple). The experiments were designed as common liquid fertilizer and diluted by groundwater (CF), fermented livestock liquid fertilizer and diluted by groundwater (LF), fermented livestock liquid and diluted by groundwater (LL), fermented livestock liquid fertilizer and diluted by effluent (LFE), and the treatment of no liquid fertilizer (CK). The results showed that the highest values for fruit weight, width and length were obtained by the treatment of LF, and the sugar degree value was registered by the treatment of LFE. Hence, the treatment of LF was superior compared to CF. Analysis of microbiology in LF, CF and LL showed that the microorganisms' species were most abundant in the LF compared to the other two samples. The main microorganisms were *Bacillus mycoides* and *Bacillus subtilis*. Anthracnose of mango was inhibited in specific isolates of the *B. subtilis* in LF. The results provided the primary information on reusing of livestock waste for crops.

Key words: Livestock liquid, effluent, tomato, wax apple

INTRODUCTION

In Taiwan, the main livestock farming was concentrated at Pingtung, Yunlin and Chianghua counties. The treatment of livestock waste was commonly provided by the three-stage system including solid-liquid separation, anaerobic and aerobic fermentation in the field area of livestock farming. On completion of the treatment, the effluent was drained to rivers. However, part of the river was still polluted occasionally. The biogas was produced after anaerobic fermentation of livestock waste (Chen et

al., 2008; Weiland, 2010; Bao et al., 2019), and then the residual solid and liquid were used as fertilizer to farmland (Holm-Nielsen et al., 2009).

In addition to the influence on plant diseases and insects, nutrients absorption imbalance will influence plant growth. The 16 main kinds of nutrient elements are necessary for plants (Kholmanskiy et al., 2019). To sustainably obtain the balanced nutrition, fertilization is necessary.

*Corresponding author. E-mail: yonghong@mail.npust.edu.tw; Tel: +886-8-7703202.

If the appropriate ingredients of liquid fertilizer were modified and supplied to crops, it will be rapidly adsorbed by crops and increase the quality of fruits. There are great amount of minerals, amino acids and humic acids in the livestock liquid that can promote the growth of plants (Richardson and Ternes, 2011); however, the negative influence of heavy metals and environmental hormones should be evaluated (Kelessidis and Stasinakis, 2012). This study was conducted to evaluate irrigated water that was replaced by livestock liquid to produce the liquid fertilizer. On the other hand, after application, the effect of different liquid fertilizers on tomato and wax apple were compared. In this experiment, circular agriculture and appropriate fertilization has been achieved.

MATERIALS AND METHODS

Experimental design and production of liquid fertilizer

This experiment was designed for the production of liquid organic fertilizers by livestock liquid. The materials used include: drainage water (or livestock liquid) (300 L), soybean powder (9 kg), rice bran (9 kg), phosphate rock powder (9 kg), seaweed powder (9 kg), molasses (18 kg) and microorganisms (3 kg). The hydrolysis of soybean powder will produce high polypeptide and amino acids and then offer abundant nitrogen fertilizer to plants (Kanu et al., 2009; Zhao et al., 2012). Phosphate rock powder (tricalcium phosphate) is commonly a kind of phosphorus fertilizer, or simply a phosphorus source in fertilizer (Xu et al., 2019). Vijayakumar et al. (2018) showed that application of a lower concentration (20%) of seaweed liquid fertilizer (SLF) will increase fresh weight, leaf area, protein, carbohydrate and lipids of *Capsicum annum* L. Hence, the seaweed was used to supply the potassium in liquid fertilizer. The productive methods are described as follows: At first, the 300 L plastic barrels were arranged, and the groundwater (or fermented livestock liquid) along with prepared materials were all put into the barrel. Thereafter, the liquid fertilizer was stirred by electric stirrer two times every day for 14 days. The stirring duration was 5 min every time. After 30 days, the crop experiments began. The experimental designs were described in Table 1. The tomato (CK, CF, LL, LF, LFE) and wax apple (CF, LF) experiments began from young fruit stage of the two kind of crops. The five irrigative times, the 1,500 L every time, and hence, the total liquid fertilizer amount was 7,500 L. The soil and leaves were sampled and analyzed before and after experiments. The quality of fruits was also estimated at the harvest stage.

Analysis of soils and plants

Pretreatment and analysis of soil

After taking back soil samples, these were air dried and grinded. Thereafter, the samples were shaken through a 2 mm sieve. Further, the soil was analyzed for the following indicators: (1) pH value: water : soil = 1:1, measured by a pH meter (McLean, 1982); (2) Organic matter content was measured by wet oxidation method described by Nelson and Sommer (1982); (3) calcium (Ca), magnesium (Mg), potassium (K) content by extraction of the Ca, Mg, K from the soil with 0.1 N HCl. The content of Ca, Mg and K was measured by inductively coupled plasma spectrometer (Jobin Yvon-2000) (Baker and Suhr, 1982); (4) phosphorus (P) was

measured by molybdenum blue method (Bray No.1) (Murphy and Riley, 1962); (5) iron (Fe) and manganese (Mn) microelements have been extracted from the soil by using 0.1 N HCl. Their contents were measured by an atomic absorption spectrometry (AAS) (Cope and Evans, 1985).

Analysis of leaves

Dust and chemical residuals on the leaves were cleaned by tap water; leaves were put into an oven (70-75°C), then after 2 to 3 days were grinded and put into a bottle (Chang, 1981). The leaves were resolved by concentrated sulphuric acid and analyzed by the following methods: (1) nitrogen (N) was measured by Kjeldahl method. (2) P was measured by molybdenum yellow method (Bray No.1). (3) K, Ca, Mg were measured by inductively coupled plasma spectrometer (Jobin Yvon-2000), and (4) Fe and Mn concentrations were measured by inductively coupled plasma spectrometer (Jobin Yvon-2000).

Analysis and application of microbiology in liquid fertilizer

Microbiological analysis in the different liquid fertilizers

After sampling 1 ml of liquid fertilizers, 9 ml distilled water was added and the mixture was shaken. Then, 1 ml mixture was sampled and 9 ml distilled water was added. After multiple dilutions, the liquid was spread on the surface of solid medium (potato dextrose agar (PDA)). Three replicates were made; afterwards, the solid medium was placed to the growth chamber at a constant temperature (27°C). After apparently observing the microorganisms, the total amount of microorganisms was calculated averagely for three replications. The calculation formula employed was as below:

$$\text{Average amount of microorganisms} = \frac{\text{Average microorganisms of three replications}}{\text{Dilutive fold } 10} \text{ (CFU/ml)}$$

Where CFU = Colony forming units.

Test of microorganisms isolated from liquid fertilizer for the antibiosis of mango anthracnose

Anthracnose is a kind of important disease that affect mango (Liu et al., 2014). Some methods were found to effectively inhibit anthracnose (Jiao et al., 2018). At first, the processing groups were set as follow: The isolated microorganisms strain from liquid fertilizer was inoculated in the medium containing agar located at the 1/4 right district. The medium were cultured in the growth chamber at a temperature of 30°C for 1 day. Thereafter, the anthracnose strain was inoculated on the 1/4 left district. The medium was continuously placed in the growth chamber and the temperature was set at 30°C. On the other hand, the treatment of check (CK) was set in such a way that only inoculating anthracnose strain was found on the 1/4 left district of medium; there were no isolated microorganisms strain on the medium. When the hyphae of anthracnose strain in the CK treatment was spread to the 1/4 right district of medium, the inhibition zones of processing groups were investigated.

Statistical analysis

Windows SPSS 10.0 was applied to treat the data statistically for variables analysis. Duncan's multiple range tests were used in differentiating the variances. It was significantly different if $P < 0.05$.

Table 1. The experimental treatments of livestock liquid fertilizer.

Treatment	Description
CK	The treatment without liquid fertilizer
CF	Common liquid fertilizer and diluted by groundwater for 400X
LL	Fermented livestock liquid and diluted by groundwater for 10X
LF	The liquid fertilizer was produced by livestock liquid and diluted by water for 400X
LFE	The liquid fertilizer was produced by livestock liquid and diluted by effluent for 400X

Table 2. The variation of soil properties in tomato garden before and after experiments.

Treatment	pH	² OM (%)	³ mg kg ⁻¹							
			P	K	Ca	Mg	Fe	Mn	Cu	Zn
¹ B.E.	6.77 ^{a3}	2.50 ^c	128 ^a	276 ^b	3045 ^a	401 ^b	198 ^{ab}	121 ^b	10 ^a	20 ^b
CK	6.75 ^a	2.45 ^c	135 ^a	266 ^b	3334 ^a	382 ^b	244 ^a	114 ^b	14 ^a	15 ^b
CF	6.12 ^a	3.60 ^b	134 ^a	345 ^b	3202 ^a	620 ^a	162 ^b	221 ^a	7.9 ^a	71 ^a
LL	6.46 ^a	4.31 ^a	124 ^a	370 ^{ab}	3492 ^a	661 ^a	284 ^a	246 ^a	17 ^a	22 ^b
LF	6.15 ^a	4.97 ^a	160 ^a	395 ^a	3064 ^a	559 ^a	293 ^a	245 ^a	20 ^a	50 ^a
LFE	6.11 ^a	3.99 ^b	143 ^a	383 ^{ab}	3157 ^a	560 ^a	175 ^b	241 ^a	16 ^a	33 ^a

¹B.E.: Before experiment; ²OM: organic matter; ³Values within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

Table 3. The variation of nutrients in the leaves of tomato before and after experiments.

Treatment	N		P		K	Ca	Mg	Fe	Mn	Cu	Zn
	g kg ⁻¹	%	g kg ⁻¹	%							
¹ B.E.	6.77 ^{a2}	2.50	0.35 ^b	0.36 ^b	276 ^a	3045	401 ^a	198 ^a	121 ^b	10	20 ^a
CK	2.25 ^a	0.399 ^a	2.03 ^a	0.399 ^a	266 ^a	3334 ^a	380 ^a	151 ^a	148 ^b	1325 ^a	124 ^a
CF	2.00 ^a	0.400 ^a	1.90 ^a	0.400 ^a	345 ^b	3202 ^a	4465 ^a	168 ^a	367 ^a	1308 ^a	120 ^a
LL	2.21 ^a	0.360 ^a	2.19 ^a	0.360 ^a	370 ^{ab}	3492 ^a	3943 ^a	153 ^a	371 ^a	1263 ^a	131 ^a
LF	2.69 ^a	0.391 ^a	1.91 ^a	0.391 ^a	395 ^a	3064 ^a	4482 ^a	150 ^a	393 ^a	1307 ^a	131 ^a
LFE	2.97 ^a	0.389 ^a	1.90 ^a	0.389 ^a	383 ^{ab}	3157 ^a	4230 ^a	159 ^a	381 ^a	1265 ^a	120 ^a

¹B.E.: Before experiment; ²Values within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

RESULTS AND DISCUSSION

Experimentation of liquid fertilizer by fermented livestock liquid for crops

Table 2 shows that the soil pH of tomato was 6.77 before experiment. However, it was reduced after experiment in all treatments. It was possible that the application of chemical fertilizer and fermented livestock liquid led to the reduction of pH. The reason why the application of LF was reduced to the lowest pH among all treatments may be stronger acidity in fermented livestock liquid (data not shown). The soil organic matter, phosphorus, potassium, calcium and magnesium were all higher in the entire treatments after experiments. In addition to supplementation of essential nutrients, either with

increasing the available elements; however, the increase of microelements was limited. Table 3 shows that the nitrogen, phosphorus, calcium, magnesium, manganese, copper and zinc in the leaves of tomato were slightly higher after all treatments. Regarding the fruits quality of tomato, effects of the treatments of fermented stock liquid (LF and LFE) were higher than that of non-fermented (Table 4). The fruit weight, fruit width and fruit length were all excellent in the treatment of LF; however, soluble solid was highest in the LFE. In general, after the production of liquid fertilizer by fermented livestock liquid, it was then applied to tomato in a bid to increase its quality. Vijayakumar et al. (2018) showed that the usage of seaweed liquid fertilizer can increase the quality of *Capsicum annum* L.

In the wax apple comparative experiment, the focus

Table 4. The compared fruit quality of tomato at harvesting stage.

Treatment	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Soluble solids (°Brix)
CK	12.8 ^{b1}	3.9 ^a	2.3 ^a	7.3 ^b
CF	13.8 ^{ab}	4.1 ^a	2.2 ^a	7.8 ^a
LL	14.7 ^a	4.2 ^a	2.4 ^a	7.3 ^b
LF	15.9 ^a	4.2 ^a	2.5 ^a	7.5 ^{ab}
LFE	12.9 ^b	4.0 ^a	2.3 ^a	7.9 ^a

¹Values within columns within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

Table 5. The variation of soil properties in wax apple orchard before and after experiments.

Treatment	pH	² OM (%)	P	K	Ca	Mg	Fe	Mn	Cu	Zn
¹ B.E.	6.76 ^{a3}	1.51 ^a	280 ^a	350 ^a	6109 ^a	372 ^a	128 ^a	198 ^a	6.9 ^a	23 ^a
CF	6.74 ^a	1.74 ^a	291 ^a	361 ^a	6115 ^a	371 ^a	120 ^a	177 ^a	6.8 ^a	19 ^a
LF	6.75 ^a	1.76 ^a	293 ^a	359 ^a	6130 ^a	376 ^a	130 ^a	185 ^a	7.1 ^a	22 ^a

¹B. E.: Before experiment; ²OM: organic matter; ³Values within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

Table 6. The variation of nutrients in the leaves of wax apple before and after experiments.

Treatment	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn
¹ B.E.	1.82 ^{a2}		1.72 ^a	7332 ^a	2979 ^a	308 ^a	41 ^a	6 ^a	18 ^a
CF	1.85 ^a	0.121 ^a	1.72 ^a	7312 ^a	2888 ^a	311 ^a	45 ^a	10 ^a	20 ^a
LF	1.87 ^a	0.125 ^a	1.74 ^a	7318 ^a	2797 ^a	314 ^a	44 ^a	12 ^a	22 ^a

¹B.E.: Before experiment; ²Values within columns within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

Table 7. The compared fruit quality of wax apple at harvesting stage.

Treatment	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Soluble solid (°Brix)
CF	101.1 ^{a1}	6.1 ^a	6.7 ^a	8.5 ^b
LF	107.4 ^a	6.2 ^a	6.8 ^a	11.9 ^a

¹Values within columns within columns followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

was only on the production and application of liquid fermented livestock fertilizer and common fertilizer (LF and CF). Table 5 shows that soil pH was reduced among different treatment. All nutrients were increased after treatments. The elements in the leaves of wax apple (Table 6), fruit weight, fruit width and fruit length along with soluble solid for LF were all higher than CF (Table 7). Lin and Chiu (2019) showed that the quality of wax apple was influenced by the absorption of abundant nutrients and water. In this experiment, we found that when the

appropriate liquid fertilizer was irrigated, the quality of wax apple was increased.

The water can be replaced by fermented livestock liquid for the production of liquid fertilizer; thereafter, it can be applied to tomato after diluting 400 times by irrigation water of fermented livestock liquid during fruit-setting stage. The fermented livestock liquid was used in producing liquid fertilizer, and then diluted 400 times by irrigation water for wax apple at fruit-setting and fruit mature stages.

Table 8. The microorganisms' phases in different liquid fertilizer were analyzed.

Treatment	C-1	C-2	C-3	C-4
	CFU/ml			
LL	-	-	-	-
CF	-	-	-	-
LF	230	310	190	47

*C-1: Fungus, C-2: *Bacillus mycoides*, C-3 and C-4: *Bacillus subtilis*.

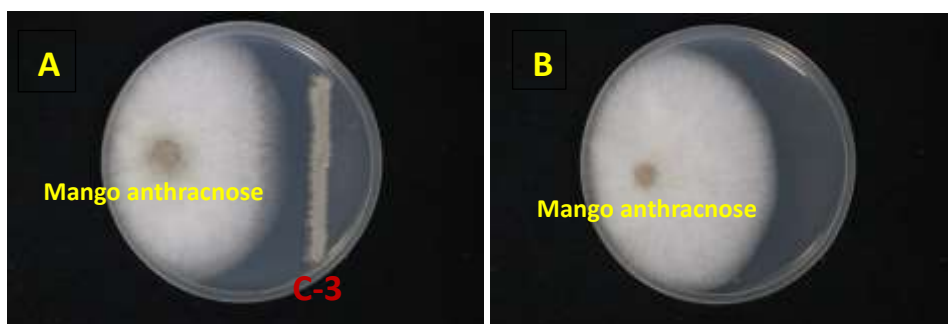


Figure 1. *Bacillus subtilis* (C3) strain screened from fermented livestock liquid fertilizer (LF) chosen for the antibiosis test of mango anthracnose (A: treatment, B: check).

Analysis and application of microorganisms in liquid fertilizer

In Table 8, it is shown that the microbiology phase in LF was most abundant among the treatments of LL, LF and CF; also, fungus and bacteria (*Bacillus mycoides* and *Bacillus subtilis*) were the main microorganisms. Additionally, the microorganisms were the most abundant in the treatment of WF. The C-3 was chosen for the inhibitive experiment of mango anthracnose. The result showed that anthracnose was inhibited by C-3 (Figure 1). Hence, the microbiology of fermented livestock liquid for the inhibition of pathogen can be an advanced research. Duan et al. (2019) reported that *B. subtilis* is a kind of microorganism that can inhibit some plant pathogens.

The results showed that the liquid fertilizers could be produced by livestock liquid, and the quality of tomato and wax apple could be promoted by appropriate dilution of specific liquid fertilizers.

Conclusion

The fermented livestock liquid contains some beneficial materials that can promote the growth of plants. In addition to its use in direct irrigation of crops, it can be regarded as the medium of liquid fertilizer products. From this experiment, the quality of tomato and wax apple were both increased when the fermented livestock liquid

fertilizer (LF or LFE) was applied. On the other hand, the microbial phase was abundant in the treatment of LF and provided an important clue for advanced research in the screening of beneficial microorganisms for fermented livestock liquid.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors appreciate Professor Yu-Chia Chung who serves in National Sun Yat-sen University, Kaohsiung, Taiwan, for detail reading of this article.

REFERENCES

- Baker DE, Suhr NH (1992). Atomic absorption and flame emission spectrometry. In A.L. Page, R. H. Miller and D. R. Keeney. (eds.) Methods of Soil Analysis, Part 2. Agronomy Monograph No. 9. 2nd edition. ASA-SSSA, WI. pp. 13-26.
- Bao W, Yang Y, Fu T, Xie GH (2019). Estimation of livestock excrement and its biogas production potential in China. Journal of Cleaner Production 229(5):1158-1166.
- Chang SH (1981). Current plant analysis in our province. Fertilizer demand diagnoses technology. Special Issue No. 13, Taiwan Agricultural Research Institute, pp. 53-59.

- Chen Y, Cheng JJ, Creamer KS (2008). Inhibition of anaerobic digestion process: a review. *Bioresource Technology* 99(10):4044-4064.
- Cope JT, Evans CE (1985). Soil testing. *Advances in Soil Science* 1:201-228.
- Duan M, Zhang Y, Zhou B, Wang Q, Gu J, Liu G, Qin Z, Li Z (2019). Changes in antibiotic resistance genes and mobile genetic elements during cattle manure composting after inoculation with *Bacillus Subtilis*. *Bioresource Technology* 292:122011.
- Holm-Nielsen JB, Al Seadi T, Oleskowicz-Popiel P (2009). The future of anaerobic digestion and biogas utilization. *Bioresource Technology* 100(12):5478-5484.
- Jiao GS, Kim S, Moayeri M, Thai A, Cregar-Hernandez L, McKasson L, O'Malley S, Leppla SH, Johnson AT (2018). Small molecule inhibitors of anthrax edema factor. *Bioorganic and Medicinal Chemistry Letters* 28(1):134-139.
- Kanu PJ, Kanu JB, Sandy H, Kande BA, Mornya PMP, Huiming Z (2009). Optimization of enzymatic hydrolysis of defatted sesame flour by different proteases and their effect on the functional properties of the resulting protein hydrolysate. *American Journal of Food Technology* 4(6):226-240.
- Kelessidis A, Stasinakis AS (2012). Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. *Waste Management* 32(7):1186-1195.
- Kholmanskiy A, Smirnov A, Sokolov A, Proshkin Y (2019). Modeling of extraction mechanism of mineral elements by plants. *Current Plant Biology*, P. 9.
- Lin YH, Chiu JY (2019). Studies on the sod culture and the management of soil moisture for the improvement of wax apple quality. *African Journal of Biotechnology* 18(3):719-725.
- Liu K, Wanga X, Young M (2014). Effect of bentonite/potassium sorbate coatings on the quality of mangos in storage at ambient temperature. *Journal of Food Engineering* 137(1):16-22.
- McLean EO (1982). Soil pH and lime requirement. In A.L. Page, R.H. Miller and D.R. Keeney (eds.) *Methods of Soil Analysis, Part 2. Agronomy Monograph No.9. 2nd edition. ASA-SSSA, WI.* pp.199-224.
- Murphy J, Riley JP (1962). A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta* 27(1):31-36.
- Nelson DW, Sommer LE (1982). Total carbon, organic carbon and organic matter. In: A.L. Page, Miller and D.R. Keeney (eds.) *Method of Soil Analysis, Part 2. Agronomy Monograph No. 9. 2nd edition. ASA-SSSA, WI.* pp. 383-411.
- Richardson SD, Ternes TA (2011). Water analysis: emerging contaminants and current issues. *Analytical Chemistry* 83(9):4614-4648.
- Vijayakumar S, Durgadevi S, Arulmozhi P, Rajalakshmi S, Gopalakrishnan T, Parameswari N (2018). Effect of seaweed liquid fertilizer on yield and quality of *Capsicum annum* L. *Acta Ecologica Sinica* 39(5):406-410. <https://www.sciencedirect.com/science/article/abs/pii/S1872203218301884?via%3Dihub>
- Weiland P (2010). Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology* 85(6):849-860.
- Xu JC, Huang LM, Chen C, Wang J, Long XX (2019). Effective lead immobilization by phosphate rock solubilization mediated by phosphate rock amendment and phosphate solubilizing bacteria. *Chemosphere* 237(1):1-9.
- Zhao Z, Liao D, Sun J, Tong Z, Huang K, Sun G, Wu Z (2012). Kinetic model of enzymatic hydrolysis of protein about the protein of silkworm pupae – Alcalase system. *International Conference on Biomedical Engineering and Biotechnology* pp. 356-359.