Kasetsart J. (Nat. Sci.) 44 : 808 - 815 (2010)

Effects of Bio-Extracts on the Growth of Chinese Kale

Chalermwut Noisopa[1], Benjaphorn Prapagdee[1],
Chalie Navanugraha[2] and Rungjarat Hutacharoen[1]*

ABSTRACT

The effectiveness of three bio-extracts: Chinese kale, Chinese cabbage and soybean on Chinese kale growth was compared. The nutrient composition and chemical properties of the three bio-extracts were investigated. The various concentrations of each bio-extract were applied daily to the supporting media, to enhance Chinese kale growth. The height, fresh weight and dry weight of the Chinese kale were measured for both stems and leaves. The results showed that the acidic condition of all bio-extracts had a high electrical conductivity (EC). However, the nutrient concentration in each bio-extract was noted to be less than that of the complete nutrient solution. Furthermore, soybean bio-extract performed the best in the most acidic conditions, had the highest EC values, and the greatest amount of total nutrients. Chinese kale and Chinese cabbage bio-extracts performed similarly in terms of chemical characteristics. With regard to growth enhancement using these bio-extracts with determined concentrations, a complete nutrient solution gave the best height and both fresh and dry weights, and showed a significant statistical difference from the other two bio-extracts. Soybean bio-extract was the most effective bio-extract to enhance growth.
Keywords: bio-extract, Chinese kale, Chinese cabbage, soybean, growth promotion

INTRODUCTION

Currently, bio-extracts, or bio-fermented or bio-decomposed matter, play an important role in the promotion of self-sufficiency agriculture due to their low cost, reduced impact on soils and moderate chemical fertilizer input. In contrast, agricultural residue from chemical fertilizers and runoff can pollute surface and water ecosystems after harvest. Therefore, bio-extracts or bio-ferments are one option to combat the pollution problem. Furthermore, bio-extracts and bio-ferments are solution compounds produced by introducing plant and animal residues to sugar or molasses, which are the carbon and energy sources for the microorganism fermentation and decomposition processes. The decomposed compound (a brown liquid), which contains many organic compounds, gives immense benefits, with an effective enhancement of cultivation. Even though bio-extracts can be used in the place of an equivalent portion of chemical fertilizer, albeit with insufficient macronutrients for plant growth, the small number of microorganisms present in the bio-extract allows a transformation of unavailable nutrients to available nutrients for continual plant growth. However, even with more available nutrients, bio-extracts are still composed

1 Faculty of Environment and Resource Studies, Mahidol University, Salaya, Nakhon Pathom 73170, Thailand.
2 Faculty of Environment and Resource Studies, Mahasarakham University, Kantarawichai, Maha Sarakham 44150, Thailand.
* Corresponding author, e-mail: enrhu@mahidol.ac.th

Received date : 10/02/10 Accepted date : 25/05/10

of other ingredients and in particular, plant hormones that regulate plant growth (Department of Agriculture, 2004). Pathanapibul (2003) found that plant or fish bio-extracts with complete nutrient supplements produced better growth of Chinese cabbage, lettuce, and sweet pepper in a soil-free culture system compared to complete nutrient solution alone. Moreover, several studies noted that fish bio-extracts contain greater amounts of nutrients and plant hormones or plant growth regulators than plant bio-extracts (Chaisit and Sudprasong, 2000; Veerasri, 2000; Department of Agriculture, 2003; Pathanapibul, 2003). Therefore, a mixture of fish bio-extract and complete nutrient supplement should have greater capability for growth enhancement than a mixture of plant bio-extract or complete nutrient supplement alone. In contrast, the results showed that a mixture of plant bio-extract and complete nutrient supplement enhanced the growth of plants more than a mixture of fish bio-extract and complete nutrient supplement. The study suggests that not only the amount of nutrients in the bio-extract, but also the components of the compounds in the bio-extract should be considered in growth enhancement. Furthermore, this suggestion was also supported by Cho (2004), who stated that "Bio-ferments from the lateral bud of any type of plant are most useful when used on itself". This means that bio-extracts produced from the residue of one particular plant show better growth of that same plant than bio-extracts produced from the residue of another plant. The same plant has the same essential requirements and contains easier-to-absorb forms of nutrients and some compounds, such as hormones, vitamins, and enzymes.

Thus, the current study attempted to investigate the efficiency of growth enhancement using bio-extracts produced from the same and different kinds of plants. Chinese kale was selected as the representative vegetable for growth enhancement, as it is the most popular vegetable in terms of consumption and has high nutritional value, especially calcium (Ca), phosphorus (P), and vitamin C. The results are expected to be used in the promotion of self-sufficiency agricultural strategies and as a solution to pollution control and environmental remediation in remote areas.

MATERIALS AND METHODS

Growth media preparation

Sand (diameter: 0.2-2.0 mm, Department of Soil Science, 1998) and coconut coir (size: less than 4.0 mm) were prepared using a sieve. The prepared sand and coconut coir was then mixed and stirred to a homogeneous mixture at a ratio of 1:2 (sand:coconut coir) by volume. The growth medium was tested to find the appropriate nutrient solution volume for daily watering. The appropriate volume of nutrient solution is related to the performance of the drainage of the growth medium. The pouring volume of the complete nutrient solution and the bio-extracts also need to be considered. This method comprises two steps: Firstly, the standard curve of the percentage of saturated water at 100% was investigated, then the percentage of saturated water at 100% was used to create the standard curve drainage rate; Secondly, the average drainage rate was used to determine the quantity of growth media required and the rate of addition of the complete nutrient and bio-extract. In the current study, each pot or experiment unit used 1,100 g of bio-extract and 134 g of complete nutrient solution as starting growth medium. The drainage volume of each amount was recorded and substituted every day to allow 100% saturation throughout the entire experiment.

Bio-extract preparation

Stems and leaves of Chinese kale, leaves of Chinese cabbage and seeds of soybean were cut or ground into small pieces and mixed with molasses at the ratio of 3:1. Clean water (10 L) was added to each 4 kg of mixture. The mixture was left to ferment in a 20-L closed bucket (anaerobic conditions) for 30 d (Department of Agriculture, 2001). Then, the product, or bio-extract, was released into plastic bottles wrapped in foil and kept at 4°C as a stock solution for experimentation.

Chemical characteristics of bio-extracts

The three bio-extracts were analyzed for pH, EC, total nitrogen and total P, according to the methods of Sirisigha (2006). In addition, total K, total Ca, total Mg and total S were analyzed by the methods described by Dean (2005).

Chinese kale seedling and nutrient solution preparation

The growth media was poured into a culture tray (108 holes). Three seeds of Chinese kale were placed into each hole. During the dormancy period, the seeds were watered with clean water. During the germination period (7 d after seeding), the seeds were fed with complete nutrient solution (Machlis and Torrey, 1965; Pathanapibul, 2003). The Chinese kale seedlings (two proper leaves had formed at around 14 d after seeding) were then transplanted to plant pots filled with growth medium, one pot for each Chinese kale seedling. The complete nutrient solution preparation procedure followed the method prescribed in Pathanapibul (2003), modified from Machlis and Torrey (1965).

Determination of Chinese kale growth enhancement

The growth of Chinese kale including height, fresh weight and dry weight was monitored at periods of 7, 14, 21, 28 and 35 d after transplantation.

Experimental design

The 11 treatments were designed following a complete randomized design (CRD). Each treatment used four replicates and each replicate used five Chinese kale specimens. Details of each treatment are as follows:

T1: complete nutrient as positive control experiment.

T2: Chinese kale bio-extract and filtered water at a ratio of 1:500.

T3: Chinese kale bio-extract and filtered water at a ratio of 1:250.

T4: Chinese kale bio-extract and filtered water at a ratio of 1:100.

T5: Chinese cabbage bio-extract and filtered water at a ratio of 1:500.

T6: Chinese cabbage bio-extract and filtered water at a ratio of 1:250.

T7: Chinese cabbage bio-extract and filtered water at a ratio of 1:100.

T8: Soybean bio-extract and filtered water at a ratio of 1:500.

T9: Soybean bio-extract and filtered water at a ratio of 1:250.

T10: Soybean bio-extract and filtered water at a ratio of 1:100.

T11: only filtered water, as the negative control.

RESULTS

Chemical characteristics of bio-extracts

The pH values of all three bio-extracts (Chinese kale, Chinese cabbage and soybean) were acidic, which caused *in situ* microorganism growth in the fermentation process. Such a process has been reported to produce several kinds of organic acids (Department of Agriculture, 2001; Department of Agriculture, 2004). Chinese kale bio-extracts had the highest pH value (5.24), while soybean had the lowest pH value (4.56). This may have been because soybean contains a greater amount of carbohydrate than both Chinese kale and Chinese cabbage (Ruanjai, 2002). It was noted that this amount of carbohydrate can be further digested by the remaining microorganisms in such bio-extracts and converted to organic acids with lower pH values (Kanthachote, 2002; Bamforth, 2005). In terms of electrical conductivity (EC), all bio-extracts had quite high values of 14.17, 13.52 and 26.60 dS m^{-1} for Chinese kale, Chinese cabbage and soybean, respectively. Such high EC values may be the cause of the organic compounds found in bio-extracts, such as organic acids, vitamins, enzymes, hormones and minerals. Similarly, soybean bio-extracts with a high organic compound content displayed high EC values. These results are similar to the report by the Department of Agriculture (2003). In addition, total N, total P, total K, total Ca, total Mg and total S were found to be highest in the soybean bio-extract, as shown in Table 1, which was a similar result to the findings of the Department of Agriculture (2004).

Efficiency of diluted bio-extracts on Chinese kale growth

The efficiency of diluted bio-extracts in various ratios with filtered water on Chinese kale growth was appraised through the indicators of height, fresh weight and dry weight.

Height

Table 2 shows that in all treatments, there was no significant ($P > 0.01$) difference in the height of Chinese kale at 7 d cultivation. The height of Chinese kale in T1 (complete nutrient solution) showed a significant ($P < 0.01$) difference from other treatments (T2-T11) and obvious significant growth began at 14-21 d of cultivation. Treatments T10 and T9, with diluted soybean bio-extracts at 1:100 and 1:250, respectively, showed significant ($P < 0.01$) differences in height from treatments T2-T8 and T11. In contrast, there was no significant ($P > 0.01$) difference among T2-T8 and T11 at 21 d of cultivation. At 28 and 35 d of cultivation, T1 showed the same significant ($P < 0.01$) difference compared to T2-T11. T10 showed a significant ($P < 0.01$) difference between T2-T9 and T11, while T8 and T9 showed no significant ($P > 0.01$) difference. The complete nutrient solution (T1) gave the tallest height (33.52 cm), followed by the diluted soybean bio-extracts with ratios of 1:100 (T10), 1:250 (T9) and 1:500 (T8) with heights of 20.02 cm, 15.05 cm and 12.97 cm, respectively.

Weight

For the total fresh weight of Chinese kale at 7-14 d of cultivation, only T1 showed a significant ($P < 0.01$) difference from other treatments (T2-T11). At 21 to 28 d of cultivation, T1 and T10 showed a significant ($P < 0.01$) difference from T2-T8 and T11. At the end of the experiment (35 d), T1, T10 and T9 showed a significant ($P < 0.01$) difference from T2-T8 and T11. The final wet weight was 156.47 g for T1, 32.73 g for T10, and 13.94 g for T9, as shown in Table 3.

Table 1 Chemical characteristics and amount of nutrients in each bio-extract.

Characteristic	Chinese kale bio-extract	Chinese cabbage bio-extract	Soybean bio-extract
pH	5.24	5.06	4.56
EC (dS m^{-1})	14.79	13.52	26.60
Total N (mg/mL)	1327.20	1092.00	4571.28
Total P (mg/mL)	209.15	221.94	974.49
Total K (mg/mL)	143.15	117.40	278.60
Total Ca (mg/mL)	50.65	51.68	66.49
Total Mg (mg/mL)	36.91	31.62	87.62
Total S (mg/mL)	10.12	14.90	68.00

From Table 4, for the total dry weight of Chinese kale at 7, 14 and 21 d of cultivation, only T1 showed a significant ($P < 0.01$) difference from the other treatments (T2-T11). At 28 d of cultivation, T10 also showed a significant ($P < 0.01$) difference. In addition, T9 showed a significant ($P < 0.01$) difference after 35 d of cultivation. At the end of the experiment, T1, T10,

Table 2 Average height (cm) of Chinese kale at different periods of growth.

Treatment	Cultivation (d)				
(Bio-extract and dilution ratio with water)	7	14	21	28	35
T1: Complete nutrient solution	8.87a	17.97a	24.30a	27.55a	33.52a
T2: Chinese kale bio-extract, 1:500	7.00a	8.62b	8.62d	8.65d	8.65d
T3: Chinese kale bio-extract, 1:250	7.15a	8.45b	8.55d	8.57d	8.60d
T4: Chinese kale bio-extract, 1:100	7.50a	8.37b	8.40d	8.42d	9.17d
T5: Chinese cabbage bio-extract, 1:500	7.32a	7.60b	8.00d	8.02d	8.17d
T6: Chinese cabbage bio-extract, 1:250	7.52a	8.05b	8.32d	8.37d	8.80d
T7: Chinese cabbage bio-extract , 1:100	6.87a	8.27b	8.35d	8.42d	8.45d
T8: Soybean bio-extract, 1:500	7.20a	9.00b	9.05d	11.55c	12.97c
T9: Soybean bio-extract, 1:250	6.85a	8.87b	10.75c	13.37c	15.05c
T10 : Soybean bio-extract, 1:100	6.47a	8.20b	14.32b	17.95b	20.02b
T11: Filtered water	7.40a	7.62b	7.62d	7.62d	8.17d
F-test	ns	**	**	**	**
CV %	12.65	16.29	9.74	14.33	13.30

ns = no significant statistical difference, ** = significant statistical difference at 99%.
Means within the same column followed by the same superscript letter were not significantly different at P < 0.01 by Duncan's new multiple range test.

Table 3 Total fresh weight (g) of Chinese kale (stems and leaves) at various periods of cultivation.

Treatment	Cultivation (d)				
(Bio-extract and dilution ratio with water)	7	14	21	28	35
T1: Complete nutrient solution	2.20^{a}	11.69^{a}	37.99^{a}	82.99^{a}	156.47^{a}
T2: Chinese kale bio-extract, 1:500	1.05^{b}	1.28^{b}	1.30^{c}	1.65^{c}	1.78^{d}
T3: Chinese kale bio-extract, 1:250	1.19^{b}	1.23^{b}	1.34^{c}	1.66^{c}	1.79^{d}
T4: Chinese kale bio-extract, 1:100	1.12^{b}	1.23^{b}	1.40^{c}	1.64^{c}	1.83^{d}
T5: Chinese cabbage bio-extract, 1:500	1.02^{b}	1.24^{b}	1.26^{c}	1.60^{c}	1.82^{d}
T6: Chinese cabbage bio-extract, 1:250	1.21^{b}	1.32^{b}	1.28^{b}	1.74^{c}	1.83^{d}
T7: Chinese cabbage bio-extract, 1:100	1.02^{b}	1.26^{b}	1.36^{c}	1.48^{c}	1.76^{d}
T8: Soybean bio-extract, 1:500	0.92^{b}	1.32^{b}	1.45^{c}	3.61^{c}	4.95^{d}
T9: Soybean bio-extract, 1:250	1.08^{b}	1.32^{b}	2.35^{bc}	6.93^{c}	13.94^{c}
T10: Soybean bio-extract, 1:100	1.00^{b}	1.31^{b}	4.97^{b}	15.68^{b}	32.73^{b}
T11: Filtered water	0.99^{b}	1.19^{b}	1.12^{b}	1.32^{c}	1.54^{d}
F-test	**	**	**	**	**
CV %	21.12	19.11	38.52	38.03	27.58

** = significant statistical difference at 99%.
Means within the same column followed by the same superscript letter were not significantly different at P < 0.01 by Duncan's new multiple range test.

T9 and T8 had total dry weights of 17.39, 3.33, 1.57 and 0.84 g, respectively. The study also considered the fresh and dry weight of the stems and leaves of Chinese kale and T1 and T10 at 21-35 days, which showed a significant ($P < 0.01$) difference from T2-T9 and T11.

DISCUSSION

The three bio-extracts (Chinese kale, Chinese cabbage and soybean) did not enhance Chinese kale growth as much as the complete nutrient solution (T1). More importantly, it was found that the intended nutrients in each bio-extract, in particular from the same kind of vegetable, did not influence growth enhancement. A problem thus may arise when trying to avoid acidic conditions associated with such bio-extracts (Table 1), as the added filtered water (pH 6.8) diluted the solution; so the essential nutrients and the expected supported organic compounds, such as hormones and vitamins, in particular nitrogen (N), were insufficient for growth enhancement. Many studies (MuangThong, 1989; Jones, 1998; Yongyut, 2000) mentioned that a lack of nitrogen can stunt Chinese kale growth or even cause it to become a dwarf plant. The availability of nutrients in the bio-extract, for example, available forms of nitrogen (NO_3^- and NH_4^+), available forms of phosphorous (H_2PO^- and HPO_4^{2-}) and available forms of potassium (K^+) could promote plant growth (Omsub, 2002). However, Pathanapibul (2003) reported that using only bio-extracts did not enhance plant growth, because the amount of macronutrients and micronutrients in the bio-extract was low and insufficient for plant growth. This finding was similar to the study by the Department of Agriculture (2004). Moreover, Khaliq *et al*. (2007) showed that using only EM (effective microorganisms, the production of which is similar to that of bio-extracts) for cotton production did not enhance the yield of cotton. In contrast with the study by Vetayasuporn (2004), EM promoted the growth of oyster mushrooms (*Pleurotus ostreatus*) by around two to four times, compared to that without EM treatment. Several groups of EM are able to degrade the complex organic compounds into simple molecules that can

Table 4 Total dry weight (g) of Chinese kale (stems and leaves) at various periods of cultivation.

Treatment (Bio-extract and dilution ratio with water)	Cultivation (d) 7	14	21	28	35
T1: Complete nutrient solution	0.14^a	0.60^a	3.32^a	8.04^a	17.39^a
T2: Chinese kale bio-extract, 1:500	0.09^b	0.16^b	0.24^b	0.35^c	0.38^d
T3: Chinese kale bio-extract, 1:250	0.10^b	0.16^b	0.23^b	0.35^c	0.39^d
T4: Chinese kale bio-extract, 1:100	0.10^b	0.15^b	0.25^b	0.33^c	0.39^d
T5: Chinese cabbage bio-extract, 1:500	0.09^b	0.15^b	0.23^b	0.35^c	0.38^d
T6: Chinese cabbage bio-extract, 1:250	0.11^b	0.16^b	0.22^b	0.36^c	0.39^d
T7: Chinese cabbage bio-extract, 1:100	0.09^b	0.15^b	0.24^b	0.34^c	0.37^d
T8: Soybean bio-extract, 1:500	0.09^b	0.16^b	0.25^b	0.57^c	0.83^{cd}
T9: Soybean bio-extract, 1:250	0.09^b	0.17^b	0.35^b	0.71^{bc}	1.57^c
T10: Soybean bio-extract, 1:100	0.09^b	0.17^b	0.54^b	1.35^b	3.32^b
T11: Filtrated water	0.09^b	0.12^b	0.20^b	0.29^c	0.35^d
F-test	**	**	**	**	**
CV %	17.05	41.09	42.48	43.95	28.99

** = significant statistical difference at 99%.

Means within the same column followed by the same superscript letter were not significantly different at $P < 0.01$ by Duncan's new multiple range test.

be utilized easily by the mushroom. The findings indicated that the type of plants and the group of microorganisms in the bio-extract had an effect on the efficiency of the with regard to the enhancement of plant growth.

In a comparison between the three bio-extracts, Chinese kale (or its bio-extract), Chinese cabbage and soybean, the soybean bio-extract with a greater nutrient content (Table 1) produced better growth enhancement. This outcome might be associated with the composition of the available nutrients in the soybean bio-extract suitable for the growth of Chinese kale (Machlis and Torrey, 1965). However, the quantity of nutrients in the soybean bio-extract was quite low and insufficient to support plant growth. In addition, soybean may contain some specific groups of microorganisms that can promote plant growth as plant growth-promoting bacteria (PGPB). Several investigations reported that PGPB can produce plant growth regulators, such as indoleacetic acid (IAA), gibberellic acid, cytokinins and gaseous phytohormone (ethylene) (Ahmad *et al.*, 2008). Moreover, some groups of PGPB can fix nitrogen gas, dissolve unavailable phosphate and produce siderophore as an iron chelator, as well as deter plant pathogens (Compant *et al.*, 2005).

Furthermore, Chonthicha and Thanpisit (2005) found that growth in the stem length and diameter of French marigolds was not significant after three weeks of cultivation using fish bio-extract and complete nutrient solution in a soil-free culture. In the initial phase of French marigold growth, it was found that the available forms of nutrients for growth enhancement could be supported using animal bio-extracts. Soybean bio-extract was the most acidic of all of bio-extracts (Table 1), but was the most suitable bio-extract for growth enhancement in the study. Chinese kale is tolerant to acidic conditions of pH 5.5–6.8 (Maynard and Hocmuth, 2007), so the acidity of 1% of soybean bio-extract may not cause any effect on Chinese kale growth, because it was diluted with neutral filtered water. However, it appeared that the amount of essential nutrients in the best diluted soybean bio-extract was not enough for Chinese kale growth enhancement. The growth rate of Chinese kale in the initial stage (7-14 d cultivation) was lower than T1 (complete nutrient solution). All Chinese kale growth rates increased slowly and showed significant (P<0.01) differences after 21-28 d of cultivation. However, the use of bio-extract for cultivation compared to chemical fertilizer is environmentally friendly, low cost and an appropriate way to dispose of agricultural wastes. Further study of different combinations of soybean bio-extract and fish bio-extract is warranted, and the optimal dilution ratios of these bio-extracts still need to be investigated.

CONCLUSION

The soybean bio-extract had the highest nutrient content, EC and pH values under acidic conditions, whereas the Chinese kale and Chinese cabbage bio-extracts had similar, but lower, nutrient contents. Considering only bio-extract solutions at ratios of 1:500, 1:250 and 1:100, soybean bio-extract gave the most efficient growth enhancement. Moreover, soybean bio-extract T10 (dilution ratio 1:100 or 1%) was the most efficient for growth enhancement. The study did not find any significant trend in growth enhancement with regards to bio-extracts produced from the same type of vegetable (residue from itself), in particular Chinese kale.

ACKNOWLEDGEMENTS

The authors thank Mr Paul Edward Watson for critical reading of this manuscript. This research was partially supported by the Alumni Association of the Faculty of Graduate Studies, Mahidol University, with special thanks to C. Noisopa.

LITERATURE CITED

Ahmad, F., I. Ahmad and M.S. Khan. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. **Microbiol. Res.** 63: 173-181.

Bamforth, C.W. 2005. **Food, Fermentation and Microorganisms.** Blackwell Science, Oxford. 216□pp.

Chaisit, T. and S. Sudprasong. 2000. Bio-extract. **Thai. Resch. Fund J.** 17(3): 48-57.

Cho, H.K. 2004. **Natural Farming: Concepts, Principles and Native Microorganisms.** 3rd ed., Trio Advertising & Media, Chiang Mai. 146 pp.

Chonthicha, V. and P. Thanpisit. 2005. The effects of bio-extract with nutrient solution on French Marigolds in hydroponic cultivation system. **Thammasat Uni. Sci. Technol. J.** 13(3): 53-62.

Compant, S., B. Duffy, J. Nowak, C. Clement and E.A. Barka. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and future prospects: Minireview. **Appl. Environ. Microbiol.** 71: 4951-4959.

Dean, J.R. 2005. **Practical Inductively Coupled Plasma Spectroscopy.** John Wiley & Sons, Chichester. 184 pp.

Department of Agriculture. 2001. **Bio-extract and Bio-composting.** Office of Research for Agriculture Development Area 5, Bangkok. 28 pp.

Department of Agriculture. 2003. **Hormones and Nutrients in Bio-extract.** 2nd ed., Ministry of Agriculture and Cooperative, Bangkok. 133 pp.

Department of Agriculture. 2004. **Basic Scientific Data of Bio-extract Solutions: Part 1.** Quick Printed Offset, Bangkok. 51 pp.

Department of Soil Science. 1998. **Introduction to Soil Science.** 8th ed., Kasetsart University Publisher, Bangkok. 547 pp.

Jones, J.B. 1998. **Plant Nutrition Manual.** CRC Press, Florida. 117 pp.

Kanthachote, D. 2002. **Microbial Ecology.** Odeon Store Printing, Bangkok. 206 pp.

Khaliq, A., M.K. Abbasi and T. Hussain. 2006. Effect of integrated use of organic and inorganic nutrient sources with effective microorganism (EM) on seed cotton yield in Pakistan. **Bioresour. Technol.** 97: 967-972.

Machlis, L. and J.G. Torrey. 1965. **Plants in Action: A Laboratory Manual of Plant Physiology.** W.H. Freeman, California. 282 pp.

Maynard, D.M. and G.J. Hocmuth. 2007. **Knott's Handbook for Vegetable Growers.** 5th ed., John Wiley & Sons, New Jersey. 621 pp.

MuangThong, T. 1989. **Principles of Vegetable Cultivation.** 2nd ed., Siam Computer Graphic, Bangkok. 210 pp.

Omsub, N. 2002. Bioextract. **Entomol. Zool. Gazett.** 24(2): 154-158.

Pathanapibul, S. 2003. **The Efficiency of Bio-extract on Some Kinds of Vegetable in Hydroponic System.** M.S. Thesis, Kasetsart University, Bangkok.

Ruanjai, K. 2002. **Miracle of Home-grown Vegetable.** 2nd ed., Europa Place, Bangkok, 160 pp.

Sirisigha, K. 2006. **Chemistry of Water and Wastewater and Analysis.** 4th ed., Chandrakasem Rajabhat University, Bangkok. 377 pp.

Veerasri, W., 2000. Organic agriculture and environment by bio-extract techniques. **Thai. Resch. Fund J.** 7(2): 43-50.

Vetayasuporn, S. 2004. Effective microorganisms for enhancing *Pleurotus ostreatus* (Fr.) kummer production. **J. Biol. Sci.** 4(6): 706-710.

Yongyut, O. 2000. **Plant Nutrition.** Kasetsart University Publisher, Bangkok. 424 pp.