The effects of bio-extract from water hyacinth (*Eichhornia crassipes* (C. Mart.) Solms) and golden apple snail (*Pomacea canaliculata* Lamarck) on photosynthetic pigment and ascorbic acid contents of Chinese cabbage (*Brassica chinensis* var. *pekinensis* Rupr.) grown in hydroponic culture

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ABSTRACT

This study was aimed to compare the effects of bio-extract from water hyacinth, bio-extract from golden apple snail and the mixture of bio-extract on growth and some physiological characteristics of Chinese cabbage that grown in Dynamic Root Floating Technique (DRFT) of hydroponic culture. The bio-extract that filled in nutrient solution was 1: 500 and 1:1000 levels. Data were recorded on days 0, 7, 14, 21, 28 and 35 days after bio-extract treatments. Results of the study revealed that Chinese cabbage that grown in bio-extract from golden apple snail at the ratio of 1: 1000 had the tendency of producing higher total dry weight. However, the bio-extract from golden apple snail at the ratio of 1: 500 had the tendency of producing higher total chlorophyll, carotenoids and β-carotene contents. On the other hand, bio-extract from water hyacinth at the ratio 1:500 resulted in increased ascorbic acid contents. Our results indicated that bio-extract affects the growth of Chinese cabbage and can be used as a source of nutrients for crops to reduce used chemicals in hydroponics culture.

Keyword : bio-extract, *Brassica chinensis* var. pekinensis Rupr, hydroponic

INTRODUCTION

Currently, bio-extracts or bio-ferment or EM (effective microorganism), play an important role in the promotion of self-sufficiency agriculture, reduced use of chemical fertilizers and synthetic hormones. Residue from chemical fertilizers can pollute surface and water ecosystems. Furthermore, bio-extracts has low cost and can be produced by a part of plants and animals mixed with sugar or molasses, which are the carbon and energy sources for the microorganism fermentation and decomposition processes. The decomposed compound, which contains many organic compounds, gives immense benefits, with an effective enhancement of cultivation (Department of Agriculture, 2004). The bio-extract can be produced from weed and pest. Weeds and invasive species are spread quickly and damage to water sources and water

transportation. Water hyacinth is one of invasive water weeds. It's growth forms are dense monocultures which change the physical and chemical aquatic environment and subsequently causing water pollution (Aboul-Enein et al, 2011). However, the water hyacinth has many uses such as using in a composting and planting material, increaseing growth in *Brassica chinensis* (Buachum and Thowattana, 2015), Chinese Kale (*Brassica Oleracea*) (Srijaroen et al, 2009). In addition, rice plantation always faces with many pests that cause damage to agricultural production. One of them is the golden apple snaill. The golden apple snail is highly adapted for survival and has a high growth and reproductive rate. It's capable of destroying rice fields which has a negative impact on rice (Ngernsoungnern and Ngernsoungnern, 2016). Bio-extracts from the golden apple snail has been demonstrated to significantly increase growth of plant such as tomato (Mungkunkamchao et al, 2010), Pathum Thani 1 rice (Ruchirasak and Koetnoon, 2014). This research aimed to use EM from water hyacinth and golden apple snail to stimulate crop production by filled with nutrient solution in hydroponics culture. Hydroponic culture is a technique used to apply nutrient solutions to plant. It offers the advantages of better control of climate and pest factors, higher yield per unit area, and easy processing of harvested product. Furthermore, hydroponic production increases crop quality and productivity, which results in higher competitiveness and economic incomes (Asao, 2012, Ikeda, 2007). From this research, the information obtained will be the basis for the agricultural crops of interest in hydroponics culture. There are many benefits to reduce the use of chemicals and reduce the cost of growing crops in this system.

The main objective of this study was to investigate the efficacy of bio-extract from water hyacinth, golden apple snail and the mixture of bio-extracts on growth and photosynthetic pigments such as total chlorophyll, carotenoids, β-carotene and ascorbic acid contents of Chinese cabbage (*B. chinensis* var. *pekinensis* Rupr.) when grown in a hydroponics system.

METHODS

1. Fermented bio-extract process

Fermented bio-extract was prepared by using water hyacinth and golden apple snail as the starting raw material, mixing with molasses and water in the ratio of 3:1:10 (weight by weight). Microbial inoculum, Por Dor 2, developed by Department of Land Development in Thailand was added to enhance the fermentation process. The mixture was subsequently kept in a tightly closed vessel and left to stand at room temperature for 30 days, set in the shade. The mixture of bio-extract was prepared by mixing bio-extract from golden apple snail and bio-extract from water hyacinth in the equal ratio.

2. Experimental design

A hydroponic experiment was carried out in a nursery of Biology Department, Faculty of Science, Burapha University, Chonburi province, Thailand. The 7 treatments were designed following a factorial complete randomized design (CRD). Each treatment used three replicates and each replicate used three Chinese cabbage plant. Details of each treatment are as follows:

T1: Complete nutrient as positive control (full strength of Hoagland solution)

T2: Golden apple snail bio-extract: nutrient solution (half strength) at a ratio of 1:500

T3: Golden apple snail bio-extract: nutrient solution (half strength) at a ratio of 1:1000

T4: Water hyacinth bio-extract: nutrient solution (half strength) at a ratio of 1:500

T5: Water hyacinth bio-extract: nutrient solution (half strength) at a ratio of 1:1000

T6: Mix bio-extract: nutrient solution (half strength) at a ratio of 1:500

T7: Mix bio-extract: nutrient solution (half strength) at a ratio of 1:1000.

3. DRFT (Dynamic Root Floating Technique) hydroponic system management

Each experimental system consisted of 21 foam boxes (45 cm × 30 cm× 60 cm). Each cultivation foam box has 18 channels, each channel was 10 cm apart. The foam box had solid foam sheet (40 cm × 53 cm × 2.5 cm) that placed deep 3 cm and build the dam high 1 cm for nutrient solution retention. An installed electric pump was set opposite to the dam for recirculation of nutrient solution.

4. Plant materials

Chinese cabbage (*B. chinensis* var. *pekinensis* Rupr.) seeds were sown in styrofoam trays within polyurethane sponge as the seedling growing medium. The seeds were watered until wet to ensure healthy seedling germination and growth. Two week-old seedlings were transplanted for hydroponic cultivation. Chinese cabbage seedlings were grown in hydroponics with Hoagland's solution. The complete nutrient solution preparation procedure followed the method prescribed in Pathanapibul (2003), modified from Machlis and Torrey (1965). One week after transplanting, nutrient solution and bio-extract were changed and pH value and EC value were maintained at 6 and 1.5-2.3 mS/cm, respectively (Sirinupong, 2013).

5. Data collection

The growth and physiology responses were determined on days 0, 7, 14, 21, 28 and 35 after transplantation. At harvest period, three plants were taken from each plot for measuring growth and physiological parameters.

The growth responses were monitored by measuring total dry weight per plant. Shoots and roots of the Chinese cabbage were dried in a hot air oven at 80 °C for 72 hours before measuring total dry weight.

Physiology responses were monitored by measuring total chlorophyll contents and carotenoids contents. The third leaf from the shoot apex from each plant in each treatment was collected for chlorophyll and carotenoid extraction and concentration determination. Chlorophyll and carotenoid concentrations were determined in 80% acetone extract using spectrophotometer. Absorbance was measured against an 80% acetone blank at 663.2, 646.8, 470 nm. The values of total chlorophyll and carotenoid was determined according to Lichtenthaler (1987) using the following formaula :

Total Chl = $7.15A_{663.2}+ 18.71A_{646.8}$

Carotenoids = $(1000A_{470}-1.82C_a-85.02C_b)/198$

Where, A663.2 was absorbance at 663.2 nm and A646.8, A470 were absorbance at 646.8 and 470 nm, respectively.

The β-carotene in the sample was extracted according to the method described by Volker et al., (2002) with slight modification. Leaf samples (1.5 g) of Chinese cabbage were mixed with hexane: ethanol: acetone (1.5 ml: 0.75 ml: 0.75 ml). The mixture was spun at 5000 rpm for 10 minutes. The upper layer was pipetted out about 1 ml into the vial and filled Hexane 0.9 ml to it. The absorbance of the extract was determined at wavelength of 449 nm by a UV vis-spectrophotometer (Biochrome; model Libra S11). For identification and quantification of β-carotene, comparison with β-carotene standard curve was performed.

In order to measure the ascorbic acid contents in leaf, samples (100 g) was extracted according to the method described by Muednon (2008). Samples leaf of Chinese cabbage was added 0.07 M oxalic acid in 0.02 mM EDTA, shaker at 200 rpm for 30 minutes and then filterd through the Whatman filter paper No.2. The content of ascorbic acid was determined by Molybdenum blue method analysis.

6. Statistical analysis

A one-way analysis of variance at the significant level of 95% was applied. The significance of differences between means was analyzed using Duncan's Multiple Range Test (DMRT). The experimental data were statistically analyzed using SPSS software version 17.0.

RESULTS AND DISCUSSIONS

Total dry weight

Total dry weight of Chinese cabbage was higher in bio-extract from golden apple snail filled in nutrient solution at the ratio of 1: 1000 treatment. The total dry weight was 7.152±0.379 g but showed no statistical significance when compared with another bio-extract treatment. The bio-extract from apple snails was derived from fermented animal. The bio-extract derived from animal have higher concentration of nitrogenous nutrients than bio-extract from plant (Rasmithamwong, 2007). In addition, Sirinupong (2013) reported that nitrogenous nutrients are the structure of organic compounds in plants, such as chlorophyll, amino acid, protein and hormone that related to the growth of plant. A similar effect of bio-extract from animal gave significantly

higher on the yield of lettuce grown in hydroponics system than bio-extract derived from plant observed by Inkham and Ruamrungsri (2014). The treatment effects and results of statistical analyze for total dry weight presented in Table 1.

Table 1 Total dry weight of Chinese cabbage treated by bio-extract filled in nutrient solution (half strength) increase over 35 days experimental period.

Treatment	Total dry weight (gram) (Mean±Standard error)					
	0 day	7 day	14 day	21 day	28 day	35 day
Control	0.021±	0.057±	0.250±	1.016±	2.704±	3.601±
	0.001^{A}	0.008^{A}	0.040^{A}	0.044^{A}	0.543^{A}	0.229^{A}
Golden apple snail	0.023±	0.055±	0.414±	1.833±	3.298±	6.792±
1: 500	0.001^{A}	0.009^{A}	0.031^{ABC}	0.271^{ABC}	0.228^{A}	1.256^{AB}
Golden apple snail	0.025±	0.051±	0.439±	1.057±	3.658±	7.152±
1:1000	0.001^{AB}	0.004^{A}	0.093^{BC}	0.043^{AB}	0.531^{A}	0.379^{B}
Water hyacinth	0.033±	0.070±	0.292±	1.291±	2.604±	4.251±
1: 500	0.002^{C}	0.010^{A}	0.005^{AB}	0.234^{ABC}	0.420^{A}	0.312^{AB}
Water hyacinth	0.029±	0.058±	0.366±	1.812±	3.594±	5.768±
1: 1000.	0.008^{BC}	0.001^{A}	0.053^{AB}	0.050^{ABC}	0.557^{A}	0.601^{AB}
Mixture bio-extract	0.021±	0.064±	0.476±	1.881±	3.430±	6.433±
1:500	0.000^{A}	0.015^{A}	0.049^{BC}	0.445^{BC}	0.523^{A}	1.441^{AB}
Mixture bio-extract	0.029±	0.047±	0.595±	2.035±	4.068±	5.864±
1:1000	0.004^{BC}	0.005^{A}	0.076^{C}	0.366^{C}	0.334^{A}	1.100^{AB}

The data represent the mean ±SE (n=3). Different letters indicate significant differences among treatment at $p < 0.05$.

Photosynthetic pigments.

The content of total chlorophyll, carotenoid and β–carotene in leaf Chinese cabbage cultivars under 6 week showed the highest in plant grown in bio-extract from golden apple snail filled in solution at the ratio of 1 : 50 0. Total chlorophyll was 0.278±0.006 mg/g fresh weight. Carotenoid was 0.094±0.003 mg/g fresh weight. β-carotene was 1.385±0.019 mg^{-1}100 g fresh weight but showed no significantly different when compared with another bio-extract treatment. The treatment effects and results of statistical analyze for presented in Table 2. The bio-extract from golden apple snail has macro-nutrients such as nitrogen, phosphorus and potassium, micro-nutrients like calcium and also plant hormone that has a beneficial to plants. (Department of Land Development in Thailand, 2006). These nutrients affect the growth and leaf color intensity (Pumprasert and Phuangchik, 2005). Nitrogen can alter carotenoid and chlorophyll composition and accumulation in leaf. Nitrogen influenced β-carotene, total carotenoids and total chlorophyll in leaf. (Kopsell et al, 2007). Chlorophyll is one of the most important pigments present in green plants, including chlorophyll a and chlorophyll b which are the major light harvesting pigments. It traps and converts

sunlight into chemical energy making processes in photosynthesis in plants. (Sánchez et al, 2014, Chatterjee and Kundu, 2015). Carotenoids are found in the chloroplasts of photosynthetic tissues. Their color is yellow to red range, the main carotene is β-carotene (Ruiz-Sola and Rodriguez-Concepcion, 2012). Carotenoids play an important role in the light-harvesting complex and in protection of the photosystems, function in light harvesting, quenching of excited chlorophyll (Frank et al., 1996). Chlorophyll and carotenoid concentration correlate with the photosynthetic potential of plants giving some indication of the physiological status of the plant (Gamon and Surful, 1999). In this research, Chinese cabbage grown in bio-extract from golden apple snail filled in solution at the ratio of 1: 500 had the tendency of producing higher total chlorophyll, carotenoid and β-carotene. A similar effect of bio-extract from golden apple snail at the ratio of 1: 500 as a foliar spray of tomato, cv. Delta, significantly increased the SPAD chlorophyll meter reading (SCMR), compared to no foliar spray application by Mungkunkamchao et al. (2010).

Table 2 Total chlorophyll, carotenoid, β-carotene, ascorbic acid content after 35 days experimental period.

Treatment	**Total chlorophyll (mg/g fresh weight)**	**Carotenoid (mg/g fresh weight)**	**β-carotene (mg^{-1}100 g fresh weight)**	**Ascorbic acid (mg^{-1}100 g fresh weight)**
Control	0.274 ± 0.024^{A}	0.082 ± 0.007^{AB}	1.346 ± 0.032^{BC}	27.456 ± 1.160^{A}
Golden apple snail 1: 500	0.278 ± 0.006^{A}	0.094 ± 0.003^{B}	1.385 ± 0.019^{C}	34.912 ± 0.316^{BC}
Golden apple snail 1: 1000	0.252 ± 0.031^{A}	0.083 ± 0.008^{AB}	1.323 ± 0.023^{ABC}	31.930 ± 0.351^{AB}
Water hyacinth 1: 500	0.227 ± 0.009^{A}	0.069 ± 0.003^{A}	1.250 ± 0.028^{A}	49.035 ± 3.137^{D}
Water hyacinth 1: 1000.	0.241 ± 0.024^{A}	0.080 ± 0.007^{AB}	1.252 ± 0.039^{A}	45.351 ± 2.198^{D}
Mixture bio-extract 1:500	0.277 ± 0.009^{A}	0.090 ± 0.003^{B}	1.344 ± 0.034^{ABC}	38.772 ± 0.575^{C}
Mixture bio-extract 1:1000	0.246 ± 0.011^{A}	0.078 ± 0.003^{AB}	1.260 ± 0.016^{AB}	35.439 ± 0.916^{BC}

The data represent the mean ±SE (n=3). Different letters indicate significant differences among treatment at $p < 0.05$.

Ascorbic acid content

The maximum ascorbic acid content in Chinese cabbage's leave was recorded in nutrient solution filled with bio-extract from water hyacinth at the ratio of 1:500. The ascorbic acid content was 49.035 ±0.001 mg^{-1}100 g fresh weight but showed no significant different from bio-extract from water hyacinth filled in nutrient solution at the ratio 1:1000. The treatment effects and results of statistical analyze for ascorbic acid presented in Table 2. Ascorbic acid acts as a powerful antioxidant in plants and increases in tolerance to oxidative and photo-oxidative stress. Chemically, the vitamin C occurs in three isomeric forms: L-ascorbic acid, Darabo-ascorbic acid and L-arabo-ascorbic acid. Ascorbic acid tends to be more concentrated in leaves (Mahmoud et al., 2013, Smirnoff, 2005). Furthermore, Ascorbic acid is involved in diverse physiological functions, such as cell cycle regulation, cell wall synthesis, cell expansion, electron transfer and plant morphology modulation (Debolt, et al, 2007).

Bio-extract contributes to an increase in ascorbic acid content. A similar effect of bio-extract on the ascorbic acid observed by Altintas and Acikgo. (2012).
The organic liquid fertilizers (bio-extract) was originated from vegetable sources and from animal and vegetable sources were commercially available products (Biofarm). Suspensions of all fertilizers were watering into the soil. The application of organic liquid fertilizers resulted in the ascorbic acid content in dry and fresh fruit of bell pepper but showed no statistically significant when compared with mineral fertilizer. Moreover, Leksono and Yanuwiadi. (2014) reported that the liquid organic fertilizer (bio-extract) increased vitamin C content in apple fruits. Application of the liquid organic fertilizer were conducted every two weeks untill harvest. This result indicates that the bio-extract was a good source of nutrients that plants can be utilize for growth. The bio-extract from water hyacinth was mixed with molasses or sugar juice and fermented with local microorganism. Water hyacinth has the macro-nutrient nitrogen, phosphorus, potassium and the micro-nutrients magnesium calcium and sodium (Khaket et al., 2012). So, these nutrients can affect the metabolism of plants.

CONCLUSION

The bio-extract from golden apple snail that filled in nutrient solution at the ratio of 1: 1000 gave the most efficient growth enhancement and bio-extract from golden apple snail that filled in nutrient solution at the ratio of 1: 500 enhanced total chlorophyll, carotenoid and β-carotene content of Chinese cabbage. Moreover, bio-extract from water hyacinth filled in nutrient solution at the ratio of 1: 500 had increased ascorbic acid content of the Chinese cabbage.

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