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Application of electrochemically activated solution to control pathogens of hydroponic solution

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Abstract. Hydroponic crop production systems are used worldwide to grow flowers, foliage, litter, and fruit and vegetable crops. The pathogens sources problem in hydroponic solution is an urgent issue. Many methods to kill bacteria and fungi in hydroponic solutions have been studied. However, some methods gave low efficiency, others gave high efficiency but it caused secondary environmental pollution. In this study we were forced on determine the ability to control pathogens in hydroponic solution of electrochemically activated solution (EAS). Different volumes of EAS were added into hydroponic solutions and mixed for 30, 45, 60, 120 or 180 min. The most effective mixed solution (mixed ratio and time) was selected to apply to cultivate *Brassica juncea*. The results show that EAS has the ability to control pathogens in hydroponic solution effectively. The mixed ratio of 1.5:500 with 90 min of exposure time were recommended to use to control pathogens in hydroponic solution. The mixed EAS - hydroponic solution did no negative effect to the growth of *Brassica juncea* in comparison with original hydroponic solution.

Keywords: electrochemically activated solution, hydroponic solution, *Brassica juncea*.

Classification numbers: 1.3.1, 3.6.2.

1. INTRODUCTION

Hydroponic and soilless systems of plant production are used worldwide to grow flower, foliage, bedding, and vegetable crops. Plants are grown using nutrient solutions with or without solid substrates for root growth. These systems have become popular in many countries in the world [1, 2]. Plant nutrition and the physical environment can be tightly controlled by the grower, resulting in higher yields, better quality, and control of crop scheduling [3]. All the elements in the nutrient solution are readily available to plant, so competition for nutrients can be reduced, and greater plant densities can be used. Thus, the proportion of glasshouse vegetables produced in hydroponic of soilless systems has been increasing in Europe, the U.S, Canada and some developed countries in Asia, in particular for tomatoes (*Lycopersicon esculentum Mill.*), cucumber (*Cucumis sativus L.*), lettuce (*Lactuca sativa L.*), spinach (*Spinacia oleracea L.*) and vegetable mustards (*Brassica juncea*).

However, soilless systems are not free of disease problems and certain types of disease have become more prominent and devastating. Several characteristics of the soilless systems can in fact exacerbate the disease situation. First, in soilless greenhouse systems, the plants are genetically identical, and can be uniformly susceptible. In addition, the dense planting can favor the movement of pathogens from infected to healthy plants. Second, the physical environment, especially temperature and moisture, can be favorable for the pathogen. Third, in closed systems with recirculating water, pathogens can be easily spread from one plant to the other [4], especially zoosporic fungi that produce swimming spores. Fungal diseases play a major role in the current deficit of food supply worldwide [5]. A small amount of contamination can lead to substantial infection and disease loss. For example, as few as 20 zoospores of *Pythium aphanidermatum* (Edson) Fitzp. introduced into 100 L of nutrient solution in a nutrient film technique system caused significant yield losses in cucumber production [6]. Closed systems are becoming more widespread [7] because of ground-water pollution problems associated with open systems. Finally, soilless substrates lack the microbial diversity and biological "buffering" found in natural soils. In natural soils, many soilborne pathogens are limited by antagonism from other microorganisms, and are subject to nutrient competition and fungistasis [8]. Thus, if a pathogen is introduced into a natural soil, its spread is much slower than if introduced into a substrate that is essentially sterile. Without competition from other microbes, the pathogen may quickly become established in the substrate and cause severe disease.

Many disease control methods in soilless systems have been studied and applied. The use of chemical pesticides is a main component of any disease control program in agriculture and horticulture. In Australia, some growers add fungicides or chlorinate the water/nutrient solution to reduce the risk of root diseases [9]. However, these are mostly used as protectants against foliar diseases. However, no pesticides are currently registered for use against root diseases in hydroponic systems in the United States or Canada [1], for food or horticultural crops. One reason is the possibility of phytotoxicity of pesticides when applied to hydroponic systems. Soil provides a buffer, which may bind pesticides and reduce their uptake by the plant. Microbial breakdown of pesticides also occurs in soil but may be reduced in hydroponic systems. Therefore, there may be a residue problem if the pesticides are taken up by the plant. In addition, greenhouse vegetables such as cucumbers, salad vegetables are harvested daily, so the normal preharvest pesticide-free period cannot be observed.

Another mean of disease control is to disinfest the nutrient solution by the use of solar energy [10] or UV radiation [11,12] or filtration [13]. Ozonation is currently used in the Netherlands as a method of disinfesting recirculation water in closed systems [14]. However, there are still questions about the effectiveness of these methods against resistant fungal propagules and these methods require machines, devices and equipment which cost a lot and not easy to apply widely on the small farms.

In recent years, electrochemically activated solution (EAS) was becoming a widely known strong disinfection solution which has potential to replace other classical sterilization methods due to its strong sterilization potency, low cost and its safety to the human and environments [15, 16]. It has been widely used in many fields of human life including food processing industry. However, the study of applying EAS in agriculture has not been properly carried out.

The purpose of this experiment was to apply EAS to kill microorganism in hydroponic solution, determine the effects of EAS on hydroponic solution properties and the growth of *Brassica juncea* in the mixed of EAS – hydroponic solution.

2. MATERIALS AND METHODS

2.1. Materials

Fresh *Brassica juncea* seeds used in the experiment were obtained from VinEco Agricultural Investment, Development and Productions LLC. Seeds that are uniform in shape and size were selected for the experiment and kept at 4 ^{o}C until used.

The salt used to prepare EAS is 99.9 % pure NaCl from Thailand. The supplies used to prepare the hydroponic solution include: Phu My urea fertilizer - PVFCCo, N: 46.3 %; Super phosphate fertilizer – LAFCHEMCO, P2O5: 20 %; Phu My Kali fertilizer - PVFCCo and KNO_3 - Merck CAS No. 7757-79-1, ≥ 99.0 %.

2.2. Solutions preparation

2.2.1. EAS solution

EAS was prepared using an EAS generator made in our laboratory. Clean water is led into the cathode divider to mix with the cathode and then fed to the cathode cavity of the electrochemical module. At the same time, a salt solution with a concentration of 50 g/L was introduced into the anode cavity of the electrochemical module. The entire solution leaving the anode cavity is mixed with a small portion of the catholic solution so that, after mixing with the catholic solution, EAS solution with a TDS not exceeding 1 g/L can be obtained. EAS was stored in polypropylene containers, and immediately used for the measurement.

2.2.2. Hydroponic solution

Hydroponic solution number 1 (HS1). This solution was prepared by dissolving 52 g of urea fertilizer, 80.5 g of phosphate fertilizer, 7.56 g of red potassium and 100.6 g of KNO_3 in 180 liters water. Hydroponic solution number 2 (HS2) was used from day 25 after sowing. This solution was prepared by dissolving 104.5 g of urea fertilizer, 161 g of phosphate fertilizer, 7.56 g of red potassium and 212.7 g of KNO_3 in 180 liters water.

Table 1. Properties of EAS, HS1 and HS2 solutions.

Solution	EAS	HS1	HS2
pH	6.8	7.0	7.0
ACC (ppm)	489	0	0
ORP (mV)	910	210	258
TDS (ppm)	950	672	1342
NO_3^- (ppm)	-	55.8	156.3
Total coliform (log CFU/g)	0	6.42	6.21

The pH value, total dissolved solids (TDS) and oxidation reduction potential (ORP) of EAS were measured by the HACH SenSion-156 device. The available chlorine concentration (ACC) in EAS was determined by an iodometric method (SMEWW 4500- Cl.B) and a photometric method with HACH DPD reagent (USA) on a measuring equipment DR 2800 (HACH - USA). Nitrate (NO_3^-) was determined by ISO 7890-3:1988 (E) method and total coliform was

determined by ISO 9308-1:2014 method. The properties of EAS, HS1 and HS2 were shown in Table 1.

2.2.3. Experimental solutions

0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5 or 3 liters of EAS was added into 500 liters of HS1 or HS2 to have experimental solutions with difference ACC. Mixed solutions were stirred well in 30 min. The properties of mixed solution were tested to determine the initial effects of EAS to hydroponic solutions. To determine the effect of exposed time to the properties of solutions, mixed solutions (of EAS and HS1) were stirred well in 30, 45, 60, 90, 120 or 180 min. The properties of solutions were determined. From the received results, the most suitable mixed ratio and exposed time was selected based on the ability to kill bacteria and the effects on the quality of hydroponic solution. This solution was applied for the growing experiment.

2.3. Production of *Brassica juncea*

In each of experiments, there were 120 baskets for hydroponics (20 barrels x 6 baskets/barrel). Germinated seeds were sown in the growing media. HS1 was irrigated into the barrels. At day 25, HS2 was used instead of HS1. Hydroponic water was changed periodically every 10 days. Vegetable was harvested on the 50^{th} day. Each experiment was repeated 3 times.

2.4. Determination of the *Brassica juncea* growth

The morphological measurements of *Brassica juncea* were performed right after harvest. Plant height and leaf diameter were measured by a ruler, quantity of leaf was counted.

Fresh and dry weight were measured by precision scale. Fresh weight was measured right after harvest. Dry weight measured after the plants were dried at 105 ^{o}C for 48 h.

2.5. Statistical analysis

All trials were replicated three times under the same experimental conditions and using seeds from the same source.

Reported plate count data represent the mean values obtained from three individual trials, with each of these values being obtained from duplicated samples. Data were subjected to analysis of variance using the Microsoft Excel 2019 program. Significant differences in plate count data were established by the smallest difference at 5 % significance level.

3. RESULTS AND DISCUSSION

3.1. Effect of EAS on the hydroponic solutions

30 min after added EASs with different ratio into hydroponic solutions (HS1 and HS2) there were changes in the quality parameters of the hydroponic solutions. There was slight increasing in the TDS and ORP value of hydroponic solutions when raising the ACC of added EAS. The TDS of mixed solutions were 648 - 678 ppm (with HS1) and 1.343 - 1.378 (with HS2). The ORP of mixed solutions were 359 - 395 mV (with HS1) and 451 - 419 (with HS2). We witnessed the opposite trend with pH and total coliform value. pH of mixed solutions was

fallen down slight when the amount of EAS added increase. At the amount of EAS added of 3 liters, the pH of mixed HS1 and HS2 were dropped to 6.9 and 6.6, respectively. A significant decrease in the total coliform of mixed solutions. With both hydroponic solutions the total coliform in mixed solution were under detected level after 30 min added more than 2.5 liters of EAS. With lesser amount of added EAS (from 0.25 to 2 liters), the total coliform in mixed solutions were 1.38 to 3.98 log CFU/ml. However, there was no obvious difference in the nitrate concentration between the mixed solution and original solutions. The quality parameters of mixed hydroponic solutions are presented in Table 2.

Based on the collected data, the exposed time experiments were performed with the EAS added ratios of 0.25 to 2 liter per 500 liters of HS1. The results of exposed time experiments on HS1 are shown in Figure 1.

Table 2. The quality parameters of mixed hydroponic solutions.

Solutions	Volume of added EAS (liter)	ACC after mixed (ppm)	After 30 min of mixing					
			ACC (ppm)	pH	TDS (ppm)	ORP (mV)	Nitrate (ppm)	Total coliform (log CFU/ml)
HS1	0	0.00	0	7.1	672	210	55.8	6.42
	0.25	0.24	0.07	7.1	648	359	55.6	2.93
	0.5	0.49	0.34	7.1	656	367	55.5	2.66
	0.75	0.73	0.56	7	658	372	55.7	2.34
	1	0.98	0.83	7	659	379	55.8	2.15
	1.5	1.47	1.28	7	662	382	55.7	1.91
	2	1.96	1.79	6.9	668	388	55.9	1.38
	2.5	2.45	2.27	6.9	675	395	56.0	0
	3	2.93	2.76	6.9	678	395	55.9	0
HS2	0	0.00	0	7	1342	258	156.3	6.21
	0.25	0.24	0.06	7	1343	419	156.3	3.98
	0.5	0.49	0.32	7	1346	418	156.2	3.26
	0.75	0.73	0.69	7	1349	421	156.4	2.71
	1	0.98	0.81	6.9	1351	427	156.5	2.45
	1.5	1.47	1.25	6.9	1359	431	156.5	1.84
	2	1.96	1.77	6.9	1364	438	156.7	1.6
	2.5	2.45	2.25	6.8	1371	444	156.4	0
	3	2.93	2.74	6.6	1378	451	156.5	0

After series of exposed time (30; 45; 60; 90; 120 and 180 min), there was no significant changes in Nitrate value of mixed solutions and just slightly drop on the pH value. However, there were decreases on the value of TDS and ORP of mixed solutions when the exposed time increased. The reason is that ORP in mixed solutions tends to react with oxidized components in solution. In addition, the TDS depletion of the mixed solution can also be attributed to the active ingredients being gradually converted to less dissociated molecular form. Longer exposed time did give positive impact to the reduction of total coliform in mixed hydroponic solutions. In all

the experiment with different mixed ratio, the coliform counts of mixed solution were dropped dramatically in the first 30 min. This is because the strong oxidants in the EAS solution can induce oxidation of sulfhydryl compounds on the cell surface [11] and other key metabolic systems [12 - 14], leading to the Inhibits growth of bacterial cells. However, in the next periods, along with the decease of ACC in solution the decrease rate of total coliform was much slower and declined though the time. This can be explained by a decrease in the concentration of oxidants and other disinfectant factors. At the mixed ratio from under 1 : 500, after 90 min of exposed the coliform counts of mixed solution were nearly unchanged. But with higher mixed ratio from above 1.5 : 500 the coliform counts of mixed solutions were dropped dramatically to under detected level.

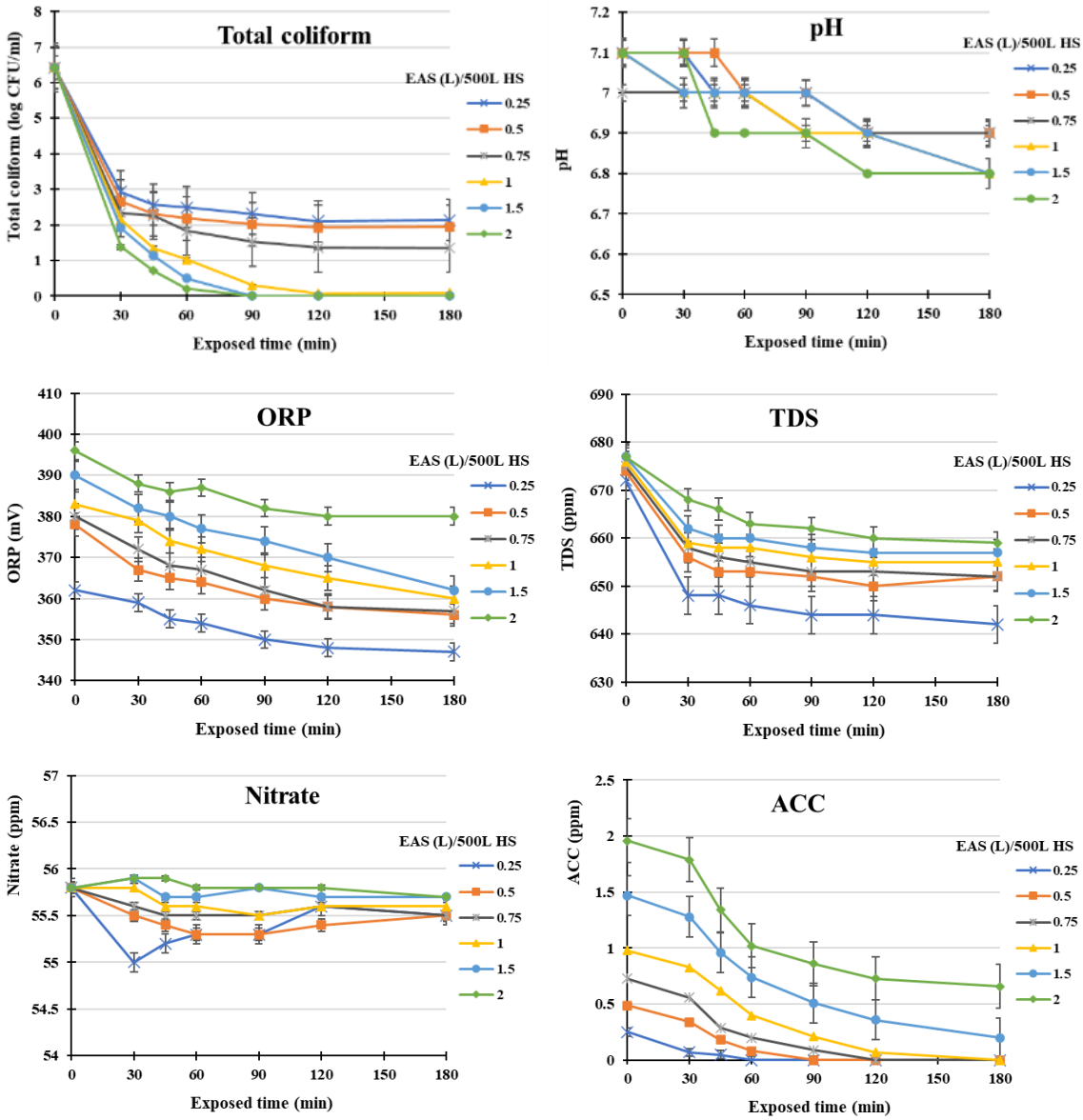

Figure 1. The effect of exposed time to the quality parameters of mixeda EAS - hydroponic solution.

To control efficiently the pathogens in hydroponic solution with EAS, the mixed ratio of 2.5 : 500 with 30 min of exposed time or mixed ratio of 1.5 : 500 with 90 min of exposure time could be applied. However, the mixed ratio of 1.5 : 500 with 90 min of exposure time was recommended because of its lesser changes on the properties of hydroponic solution. Since the organic contents in the mixed solution may affect the sterilization ability of EAS, for different types of hydroponic solutions, specific studies on mixed ratio and exposure time are required.

3.2. Effect of mixed EAS - hydroponic solution on the *Brassica juncea* growth

The mixed ratio of 1.5 : 500 with 90 min of exposure time was selected for the cultivate experiment. *Brassica juncea* grown by selected mixed EAS - hydroponic solutions were harvested at day 50th. The morphological, fresh and dry weight of harvested *Brassica juncea* were measured, the results are shown in Table 3.

Table 3. The morphological, the fresh and dry weight of *Brassica juncea*.

Hydroponic solutions	Plant height (cm)		Leaves/plant		Leaf diameter (cm)		Fresh weight (gram/plant)		Dry weight (gram/plant)	
	Min/Max	Ave	Min/Max	Ave	Min/Max	Ave	Min/Max	Ave	Min/Max	Ave
Original	8.9 - 15.7	11.78	4 - 6	5	2.2 - 3.1	2.7	2.2 - 2.7	2.5	0.13 - 0.18	0.16
EAS mixed	9.0 - 15.9	11.83	4 - 6	5	2.3 - 3.3	2.8	2.2 - 2.8	2.5	0.13 - 0.18	0.17

Average plant height of *Brassica juncea* grown with original and mixed with EAS hydroponic solutions were 11.78 and 11.83 cm, respectively. 2.7 and 2.8 cm were the average leaf diameter of *Brassica juncea* grown with original and mixed with EAS hydroponic solutions, correspondingly. And both experiments gave the same average number of leaves per plant, at 5 leaves per plant.

Average fresh weight of *Brassica juncea* grown with original and mixed with EAS hydroponic solutions was both 2.5 gram per plant. And the figure of average dry weight was 0.16 and 0.17 gram per plant, respectively. Overall, the morphological, fresh and dry weight of *Brassica juncea* grown with original and mixed with EAS hydroponic solutions was similar. This result showed that cultivate *Brassica juncea* using mixed EAS - hydroponic solution was not affected to the growth of plants in comparison with original hydroponic solution.

4. CONCLUSIONS

The electrochemically activated solutions were successfully applied for bacterial control in hydroponic solutions. When increased the volume of EAS added (higher ACC of mixed solution) or the exposure time, the TDS and ORP index of hydroponic solution were raised slightly. While pH value was slightly dropped and the total coliform counts decreased significantly. The total coliform counts of mixed solutions reduced over the exposed time. However, the increasing rate of killing ability of EAS was decreased (along with ACC of solutions). Meanwhile, there was no obvious change in the nitrate concentration. EAS had been shown to be used to control pathogens in hydroponic solutions, replacing other harmful chemicals. The most effectivest mixed ratio EAS : hydroponic solution and exposure time for

pathogens control were 2.5 : 500 with 30 min or 1.5 : 500 with 90 min. The mixed ratio of 1.5 : 500 with 90 min of exposure time were recommended because of it lesser changes on the properties of hydroponic solution. However, the organic contents in the solution may affect the sterilization ability of EAS, for different types of hydroponic solutions, specific studies on mixed ratio and exposure time are required. The mixed EAS - hydroponic solution caused no negative effect to the growth of *Brassica juncea* in comparison with original hydroponic solution.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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