

Ultra-Fine Bubble Generators for Advanced Versatile Applications to Agriculture, Aquaculture and Food Safety

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An emerging technology of Ultra-Fine bubbles (UFB) of micro and nano sizes called fine (<100µm), and ultra-fine (<1µm) bubbles is a very innovative technology for rapidly expanding versatile applications to such as, agriculture, aquaculture, food safety, sterilization, waste water treatment, automobile gas mileage improvement, cancer treatment and so on.

Those UFB have very unique characteristics which are vastly different from conventional simple macro bubbles with larger diameters. UFB float-up speeds are proportional to diameter square, and electrically charged up negative.

This enables nano bubbles of, for example, 100nm dia. having almost 30 bars inside the bubble to stay pretty long in the water without any merging into micro or macro bubbles.

By making use of these characteristics, UFB water of such as air, oxygen, nitrogen, ozone and so on can have versatile outstanding effects in various applications, for example, oxygen free water produced by nitrogen UFB injection into water can preserve fish for one week as fresh without any chemicals by suppressing activity of aerobic bacteria, which is now being used at Tokyo Tsukiji fish market.

RMUTL has prepared commercially available micro and nano bubble generators imported from Aura Tec, Japan, and developed by ourselves various kinds of less-expensive, but high performance UFB generators based on the high pressure gas dissolution methods. According to the analyses by UFB analyzer, Nano Sight, measured at Kyoto University, the density is found favorable, as high as approx. 10⁸/cm³ as shown in Fig.1.

With almost 20 micro/nano bubble generators of several kinds now possessed by RMUTL, RMUTL is planning to expand more innovative researches in various application fields, in particular, food safety.

In the presentation, more applications will be presented relating to micro/nano bubble generators.

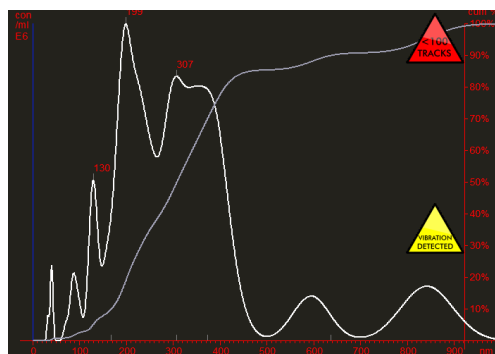


Fig.1

RESULTS:
 Size Distribution: Mean: 348 nm, Mode: 199 nm, SD: 192 nm
 Cumulative Data (nm): D10: 188, D50: 308, D90: 637, D70: 374
 User Lines: 0 nm, 0 nm
 Total Concentration: 7.51 particles / frame, 1.07E8 particles / ml
 Selected Concentration: 0.00 particles / frame, 0.00E8 particles / ml
 Fitted Curve: Mean: 0 nm, SD: 0
 Completed Tracks: 50
 Drift Velocity: 208 nm/s



Characterizations of electrical filament discharge plasma in air bubbles under water

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Through the hydroponic system, farmers can plan to grow the amount of production volume considering the market demand. Since mixed nutrients in the water are supplied directly to the root system of plants, the growth rate is faster than a plant on the soil, and this system can lead to higher competitiveness and economic incomes. However, hydroponic have problems with fungi and pathogens. Since circulated hydroponic solutions are applied in the system may cause microbial contamination which will bring about serious damages to entire cultivation plants. Moreover, the nutrient solution is an aqueous concentrations of some ions in water. This can be a major problem to the environment. An interesting technology to solve these problems inherent in circulated nutrient solutions is discharge plasma gases underwater. The discharge plasma gases underwater are found to be effective both to the growth rates of plants and the associated bacterial activities, because these plasma gases can generate chemically active species, such as O, O₃ and OH which work to inactivate the pathogenic, fungi and bacteria. Also plasma gases can produce NO_x in the solution which is absorbed by the roots of the plants helping their rapid growth. The electrical filament discharge plasma in air bubbles underwater for hydroponic system has been developed by RMUTL (Fig.1) with a low cost high voltage power supply, namely, a commercially available transformer for neon light (KODERA, CR-N16) operated at 15 kHz. This presentation will discuss on the discharge characteristics of air discharge plasma through multiple bubbles as shown in Fig. 2. In addition to measuring current-voltage waveforms (Fig. 3) as well as optical emission spectra from plasma, the effects of chemical products in the water were investigated.

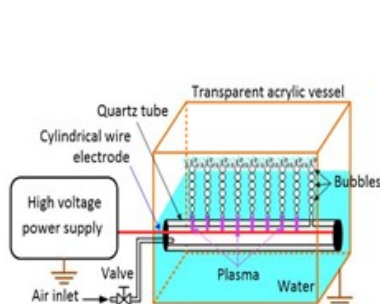


Fig.1 The schematic of the experimental

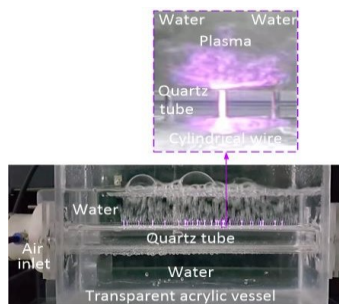


Fig. 2 Typical of air discharge plasma through multiple bubbles under water.

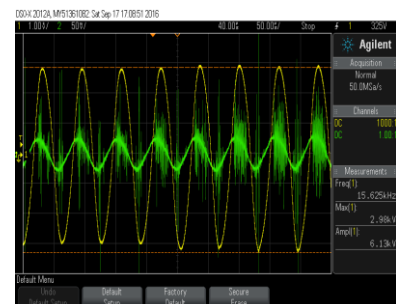


Fig. 3 Typical waveform of voltage (yellow line) and current (green line) during air discharge plasma.

Effects of Micro/Nano Bubbles on Seed Germination and Growth of Crop in Hydroponics System

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In the recent year, hydroponic system is widely technology for plant production in the world. The plant can grow by using mineral nutrition solution in the water without the use of soil. Plant production in hydroponic system offers numerous advantages such as the plant growth is very fast, increases yield and fruit quality. However, hydroponics system in Thailand where tropical condition that has the problem of contamination by pathogens in the nutrient solution, and moreover, the diseases specific to hydroponics have been reported. For example, zoospore-producing micro-organisms.

The new technology, i.e., micro/nano bubble technology, is being studied and found to be very effective to the agriculture as well. For example, micro/nano bubbles were used to enlarge the mass transfer of the ozone gas into the water and eventually increase the dissolved oxygen concentration, which is found to be very efficient to wastewater purification.

The effective applications of micro/nano bubbles are reported to range in the versatile fields, such as, purification of wastewater, water quality improvement, sterilization, de-colorization, cleaning of contaminated water, and promotion of the physiological activities of living organisms, micro/nano bubbles applications to soilless culture may solve these problems by their biological effects among versatile application fields, such as, improvements of germination of plant seeds, control of growth rate of the plants, control of pests and pathogens.

The micro/nano bubble generators were developed by RMUTL with the equipment imported from Aura Tec, Japan.

In the presentation will apply micro/nano bubble generators to seed germination and plant growth under hydroponic system. The results will be presented at the conference.



Fig.1 Effect of micro/nano bubbles on seed germination



Fig. 2 Effect of micro/nano bubble on plant growth in hydroponics system

MNB-01

Effects of Micro/Nano Bubbles on The Growth and Survival of Carp (Cyprinus Carpio) at Different Stocking Density

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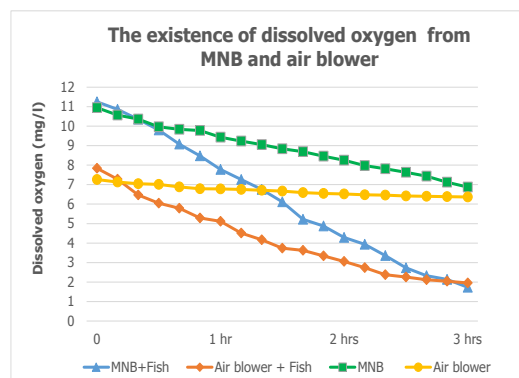
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Dissolved oxygen concentration is the most important parameters for aquaculture relating, particularly, to fish growth and live fish transport. Micro/nano bubble (MNB) method, which is an emerging technology of Ultra-Fine bubbles (UFB) containing micro and nano size bubbles, has been developed at RMUTL, and is found to be an efficient way to dissolve much oxygen into the water. This study presents some preliminary results relating to the use of MNB as an aerator in the fancy carp culture compared with a conventional air blower for different fish stocking densities. The dissolved oxygen (DO) levels in the water by using the MNB generator of RMUTL version 5 type with a 99.5% pure oxygen gas was first studied. After oxygenating under the condition of a tap water temperature of 26.7 deg C, a water flow rate of 2 l/min with an oxygen gas pressure of 0.4kg/ cm², and a flow rate of 0.5 l/min, the DO level increased in 30 minutes up to 35.6 mg/l from 7.6mg/l averaged tap water level. After 24 hours left, the DO level was found to decrease to 7.65 mg/l. By using this high DO water as well as the water with a conventional aerator, DO comparisons were made for the tap and MNB containing water of initial level of 11mg/l, with and without fishes in the fish tanks. As shown in Fig.1 the DO was kept high for the MNB water, and the DO of fish tank water without fishes decreased to the normal DO levels in 3 hours. This may be due to the fact that the MNB of very fine miniscule bubbles are vastly different in property from big bubbles produced by the air blower.

Also, the application of MNB technology to fish culture was examined. Fancy carps were stocked with 50 and 100 fishes/m³ in 200 litres tanks aerated with the air blower (case 1) and the MNB generator (case2), respectively. Their lengths and weights were measured every week as well as water quality parameters (pH, dissolved oxygen, temperature, ammonia and nitrite levels). The results show that DO level in case 2 was higher than in case 1, as predicted.

Moreover, the new MNB aeration system can also provide a positive effect on growth rate of fancy carps, particularly when fishes were stocked with 50 fishes/m³. Water quality was found generally acceptable for carp growth for all parameters examined. The live fish transportation is another essential process of aquaculture necessary for healthy live fish shipping to the destination. Fancy carps of size 7-8 cm were transported in plastic bags with oxygenating by pure bottled oxygen and oxygen MNB water, and these two aerating systems were compared as case 1 and case 2, respectively. Fishes with 1, 2, 3 and 4 fishes/litre were contained in plastic bags. One-fourth of these bags contain water and fish, and three-fourth contains oxygen in case 1 and pure air in case 2. Water quality and survival rates were recorded after 1, 3, 6, 12, 24, 48, 72 and 96 hours.



Results showed that 100% fishes in both cases could survive during experiments. DO levels in the plastic bag with oxygenating by pure bottled oxygen were higher than those of MNB water. This means that the MNB system may be good enough for live transport in a close system with less oxygen consumption. Further studies in fish containers in an open system will be needed to clarify to what extent and for which aquaculture system the MNB method can be most efficiently applicable.

The MNB is found to be a noticeable emerging technology to aquaculture that will play an increasingly important role in economy of Thailand. However, it is necessarily requested to seek the suitable way to use this potential technology for Thai farmers in the long run.

MNB-02

Growth Performance of Asian Sea Bass (*Latescalcarifer Bloch*) Using Micro Bubbles in Aquaponic System

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Nowadays, an emerging technology of Ultra-Fine bubbles (UFB), i.e., micro and nano sizebubbles (MNB), has become an alternative way to enhance fish production in high stocking density, due to its tiny bubbles of diameter less than 100 μm including nano sizes. In a successfully fish culture, consideration are generally given to the water rearing component and DO concentration in water to produce optimal fish growth. Today, Third water, called an artificial water, is one of the important technology with a high potential cultivation of aquaculture because the water has properties that do not exist in nature and lacks the infectious agents often found in seawater and fresh water.

This study aims to compare the resources of water supply under rearing conditions on the growth performance of Asian sea bass (*Lates calcarifer Bloch*) fishes using MNB in the aquaponic system. The experiment was designed on a completely randomized model with four groups of water resources including, freshwater (control1), third water (group 1), low salinity water (4 ppt: group 2) and seawater (30 ppt: group 3), respectively. There were two replicates per group. Asian sea bass of average body weight (32.65 ± 1.26 g) were acclimatized to experimental conditions for 15 days before they were randomly allotted to each group. The stocking density of each group was fixed to be 50 fishes m^{-3} in a fish tank (2,000 liters). During 60 days of experimental period between July and August, 2016, all fishes were fed 3-5% of their body weight using a commercial diet consisting of 45% protein and 15% lipid at the time of 9:00 and 17:00, twice a day.

The results indicated that Asian sea bass in group 3 (sea water) had the highest values of final mean weights, followed by the fishes in group 2 and group 1, respectively. However, there were no significant difference between each groups ($P > 0.05$), compare between group1 to group2, group1 to group3, and group2 to group3, respectively. The lowest of final mean weight was found for fishes in the controlled group, i.e., freshwater (Fig.1). Average values of pH (5.43-8.66), dissolved oxygen (5.89-6.40 mg/l), conductivity (0.128-0.301mS/cm), total dissolved solids (0.218-0.256g/l), water temperature (29.45-31.11 $^{\circ}\text{C}$), turbidity (43.25-49.71NTU), and nitrates (0.23-0.25 mg/l) were respectively measured during the experiments in all the fish tanks.

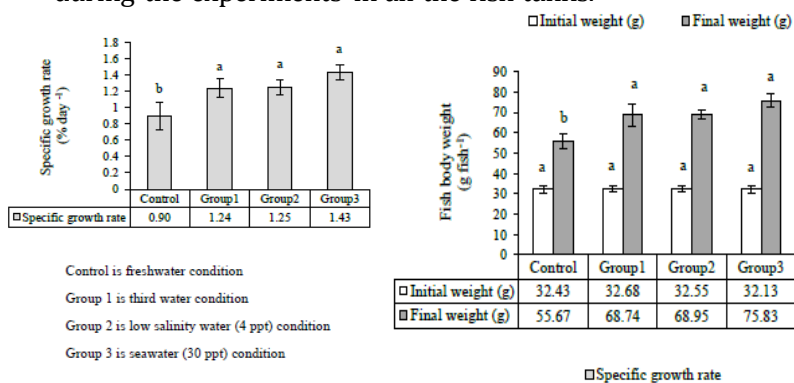


Fig. 1. Growth performance of Asian sea bass (*Lates calcarifer Bloch*) fish using micro bubbles under culture conditions, an aquaponic system



MNB-03 Study of Micro/Nano Bubble Efficiency Effecting to Water Quality in Tilapia Fish Pond.

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Farming of Tilapia fishes in Thailand is worth six billion bahts per year and still growing. In Thailand, aquaculture is an important element in the overall production of foods needed to supply the country's population. However, farmed fishes are prone to mass kills resulting from a lack of oxygen in the water or too excessive nutrient levels. When either or both of these problems exist on a fish farm, there can be big losses of up to 70% of the fish stock in a given pond. For farmers with relatively low margins, this could be a serious fatal impact to their livelihoods.

The aim of this research was to monitor the effectiveness of a micro/nano bubble(mnb) generator to water with an air mnb injection. In this preliminary research, the water parameters: DO, BOD and turbidity, were investigated during experiments. It is found that the water parameters were significantly different between with and without the aeration by a micro/nano bubble generator. Parameters from both sources are found to show the same trend of evolution (Fig.1). The further work, the effectiveness of the micro/nano bubble generator to water with air will be studied in the bigger ponds.

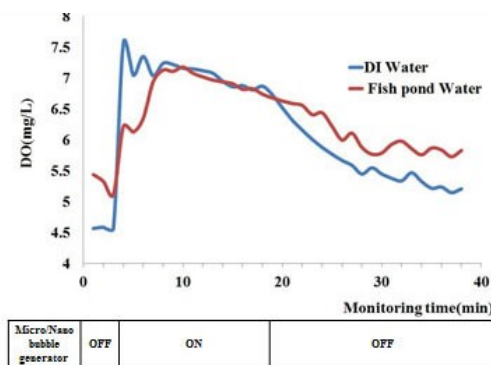


Fig. 1 Comparison of DO in DI water & fish pond water for various time.

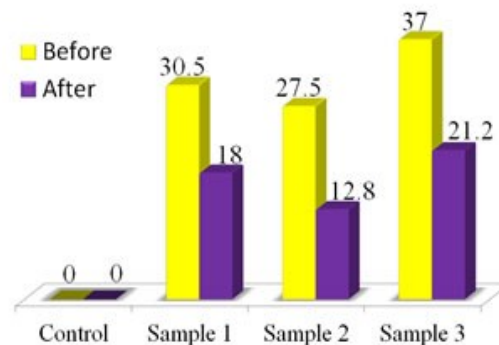


Fig.2 Comparison of BOD before and after applying the mnb generator with air.

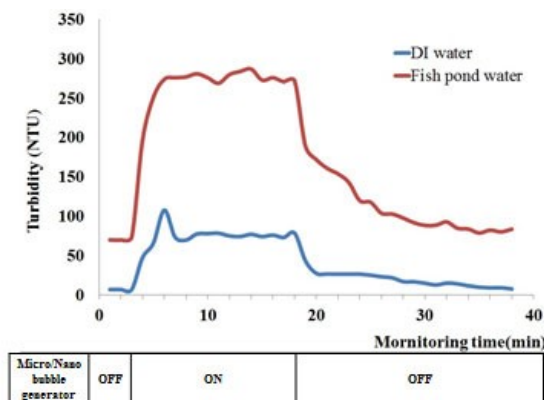


Fig. 3 Comparison of turbidity in DI water & fish pond water for various time.

DO and turbidity of both deionized and fish pond water were monitored during the injection of air micro/nano bubbles. In case of BOD, it was measured before and after applying the air mnb injection. It is found that the water parameters were significantly different between with and without the aeration by air micro/nano bubble injection. Parameters from both sources are found to show the same trend of the evolution.

MNB-04

Application of Microbubbles to Reduce Microorganisms on Thai Vegetables

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Abstract The objective of this study is to apply the micro bubbles technology in washing process to reduce microorganisms on fresh Thai vegetables (morning glory, ginger and lettuce). Fresh vegetables were washed with micro bubbles water (MB) or micro bubbles water combined with 80 ppm sodium hypochlorite (NaOCl) (MB+Cl₂) for 5 minutes. The results showed that after washed with MB, the microbial reduction were 73.08, 67.64 and 69.80%. While washed with MB+Cl₂ gave a higher efficacy, the microbial reduction were 97.54, 96.98 and 99.59%, on these three Thai vegetables. Additionally, the effect of different water flow rates on microbial reduction on fresh produce was investigated. Fresh lettuce was washed for 2 minutes. The results show that 7.5 L/min of water flow rate had the highest efficacy 81.80%, while 2.5 and 5.0 L/min showed 72.45 and 76.55%, respectively. These washing protocols could be implemented in the washing step to enhance the safety of fresh vegetables production.

Keywords: Micro bubbles, Washing, Microbial reduction, Sodium hypochlorite, Water flow rate, Fresh produce

Introduction Micro bubbles are defined as the small bubbles with diameter less than 10-200 μm (1). There have been several studies on the use of micro bubbles technology in washing process for reducing microorganisms from the vegetables (2).

Materials and Methods Three types of Thai vegetables were purchased from a local market. MB was generated by micro bubbles generator (Micro star FS101-1 (Fuki manufacturing Co, Ltd., Japan). NaOCl 80 ppm

were prepared and measured the concentration by starch-iodide titration method. In washing process, 1 kg of vegetable was washed in 35 liters of wash water. The vegetables samples were taken for microbial analysis. Data were analyzed using the Student's t-test (P < 0.05).

Results and Discussions The efficacy of MB and MB+Cl₂ show in Fig.1. These results indicate that the combined MB with 80 ppm NaOCl was more effective as compared to washing with MB only. The effect of different water flow rates on microbial reduction on fresh lettuce show in table 1. According to the results, the increase of water flow rate exhibited better washing efficacy.

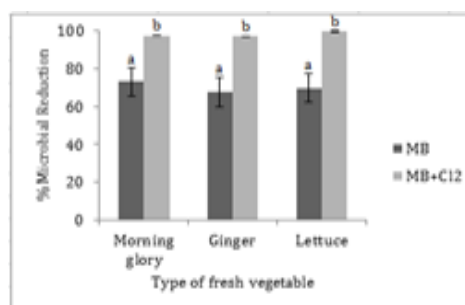


Fig. 1 Microbial reduction on three Thai vegetables as affected by MB and MB+Cl₂

Acknowledgement: This study was supported by The Graduate School, Kasetsart University.

Keywords: Micro bubbles, Washing, Microbial reduction, Sodium hypochlorite, Water flow rate, Fresh produce

Flow rate (L/min)	Washing time (min)	Microorganisms population (log ₁₀ CU/ml)	
		Before	After
2.5	2	6.8±0.18	6.25±0.04
5	2	6.81±0.10	6.18±0.52
7.5	2	7.00±0.17	6.26±0.64

Table 1 Microorganisms population on fresh lettuce.

Reference:

- 1) Parmar, R. and S. K. Majumder. 2013. Microbubble generation and microbubble-aided transport process intensification-A state-of-the-art report. Chem. Eng. Process. 64: 79-97.
- 2) Lee, W.J., C.H. Lee, J.Y. Yoo, K.Y. Kim and K.I. Jang. 2011. Sterilization Efficacy of Washing Method Using Based on Microbubbles and Electrolyzed Water on Various Vegetables. J. Korean Soc. of Food Sci. Nutr. 40 (6): 912-917.

MNB-05

Feasibility of Dirt Removal and Decontamination of Agricultural Product by Micro- and Nano bubble Treatment

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In the recent year, hydroponic system is widely technology for plant production in the world. The plant can grow by using mineral nutrition solution in the water without the use of soil. Plant production in hydroponic system offers numerous advantages such as the plant growth is very fast, increases yield and fruit quality. However, hydroponics system in Thailand where tropical condition that has the problem of contamination by pathogens in the nutrient solution, and moreover, the diseases specific to hydroponics have been reported. For example, zoospore-producing micro-organisms.

The new technology, i.e., micro/nano bubble technology, is being studied and found to be very effective to the agriculture as well. For example, micro/nano bubbles were used to enlarge the mass transfer of the ozone gas into the water and eventually increase the dissolved oxygen concentration, which is found to be very efficient to wastewater purification.

The effective applications of micro/nano bubbles are reported to range in the versatile fields, such as, purification of wastewater, water quality improvement, sterilization, de-colorization, cleaning of contaminated water, and promotion of the physiological activities of living organisms, micro/nano bubbles applications to soilless culture may solve these problems by their biological effects among versatile application fields, such as, improvements of germination of plant seeds, control of growth rate of the plants, control of pests and pathogens.

The micro/nano bubble generators were developed by RMUTL with the equipment imported from Aura Tec, Japan.

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Fig.1 Development of MNB generator by RMUTL research team



Fig.2 MNB experimental study for food application



Fig.3 Potential use of MNB for enzyme production and starch modification during grain malting for brewing.

MNB-06

Preliminary Study of Micro/Nano bubble Technology on Food Safety in Agricultural Product: A Case Study of Chiang Mai Strawberries

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Strawberries are one of the most popular agricultural product in Chiang Mai. However, strawberries are prone to several pests therefore, pesticides are used heavily in strawberry production. Pesticide residue in strawberry effect consumer's health. The groups of pesticide frequently used for strawberry production are organophosphate and carbamate. Even though the pesticides generally decompose after application, the residue may be found on the berries. Constant exposure to low level of pesticide effect consumer's health therefore, it is necessary to clean the berries before consumption.

Generally, normal water cannot eliminate all pesticide residue. However, micro/nano bubble water (MNB) has been studied and found to be effective in elimination of pesticide residues in some agricultural products.

In this recent study, pesticide residue was not found in the strawberries. Therefore we cannot yet conclude the effect of MNB in pesticide residue elimination. However, this preliminary study proved that the test kit used for pesticide residue detection is effective. (Figure 1) Further study will be carried out by using organophosphate insecticide (Deca 40% W/V EC) as a positive control.

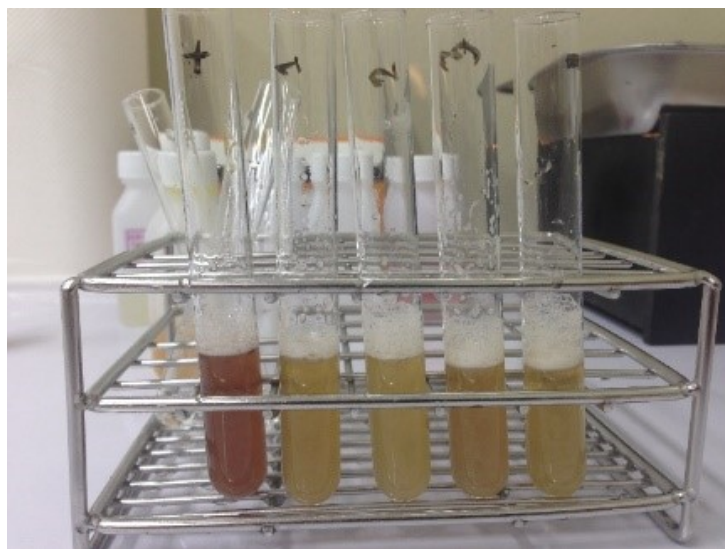


Figure 1 GT test kit for organophosphate and carbamate pesticides