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Message from the President of the Association of Researchers of Thailand

Dear All Members

Hello to all members of the Association of Researchers of Thailand. The world and Thailand are now facing the Covid-19 pandemic and this we need to largely adjust ourselves now and after the Covid-19 with the “New Normal” protocol in all aspects, including in the research industry, for example, vaccine research related to Covid-19 in which the research process has been accelerating faster than usual. On the Facebook page of the Association of Researchers of Thailand, we publish information about the situation about Covid-19 every day that members can follow. Due to the current situation, the Association is unable to hold an Annual General Meeting this April since there has been the Government Emergency Decree issued that we need to follow. However, once the situation returns to normal, the Association will arrange the ordinary meeting again. Please follow our updates from the communication channels of the Association.

Lastly, we hope that all members of the Association of Researchers of Thailand will be safe from the crisis of the Covid-19 pandemic, and we wish everyone good health.

Associate Professor Dr. Phiphat Nonthanathorn
the President of the Association of Researchers of Thailand

This International Journal of Science and Innovative Technology (IJSIT) is the 3rd year, vol.1 issue. The Journal still receives honors from numerous academics and researchers that have continuously submitted the works for publication, the research works that can be further extended to innovations in order to propel Thailand to international recognition. And this is aiming for sustainable development of many activities of the Association of Researchers of Thailand that are organized in cooperation with external agencies, leading into rapid cooperation in research and various academic fields in the forms of online format which is a form of Digital Transformation in the period of this New Normal of an epidemic of COVID 19 such as INTERNATIONAL WEBINAR ON “IMPACT AND STRATEGIES FOR TOURISM, SERVICE MANAGEMENT, AND ACADEMIC SECTOR”. And in this issue, there are also interesting guest articles on Biodiversity following the Covid-19 Virus Outbreak to the Adaptation of Humanity. On this occasion, the Editorial team would like to thank the professors, academics, and researchers for your contribution to submitting important articles for the further development of innovation for the actual application. And the International Journal of Science and Innovative Technology (IJSIT) will play an important role in disseminating knowledge, research works for application in solving problems and for continuous development and to provide sustainable international connections for Thailand

Assistant Professor Dr. Sujinna Karnasuta

Editor in Chief


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Visible-Light Photocatalysis with WO₃/TiO₂ Nanocomposite Fiber Sponges Prepared via Solution Blow-Spinning Process

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Saengdeon Doungdaw^{1,*}, and Siriporn Larпкиattaworn^{1,*}

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Abstract

Three-dimensional nanocomposite fiber sponges consisting of tungsten trioxide (WO₃) and titanium dioxide (TiO₂) as visible-light photocatalysts were successfully prepared by solution blow-spinning process in combination with a calcination process (450 °C). The precursor solution of tungstic acid (H₂WO₄)/tetrabutyl titanate (Ti(OBu)₄)/polyvinyl pyrrolidone (PVP) with various molar percentage ratios of tungsten to Ti (i.e., 0.5, 1.0, 3.0, and 5.0) in 75/25 w/w mixture of ethanol and acetic acid were prepared and blown-spun. Smooth fiber morphologies and average fiber diameters in a range of 480 nm to 645 nm before calcination and 351 nm to 479 nm after calcination were observed by a scanning electron microscope (SEM). The presence of WO₃ within the nanocomposite fiber sponges was found by using X-ray diffractometer (XRD). The photocatalytic properties of the WO₃/TiO₂ nanocomposite fiber sponges were investigated by photocatalytic degradation of methylene blue solution under both UV- and visible-light radiation. Results showed that the WO₃/TiO₂ nanocomposite fiber sponges at molar percentage ratios of 5.0 exhibited the highest activity under both UV- and visible-light radiation.

Keywords: Photocatalyst, WO₃/TiO₂, Nanocomposite fiber sponges

Introduction

Titanium dioxide is one of the most popular photocatalysts which have been widely investigated because of its advantages such as non-toxicity, low cost, chemical stability, and high photocatalytic property (Li et al., 2005; Zhang et al., 2006; Ye et al., 2013). Recently, it has been extensively used in a variety of fields, including air purification, self-cleaning surfaces, and water purification (Luevano-Hipolito et al., 2014; Elahifard et al., 2007; Wang and Lim, 2013). The photocatalytic activity mechanism of TiO₂ involved with converting solar energy to chemical energy by generating the photoinduced electrons and holes. As a result, superoxide radical anions and hydroxyl radicals generated from the reaction between the holes and water molecules could decompose organic matter (Zeng et al., 2007; Ismail & Bahnemann, 2011). However, the use

of TiO₂ is still limited in some aspects due to its wide electronic band gap value of 3.2 eV, requiring only UV light to activate. Moreover, the rapid photo-generated electron-hole recombination rate of TiO₂ also reduces its photocatalytic activity (Hayden, Allam and El-Sayed, 2010; Linsebigler, Lu and Yates, 1995).

To overcome these limitations, several previous researchers have paid attention to extend TiO₂ adsorption in the visible spectrum and to reduce recombination rate of photo-generated electron-hole pairs by doping with metals/non-metals, surface modification, loading of metal nanoparticles, and combining with other semiconductor oxides such as CdS, Fe₂O₃, Bi₂O₃, WO₃, etc. The coupled semiconductor oxides have been widely reported to improve the photocatalytic activity, in comparison to that of individual semiconductors (Luevano-Hipolito et al., 2014; Laursen et al., 2012; Kment

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et al., 2017). Among them, WO_3 has attracted much attention for enhancing the photocatalytic activity of TiO_2 , owing to its advantages such as low cost, smaller band gap (2.7 eV), and high stability in water (Yadav et al., 2017; Mugunthan, Saidutta and Jagadeeshbabu, 2018; Ofori et al., 2015).

Nowadays, nanofibers have received a great deal of attention in the field of environment such as air purification and water purification due to excellent characteristics such as high surface area to volume, and high porosity (Doshi and Reneker, 1995; Khil et al., 2003). Various WO_3/TiO_2 nanofibers, mostly based on electrospinning process, were reported (Chen et al., 2016; Chakornpradit, Phiriyawirut and Meeyoo, 2017; Szilagyi et al., 2013). However, the WO_3/TiO_2 nanofibers typically were manufactured in a flat form (two-dimensional or 2D). In this work, three-dimensional photocatalysts consisting of WO_3 and TiO_2 with various molar percentage ratios of tungsten to Ti (i.e., 0.5, 1.0, 3.0, and 5.0) prepared by solution blow-spinning process was investigated.

Experimental Procedure

Polyvinyl pyrrolidone (PVP; with a molecular weight about 1,300,000), tungstic acid (H_2WO_4 ; $\geq 99\%$), tetrabutyl titanate ($\text{Ti}(\text{OBU})_4$; $\geq 97\%$), and methylene blue (MB; dry content $\geq 82\%$) were purchased from Sigma-Aldrich (Switzerland). Ethanol (99%) and acetic acid (99.8%) were purchased from Lab-Scan Asia (Thailand).

All of the chemicals were of analytical grade and used without further purification.

As for the preparation of TiO_2 fiber sponges, the precursor solution of $\text{Ti}(\text{OBU})_4/\text{PVP}$ was prepared by a sol-gel method and blown-spun as previously described by Wang et al., 2017. Briefly, $\text{Ti}(\text{OBU})_4$ and PVP with 2:1 mass ratio were mixed in a mixture of ethanol and acetic acid (75/25 w/w). The concentration of PVP in the solution was fixed at 7 wt%. The solution was continuously stirred using magnetic stirring for 6 h at room temperature, and then was blown-spun by using a specific coaxial needle under a gas pressure of 69 kPa (airflow velocity is about 21 m/s). The flow rate of the solution and the distance between the tip to the porous and air permeable-cage collector were set at 3 mL/h and 20 cm, respectively. After spinning, the $\text{Ti}(\text{OBU})_4/\text{PVP}$ fiber sponges were immediately calcined at 450 °C for 200 min in air. Finally, the fiber sponges were irradiated with UV radiation (12 W) for 2 h prior to further use.

As for the preparation of WO_3/TiO_2 nanocomposite fiber sponges, the preparation process was similar to that of TiO_2 fiber sponges, except that the H_2WO_4 with various molar percentage ratios of tungsten (W) to Ti (i.e., 0.5, 1.0, 3.0, and 5.0) was dispersed in 75/25 w/w mixture of ethanol and acetic acid under magnetic stirring for 48 h and sonicated in ice bath for 5 min prior to the addition of $\text{Ti}(\text{OBU})_4$ and PVP.

The particle size of H_2WO_4 after dispersion in mixed solvent was observed by using Zetasizer Nano

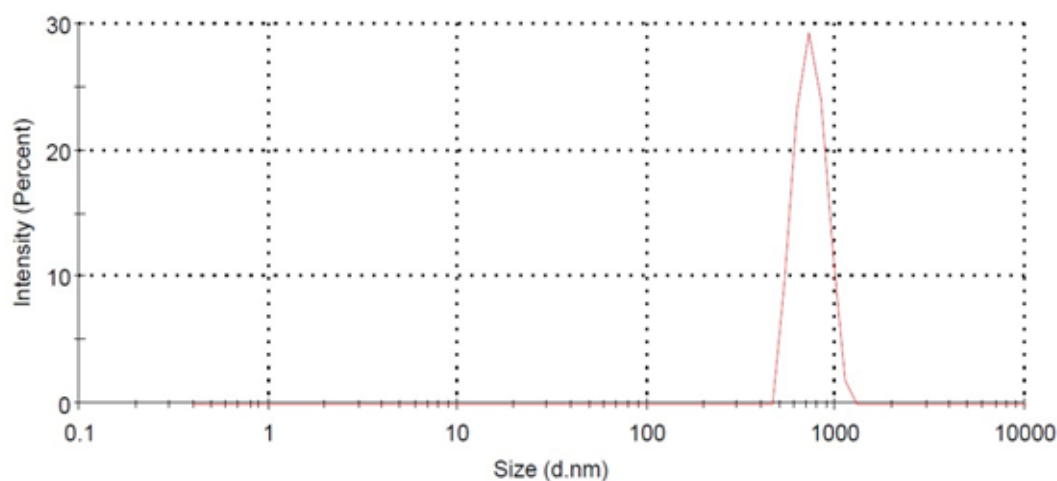


Figure 1. Particle size distribution of H_2WO_4 dispersed in 75/25 w/w mixture of ethanol and acetic acid.

ZS (Malvern Instrument). The morphology and size of the as-prepared fiber sponges were investigated using a Field Emission Scanning Electron Microscope (FE-SEM, JEOL, JSM-6304F). X-ray diffraction patterns of the as-prepared fiber sponges were recorded using a Rigaku SmarLab X-ray diffractometer (XRD).

The photocatalytic activity of the as-prepared fiber sponges was evaluated by monitoring degradation of MB under both UV- and visible-light radiation. The as-prepared fiber sponges were immersed (1g/L) in MB at a concentration of 10^{-5} mol/L, and irradiated with UV radiation (12W) or irradiated with visible-light radiation (LED lamp 18W). The changes of MB concentration during the test were measured by UV-Vis spectrometer (Shimadzu, UV-1700) at a wavelength of 660 nm.

Results and Discussion

H_2WO_4 dispersed in 75/25 w/w mixture of ethanol and acetic acid was evaluated in terms of particle size and size distribution as shown in Figure 1. The aver-

age diameter of H_2WO_4 prepared was 735.4 nm and the polydispersity index value of 0.353 were observed.

The prepared precursor solutions of $\text{Ti}(\text{OBu})_4/\text{PVP}$ and $\text{H}_2\text{WO}_4/\text{Ti}(\text{OBu})_4/\text{PVP}$ with various molar percentage ratios of tungsten to Ti (i.e., 0.5, 1.0, 3.0, and 5.0) were blown-spun by using a home-made equipment, which was assembled with an inner nozzle for allowing airflow and a concentric outer nozzle for injecting of prepared precursor solution as presented in Figure 2a. Under the pressure, the ejected precursor solution was rapidly stretched and solidified during traveling in air. As a result, the nanocomposite fiber sponges were collected in an air-permeable cage-like collector as represented in Figure 2b.

The morphologies of the $\text{Ti}(\text{OBu})_4/\text{PVP}$ and a series of $\text{H}_2\text{WO}_4/\text{Ti}(\text{OBu})_4/\text{PVP}$ fiber sponges with various molar percentage ratios of tungsten to Ti (i.e., 0.5, 1.0, 3.0, and 5.0) before and after calcination treatment at 450°C for 200 min are presented in Figure 3. Before calcination treatment (figures on the left column), the

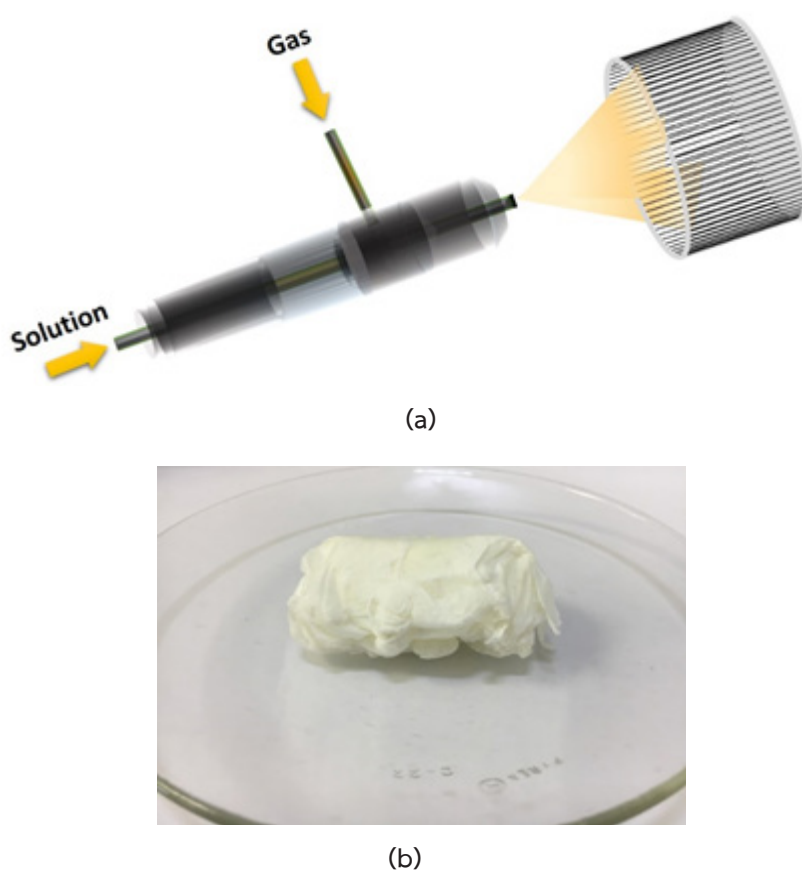


Figure 2. Schematic diagrams of (a) the solution blow-spinning and (b) photograph of the precursor $\text{H}_2\text{WO}_4/\text{Ti}(\text{OBu})_4/\text{PVP}$ fiber sponges obtained from the solution blow-spinning process.

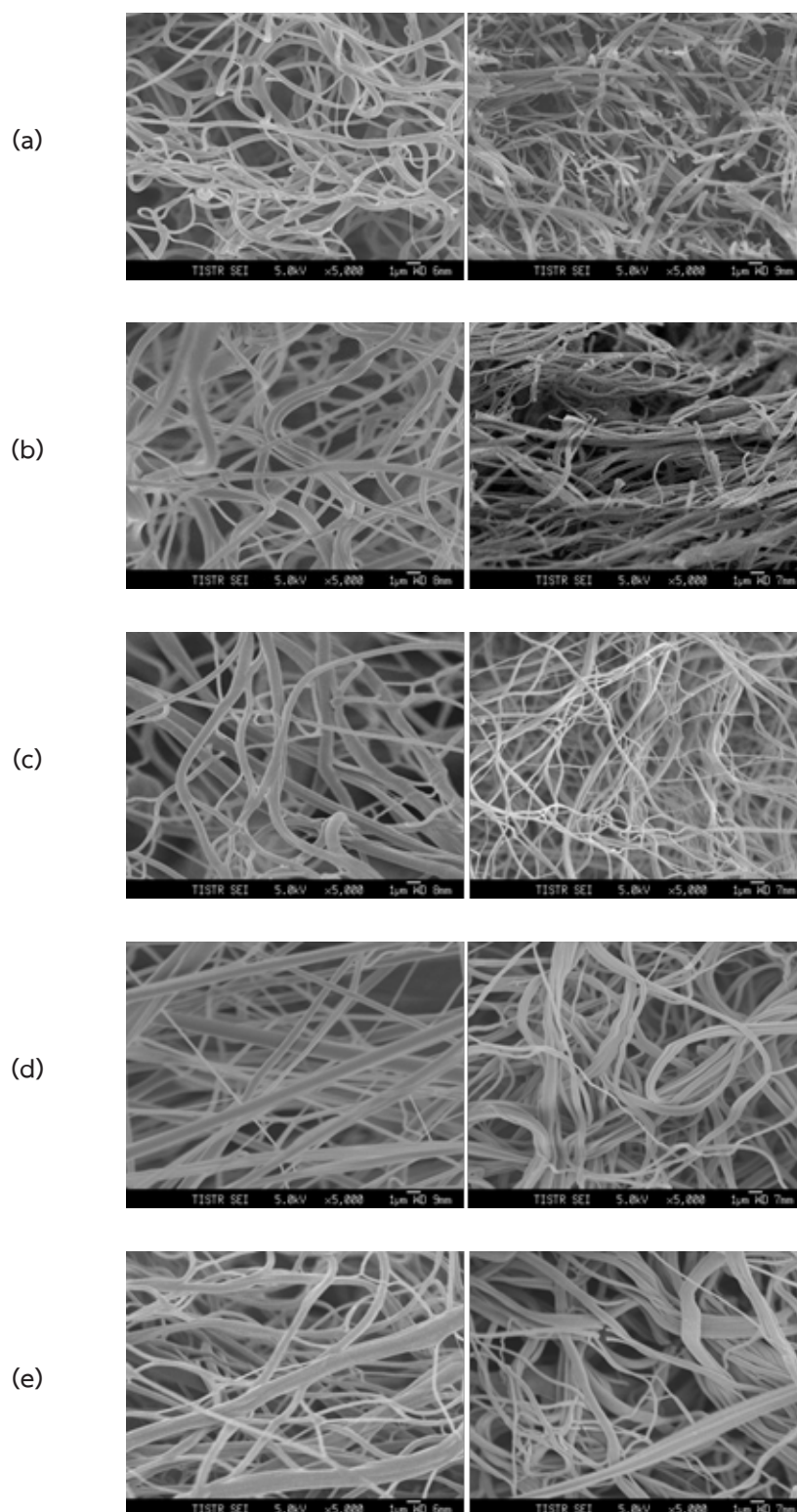


Figure 3. SEM images representing the surface morphologies of (a) $\text{Ti}(\text{OBu})_4/\text{PVP}$ fiber sponges, (b-e) the $\text{H}_2\text{WO}_4/\text{Ti}(\text{OBu})_4/\text{PVP}$ fiber sponges of tungsten to Ti molar ratios 0.5, 1.0, 3.0, and 5.0, respectively. Left column presents the fiber before calcination and right column presents the fibers after calcination at 450 °C for 200 min.

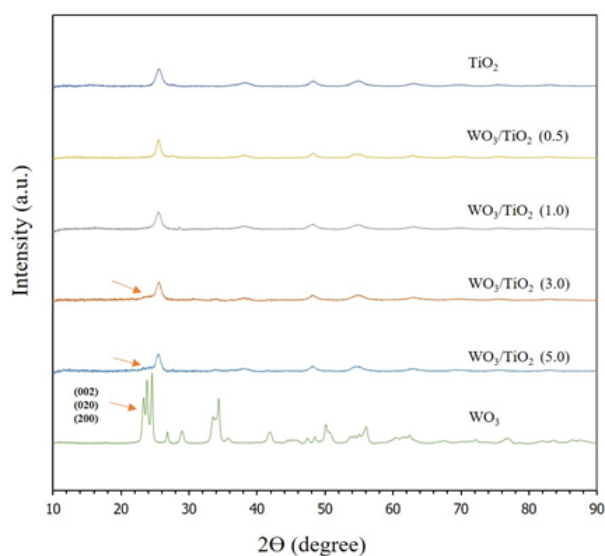


Figure 4. XRD patterns of TiO_2 fiber sponges and the WO_3/TiO_2 fiber sponges after calcination treatment.

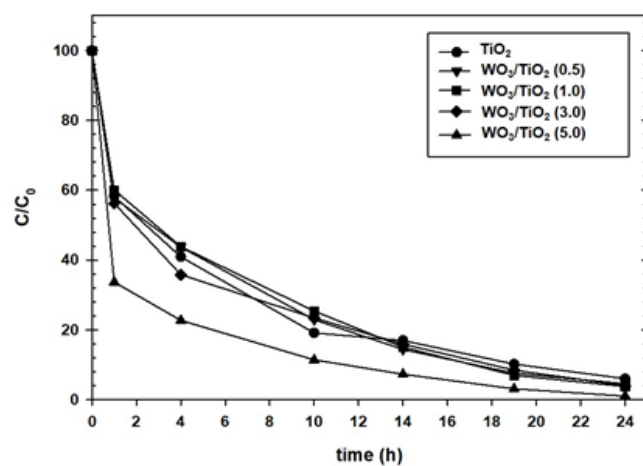


Figure 5. Photocatalytic degradation of methylene blue solution under UV radiation.

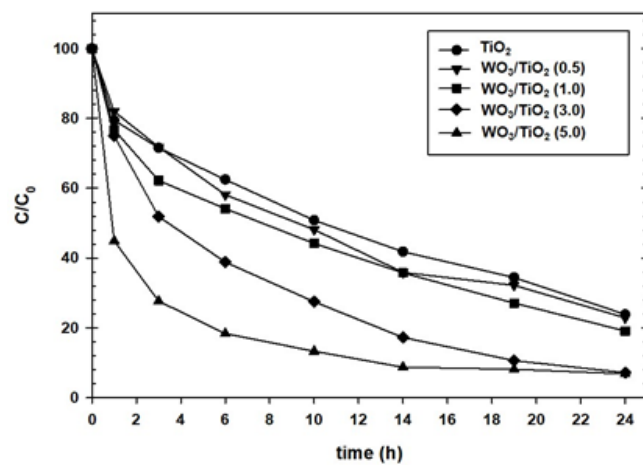


Figure 6. Photocatalytic degradation of methylene blue solution under visible-light radiation.

nanofiber sponges with smooth surface were observed on the $\text{Ti}(\text{OBU})_4/\text{PVP}$ and a series of $\text{H}_2\text{WO}_4/\text{Ti}(\text{OBU})_4/\text{PVP}$ fiber sponges. The average fiber diameter of $\text{Ti}(\text{OBU})_4/\text{PVP}$ fiber sponges was 430 nm, while the average fiber diameters of 480, 540, 573, and 645 nm were found in $\text{H}_2\text{WO}_4/\text{Ti}(\text{OBU})_4/\text{PVP}$ fiber sponges at molar percentage ratios of tungsten to Ti of 0.5, 1.0, 3.0, and 5.0, respectively. The increase in fiber diameter observed was due to the increase in the amount of H_2WO_4 loading in the fibers. After calcination treatment (figures on the right column), the average fiber diameters of fibers blown-spun from $\text{Ti}(\text{OBU})_4/\text{PVP}$ precursor solution was 340 nm, while the average fiber diameters of 351, 386, 414, and 479 nm were obtained from $\text{H}_2\text{WO}_4/\text{Ti}(\text{OBU})_4/\text{PVP}$ precursor solution of tungsten to Ti molar percentage ratios of 0.5, 1.0, 3.0, and 5.0, respectively. The smaller fiber diameters were observed in all cases, suggesting that the removal of PVP occurred during the calcination process.

The nanocomposite fiber sponges containing WO_3 and TiO_2 were further investigated to confirm the presence of WO_3 within by using X-ray diffractometer (XRD). After calcination treatment, hydrate water in H_2WO_4 was released, resulting in the formation of high crystalline WO_3 (Ke et al., 2018). XRD patterns revealed the peaks at 23.1° , 23.6° , and 24.4° , corresponding to the crystal planes (0 0 2), (0 2 0), and (2 0 0) of monoclinic WO_3 (Figure 4). The existence of weak peaks observed at 23.1° , 23.6° , and 24.4° confirmed that WO_3 were successfully formed in the nanocomposite fiber sponges. However, the characteristic peaks of WO_3 were not clearly seen in the nanocomposite fiber sponges with tungsten to Ti molar ratios 0.5 and 1.0 due to the low amounts of WO_3 within.

The photocatalytic activities of nanocomposite fiber sponges consisting of WO_3 and TiO_2 with various molar percentage ratios of tungsten to Ti (i.e., 0.5, 1.0, 3.0, and 5.0), in comparison to the TiO_2 nanofiber sponges, were further evaluated by photocatalytic degradation of methylene blue solution under both UV- and visible-light radiation. In Fig. 5, under UV radiation, the degradation of methylene blue solution using the TiO_2 nanofiber sponges or a series of nanocomposite fiber

sponges consisting of tungsten and TiO_2 were observed to increase progressively with increasing UV exposure time. After 1 h of experiment, the concentration of methylene blue was about 60% as found in all samples except nanocomposite fiber sponges at tungsten to Ti molar percentage ratio of 5.0, which was about 33%. After 24 h, the methylene blue solution was almost diminished, especially in nanocomposite fiber sponges at tungsten to Ti molar percentage ratio of 5.0 (about 1% left), while the remaining methylene blue were found in a range of about 4% to 6% for the rest of photocatalyst samples.

Figure 6 shows the methylene blue degradation profiles of the photocatalysts under visible-light radiation. The photocatalytic degradation rate of methylene blue solution under visible-light radiation was found to be slower than under UV radiation, however, the similar trend was observed. The photocatalytic degradation performance was found to increase with increasing WO_3 content. Interestingly, WO_3/TiO_2 (5.0) showed significantly higher performance in both UV- and visible light irradiation compared to the others. A sharp decrease in methylene blue concentration (around 55 -65%) was observed in the first hour. The faster degradation of methylene blue observed under UV radiation might be ascribed to the fact that the photogenerated electrons from TiO_2 could be transfer to the conduction band of WO_3 under UV radiation, leading to an increase in the lifetime of the photogenerated electrons and holes (Luevano-Hipolito et al., 2014).

Conclusion

Visible-light photocatalyst nanocomposite fiber sponges were successfully prepared from tungsten trioxide (WO_3) and titanium dioxide (TiO_2) by solution blow-spinning process. After calcination treatment, hydrate water of H_2WO_4 within nanocomposite fiber sponges was removed, resulting in formation of high crystalline WO_3 . The presence of WO_3/TiO_2 at tungsten/Ti molar ratio of 5.0 showed the best photocatalytic performance in degradation of methylene blue solution under both UV- and visible-light radiation.

References

- Chakornpradit, P., Phiriyawirut, M., & Meeyoo, V. (2017) Preparation of TiO_2/WO_3 Composite Nanofibers by Electrospinning. *Key Engineering Materials*, 751, 296-301.
- Chen, Z., Zhao, J., Yang, X., Ye, Q., Huang, K., Hou, C., Zhao, Z., You, J., & Li, Y. (2016) Fabrication of TiO_2/WO_3 composite nanofibers by electrospinning and photocatalytic performance of the resultant fabrics. *Industrial & Engineering Chemistry Research*, 55, 80-85.
- Doshi, J., & Reneker, D. H. (1995) Electrospinning process and applications of electrospun fibers. *Journal of Electrostatics*, 35, 151-60.
- Elahifard, M.R., Rahimnejad, S., Haghighi, S., & Gholami, M.R. (2007) Apatite-coated $\text{Ag}/\text{AgBr}/\text{TiO}_2$ visible-light photocatalyst for destruction of bacteria. *Journal of American Chemical Society*, 129, 9552-9553.
- Hayden, S.C., Allam, N.K., & El-Sayed, M.A. (2010) TiO_2 nanotube/ CdS hybrid electrodes: extraordinary enhancement in the inactivation of *Escherichia coli*. *Journal of American Chemical Society*, 132, 14406-14408.
- Ismail, A.A., & Bahnemann, D.W. (2011) Mesoporous Pt/TiO_2 Nanocomposites as highly active photocatalysts for the photooxidation of dichloroacetic acid. *The Journal of Physical Chemistry C*, 115, 5784-5791.
- Ke, J., Zhou, H., Liu, J., Duan, X., Zhang, H., Liu, S., & Wang, S. (2018) Crystal transformation of 2D tungstic acid H_2WO_4 to WO_3 for enhanced photocatalytic water oxidation. *Journal of Colloid and Interface Science*, 514, 576-583.
- Khil, M. S., Cha, D. I., Kim, H. Y., Kim, I. S., & Bhattarai, N. (2003) Electrospun nanofibrous polyurethane membrane as wound dressing. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 67, 675-679.
- Kment, S., Riboni, F., Pausova, S., Wang, L., Wang, L., Han, H., Hubicka, Z., Krysa, J., Schnuki, P., & Zboril, R. (2017) Photoanodes based on TiO_2 and $\alpha\text{-Fe}_2\text{O}_3$ for solar water splitting-superior role of 1D nanoarchitectures and of combined heterostructures, *Chemical Society Reviews*, 46, 3716-3769.
- Laursen, A., Kegnas, S., Dahl, S., & Chorkendorff, I. (2012) Molybdenum sulfides-efficient and viable materials for electro- and photoelectrocatalytic hydrogen evolution, *Energy & Environmental Science*, 5, 5577-5591.
- Li, D., Haneda, H., Hishita, S., & Ohashi, N. (2005) Visible-light-driven N-F-codoped TiO_2 photocatalysts. 2. Optical characterization, photocatalysis, and potential application to air purification. *Chemistry of Materials*, 17, 2596-2602.
- Linsebigler, A.L., Lu, G., & Yates, J.T. (1995) Photocatalysis on TiO_2 surfaces: principles, mechanisms, and selected results, *Chemical Reviews*, 95, 735-758.
- Luevano-Hipolito, E., Martínez-de la Cruz, A., Lopez-Cuellar, E., Yu, Q.L., & Brouwers, H.J.H. (2014) Synthesis, characterization and photocatalytic activity of WO_3/TiO_2 for NO removal under UV and visible light irradiation. *Materials Chemistry and Physics*, 148, 208-213.
- Mugunthan, E., Saidutta, M.B., & Jagadeeshbabu, P.E. (2018) Visible light assisted photocatalytic degradation of diclofenac using $\text{TiO}_2\text{-WO}_3$ mixed oxide catalysts. *Environmental, Nanotechnology & Monitoring Management*, 10, 322-330.
- Ofori, A.O., Sheikh, F.A., Appiah-Ntiamoah, R., Yang, X., & Kim, H. (2015) A Simple Method of Electrospun Tungsten Trioxide Nanofibers with Enhanced Visible-Light Photocatalytic Activity. *Nano-Micro Letters*, 7, 291-297.
- Szilagyi, I. M., Santala, E., Heikkilä, M., Pore, V., Kemell, M., Nikitin, T., Teucher, G., Firkala, T., Khriachtchev, L., Räsänen, M., Ritala, M., & Leskelä, M. (2013) Photocatalytic Properties of WO_3/TiO_2 core/shell nanofibers prepared by electrospinning and atomic layer deposition. *Chemical Vapor Deposition*, 19, 149-155.
- Wang, H., Zhang, X., Wang, N., Li, Y., Feng, X., Huang, Y., Zhao, C., Liu, Z., Fang, M., Ou, G., Gao, H., Li, X., & Wu, H. (2017) Ultralight, scalable, and high-temperature-resilient ceramic nanofiber sponges. *Science Advances*, 3, 1-9.

- Wang, X.P., & Lim, T.T. (2013) Highly efficient and stable Ag-AgBr/TiO₂ composites for destruction of Escherichia coli under visible light irradiation. *Water research*, 47, 4148-4158.
- Yadav, M., Yadav, A., Fernandes, R., Popat, Y., Orlandi, M., Dashora, A., Kothari, D.C., Miotello, A., Ahuja, B.L., & Patel, N. (2017) Tungsten-doped TiO₂/reduced Graphene Oxide nano-composite photocatalyst for degradation of phenol: A system to reduce surface and bulk electron-hole recombination. *Journal of Environmental Management*, 203, 364-374.
- Ye, L., Pelton, R., Brook, M.A., Filipe, C.D.M., Wang, H.F., Brovko, L., & Griffiths, M. (2013) Targeted disinfection of E. coli via bioconjugation to photoreactive TiO₂. *Bioconjugate Chemistry*, 24, 448-455.
- Zeng, Y., Wu, W., Soowohn, L., & Gao, J.H. (2007) Photocatalytic performance of plasma sprayed Pt-modified TiO₂ coatings under visible light irradiation. *Catalysis Communications*, 8, 906-912.
- Zhang, X., Fujishima, A., Jin, M., Emeline, A.V., & Murakami, T. (2006) Double-layered TiO₂-SiO₂ nanostructured films with self-cleaning and antireflective properties. *The Journal of Physical Chemistry B*, 110, 25142-25148.

Outside Cold Air Temperature Impact to Energy Consumption in Air Conditioning System Bangkok Metropolitan Area in 2019

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Abstract

Electrical consumption data from Metropolitan Electricity Authority (MEA) was collected and compared between normal warm weather in November, 2019 and cold weather in December, 2019 for seven days. Questionnaire asking target group as working people, elderly people and students and classified building type into office, single house, townhouse condominium or apartment with at least 100 samples each. Then, 10 personal interviews was focus group for each building type. It was found that air temperature in November 2019 was 23-33 degree Celsius while 18-28 degree Celsius in December (AccuWeather, 2020: TMD, 2019). In December 2019, the outside air temperature was in comfort zone during the daytime while in the night time it was below thermal comfort. It was found that nobody turned on air condition in the building both in the office during the daytime and in their residences during the night time. Calculating electrical cost at 4.22 baht per kilowatt-hour (MEA, 2020) the energy reduced roughly about 1,000 MW per hour which cost about 4,220,000 baht hourly, 101,280,000 baht a day, or 708.96 million baht a week. It equivalent to 1,000,000 ton of split-type air condition or 500,000 ton of chilled water air condition system.

Keywords: Energy consumption, Cool weather, Cooling load, Metropolitan Electricity Authority (MEA), Enthalpy

Introduction

Business and residential sectors have majority electrical energy consumption in Bangkok area, about 69 percent of total consumption. Bangkok has lack of database as how many tons of air-conditioning installed and operated in Bangkok Metropolitan area. To calculate back during the cold weather compared with normal weather with required air-conditioning system, we would estimate actual requirement and Cooling Load demand. During December 2019, the air temperature had reduced rapidly for about 2 weeks. It was an opportunity to explore how people in Bangkok area behave. Moreover, it would be possible to gather residential building performance as the infiltration effect to users.

Most houses, townhouse, and condominium in Thailand have a lot of air leakage construction joint detail since it has no need to design an air tight system. People will stay indoor most of the day almost 24 hours. Stay in their rooms during the night, stay in office building during the day, and stay in car, bus, and train during travelling. Therefore, to make comfort built environment for people is more important. To design energy efficient building, the air tight construction details are very important but it also needs the appropriate mechanical system to circulate indoor and outdoor fresh air. It requires extra operation cost which most residents in Thailand have more concerned.

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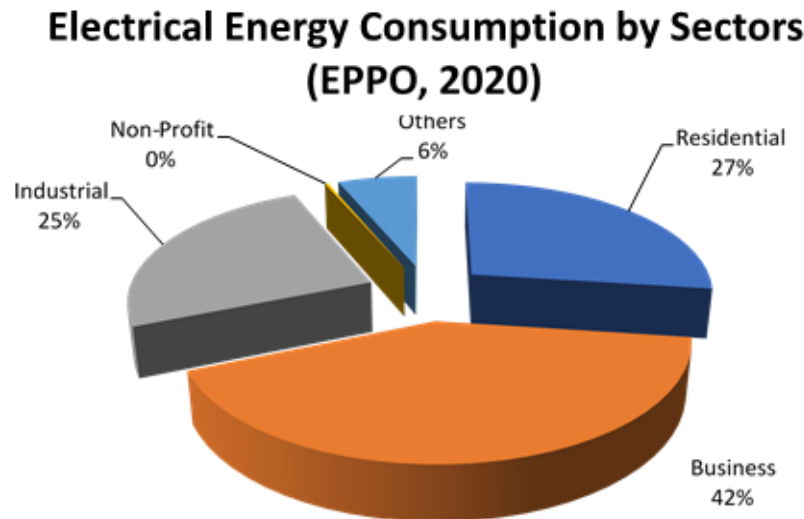


Figure 1. Electrical Energy Consumption by Sectors (EPPO, 2020).

Data from Energy Policy and Planning Office (EPPO, 2020), Ministry of Energy has shown that more than half of energy budget in urban area are office and residential buildings. If we promote energy conservation to those sectors, it can reduce a significant number of energy budgets. During daytime, people are at work places in their office buildings. Buildings have heat gain all day long. It is a reason that the energy consumption is almost double compared to residential buildings which operate during night time without solar radiation heat gain. Even energy conservation regulation has been enforced since 1992 but most architects still in a process of develop energy conversion design experiences. From then, it is about 25 years. Small number of architects can design energy conservation buildings. Building life is another influence factor that costs energy consumption until the end of their structure which is about 30 years or more. Then, the portion of energy conservation buildings are still very less in urban energy budget consumption.

Installing air condition systems in residential buildings are mostly estimated by local contractors which using traditional cooling load calculation as 1,000 to 1,200 Btu per square meter. Normally, they would install an oversize air conditioning system for the only reason that they would not be blamed if it is not cool fast enough. This situation will effect high energy consumption and also cause health problem since high hu-

midity and cool indoor air temperature inside a room. Fungus and bacteria would have good environmental condition to grow and enjoy propagation.

Methodology

This research collected electrical consumption data from Metropolitan Electricity Authority (MEA) by comparing the amount of electrical consumption between normal warm weather and cold weather in the same working day (Monday through Friday) and holiday for seven days. Then, researcher conducted Google questionnaire asking target group as working people, elderly people and students. Questionnaire classified building type into office, single house, townhouse condominium or apartment with at least 100 samples each. Researcher conducted 10 personal interviews as focus group for each building type.

Results

Using questionnaire (December 4th-10th, 2019) asking working people, elderly people, and students, It is found that not even single person turned on air condition in the building both in the office during the daytime and in their residences during the night time. During a week of December 4th to 10th, people never turned on air conditioning system because the weather is very cold more than their comforts. Even during the night time, when they sleep, they closed all windows

and doors but they still put the blanket on and they still feel very very cool. This happened because construction in Thailand has a lot of infiltration details. Those infiltrations occurred since they need natural ventilation which is normally practice in hot and humid climate. Therefore, when the weather is very cold, cold air outside building can easily leaks into rooms and indoor spaces.

Comparison on Wednesday November 6th and December 4th, 2019 in Figure 2 (a) showed the parallel curves during working day. Graph profile has lowest consumption on 4:00 -5:00 am because of low ambient outside air temperature and all inside materials has the same temperature as inside air temperature. Then, it raised from 6:00 am because of people begin to weak up and use all appliances until peak load at 11:00 am and 14:00 pm. During office hours, water cool chiller would be turned on around 7:00 am (about 2 hours before start working) till 4:30 pm (half an hour before the end of working hour). During lunch time, most split type air condition system would be turned off according to national energy conservation policy. Then, electrical consumption dropped between noon to 1:00 pm. Then, after working hour, people travel back to their residences turning on their split type air conditioning systems. Energy demand is still high constantly. Graphs still remained flat until 11:00 pm because of most split type air condition systems cool down indoor air, room elements (wall, ceiling, and floor), and all furniture. When all room components have their temperature close to indoor air temperature set point, cooling load has reduced. Therefore, consumption graphs dropped down until people weak up and sun rise again in the next day.

Comparison on Thursday November 7th and December 5th, 2019 (the national holiday) in Figure 2 (b) showed flat curve of December 5th and normal consumption curve on November 7th which is working day. Graph profile has shown the different electrical consumption between working and holiday demand. During night time and after working hours, the different between working day and holiday is about 1,000-2,000 MW. From 6:00 pm – 9:00 pm electrical consumption slightly increased since Thai people celebrating King

Rama 9 Birthday (December 5th). Then, energy demand reduced. The different during working hours of working day and holiday started from 8:00 am till 5:00 pm which was about 3,000 MW. It can be analyzed that the amount of energy consumption from air condition system in office building has a lot more demand than residential systems. If we assumed than office use split type air conditioning system, it would be 250 million ton of split type or about 125 million ton of chill water air conditioning system.

Comparison on Friday November 8th and December 6th, 2019 in Figure 3 (a) showed the slightly parallel curves during working day. Graph profile in December 6th has lowest consumption on 0:00 -5:00 am because people began to turn off all resident air condition systems according to outside cool air temperature. It would take about 2 days for building materials cool down from cold weather since most building envelopes are heavy with high mass and high heat capacity property such as masonry and reinforced concrete. Graph profiles during working hours are still the same shape with about 1,500 MW different. This can be analyzed that low outside ambient air temperature increased air conditioning efficiency and reduce all cooling load from hot air infiltration. Outside cool air has low enthalpy with low relative humidity (low grain of moisture also).

Comparison on Saturday November 9th and December 7th, 2019 in Figure 3 (b) showed the smoothly parallel curves during holiday. Graph profile illustrated the outside low enthalpy effected air conditioning load. Most resident air conditioning systems have been turned on according to city people behaviors. It rose up during 8:00 am till 5:00 pm and slightly consumed until midnight because of tomorrow is still another holiday. They do not have to hurry weak up to work. The electrical consumption has about 1,000 MW different.

Comparison on Sunday November 10th and December 8th, 2019 in Figure 3 (c) also showed the smoothly parallel curves during holiday. Compared with Saturday in November, the peak load was about 5,000 MW while it was 4,000 MW on Sunday. It is 1,000 MW lower because of people stay home and relaxes. In December, peak load on Saturday was about 5,000 MW

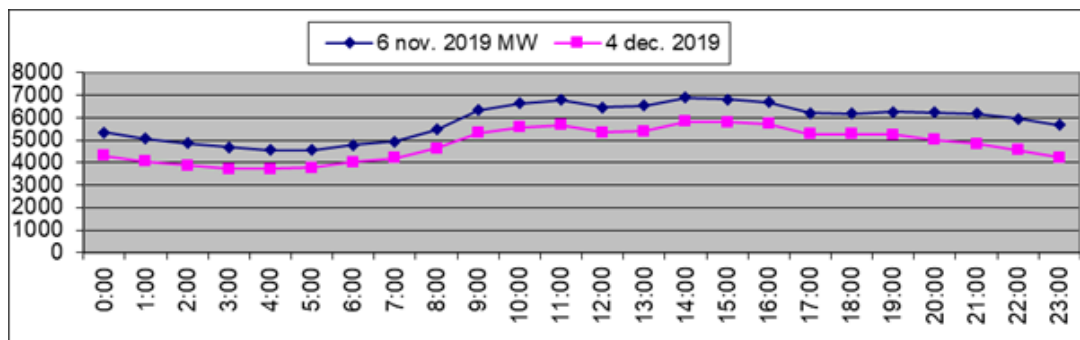
while it was 4,000 MW on Sunday. It is also 1,000 MW different. People behavior remains the same between Saturday and Sunday activities. It can be analyzed that outside cool weather and low enthalpy reduce energy budget and cooling load at the same time.

Comparison on Monday November 11th and December 9th, 2019 in Figure 3 (d) showed the similar curves during working day. It has closed to 7,000 MW peak load in November and 5,500 MW in December about 1,500 MW different. After office hours, the different was about 1,000 MW. In December, energy demand had rapidly reduced from 9:00 pm till 5:00 am since all people turned off all resident air conditioning systems.

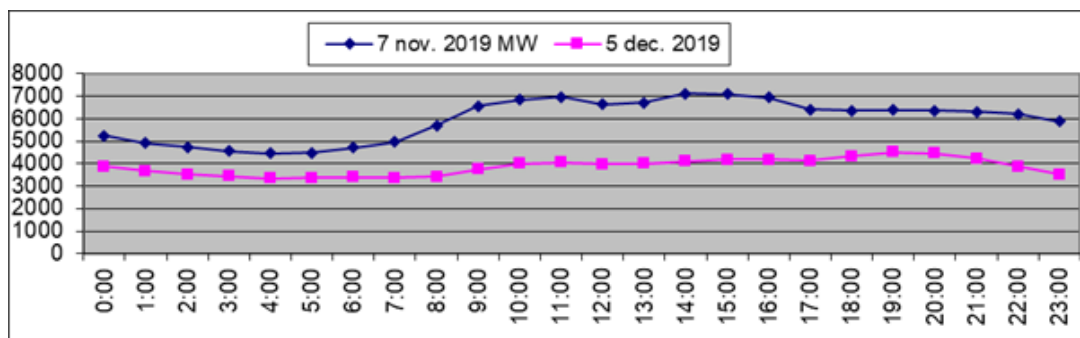
Comparison on Tuesday November 12th and December 10th, 2019 in Figure 3 (e) also showed the similar curves during working day in November while it has lower curve in December. The different consump-

tion during working hours was about 1,500 MW while it was 1,000 MW after working hours. All graphs are similar to Monday.

Electrical consumption from Metropolitan Electricity Authority (MEA, 2020) illustrated that 1,000 MW per hour roughly in average has been reduced during December 4th to 10th, 2019, compared to the same day in November 2019. December 4th, 6th, 9th, and 10th were working days. The electrical consumption was about 1,000 MW different in every hour (Figure 2 (a), Figure 3 (a), (d), (e)). December 5th, 7th and 8th were holidays. It was about 4,000 MW consumption average (Figure 2 (b), Figure 3 (b), (c)). Electrical consumption different was remain similar to those working days which is 1,000 MW hourly. Peak load ranged from 9:00 am till 9:00 pm which was about 5,000 MW consumption in working days. Then, peak load during day time in holi-

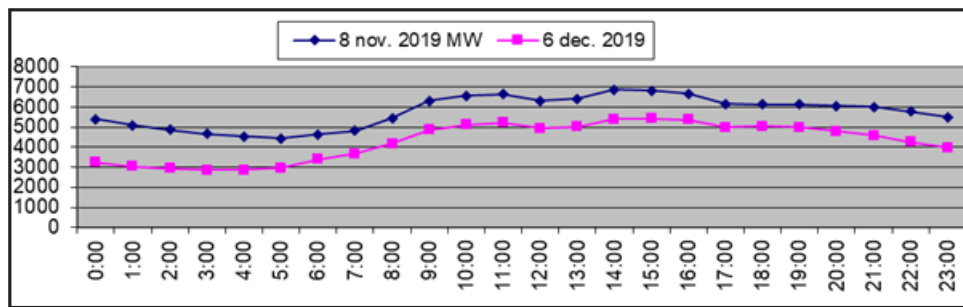


(a) Wednesday

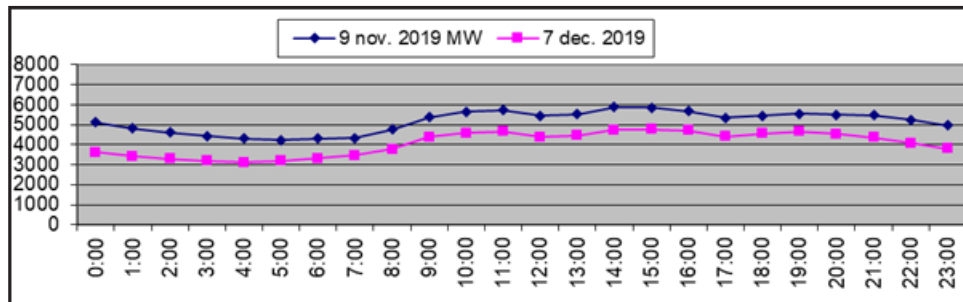


(b) Thursday

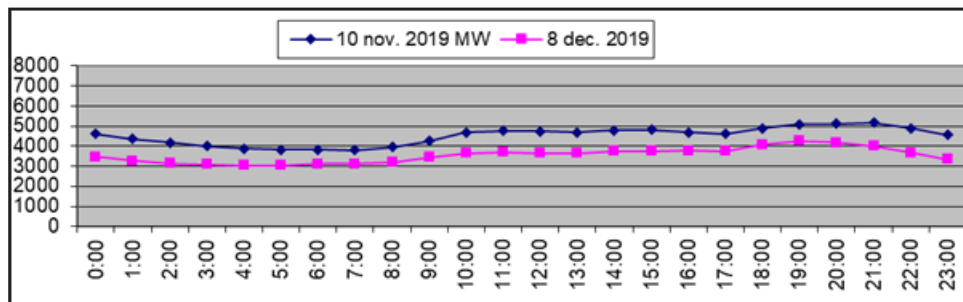
Figure 2. Electrical consumption in Bangkok area comparison between November 6th-7th (warm climate) and December 4th-5th (cold climate), 2019. During working days from Wednesday to Thursday have shown in (a) and (b).



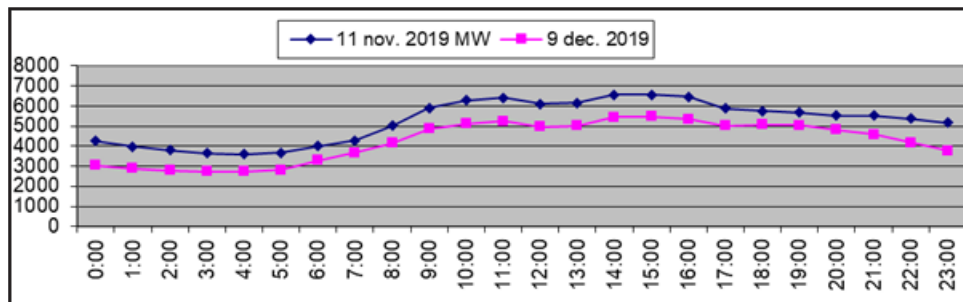
(a) Friday



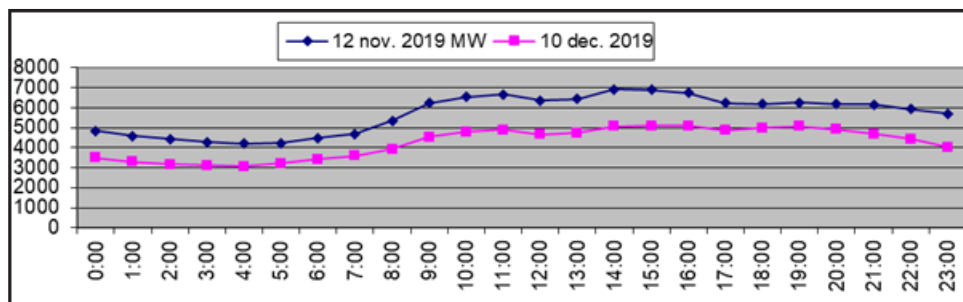
(b) Saturday



(c) Sunday



(d) Monday



(e) Tuesday

Figure 3. Electrical consumption in Bangkok area comparison between November 8th-12th (warm climate) and December 6th-10th (cold climate), 2019. During working days from Friday to Tuesday have shown in (a), (d), and (e) respectively, while Saturday and Sunday is (b) and (c).

Table 1 Electrical Consumption Summary During Cool Weather in December 2019 Compared to the Same Day with Normal Ambient Air Temperature in November 2019.

	2019 (2562)	MW different (hourly average)	Min. air temp.	Max. air temp.
Wednesday	6 nov. & 4 dec.	1,300	26 & 19 C	33 & 28 C
Thursday	7 nov. & 5 dec.	2,000	26 & 18 C	33 & 27 C
Friday	8 nov. & 6 dec.	2,000	25 & 18 C	32 & 28 C
Saturday	9 nov. & 7 dec.	1,300	24 & 17 C	31 & 27 C
Sunday	10 nov. & 8 dec.	1,000	20 & 19 C	31 & 27 C
Monday	11 nov. & 9 dec.	1,000	23 & 18 C	32 & 28 C
Tuesday	12 nov. & 10 dec.	1,000	23 & 18 C	33 & 29 C

days about 4,000 MW.

Conclusion and discussion

Air temperature in Bangkok in November 2019 was highest 31-33 degree Celsius while 27-28 degree Celsius highest in December (AccuWeather, 2019). It is 3-5 degree Celsius different. Outside air temperature in Bangkok in November 2019 was the lowest 23-26 degree Celsius while the lowest 18-19 degree Celsius in December 2019 was (TMD, 2019). It is 4-7 degree Celsius different. In December 2019, the outside air temperature was in comfort zone during the daytime while in the night time it was below thermal comfort. It was also rapidly cold, then people still got used to the previous experience of warm weather.

Calculate only electricity cost at 4.22 baht per kilowatt-hour (MEA, 2020) the energy reduced roughly about 1,000 MW per hour which cost about 4,220,000 baht hourly or 101,280,000 baht a day. It's worth 708.96 million baht a week. This is a significant number for urban planning, architect, engineer, designer, and MEA to recognize and draw actual plan to invest in environmental design reducing ambient air temperature. If we calculate back into cooling load for household using split-type air conditioning system turn on for 10 hours during night time and the rest turn on during office hours, it would be 1,000,000 ton of split-type air condi-

tion or 500,000 to of chilled water air condition system. Now we can estimate cooling load which is actual operate in Bangkok area regularly. If we renovated or replace those air conditioning systems and increase double efficiency, energy consumption will reduce in half. It would be 18,432.96 million baht saved annually. This number would help governors running economic model and its rapid payback to promote energy conservation regulation and loan funding.

To make urban area beautiful and rich nature, those amount of saving money can help to develop more green spaces in street furniture, city park, garden, open space, water pond, etc. many research results have been proposed to increase green area in urban area. If we balance green area as a surface to receive solar radiation rather than hard surfaces of construction, the ambient air temperature would reduce dramatically. It affects all cooling load in all buildings, consequently.

Acknowledgements

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References

- AccuWeather, Inc. (2019). <https://www.accuweather.com/th/th/bangkok/318849/november-weather/318849?year=2019> Accessed on 12 December 2019.
- Energy Policy and Planning Office (EPPO), Ministry of Energy. (2020). [http://www.eppo.go.th/index.php/en/en-energystatistics/electricity-statistic?orders\[publishUp\]=publishUp&issearch=1](http://www.eppo.go.th/index.php/en/en-energystatistics/electricity-statistic?orders[publishUp]=publishUp&issearch=1) Accessed on 31 March 2020.
- Meteorological Department of Thailand (TMD), Ministry of Digital Economy and Society. (2019). https://www.tmd.go.th/weather_report_3hour.php?StationNumber=48455 Accessed on 12 December 2019.
- Metropolitan Electricity Authority (MEA). (2020). <https://www.mea.or.th/profile/109/111> Accessed on 29 March 2020.

Innovative Learning Management for the local wisdom on Herb using Local Community Resource Base

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Abstract

The purposes of this research are; 1) To study local wisdom on herbs of Chaiphum province 2) to prepare learning lesson related to local herbs 3) to disseminate and promote local wisdom and local herbs, learning sources of herbs in Chaiphum province using the qualitative research method, The main informants of the research consist of 9 people, namely village philosophers living in Sai Ngam and Ban Na Si Nuan villages in Na Siao Subdistrict, Mueang District, Chaiphum Province. Tools used in the research are interview forms and field record form. Data derived from the interview are analyzed using content analysis protocol.

Findings in this research reveal that: 1) In the study of local wisdom on the herb of 90 types in Chaiphum province, plants with the most medicinal properties include; *Dracaena angustifolia* Roxb (ต้นคอนหมา), *Cryptolepis dubia* (เถาเอ็นอ่อน), Caper tree peels (เปลือกบก), Phlai herb (ว่านไพล), Camphor Tree leaves (ใบหนาด), Turmeric (ขมิ้นชัน), *Croton persimilis* root (รากเปล้าใหญ่), Bergamot peels (เปลือกมะกรูด), used to cure tendon, tendon stretch, back stiff, tendon pain, ligament pain. To use, make it into a hot compress ball. 2) Preparation of lessons about the development of local knowledge by creating a CD of Medicinal Plant Database and Medicinal Plant Documents consisting of photos, medicinal plant name, scientific name, cultivation, properties, and herbs application to be used for learning in communities, schools and for interested people. 3) In the dissemination of local wisdom and innovation of local herbs as a source for learning herbs in Chaiphum Province to disseminate knowledge to benefit those interested and can be used as a source for learning herbs, the community can learn how to use herbs as food and medicine which will help to participate in preserving herbs for sustainability.

Keywords: Innovative Learning Packages, Local herb wisdom, Community resource base

The importance of the problem

Thailand's 20-year Research and Innovation Strategy (2017 - 2036) aims at creating basic knowledge and understanding of Thai wisdom and Local knowledge in living with quality of life to recognize self-reliance, and health care (20-year Research and innovation Strategy 2017 - 2036, 2017, pages 68 - 69). National Master Plan on Thai Herb Development No. 1, 2017 - 2021 defines the strategy in developing Thai herbs, health promotion, and disease prevention in the community. The Local Government Organization has a plan to promote the uses of health herbs wisdom and primary care in integrating with local medicine wisdom promoting local herbs for health care and disease prevention (Ministry of Public Health. (2017). National Economic and Social

Development Plan 2017-2021 for agricultural development has created and transferred academic knowledge, science, technology and innovation and local wisdom in participatory agriculture to support the utilization of biological resource bases, respond to the quality of life of people and the environment of the country including supporting the development of sustainable agriculture through the participation process. In the study of alternatives and the determination for the process of study, research, development, and mechanisms for the community to be aware of health and natural products that safe for both personal and environmental, the medicinal plant is one option that is commonly used as food. The wisdom of Thai medicinal plants has always been with Thai people since ancient times until now

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although nowadays has been developed with technology to be innovative and into various herbal products but the transfer of knowledge of local wisdom, knowledge transfer will make learners understand, be able to bring into practice. Local wisdom transfers knowledge to learners automatically without learning from the institute, but it will use the Socialization Process method created by imitating and remembering to inherit within the family and it deploys demonstration as transfer method to make an example, explaining every step to learners to understand and then bring into real practice. Demonstrate and put into practice, practice repeatedly until becoming proficient because the work that will be used for a living must be actual work that can be utilized. Learners or target groups that receive wisdom knowledge transferred from knowledgeable people have a belief in keeping a promise for the ancestor to cover up the secret in the technology is not properly developed (Ratana Bua Son (1992). Teaching and learning innovation of modern teachers in order to improve the skills of learners in the 21st century currently has created teaching materials and developed into teaching and learning innovation to provide learners with important and necessary skills for learners in the 21st century to meet the goals of national curriculum according to National Education Plan 2017-2036 which link to national strategies with the goal in developing the potential of people of all ages and the creation of a learning society for all types of innovation that are transferred while providing examples at actual locations to help readers to understand clearly.

Chaiyaphum Province has natural resources and the environment with 50% forest area, which is a local herbal forest called "Phu Laen Kha". It's a natural asset suitable for the growth of herbs. There are knowledgeable people or local sages, folk doctors in the province who have the experience, herbal wisdom that has been registered and granted for professional rights for a total of 36 people. The strategy of Chaiyaphum Province is, therefore to propel for the innovation of community herbs to carry on the preservation of Thai wisdom about herbs to be recognized and to be used for planting, food and medicine processing for quality of life of people

(Chaiyaphum Farmers Council, 2017). From the result of research on the inheritance and transfer process of folk medicine wisdom leading into the health care of people in Ban Sai Ngam Village No. 3 and Village No. 12, Na Siao Sub-district, Mueang District, Chaiyaphum Province, the results reveal that for the community way of life, the folk healers have a tied relationship with the community forests. There has been herb collecting activity since the year of 1950, with knowledgeable doctors who have local wisdom in using herbs to cure the diseases. For methods in the inheritance of knowledge and local wisdom in using herbs, there are folk healers in the province that transfer knowledge to public health volunteers while there is Miang Khing (wrapped in leave ginger), a recipe containing ingredients like Black Galin-gale (กระชายดำ), Long pepper (ดีปลี), Toon Caladium (หัวทูน), Ginger (กระเทียม), Phlai (Bengal root ว่านไพล), dried chilies, Crataeva (กลุ่ม) Peels, Pipe Tamarind (มะขามเปียก), Brown sugar (น้ำตาลทรายแดง), Odized salt (เกลือไอโอดีน), Elephant garlic (กระเทียมโทน) which is the ancient formulas from Ban Anu Ban transferred to the new generation alongside the Sai Ngam village (Monthan Penporn Chai-pakdee, 2020)

Chaiyaphum Rajabhat University has introduced the King's Philosophy for the local development in accordance with the sufficiency economy philosophy for self-reliance for the dissemination of local herbs to be a community learning center in Chaiyaphum province. With a research fund from the National Research Council of Thailand 2018 on the succession process of folk medicine wisdom leading to the health care of the villagers in Ban Sai Ngam Village No. 3 and Village No. 12, Na Siao Subdistrict, Mueang District, Chaiyaphum Province. It, therefore, has been extended to the research on the learning innovation for knowledge of local herbs from the resource base of the community focusing on people to use a source of learning herbs for medicine and herbal food and preserving the environment and herbs in the community for the sustainability

Research Objectives

1. To study local wisdom on herbs in Chaiyaphum Province

2. To create a lesson about the development of local wisdom on herbs
3. To disseminate local wisdom on herbs, and herb learning center in Chaiyaphum province

Expected Benefits

1. To obtain information about local wisdom on herbs in Chaiyaphum province where management in the production and development of medicinal plant network in Chaiyaphum province are fully implemented
2. To obtain the practical guidelines in the management of learning resources with community participation in continuously driving the activities which result in the development of strong communities and a sustainable environment.
3. The community has continued to create awareness in the community to recognize and preserve the importance of local herbs in the community.

Research Extent

1. Areas of study include Sai Ngam Village, Ban Na Si Nuan, Na Siao Sub-district, Mueang District, Chaiyaphum Province
2. Sample population used in the study and providing information are folk philosopher on herbs in Sai Ngam Ban Na Si Nuan Village, Na Siao Sub-district, Mueang District, Chaiyaphum Province
3. The research period is January 2019 - December 2019

Research Methods

1. Plan to collect data, create tools for data collection according to objectives
2. Study the concepts, theories related to medicinal plants from various documents and from individuals

The researcher searches for knowledge from relevant research documents, books, academic documents and news from the website and from experienced persons, study topics of medicinal plants used in the treatment of diseases from Thai herbal textbook, research results on Thai traditional medication, and various research reports about medicinal plants.

1. Population and samples

1.1. To study and survey of data in the area, the researcher has chosen to study the use of herbs, herbs used to treat diseases in the garden areas of Baan Sai Ngam Herb, Ban Na Si Nuan, Na Siao Sub-district, Mueang District, Chaiyaphum Province which are sources of over 90 medicinal plants in an area of 9 rai. The researcher meets with Phra Kru Kittithamworakhun, a monk dean of Na Siao Sub-district an abbot of Wat Pa Phatthanatham temple at Na Siao Sub-district, Mueang District Chaiyaphum province, the grower and the planter who takes care of the herb garden in Pa Phatthanatham Temple to clarify the purpose of the study to understand and to request in exploring and studying the use of herbs for the treatment of diseases, as well as various herbs in the herb garden of Wat Pa Phatthanatham Temple and Sai Ngam Community. The re-

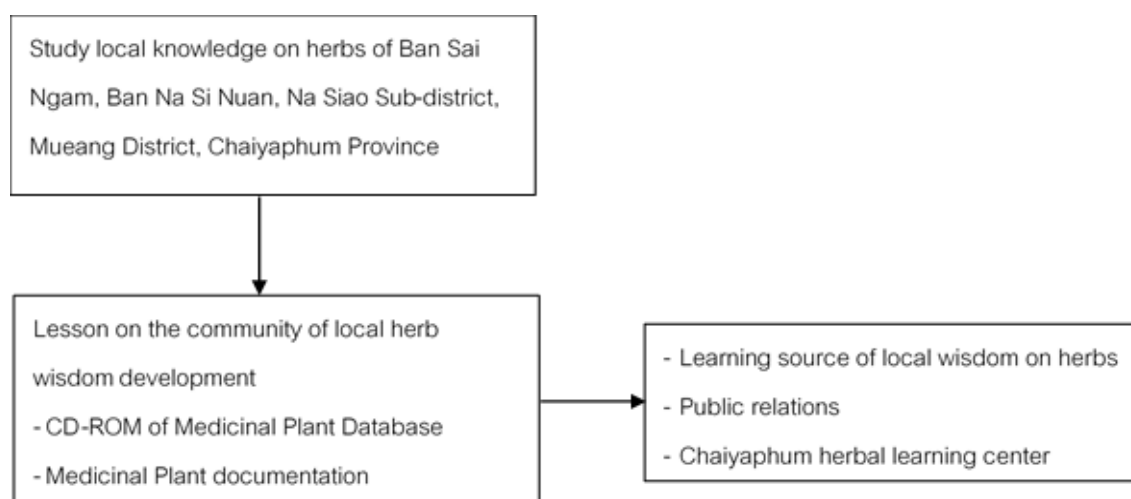


Figure 1. Research Conceptual Framework

searcher requests permission from the village headman, community leader, village health volunteer to interview the local philosophers and folk healers at Ban Sai Ngam, Na Siao Sub-district, Mueang District, Chaiyaphum Province to collect research data. The researcher used the research methods as follows

1.2 Interview with the local philosopher and folk healers 3 times 9 persons at Sai Ngam village, Na Siao Subdistrict, Mueang District, Chaiyaphum Province.

1.3 Interview with individuals or a group of people who use the Baan Sai Ngam Herb garden, about the study of various knowledge of herbs in the community, natural planting sources, uses of herbs as food and medicine, properties of each type of herbal, the method in using herbs for food and treating diseases. The interviews are conducted 3 times with 9 people.

2. Research tools

In this study, the researcher conducts qualitative research. The tools used in this study are :

1. The survey form, to survey of medicinal plants grown in Wat Pa Phattham Temple, Ban Na Si Nuan and Ban Sai Ngam, Na Siao Sub-district, Mueang District, Chaiyaphum Province to collect data of name, type, properties, and parts of medicinal plants

2. The interview form, an interview with Pra Kru Kittitham Worakhun, a monk dean of Na Siao District and is the abbot of Wat Na Siao Subdistrict, Mueang District, Chaiyaphum Province.

3. Medicinal plant database record form to disseminate knowledge about the herbs in Sai Ngam villages and herbs in Pa Phatthatham Temple, Na Siao Sub-district, Mueang District, Chaiyaphum Province.

4. Interview forms about the use of herbs for treating diseases of the herb gardens in Sai Ngam village and herbs in Pa Phatthana Tham Temple, Na Siao Sub-district, Mueang District, Chaiyaphum Province.

3. Data Collection

In this research, the researcher collects data covering all the contents according to the studied issues as follows;

1) Study documents, articles, books, and related research from academic experts to be used as research guidelines

2) In-depth interview about herbs information based on the main questions according to the needs and convenience of the informants

3) Observation, the researcher uses the principle of participatory observation to help find and evaluate information during the interview and records the required data later.

4) Field records, the researcher use both shorthand lecturing and tape recording methods during the interview. After that, the researcher transcribes the tape and collects and organizes all recorded data every time after the interview to record all the events occurred and then verify and update to be correct and complete.

4. Procedures for field data collection in the field

4.1 Community preparation, the researcher goes to study in the area full time. The researcher arranges the interview at home or depending on the convenience of samples. To become familiar with the samples for qualitative data collecting, the informants can perceive value and the benefits of research.

4.2 Observation, it's the methodology to analyze or find out the relationship of the incident with other things in which the researcher uses participant observation as an observation methodology where the researcher does not involve in the event to be interviewed. The researcher follows the interview with the informants according to the questions that the researcher creates.

4.3 Data collecting by in-depth interview uses interview guidelines as follows

4.3.1 The first in-depth interview, the researcher starts by talking about general topics in order to create a friendly atmosphere in the form of person to person conversation rather than an official interview. The researcher behaves on the basis of good manners and punctuality being a good listener, sensitive to both the emotional reaction and to a matter of information, accepts the behavior that the sample group is expressing, not much sticking with the sequence of the question while linking to the issue using conversation. Researcher exchanges with the samples for opinion, belief, life experiences which are subjective using broad questions to open issues with the sample group as open-ended

questions.

4.3.2 The researcher records the conversation during the interview, summarizes important issues that have been interviewed for informants to verify for accuracy, arranges an appointment for the next interview, gathers and categorizes information received from interviews, simultaneously transcribes the recorded interview and analyze the data to understand the content of the interview while looking for definitions that are not clear and some issues that are still missing to ask the next sample group

4.3.3 The Duration of the interview is 30-45 minutes. Each session, the number of times used for the interviews is between 2-3 times by mainly taking into account the suitability and completeness of data derived from the in-depth interview each day, 1 interview per day. The second in-depth interview is approximately 4-5 days from the first interview. This depends on the convenience of each time appointment but not for more than 15 days. The second interview will be an interview based on additional questions to gather the information that may be missing from the 1st interview. After the interview, the interview content will be reviewed so that the sample informants will be informed for information from the 1st interview to add or edit further information if needed

4.3.4 Time and place of interview will depend on the convenience of the sample informants which is usually at homes of the samples where following up of interview can be possible

4.3.5 The interview will be completed once all the information required is sufficient and the researcher expresses the appreciation for the cooperation of the sample groups for shared valuable experiences.

4.4 Data Validation Checks

The researcher uses methods in checking the reliability of the data using the following methods.

4.1.1 Data validation is conducted every time the data is studied. The researcher uses a qualitative data validation method called the triangular inspection from the data source as follows

1) Individual sources, by examining the infor-

mation of the same topic but different individuals to acquire different information and for evaluating if the data that has changed or not. If checking and receiving the same information every time, it means that the information acquired is reliable. However, if the acquired information conflicts, the researcher will repeat the interview to collect further information and verify with other informants again.

2) Source and place, by interviewing the same informants in different locations, such as interviewing the local philosopher and observing the similarities and differences of the data, if the information obtained is the same, it is considered accurate

3) Checking the same data but at different times, such as morning, afternoon, or evening. It sometimes may require defining the interval between the sessions.

Data Analysis

Data analysis is performed simultaneously with data collection. Qualitative data analysis is conducted using Content Analysis consists of 3 analysis principles (Mile and Huberman, 1994) including data organizing, data display, and conclusion, interpretation, and verification of the research results in order to obtain the ideas about local wisdom on medicinal plants. The information obtained from the in-depth interview relative to the questionnaire is collected and organized into categories, summarized, analyzed, and used for discussion according to the process of qualitative data analysis.

Results

1. In the study of local wisdom on herbs of Sai Ngam Village and Na Si Nuan Village, Na Siao Subdistrict, Mueang District, Chaiyaphum Province from the survey and sampling of medicinal plant species derived from Wat Pa Phattham, Ban Na Si Nuan Around Sai Ngam homes, consisting of 90 species, the results reveal that for herbs in the area used as medicine for external use as hot herbal compress ball the most commonly used herbs (Table 1).

In using dried ginger for cuisine as leave-wrapped ginger, it is found to be very nutritious which

contains the following ingredients;

1) The main ingredient includes 3 portion ginger, but if cooked for a lactating woman, add small fried ginger 1 portion.

2) Secondary ingredients are Black Galingale (กระชายดำ), Long pepper (ดีปลี), Toon (Caladium (หัวทูน) 1 part for each. Other parts include Ginger (กระเทียม), Phlai (Bengal root ว่านไฟล) ½ part for each

3) The flavor enhancers to add are dried chilies, Crataeva (กุ่ม) Peels, Pipe Tamarind (มะขามเปียก), Brown sugar (น้ำตาลทรายแดง), Odized salt (เกลือไอโอดีน), Elephant garlic (กระเทียมโทน)

4) Side dishes include Piper Lolot (ชะพลู) Star Gooseberry leaves (ใบมะยม), Raw Bananas (กล้วยดิบ)

2. Create lessons for the community about local wisdom development for Sai Ngam Village, Na Siao Subdistrict, Mueang District, Chaiyaphum Province with information on medicinal plants, photos, scientific names, medicinal properties, and parts of herbs used, and propagation for the benefit of visitors while focusing on the importance of the propagation of herbs in the community.

3. In the dissemination knowledge and local knowledge for learning resources, the local knowledge center is established to disseminate local wisdom in Chaiyaphum province. The learning center consists of CD-ROM of medicinal plants database, medicinal plant documentation

Table 1. The most commonly used herbs.

Common Name	Scientific Name	Properties	Parts of herbs that are used	Propagation
- Khon Mah ตันคอนหมา	Dracaena angustifolia Roxb.	Drink to Cure diabetes	- Mild leaves and flowers - Use the whole trunk for boiling with water	-Cultivation using seed -Leaving to root
- ตันกุ่มบก Sacred barnar, Caper tree, Sacred garlic pear, Temple plant กุ่มบก	Crataeva magna (Lour.) DC.[1] or Crateva religiosa Ham.[2] or Crateva religiosa G.Forst.	Thai medicine formula use: - Root, intense flavor to relieve stomach aches, nourishing the element - Leaves taste bitter and fragrant, improve sweat excretion, antipyretic and laxative properties, expel the parasite, cure tendon pain, and rheumatoid arthritis. Leaves are used for massaging - The bark is used as an antidote for the skin. - The flower tastes minty, cure eye, and throat sore - The fruit tastes bitter, cure fever - The bark tastes intense flavor to cure hiccups, improve fart and sweat excretion, cure the wasting disease. The bark of the trunk is heated with steaming hot to use to relieve pain. - The seed is used for boiling and drinking to help relieve aches.	Mild leaves and flowers can be fermented giving a sour taste. Nutritional values per 100 grams provide energy 88 kilocalories carbohydrate 15.7 grams, in 3.4 grams of protein, 4.9 grams of fiber, 1.3 grams of fat, 73.4 grams of water, Vitamin A 6,083 international units, Vitamin B1 0.08 mg., Vitamin B2 0.25 mg., Vitamin B3 1.5 mg., Vitamin C 5 mg., Calcium 124 mg, Iron 5.3 mg., Phosphorus 20 mg.	Propagated by seeds, leaving the cuttings to root, and grafting

Table 1. (Continue)

Common Name	Scientific Name	Properties	Parts of herbs that are used	Propagation
- Bergamot มะกรูด	Citrus hystrix DC.	Bergamot is high in antioxidants, help to strengthen the immune system to be healthy and resistant to disease improving appetite	1. The bark helps relieve stroke, dizziness. Used as a heart tonic by using a slice of fresh kaffir lime peels about 1 curry spoon of curry. Add camphor or a handful of borneol, brew with boiling water, leave to soak, and then drink the boiled water 1-2 times.	- Seeds in the mature bergamot fruit - Grafting
-Phlai, Cassu- munar ginger, Bengal root ว่านไพล	Zingiber montanum (J.Koenig) Link ex A.Dietr.	Relieve inflammation, cure sprains, swelling and bruising while it can help to alleviate aching	- Leaves help to relieve pain or fever. - Roots help to relieve nosebleeds. But the most important and valuable part is the mature rhizome	Seeds or rhizomes or rhizomes of the underground trunk. Parts of the rhizome as the stem for planting. Planting in the soil is not flooded, not near a source of toxic substances.
- Ngai Camphor Tree, Camphor Tree หนาด	Blumea balsamifera (L.) DC.	Relieve body aches and dizziness and it helps increase blood circulation nourishing the skin	- Roots are carminative resulting in good blood flow. Improve excretion and cure diarrhea. Relieve swelling, joint pains, bruises. Relieve aches after childbirth -Leaves nourish the body resulting in good blood flow Relieve swelling, bruises, and wounds. Relieve joint and bone pains. Cure eczema having carminative properties curing stomach aches. De-worm the parasite. Relieve the fever, headache, asthma. stop the bleeding. Increase sweat excretion and expectorate. Cure hemorrhoids -Stems help improve expectorate	An outdoor plant species that often grows in wide areas, fields, or valleys in general Propagated by seeds or fruits.

Table 1. (Continue)

Common Name	Scientific Name	Properties	Parts of herbs that are used	Propagation
- Thick-leaved croton, Thick leaf croton รากคังคี่	Croton crassifolius Geiseler (Science Synonym Name: Croton chinensis Benth.)	Help pain relief. The root helps to cure colic, flatulence, stomach ache, and abdominal distension which can be cured by using boiled roots with drinking water.	Roots can be processed by pounding to make a compress ball to relieve pain	By seeds. Found mostly in the northeast region. Grow in all soil conditions, medium humidity under full-day sunlight.
- Turmeric ขมิ้นชัน	Curcuma longa L.	Apply to blisters on the scalp. Use mature rhizome a size of 1 thumb. Wash, clean, grind and add a little alum and moistened coconut oil sufficiently for use as an external medicine	- Turmeric powder can be applied to the area of pock, ulcers from insect bites. - Mature dried rhizomes, ground into fine powder to apply to the area of the rash. /- Dried rhizome ground into powder, simmer with vegetable oil making oil to apply fresh wounds	Seedling in a planting hole covered with the soil of 5 - 10 cm thick. Or bringing the seed to cultivate before planting by exposing to dry in the shade covered with decomposed manure for about 20 days. The seed will sprout up. Therefore, brought to plant in the plot
- Croton perisimilis root รากเปล้าใหญ่	Croton oblongifolius Roxb.	Cure diarrhea nourishing blood. Relieve pain and hepatitis Relieve joint pain and muscle aches.	- Bark and leaves are used to relieve diarrhea, nourishing blood. - Boiled water of the bark is used for fever relief, curing hepatitis, joint pain, and muscle aches	Propagation by tissue culture method (Research of Duangporn Ang Sumalee)
- Dried ginger ขิงแห้ง	Zingiber officinale Roscoe.	Where the dried ginger is used as a component of traditional medicine includes Na Wa Kot cordial, Wisamphaya Yai medicine, clove pills, Fai Palaikulp medicine, and diuretics drug in the herbal drug list from the Main National Drug Information announced on 7 August 2013	Dried ginger rhizome is used as an ingredient in 5 other formulas: haematinic drug (ยาเลือดงาม), Blood tonic drugs (ยาบำรุงโลหิต) Benjakul (BJK) drug (ยาเบญจกุล) gastric juice pills (ยาปลูกไผ่ธาตุ) , Yar Tree Phikat medicine (ยาตรีพิกัต)	Dried ginger rhizome

Discussion

Results in the study of the innovative learning of local wisdom from the community resource base in Na Si Nuan Village, Sai Ngam Village, Na Siao Sub-district, Mueang District, Chaiyaphum Province are discussed as follows:

1. According to the study of species and properties of medicinal plants in Na Si Nuan Village, Ban Sai Ngam, Na Siao Sub-district, Mueang District, Chaiyaphum Province by surveying, collecting specimens of medicinal plants with taking pictures and collecting important details related to herbs in the Na Si Nuan Village, Ban Sai Ngam, Na Siao Sub-district, Mueang District, Chaiyaphum Province, the results indicate that herbs in the area that people use for the treatment of diseases and nourishing the body include the following herbal samples

1. Roselle, properties: mild fruit can be eaten for parasitic excretion
2. Black Sugarcane: Relieve leg pain, use as diuretic
3. Dung Etok (passiflora lye, passion fruit): Relieve leg pain
4. *Gymnanthemum extensum* (bitterleaf tree): Bitter taste, cure fever
5. Sweet Basil: carminative properties, nourish the wind element
6. Citronella grass: Diuretic properties
7. Pak Waan Ban Tree (*Sauropus androgynus*): can be eaten at all times
8. Pea Eggplant: Cure the diabetes
9. Plai (*Zingiber cassumunar*): carminative properties
10. Galangal: carminative properties
11. Ginger, Lesser Galangal: deodorize the fishy smell
12. Agasta Flower (Vegetable Hummingbird): mild leaf help cure a seasonal Influenza, the peels cure mouth ulcer
13. Karen Chili: carminative properties, enhance digestion, cure napping, nausea, vomiting, dizziness
14. Carambola, lemon; reduce blood fat, dissolve fat, and blood clots. Help cleansing

15. Heart-leaved moonseed (*Tinospora crispa*): inhibit aphthous ulcer and immunodeficiency symptoms, purify blood. Chew to eat for internodes of 1 finger per day

16. *Cryptolepis Dubia*: Relief disabled tendons and nourish tendons

17. *Derris Scandens*; relief fever in women

18. Chaya (spinach): nourish the four elements; Ceylon Spinach and Morning glory help nourishing eyesight

19. Indian cork (*Millingtonia hortensis*) tree: Cure asthma and allergy symptoms

20. *D. longissima* Schum: Help nourishing milk and blood; stalks cure Hemorrhoids, allergy, and asthma symptoms

21. Fa thalai chon (*Andrographis paniculata*); cure sinusitis and tonsillitis symptoms

22. Long Pepper; carminative properties: Diamnel flower (*Oldenlandia corymbosa*); diuretic properties, heal colic symptoms in the stomach. These 22 medicinal plants are in accordance with the Thailand Institute of Scientific and Technological Research. (2015) which find these plants are edible and can be used as medicine. 23. Dried ginger can be processed into leaf-wrapped ginger. Herbal food products containing the following ingredients; Dried ginger, Black Galangale (กระชายดำ), Long pepper (ดีปลี), Toon (*Caladium* (หัวทูน), Wild (Bitter) ginger (กระเทียม), Phlai (Bengal root ว่านไพล), Crataeva (กุ่ม) peels, Pipe Tamarind (มะขามเปียก), Brown sugar (น้ำตาลทรายแดง), Odized salt (เกลือไอโอดีน), Elephant garlic (กระเทียมโทน), Piper Lolot (ชะพลู), Star Gooseberry leaves (ใบมะยม), Raw Bananas (กล้วยดิบ), will be the cuisine that contains herbal ingredients with carminative, properties that help expel wind-dampness, relieve colic syndrome, nourish the milk of women after childbirth. In the use of dried ginger as herbs, it can be chosen based on the understanding and convenience that the plant can be found in the locality. This is consistent with Samittinan, T. (2001) who found that dried ginger is used as a component in traditional medicine in the traditional home medicine, such as Ya Hom Naowa Kot Kot cordial (ยาหอมเนาวโกฐ), Ya Wisumphaya Yai medicine (ยาวิสัมพยาใหญ่), Clove pills (ยาประสะกานพลู).

Sai Ngam villagers have processed products from dried ginger into "Miang Khing" (leave-wrapped ginger) sold at 160 baht per kilogram. According to Duanpenporn Chaiphakdee (2019), it is found that herbal wisdom can help to process food products generating income for the community.

2. Create lessons for the community about local wisdom development for Sai Ngam Village, Na Siao Subdistrict, Mueang District, Chaiyaphum Province with information on the name of medicinal plants, photos, scientific names, family names which are in line with the research of Kamonchat Klom-Im (2017) where information related to herb name, photos, scientific names, parts of herbs used and properties are used to create community lessons for visitors and learners of Thai traditional medicine have applied for plant propagation and herb preserving for sustainability. This is consistent with Pornchulee Archa Amrung and et.al (2018) which find that digital media of herbal products can be linked to technology as a business center for herbs.

3. In the dissemination knowledge and local knowledge for learning resources, the local knowledge center is established at Na Siao Subdistrict Health Promotion Hospital to disseminate local wisdom in Chaiyaphum province. This learning center consists of CD-ROM of medicinal plants database and medicinal plant documentation which is in line with the research of Kanitkan Pankaew et al. (2017) which find that the local wisdom learning center consists of the posters of local wisdom from different ethnic groups, learning materials, booklets and CD with information about local wisdom for schools to be distributed to the youth in the area. This is consistent with Juljerm Suriwong (2016). and Duenpenporn Chaipakdee (2016) which find that

the requirement for knowledge about medicinal plants of students, non-formal and informal education centers in Kaeng Khro District Chaiyaphum province is at the highest level.

Suggestions

1. Knowledge about the properties of herbs should be provided through online media and websites for lifelong learning.

2. Booklets to educate the public about health care for every family should be publicized.

3. Planting, caring for community herbs, and preservation of the nearby community environment should be publicized.

Suggestions for future research

1. The conservation of rare or endangered medicinal plant varieties used for the treatment of diseases should be studied.

2. Research and development of learning courses on local medicinal plants in the form of participatory action research should be conducted while school encourages teachers, students and the community with local knowledgeable people to participate in the learning and teaching activities.

3. Process of knowledge transfer on herbs for cultivation and natural herb resource inherited from local ancestors should be conducted to create knowledge about the sources of herbs in natural forests as a heritage of natural resources and for sustainable environmental conservation

4. Knowledge should be provided by organizing the training for community members to learn the processing of local herbal products to create extra careers for families and the community.

References

- Chaiyaphum Farmers Council. (2017). Chaiyaphum Farmers Council Action Plan 2018 Copies documents, n.d.
- Duenpenporn Chaiphakdee. (2019). Participation for Career Promotion of the Elderly by Adopting Local Wisdom on Herb Pickled Fish Food Product, *Journal of Humanities and Social Sciences Surin Rajabhat University*
- _____ (2016). Learning Management of Traditional Folk Massage Wisdom Using Herbs in the Community. The 3rd National Academic Conference and Research Presentation towards the 2nd Decade: Research Integration, Use of knowledge to sustainability 17 June 2016 at Nakhon Ratchasima College. (Online system). Source: http://journal.nmc.ac.th/en/admin/Journal/2559Vol4No1_5.pdf (26 March 2018)
- Jenchob Yingsumol (2012). Thai herbal encyclopedia. Bangkok: Tossaporn
- Juljerm Suriwong & Duenpenporn Chaipakdee. (2016). The Development of Training Curriculum on Medicinal plants for Students of Non-formal and Informal Education in Primary School Level. Non-Formal and Informal Education Center. Kaeng Khro District, Chaiyaphum province. *Academic Journal. Liberal Arts and Science, Chaiyaphum Rajabhat University* Year 5, issue 10, December 2016
- Kamonchat Klom-lm. (2017). *Academic Journal, Humanities and Social Sciences. Uttaradit Rajabhat University*, Year 4 Issue 2, July - December 2017 (p. 150-164)
- Kanitkan Pankaew et al. (2017). Ban Cham Pui Ethnic Group Local Wisdom Learning Center Mae Moh District, Lam-pang Province. Lampang: n.d.
- Miles, M.B., & Huberman, A.M. (1994). *Qualitative data analysis: expanded sourcebook*. (2nd ed.). Thousand Oaks, CA: Sage.
- Office of the National Economic and Social Development Council (2017). National Economic and Social Development Plan No. 12, 2017-2021. Office of the Prime Minister. Retrieved 15 November 2017, From <http://www.nesdb.go.th>
- Phenchan Karunmaiwong. (2004). Processing of Thai herbs, No.2. Bangkok, n.d.
- Pornchulee Archa-amrung et al. (2018.) Raising the capability for the center of the herb business of the lower northern provinces. ST Printing Co., Ltd. Phitsanulok
- Samittinan, T. (2001). Chue pran mai hang pra tes thai. Bangkok : Forest Research and Development Bureau. [In Thai]
- Thailand Institute of Scientific and Technological Research. (2015). Edible Plants in the Sakae Ratch Forest, Thailand Institute of Scientific and Technological Research (TISTR). "khon mah khao". [Online]. Accessed from: www.tistr.or.th. [21 Jan 2015].
- Wichian Somngam. (2014). The study of the Wisdom on Medicinal Plants preserved in the Association of Thai Traditional Medicine. Khlong Sam Sub-district, Khlong Luang District Pathum Thani Province. Pathum Thani, n.d.

Sarcoma Cancer Treatment using Extracted Cannabis Oil in Cat

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Abstract

The 6 year old male local Thai cat weight about 3.5 kilograms. He has a purulent from his right eye. The method used as a significant treatment of extracted cannabis oil applied against inflammation to sarcoma cancer to observe the changes of sarcoma tumours, germination rate, etc. The extracted cannabis oil was applied only to cat mouth by dropping as normally applied to human. This objected to reduce pain and as well as to cure sarcoma cancer. First, local university veterinarian at animal hospital diagnosed to process surgery removing right eyeball and examined biopsy. Two weeks later, the biopsy results showed that he has sarcoma cancer in his right eye. A 4 centimeter diameter lump grew up in the right eye socket. The veterinarian at private clinic recommended using extracted marijuana to reduce pain. Giving extracted marijuana oil in mouth and spraying around the lump. He calmed down to relieve the pain at the first time use. Couple weeks later, sarcoma lump become smaller until fell out.

Keywords: Thai cat, Sarcoma cancer, Extracted cannabis oil

Introduction

A Thai male cat aged around six to seven years, weighing 3.5 kilograms was examined. The cat was adopted by philanthropists. The philanthropist as a good caretaker provided all necessary vaccinations. Sarcoma cancer, in this case, had occurred at the right eye muscle of the cat. It had a Ping-Pong round ball shape, approximately a ping pong ball size. When it was touched, even gentle touch, the cat felt extremely pain, showing signs of wrestling and frustration. The extracted cannabis oil has been popular for curing cancer in human today. The Tetrahydrocannabinol (THC) and Cannabidiol (CBD) are two significant medical essences for medical treatment for human (Hasan et al., 2010). Those essences stimulate cannabinoid receptor type 1 (CB1) and type 2 (CB2) through G protein to express peripheral nervous and central nervous systems. The delta-9-THC activates CB1 receptor. Both CB1 and CB2 called endocannabinoid which involve body synthesize as pain, stress, appetite, energy, metabolism, cardiovascular function, motivation, reproduction, and sleep. The CB1 protein is

one of other protein to make a blueprint how to build and reside in DNA. In human body, applying extracted cannabis oil can cure cancer (Kittimunkong, 2019).

Background and pathogenesis

Cats are more at risk of skin cancer from sunlight exposure. Infection with some viruses, including feline immunodeficiency virus may increase the chances of getting cancer. Cancer can occur in any part or system of cat's body. Its symptoms are very varied. Many of the symptoms are also common to a large range of diseases. A diagnosis of cancer cannot be made on symptoms alone. Other signs of tumours (benign or cancerous) affecting internal organs can include loss of appetite, weight loss, lethargy and weakness, difficulty in breathing, limping and recurrent digestive problems. A biopsy (taking a small sample for examination under a microscope) may help to identify the tumour and see if it is cancerous. Reaching a definite diagnosis can sometimes be difficult – for example, biopsies do not always contain enough good quality material for diagnosis.

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There are many types of tumours and treatment is available for some non-cancerous or some cancerous tumours. It depends on where the tumour is growing. Tumour at the beginning in some area, such as eye, cannot be easily removed in animals. A cancer may spread. The possibilities for treatment depend on how far it has spread. However, quality of animal life is important. If an animal is in unrelievable pain, it is encouraged.

There are three basic types of tumour treatments as surgery, chemotherapy and radiotherapy. Surgery is often chosen for tumours of the skin or apparently internal growths. The tumour would be removed by surgery if it does not spread. Sometimes with internal growths where the size of the tumour is causing illness, surgery can relieve the symptoms.

Animals process cannabis oil faster than human body. Normally human would take 60 days of extracted cannabis oil for 90 days curing cancer. Hemp Medicine Planet (2016) recommended using 2 grams for 60 or 90 days since cancer in dog and cat would vanish within 2 weeks. Therefore, it is 1-2 grams for 2-3 months recommended dose (11-33 mg/day).

Methodology

Clinical trial method was conducted in this paper. Thai male cat with sarcoma tumors was an experiment treatment research using extracted cannabis oil. This clinical trial is an individual clinical study. This research started with collecting only 1 individual patient as a pilot study.

Research question: What are the treatment results using extracted cannabis oil for sarcoma cancer patient?

Hypothesis: If the extracted cannabis oil actually has an effect against inflammation of sarcoma cancer, what is the significant treatment?

Research objective: An individual case was set as a preliminary study. If it has shown significant result, the other samples would be conducted in the further study.

Measurement results: To observe the changes of sarcoma tumours, germination rate, etc.

Research results: Research results are comparison treatments of extracted cannabis oil with regular practice medicines. If it works, the experiment will continue to be used in all areas. If it does not work, the physical symptoms will be summarized and the experimental results will be recorded.

Literature using cannabis for animal such as dog recommended applying a quarter gram (5.5-10 mg/kg) to a gram (4-22 mg/kg) of full spectrum cannabis oil per month. It would increase immune system and rejuvenate vital organ (Hemp Medicine Planet, 2016). In this experiment, the extracted cannabis oil contains 15-18% THC and 1-3% CBD with 0.35-0.37 mg/drop or 7-7.4 mg/cc produced for human was applied to Thai cat. For a 50-60 kilogram human, it was recommended to use a gram of extracted cannabis per day. This means 20 milligram per kilogram of patient approximately. For the 3.5 kilogram Thai cat, the approximated dose was 0.7 mg per day as 0.35 mg twice a day (morning and evening) 30 minute before meal. Cat symptoms as was observed as frustration, pain, amount of secretion, and tumour size.

Results (Diagnosis and Treatment)

The street Thai cat started to develop cancer around early July 2019. His caretaker (philanthropist) noticed that his right eyeball has spilled out. He was taken to the State Animal Hospital on Phahonyothin Road, next to the state university for treatment. A veterinarian in state hospital recommended to process surgery cutting out the fleshed tissue and process an injecting treatment chemotherapy. On that day, hospital veterinarian performed surgery to remove the entire right eye ball and sew the wound. After surgery, veterinarian gave analgesics (gabapentin) and disinfectants. During the operation, the specimen was examined in a biopsy laboratory. After four weeks, government hospitals have reported the examined biopsy laboratory; it is found that the cat has sarcoma cancer.

Seven days after government hospitals reported, the wound in the surgical area was removed and a lump came out. A sarcoma lump has grown steadily as large as a duck egg over a period of two weeks. The

provided pain relieves and antibiotics medicines from government hospital are not able to alleviate the cat symptoms.

The philanthropist who owns this cat decided to bring cat to a private veterinary clinic on August 1, 2019. The clinic veterinarian examined symptoms and interviewed philanthropist. It is found that sarcoma cancer lump has rapidly developed after surgery. Luckily, cat still can eat by himself. Clinic veterinarian started treatment using extracted cannabis oil for humans. The extracted cannabis oil contains cold pressed coconut oil with concentration extracted cannabis of 0.35 milligrams per drop (Deutsch et al., 2008). The extracted cannabis oil was mixed with hemp oil half and half by volume. The 0.05 cc. (1 drop) extracted cannabis oil with hemp was taken by the 30 minutes before food, twice morning and evening. The extracted cannabis helps cat to be calm when touching the wound. In every visit, clinic veterinarian process usual wound cleaning with normal saline. Then, spray extracted cannabis oil diluted with normal saline in the ratio of 1: 5, twice a day in the morning and evening.

After using extracted cannabis oil with hemp oil for 10 days, veterinarian gave 0.05 cc. normal extracted cannabis oil (0.35 mg. per drop), 30 minutes before meals, twice in the morning and evening, together with an ointment mixed with extracted cannabis oil.

On September 10, 2019, the sarcoma lump has shrunk from 5 centimeters diameter to 1.5 centimeters and then fell out. The cat has lost his weight from original 3.5 kg to 2.6 kg. He began to slim down. Since he started sarcoma symptom, he was able to eat the same amount of food and his blood and sugar tests are normal. At the end of September, 2019, sarcoma tumour was fallen out. The cat can live normally.

Hemp Medicine Planet (2016) recommended 11-33 mg per day for dog and cat. For the 3.5 kilogram Thai cat, the approximated dose was 0.7 mg per day which means 0.2 mg per kilogram. This can illustrated that extracted cannabis oil can treat sarcoma cancer in cat.



Figure 1. First day treatment in private veterinarian clinic treating Sarcoma symptom.



Figure 2. Infection symptom in Sarcoma cancer.



Figure 3. Cleaning purulent from Sarcoma lump. Notes: cleaning sarcoma lump with normal saline



Figure 4. 3.6 centimeter diameter sarcoma lump at right eye after treatment.

Conclusion and Discussion

The extracted cannabis oil had cured sarcoma cancer a case study cat. It was found that the cat had very bad pain from sarcoma cancer. After taken extracted cannabis oil, the large, round, about 4 cm in diameter sarcoma tumour was significantly smaller, until it finally dropped out. The extracted cannabis oil can reduce inflammation in the cancer area. The cat had calmed down and can live closed to its previous daily life. Cat can eat by itself as normal every day.

According to the result study, the extracted cannabis oil can quickly help to reduce symptoms of sarcoma cancer growth. The important substances in extract cannabis oil may various. Some of those sub-

stances affected cats' body and sarcoma cancer, so further research and cases must be further conducted in detail. Therefore, studying extracted cannabis oil in various mammals and human body is needed. In order to know the body's processes for using non-active extracts or the stimulation of those extracts to enable the body to utilize immunity for each type of illness, step by step research as well as significant number of cases may be conducted. Then, the extracted cannabis oil can turn into appropriate use for various symptoms.

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References

- Blue for Pets cross. (2019). <https://www.bluecross.org.uk/pet-advice/coping-cancer-cats>. Accessed on 23 November 2019.
- Brunnauer A, Segmiller FM, Volkamer T, Laux G, Müller N. & Dehning S. (2011). Cannabinoids improve driving ability in a Tourette's patient. *Psychiatry Res*, 9 June 2011 [in press].
- Deutsch SI, Rosse RB, Connor JM, Burket JA, Murphy ME, & Fox FJ. (2008). Current status of cannabis treatment of multiple sclerosis with an illustrative case presentation of a patient with MS, complex vocal tics, paroxysmal dystonia, and marijuana dependence treated with dronabinol. *CNS Spectr* 2008;13(5):393-403.
- Hasan A, Rothenberger A, Münchau A, Wobrock T, Falkai P & Roessner V. (2010). Oral delta9-tetrahydrocannabinol improved refractory gilles de la tourette syndrome in an adolescent by increasing intracortical inhibition: a case report. *J Clin Psychopharmacol* 2010;30(2):190-2.

- Hemp Medicine Planet PTY.LTD. (2016). <http://hempmedicineplant.weebly.com/cannabis-oil-dosage-animals.html>. Accessed on 8 May 2020.
- Jakubovski E & Müller-Vahl K. (2017). Speechlessness in Gilles de la Tourette Syndrome: Cannabis-Based Medicines Improve Severe Vocal Blocking Tics in Two Patients. *Int J Mol Sci.* 2017;18(8).
- Kittimunkong, Somyot. M.D. (2019). Hemp and Cannabis Treatment. Go Green Social Venture Co., Ltd. ISBN 978-6167784797-7. April, 2019. พิมพ์ครั้งที่ 6.
- Leroux E, Taifas I, Valade D, Donnet A, Chagnon M & Ducros A. (2013). Use of cannabis among 139 cluster headache sufferers. *Cephalalgia.* 2013;33(3):208-13.
- Müller-Vahl KR, Schneider U & Emrich HM. (2002). Combined treatment of Tourette syndrome with delta-9-THC and dopamine receptor agonists. *J Cannabis Ther* 2002;2(3-4):145-54.
- Russo, EB. (2001). Hemp for headache: An in-depth historical and scientific review of cannabis in migraine treatment. *J Cannabis Ther* 2001;1(2):21-92.
- Raby, WN, Modica PA, Wolintz RJ & Murtaugh K. (2006). Dronabinol reduces signs and symptoms of idiopathic intracranial hypertension: a case report. *J Ocul Pharmacol Ther* 2006;22(1):68-75.
- Saba, Corey F. (2017). 12 January 2017 Volume 2017:8 Pages 13—20. <https://doi.org/10.2147/VMRR.S116556>. <https://www.dovepress.com/vaccine-associated-feline-sarcoma-current-perspectives-peer-reviewed-full-text-article-VMRR>. Accessed on 23 November 2019. Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA, USA.

Isolation and Molecular Characterization of Antifungal Production from Rice Fields Rhizosphere soil, Thailand

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Abstract

One hundred actinomycete strains were isolated from rice field soil Nong Bua, Nakonsawan, Thailand and twenty actinomycete strains were isolated from Happy Rice Organic Farm at Nong sua, Pathumthani, Thailand. A total of one hundred and twenty strains were tested for antifungal activities against rice blast disease (*Pyricularia* sp. CRI60007). The selected actinomycete strains were chosen from in vitro radial growth inhibition test against *Pyricularia* sp. CRI60007 that showed the radial growth inhibition percentage of the growth of the fungi (%RGI) more than 50%. The results showed eleven strains gave %RGI more than 50% and strain NK05201 gave the highest %RGI (100 ± 0.00%). Analysis of 16S rRNA gene sequence and blast analyses confirmed that the selected actinomycete, ten strains belong to the genus *Streptomyces* and one strain belong to the genus *Amycolatopsis* with similarity to *Streptomyces albidoflavus* (NK01101 (99.56%), NK05201 (99.78%), NK05203 (99.79%), NK05205 (99.79%), NK07201 (99.79%), and NK08202(99.79%)), *Streptomyces hydrogenans* (NK08203 (100%)), *Streptomyces jeddahensis* (NK08205 (99.17%)), *Streptomyces corchorusii* (NK21201 (99.86%)), *Streptomyces shenzhenensis* (HPF3303 (99.86%)), and *Amycolatopsis rhizosphaerae* (HPF2102 (99.72%)).

Keywords: Actinomycete, Antifungal, Rice blast disease, 16s rRNA gene

Introduction

Rice and wheat are two main cereal crops that supply the majority of the global daily carbohydrate intake as energy source of human. Any pathogen targeting these two crops supposed to have serious connotations for the economy (John and Jacqueline, 2003). Thailand is one of rice producers to export rice product about 45 percent of rice yield. The main effect that reducing rice yield caused of the outbreaks of rice blast disease that can damaged to rice plant in all stages of growth (Saruda et al.,2017). Rice blast disease caused by the plant pathogenic fungi *Magnaporthe grisea* (Hebert) Barr. (anamorph, *Pyricularia grisea* Sacc.) is one of the most economically harmful crop diseases (Seochang and Katherine, 2004). The disease is widespread and distributed about 85 countries around the world where rice is grown. The disease appearance and asperity depend-

ing on year, place, environmental conditions and crop management practices. Yield loss appraisals from other areas of the world have ranged from 1% to 50% (Jeffrey, 2016).

Potential use of microbes-based biocontrol-agents as supplement or displacement for agrochemicals has been reported in several reports (Shimizu et al., 2000). Actinomycetes well-known as the source of several drugs such as antibiotics, antiviral, antitumor, antiparasitic agents, and immunosuppressants, microorganisms are responsible for the production of about 23,000 bioactive secondary metabolites (Feitosa et al., 2014; Subbanna et al., 2018). Some strains of actinomycete from rice were found to have antagonism against *Magnaporthe grisea*, *Rhizoctonia solani*, *Xanthomonas oryzae* pv. *oryzae*. and *Fusarium moniliforme* (Tian et al., 2004).

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The purpose of this present study to isolate actinomycetes from rhizosphere rice that can produce antifungal activity and searching for potential strains to control *Pyricularia* sp. CRI60007 that causes rice blast on rice leaves. Characterisation of the selected strains were done based on morphological, biochemical and physiological properties and studied of hydrolytic enzyme production against fungal cell wall. Identification analysis of 16S rRNA gene sequencing were performed. The results from this study can be used for pot experiment test in further study.

Material and methods

Collection of soil sample

Soil samples were collected from Happy Rice Organic Farm at Nong sua, Pathumthani, Thailand. Samples were randomised collecting at rhizosphere soil. These samples were kept in sterile polyethylene bags without sealed and transported immediately to the laboratory (Ruttanasutja and Pathomaree, 2015).

Isolation of Actinomycetes

The soil samples were pre-treated by air-drying method for 3-5 days at room temperature. Ten grams of soil sample was accurately weighed and mashed then transferred to 90 milliliters of 0.1% Tween80, mixed well (10-1). One milliliter of resultant solution was serially diluted with 9 milliliters of 0.85% Sodium Chloride (NaCl) up to 10⁻⁵. 200 µl of each intermediate dilution was spread on Modified Zhang's Soil Starch Extract (ZSSE) agar medium that supplemented with nystatin (50 micrograms /milliliter). The plates were incubated for the growth of actinomycetes colonies at 30 ± 2 °C and observed growth of actinomycete intermittently during incubation. After 7 days incubation, colony was picked by micro-needle then cross streak on International Streptomyces Project 2 (ISP2) medium. The pure colonies of actinomycete isolates were selected and maintained on ISP2 medium at 30 ± 2 °C for 7–14 days. Stocks were preserved in 20% glycerol (w/v), and stored at -80 °C for long time preservation. (Waksman et al., 1961).

In vitro radial growth inhibition test

The antifungal activity was analysed using dual culture method. Potato Dextrose Agar (PDA) medium

plates were used, inoculated with the actinomycete isolates by single streak far from edge of petri dish two centimeters and incubated at 30 ± 2 °C for 7 days then using cork borer size 5 millimeters plug the fungi (*Pyricularia* sp. CRI60007) and placed on opposite site of actinomycete far from edge of petri dish two centimeters and incubated at 28 ± 2 °C. The inhibition zone was measured after the fungal mycelia in the control plates reached the edges of the plates. The antagonism against fungi was recorded by radial growth inhibition percentage was obtained using the formula: %RGI = 100 x (R1-R2)/R1 where R1 was the furthest radial growth distance of the fungus (control) and R2 was the radial growth distance of the fungus in dual culture with the actinomycetes (Mahadtanapuk et al., 2007).

Morphological biochemical and physiological characterization of the selected isolate

The morphological characteristics were observed using light microscopy with long working distance lens (40X) by using 14 days cultures grown at 30 ± 2 °C on ISP2 medium. The colour of aerial mycelium, substrate mycelium and soluble pigment were examined by using ISCC–NBS colour system (Kelly, 1964). Growth at different temperature was test at 4, 10, 20, 28, 30, 37, 40, 50 °C on ISP2 agar plates and visible growth was determined. Growth at different pH range 4 to 12 in ISP2 broth medium was recorded after 14 days, and growth in presence of NaCl (%) concentration 0 to 10 were studied on ISP2 agar plates. All phenotypic properties were recorded after incubation for 14 days at 30 ± 2 °C. Biochemical properties were tested using the several standard methods. Carbon source utilisation was performed by using ISP 9 medium containing different sugar types (Arai, 1975; Shirling and Gottlieb, 1966; Williams and Cross, 1971). Production of melanin pigment was determined by using ISP 6 (Pridham and Lyons, 1969). Hydrolysis of tyrosine, xanthine, hypoxanthine, adenine, and cellulose was recorded positive result by clear zone formation around the colony. Starch hydrolysis was observed as described by Cowan (1974). Urease test was determined by the indicator change phenol red changing pH yellow to pink (Gordon et al., 1974).

DNA isolation, PCR amplification and 16s rRNA gene sequencing

DNA amplification, sequencing and phylogenetic analysis of selected actinomycete strains were identified by using 16S rRNA gene. The selected strains were grown on ISP2 agar medium for 4 days at 30 °C. The colony was picked up by a sterilized toothpick and resuspended in 40 µl of TE buffer pH 8.0 as DNA template. The 16S rRNA gene was amplified and sequenced by using primers 9F (5' GAGTTTGATCITIGCTCAG3') and 1541R (5'AAGGAGGTGATCCAGCC3'). The temperature for PCR amplification and sequencing reaction followed the method of Yukphan et al., (2005). Each PCR reaction of 50 microliters in total included 25 microliters AccuPower® Taq PCR Master Mix (Bioneer), 18 microliters dH₂O, 2.5 microliters the final concentration 10 picomole of each primer and 2 microliters DNA template. The cycling conditions for the amplification of the 16s rRNA gene region were as follow: 3 minutes at 94 °C, 25 cycles at 94 °C for 1 minute, at 50 °C for 1 minute and 2 minutes at 72 °C, then followed by a final extension step for 3 minutes at 72 °C. The sequences of 16s rRNA gene was aligned with the program BioEdit Sequence Alignment Editor (version 7.0.0. Distance matrices for the aligned sequences were calculated by using the two-parameter method of Kimura (1980). The Maximum Likelihood method based on the Kimura 2-parameter model of Kumar et al., (2016) was used to construct a phylogenetic tree with the program MEGA7.

In vitro screening of the selected actinomycete isolates for hydrolytic enzyme production against fungal cell wall

Detection of Protease

Protease detection was performed using skim milk agar plate (peptonisation), skim milk broth (coagulation) was determined by following the methods described by Atlas (1997) and bouillon gelatin broth (gelatinisation).

Detection of Lipase

Lipase detection was observed by the production of clear zone in agar plates using Tween80 by following the methods described by Schoofs et al., (1997) and using phenol red by following the methods described by Singh et al., (2006).

Detection of Chitinase

Chitinase detection was detected by following the methods of Nawani et al., (2002). The positive result was determined by formation of clear zone around colony on chitin agar plates.

In vitro screening of the selected actinomycete isolates for plant growth-promoting (PGP) activities
Siderophore production

Siderophore production test of the selected actinomycete strains were screened on Chrom azurol S (CAS) agar medium. The selected actinomycete isolates were spot inoculated on the medium and incubated at 30 ± 2 °C for 7-14 days. A positive CAS reaction was observed by changing to be yellow color around the spot growth. (Alexander and Zuberer, 1991).

Phosphate solubilisation

Solubilization of Phosphate test was used Pikovskaya's agar plates. Plates were divided in four equal sectors and the selected actinomycete isolates were spot on the agar plates and incubated at 30 ± 2 °C for 3-14 days. The presence of clear zone around the spot growth indicated phosphate solubilisation activity (Gaur, 1990).

Hydrogen cyanide (HCN) production

Production of hydrogen cyanide, the selected isolates were screened by adapting the method of Lock (1948). Using ISP2 agar medium supplemented with 0.44% glycine. Actinomycetes were streaked on these modified agar plate. Whatman filter paper No.1 dipped in 2% sodium carbonate in 0.5% Picric acid solution placed in the top of the plate. Plates were sealed with parafilm and incubated at 30 ± 2 °C for 3-14 days. The orange to red color of filter paper was indicated a positive test for HCN production.

Ammonia production

The selected actinomycete isolates were tested for ammonia production in peptone water medium. The selected actinomycete isolates were inoculated into 10 milliliters of peptone water and incubated at 30 ± 2 °C for 7-14 days. After that Nessler's reagent (0.5 milliliter) was applied to each tube. The results showed brown to yellow color was determined to be a positive test for ammonia production (Cappucino and Sherman, 1992).

Results and discussion

Isolation of Actinomycetes

A total of 120 actinomycete isolates were isolated from rice field soil Nong Bua, Nakonsawan, Thailand (Thitiya and Khanungkan., 2019) 100 isolates and 20 isolates from Happy Rice Organic Farm at Nong sua, Pathumthani, Thailand.

In vitro radial growth inhibition test

Screening for antifungal activity of 120 isolates were tested against *Pyricularia* sp. CRI60007. The results of antifungal activity showed that 11 actinomycete strains were able to inhibit growth of *Pyricularia* sp. CRI60007 more than 50% including of NK01101 ($66.44 \pm 0.59\%$), NK05201 ($100 \pm 0.00\%$), NK05203 ($85.88 \pm 0.61\%$), NK05205 ($71.30 \pm 0.68\%$), NK07201 ($78.24 \pm 0.73\%$), NK08202 ($61.11 \pm 0.41\%$), NK08203 ($90.51 \pm 0.50\%$), NK08205 ($77.78 \pm 0.05\%$), NK21201 ($84.95 \pm 0.14\%$), HPF2102 ($99.53 \pm 0.03\%$), and HPF3303 ($51.39 \pm 0.31\%$) as shown in Figure 2.

Morphological biochemical and physiological characterization of the selected actinomycetes strains

The selected actinomycete strains were grouped by using ISCC–NBS colour system (Kelly, 1964) of aerial mycelium into beige yellow (NK01101, NK05201, NK05203, NK05205, NK07201, NK08202, and NK08203), light gray (NK21201 and HPF3303), white (HPF2102) and NK08205 strain was not produced aerial mycelium on ISP 2 agar medium. All strains were able to utilise galactose, glucose, mannose and arabinose while strain NK08205 and HPF3303 were able to utilise sucrose. Strain NK01101, NK05201, and NK05203 were unable to utilise raffinose and xylose while NK05205, and NK07201 were unable to utilise raffinose, and strain NK21201, and HPF2102 were unable to utilise mannitol as carbon source. The ability to use differential types of carbon source might be indicate that actinomycetes were able to survive in the different environment (Ridhi et al., 2017). Most of selected actinomycetes were able to grow at temperature 20 to 40 °C (Table 1). All of them could not grow at temperature 4 °C. The optimum temperature and pH were found to be 30 °C and pH7, respectively.

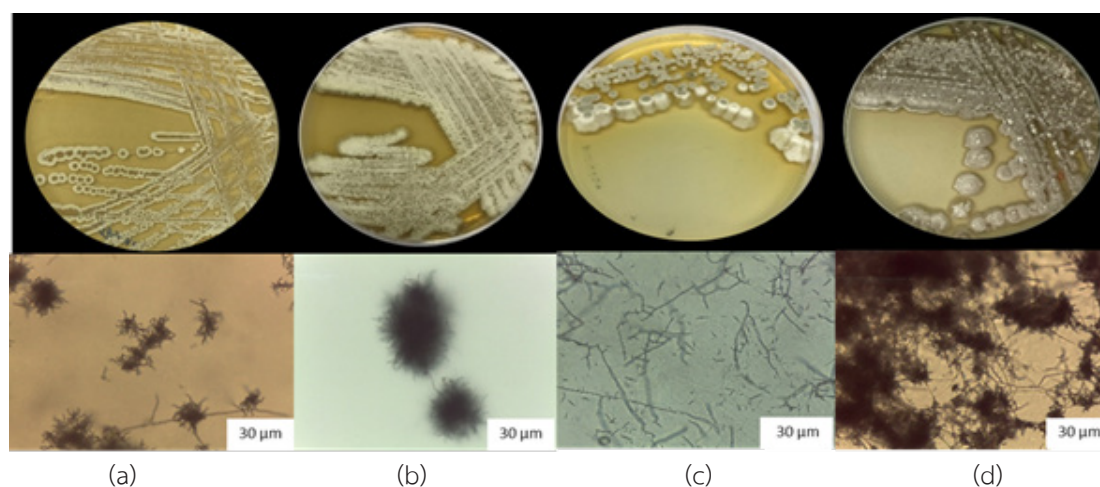


Figure 1. Morphological appearance colonies on ISP2 agar medium and spore arrangement of some selected actinomycete strains NK05201 (a), NK08203 (b), NK21201 (c), and HPF3303 (d).

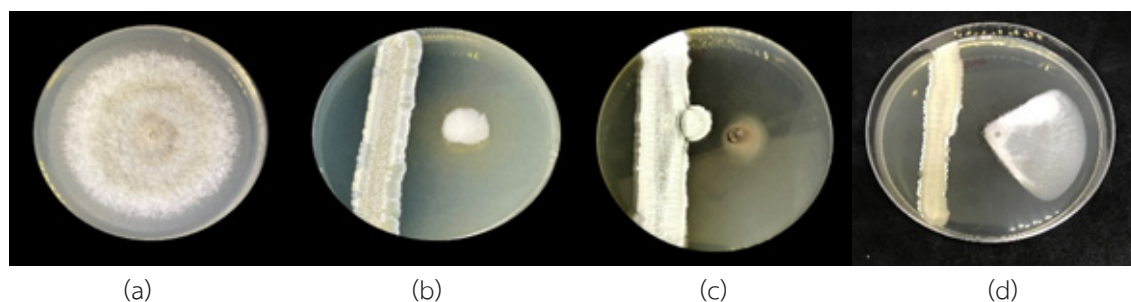


Figure 2. Antifungal activity evaluation of some actinomycete strains (a) control, (b) NK07201, (c) NK21201, and (d) HPF 3303 against *Pyricularia* sp. CRI60007.

Table 1 (Continue)

Characteristics	Actinomycete strains										
	1	2	3	4	5	6	7	8	9	10	11
Hydrolysis of											
Tyrosine	-	-	-	-	-	-	-	-	-	-	-
Xanthine	-	-	-	-	-	-	-	-	-	-	-
Hypoxanthine	+	+	+	+	+	+	+	+	+	+	+
Adenine	+	+	+	+	+	+	+	+	+	+	+
Cellulose	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	+	+	+	+	+	+	+	-
Urea	-	+	-	+	-	-	w	-	-	-	-

Strains; 1. NK01101, 2. NK05201, 3. NK05203, 4. NK05205, 5. NK07201, 6. NK08202, 7. NK08203, 8. NK08205, 9. NK21201, 10. HPF2102, and 11. HPF3303.

The symbol, + means the positive reaction or appearance of growth while symbol – means the negative or absence of growth.

Molecular identification

The 16S rRNA gene sequences and Blast analyses confirmed that selected ten strains of actinomycete belong to the genus *Streptomyces* spp. with similarity of 99.56-100 % including of *S. albidoflavus* (NK01101, NK05201, NK05203, NK05205, NK07201, and NK08202), *S. hydrogenans* (NK08203), *S. jeddahensis* (NK08205), *S. corchorusii* (NK21201), *S. shenzhenensis* and (HPF3303) with similarity percentage of 99.56, 99.78 99.79, 99.79, 99.79, 100, 99.17, 99.86 and 99.86, respectively. One strain belongs to the genus *Amycolatopsis* spp. As *A. rhizosphaerae* (HPF2102) with similarity 99.86 %. These sequences from selected actinomyces strains have been deposited in the GenBank with accession number as showed in Table 2 and the maximum likeli-

hood phylogenetic tree construction showed in Figure 2.

Several strains of the selected actinomycetes have been reported their antifungal activities such as strain NK01101, NK05201, NK05203, NK05205, NK07201, and NK08202 tend to be *Streptomyces albidoflavus*. Ahmed et al., (2018) reported that *Streptomyces albidoflavus* AS25 produced antifungal against. Strain NK 08203 tend to be *Streptomyces hydrogenans*. Rajesh and Talwinder, (2016) emphasized *Streptomyces hydrogenans* DH16 and its culture metabolites can be developed as biofungicides. Strain NK21201 tend to be *Streptomyces corchorusii*. Tamreihao et al., 2016 reported that *Streptomyces corchorusii* strain UCR3-16 showed antifungal activities against rice fungal pathogens.

Table 2 Molecular identification of the selected actinomycetes strains determined by 16s rRNA gene sequencing.

Strains	Source of isolation	Identification as	Similarity (%)	Accession
NK01101	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.56	LC516418
NK05201	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.78	LC516413
NK05203	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.79	LC516409
NK05205	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.79	LC516410
NK07201	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.79	LC516411
NK08202	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.79	LC488882
NK08203	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces hydrogenans</i>	100	LC516412
NK08205	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces jeddahensis</i>	99.17	LC477341
NK21201	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces corchorusii</i>	99.86	LC516414
HPF2102	Rice rhizosphere soil, Pathumthani, Thailand.	<i>Amycolatopsis rhizosphaerae</i>	99.86	LC516416
HPF3303	Rice rhizosphere soil, Pathumthani, Thailand.	<i>Streptomyces shenzhenensis</i>	99.72	LC516417

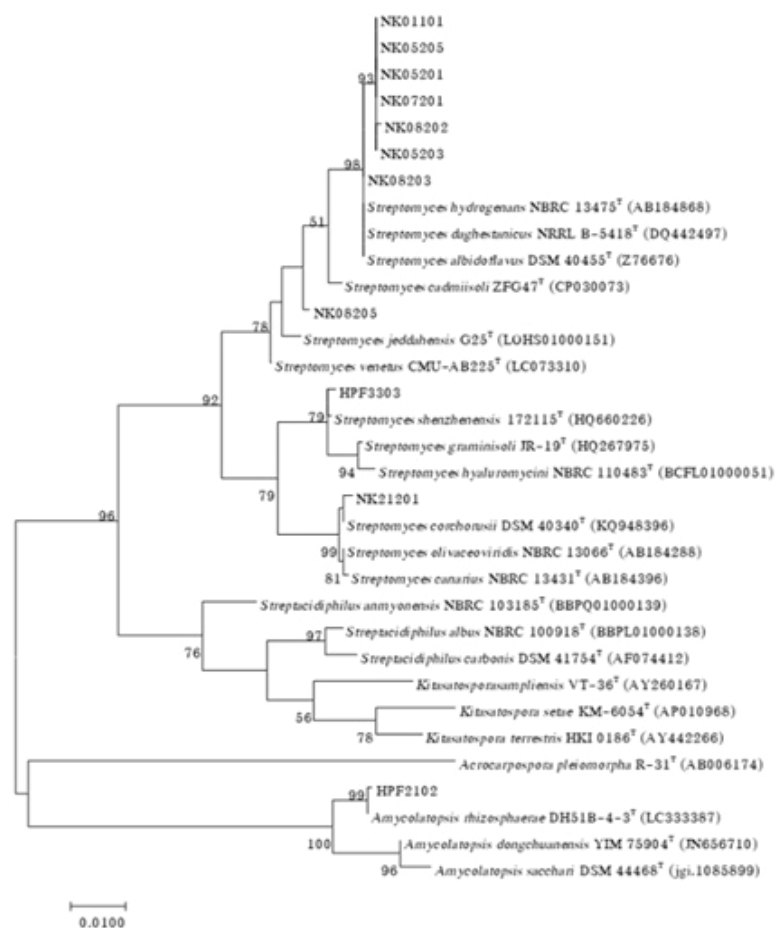


Figure 2. Maximum likelihood phylogenetic tree based on the 16S rRNA gene sequences of selected actinomycetes 11 strains and their closely related type strains.

In vitro screening of the selected actinomycete strains for hydrolytic enzyme production against fungal cell wall

The possible mechanisms of antifungal activity from actinomycetes to fungal pathogens is usually related to the production of antifungal compounds and extracellular hydrolytic enzymes including of protease, lipase, chitinase, and β -1,3-glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls (Prapagdee et al., 2008). The result of hydrolytic enzyme production against fungal cell wall showed all most of the selected actinomycete strains could be produced enzyme protease and lipase (Table 3).

In vitro screening of the selected actinomycete strains for plant growth promoting (PGP) activities

Despite their preliminary track record as BCAs and plant growth promoting (PGP) activities actinomy-

cetes. have been scarcely reported in the literature. Some reports exist for their ability to solubilize phosphate, and production of indole acetic acid (IAA), siderophores, 1-amino cyclopropane-1-carboxylic acid (ACC) deaminase (Sadeghi et al., 2012; Passari et al., 2015; Qin et al., 2015). The result of plant growth promoting (PGP) activities showed strain NK05205, NK07201, and NK08205 could be produced siderophore while strain NK05201, NK05203, NK05205, NK07201, NK08202, and NK08203 were determined to be a positive test for ammonia production. Strain HPF3303 showed positive result of hydrogen cyanide production. Strain NK21201 and HPF2102 showed positive result of phosphate solubilisation (Table 4).

Table 3 In vitro screening of the selected actinomycete strains for hydrolytic enzyme production against fungal cell wall.

Characteristics	Actinomycete strains										
	1	2	3	4	5	6	7	8	9	10	11
Protease Production											
Peptonization	+	+	+	+	+	+	+	-	w	-	-
Coagulation	+	-	-	+	+	+	+	-	w	-	-
Gelatinization	-	+	-	-	+	-	w	-	-	-	-
Lipase production											
Tween80	+	+	+	+	+	+	+	-	+	+	+
Phenol red	-	-	-	-	-	+	-	-	+	-	+
Chitinase	-	-	-	-	-	-	-	-	-	-	-

Strains.; 1. NK01101, 2. NK05201, 3. NK05203, 4. NK05205, 5. NK07201, 6. NK08202, 7. NK08203, 8. NK08205, 9. NK21201, 10. HPF2102, and 11. HPF3303. The symbol, + means the positive reaction while symbol, – means the negative reaction and w, weak.

Table 4 In vitro screening of the selected actinomycete strains for plant growth-promoting (PGP) activities

Characteristics	Actinomycete strains										
	1	2	3	4	5	6	7	8	9	10	11
Production of											
Siderophore	-	-	-	+	+	-	-	+	-	-	-
Ammonia	-	+	+	+	+	+	+	-	-	-	-
HCN	-	-	-	-	-	-	-	-	-	-	+
Phosphate solubili- sation	-	-	-	-	-	-	-	-	+	+	-

Strains.; 1. NK01101, 2. NK05201, 3. NK05203, 4. NK05205, 5. NK07201, 6. NK08202, 7. NK08203, 8. NK08205, 9. NK21201, 10. HPF2102, and 11. HPF3303.

The symbol, + means the positive reaction while symbol, – means the negative reaction and w, weak.

Conclusion

In this study the selected actinomycetes strains were chosen from in vitro radial growth inhibition test against *Pyricularia* sp. CRI60007 that showed the radial growth inhibition percentage of the growth of the fungi (%RGI) more than 50%. The results showed eleven strains gave %RGI more than 50%. Strain NK05201 gave highest %RGI ($100 \pm 0.00\%$) and followed by strain HPF2102 ($99.53 \pm 0.03\%$), NK08203 ($90.51 \pm 0.50\%$), NK05203 ($85.88 \pm 0.61\%$), NK21201 ($84.95 \pm 0.14\%$),

NK07201 ($78.24 \pm 0.73\%$), NK08205 ($77.78 \pm 0.05\%$), NK05205 ($71.30 \pm 0.68\%$), NK01101 ($66.44 \pm 0.59\%$), NK08202 ($61.11 \pm 0.41\%$), and HPF3303 ($51.39 \pm 0.31\%$) respectively. Molecular identification of the selected actinomycetes strains belong to the genus *Streptomyces* spp. with similarity of 99.56-100 % and one strain belong to the genus *Amycolatopsis* spp. with similarity 99.86%. The results from this study can be used for further study.

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References

- Ahmed Nafis, Najoua Elhida, Brahim Oubaha, Salah Eddine Samri, Timo Niedermeyer, Yedir Ouhdouch, Lahcen Hassani, Mustapha Barakate. (2018). Screening for Non-polyenic Antifungal Produced by Actinobacteria from Moroccan Habitats: Assessment of Antimycin A19 Production by *Streptomyces albidoflavus* AS25. *Int J Mol Cell Med Spring*. Vol 7 No 2:133-145.
- Alexander, D. B., and Zuberer, D. A. (1991). Use of Chrome Azurol S reagents to evaluate siderophore production by rhizosphere bacteria *Biol. Fertility Soils*. 12: 39–45.
- Atlas, R.M. (1997). *Handbook of Microbiological Media*. CRC Press, Boca Raton, Florida, USA
- Arai, T. (1975). *Culture Media for Actinomycetes*. Tokyo, Japan: The Society for Actinomycetes.
- Cappuccino, J. G., and Sherman, N. (2002). *Microbiology: A Laboratory Manual*, 6th Edn. California City, CA: Pearson Education
- Cowan, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd Edn. London: Cambridge University Press.
- Feitosa TR, Arruda FVF, Cavalcanti FJR, Baptista NMQ, Callou MJA, SILVA T, Miranda RCM, Gusmão NB. (2014). Antimicrobial activity of fungi isolated from the water of the sky high, Recife-PE supply against bacteria of clinical interest system. *Afr. J. Microbiol. Res.* 8:2999-3007
- Gaur, A. C. (1990). *Phosphate Solubilizing Microorganisms as Biofertilizer*. New Delhi: Omega Scientific Publishers.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E., and Pang, C. H. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int. J. Syst. Bacteriol.* 24: 54–63.
- Jeffrey R. Ryan, in *Biosecurity and Bioterrorism (Second Edition)*, (2016). *Biosecurity and Bioterrorism* 185-216
- John L. Sherwood and Jacqueline Fletcher. (2003). *Crop Biosecurity: Are We Prepared?* Developed by the Public Policy Board of the American Phytopathological Society (APS)
- Kelly KL. (1964). *Inter-Society Colour Council–National Bureau of Standard Colour Name Charts Illustrated with Centroid Colours*. Washington, DC:US Government Printing Office.
- Kimura M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111-120.
- Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.
- Lorck, H. (1948). Production of hydrocyanic acid by bacteria. *Physiol. Plant.* 1:142–146.
- Mahadthanapuk S, Cutler RW, Sanguanserm Sri M, Sardud V, College B, Anuntalabhochai S. (2007). Control of anthracnose caused by *Colletotrichum musae* on *Curcuma alata* matifolia Gagnep. Using antagonistic *Bacillus* spp. *American Journal of Agricultural and Biological Sciences*. 2(2):54–61.
- Nawani, N. N., Kapadnis, B. P., Das, A. D., Rao, A. S., & Mahajan, S. K. (2002). Purification and characterization of a thermophilic and acidophilic chitinase from *Microbispora* sp. V2. *Journal of Applied Microbiology*, 93(6):965–975.
- Passari, A.K., Mishra, V.K., Gupta, V.K., Yadav, M.K., Saikia, R., Singh, B.P., (2015). In vitro and in vivo plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with me-

dicinal plants. PLoS One 10, e0139468.

- Prapagdee, B., Kuekulvong, C., & Mongkolsuk, S. (2008). Antifungal Potential of Extracellular Metabolites Produced by *Streptomyces hygroscopicus* against Phytopathogenic Fungi. *International Journal of Biological Sciences*, 330–337.
- Pridham, T. G., and Lyons, A. J. (1969). Progress in clarification of the taxonomic and nomenclatural status of some problem actinomycetes. *Dev. Ind. Microbiol.* 10: 183–221.
- Qin, S., Miao, Q., Feng, W.-W., Wang, Y., Zhu, X., Xing, K., (2015). Biodiversity and plant growth promoting traits of culturable endophytic actinobacteria associated with *Jatropha curcas* L. growing in Panxi dry-hot valley soil. *Appl. Soil Ecol.* 93: 47–55.
- Rajesh K. Manhas and Talwinder Kaur. (2016). Biocontrol Potential of *Streptomyces hydrogenans* Strain DH16 toward *Alternaria brassicicola* to Control Damping Off and Black Leaf Spot of *Raphanus sativus* *Front. Plant Sci.*
- Riddhi N. Dholakiya, Raghawendra Kumar, Avinash Mishra, Kalpana H. Mody, and Bhavanath Jha. (2017). Antibacterial and Antioxidant Activities of Novel Actinobacteria Strain Isolated from Gulf of Khambhat, Gujarat. *Front Microbiol.* 2017; 8: 2420.
- Ruttanasutja, P and Pathomaree, W. (2015). Selective Isolation of Cultivable Actinomycetes from Thai Coastal Marine Sediment. *Chiang Mai J. Sci.* 42(1): 88-103.
- Sadeghi, A., Karimi, E., Dahaji, P.A., Javid, M.G., Dalvand, Y., Askari, H., (2012). Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. *World J. Microbiol. Biotechnol.* 28:1503–1509.
- Saruda Hensawang, Angkool Wangwongchaia, Usa Humphries a, and Thidarat Bunsri. (2017). Simulation of severity of rice blast disease in Prachin Buri using plant disease epidemiological model: simulation of rice blast disease. The 22nd Annual Meeting in Mathematics (AMM 2017) Department of Mathematics, Faculty of Science Chiang Mai University, Chiang Mai, Thailand.
- Schoofs, A., F.C. Odds, R. Colebunders, M. Leven and H. Goussens. (1997). Use of specialized isolation media for recognition and identification of *Candida dubliniensis* isolates from HIV-infected patients. *Eur. J. Clin. Microbiol. Infect. Dis.*, 16:296-300
- Seochan Kang and Katherine F. Dobinson. (2004). Molecular and Genetic Basis of Plant-Fungal Pathogen Interactions. *Applied Mycology & Biotechnology an International Series. Volume 4:59-97*
- Singh, R., N. Gupta and N. Kango. (2007). Production of lipase by hyper-lipolytic *Rhizopus oryzae* KG-10 on low value oil emulsions. *Res. J. Microbiol.*, 2: 671-677
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16:313–340.
- Shimizu, M., Nakagawa, Y., Sato, Y., Furuma, T., Igarosh, Y., Onaka, H., Yoshida, R. and Kunoh, H. (2000). Studies on endophytic actinomycetes and *Streptomyces* sp. isolated from *Rhododendron* and its antifungal activity. *J. Gen. Plant. Pathol.*, 66: 360-366.
- Subbanna ARNS, Rajasekhara H, Stanley J, Mishra KK, Pattanayak A. (2018). Pesticidal perspectives of chitinolytic bacteria in Molecular and Genetic Basis of Plant-Fungal Pathogen Interactions. *Applied Mycology & Biotechnology an International Series. Volume 4:59-97*
- Singh, R., N. Gupta and N. Kango. (2007). Production of lipase by hyper-lipolytic *Rhizopus oryzae* KG-10 on low value oil emulsions. *Res. J. Microbiol.*, 2: 671-677
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16:313–340.

- Tamreihaoa, K., Debananda S. Ningthoujama, Salam Nimaichanda, Elangbam Shanta Singh, Pascal Reenac, Salam Herojeet Singh. (2016). Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. *Microbiological Research* 192:260–270
- Thitiya Rattanakavil and and Khanungkan Klanbut. (2019). Actinomycetes from rice field soil, Nakonsawan and their antimicrobial activity. The 31st Annual Meeting of the Thai Society for Biotechnology and International Conference. November 10-12, 2019 Duangjitt Resort & Spa, Patong, Phuket, Thailand, pp 482-495.
- Tian, X. L., Cao, L. X., Tan, H. M., Zeng, Q. G., Jia, Y. Y., Han, W. Q., & Zhou, S. N. (2004). Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities in vitro. *World Journal of Microbiology and Biotechnology*, 20(3):303–309.
- Waksman SA. (1961). Baltimore: The Williams and Wilkins Company. The Actinomycetes, classification, identification and description of genera and species. 2: 61–292.
- Williams, S. T. & Cross, T. (1971). Actinomycetes. *Methods Microbiol* 4:295–334.
- Yukphan, P., T. Malimas, W. Potacharoen, S. Tanasupawat, M. Tanticharoen and Y. Yamada. (2005). *Neosasaia chiangmaiensis* gen. nov., sp. nov., a novel osmotolerant acetic acid bacterium in the α -Proteobacteria". *J. Gen. Appl. Microbiol.* 1(51): 301-311.

Effects of Coconut Coir Powders on the properties of Natural Rubber Composites

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Abstract

In this work, the coconut coir powders (CCP)/natural rubber (NR) composites were successfully prepared. The CCP acts as a reinforcing filler with two different sizes of fine powders (39 μm) and coarse powders (101 μm). The coconut coir powders added to the natural rubber matrix at the filler content of 25, 50, 75, and 100 phr. The effect of CCP contents on physical and mechanical properties was studied. The result revealed that the increase in CCP content has decreased the tensile strength, elongation at break and toughness of composites but increased the modulus of elasticity. The CCP/NR composite at 25 phr of filler loading shows better mechanical properties. For different sizes of fillers, the F-CCP exhibit the better mechanical properties and hardness compare to C-CCP. These properties of composites indicate that it can develop and possible apply in rubber mats.

Keywords: Coconut coir powders, Natural rubber, Mechanical properties, Composites

Introduction

Polymer composites are a combination of a polymer matrix with fillers. They have been attractively applied in industrial and academic researches due to the control of the material properties. For the past few decades, researchers have shown increasing interest in composite materials for those of biodegradable, eco-friendly, and renewable. Natural rubber (NR) as a green and renewable polymer is used extensively in many applications, including in tires, automotive parts, and rubber floor mats, because of its excellent mechanical and elastic properties. However, fillers are essential to modify the NR properties for the versatile application. The capability of the reinforcing filler depends on various factors such as surface area, the shape of fillers, and particle size. Natural fillers have been in enormous demand as a reinforcing material. These fillers act as good reinforcing agents with several specific properties such as high toughness, low cost, lightweight, good specific strength properties, and modulus, and complete burning without residue on the combustion (Ismail et

al., 2002; Luz et al., 2007; Panthapulakkal et al., 2006). The natural fibers act as reinforcing natural fillers with biodegradable and renewable properties. The probability of natural fibers such as kenaf, sisal, pineapple leaf, banana jute to produce polymer composites was studied (Faruk et al., 2012). Coconut coir is an inexpensive agricultural product that can be obtained in a large volume from the local community. It is a cheap eco-friendly fiber that is also less expensive than jute and sisal (Geethamma et al., 1998). The utilization of coconut coir as reinforcing fillers in polymer composites becomes more desirable due to their high strength and modulus properties (Macedo et al., 2010; Monteiro et al., 2008). Studies on powdered coconut coir as the filler has been appeared in the literature (Kaewduang et al., 2015; Sarki et al., 2011). This work aims to investigate the possibility of Coconut coir powders (CCP) of different particle sizes in using as reinforcement filler in the NR matrix. The effect of filler content on the properties of CCP/NR composites was investigated.

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Materials and Methods

Preparation of Coconut coir powders

The coconut coir was collected from the shell of the matured coconut and was broken into small pieces. The coconut coir was oven-dried at 100 °C overnight for removing moisture. After drying, a portion of the coconut coir powders (CCP) was milled into fine particles by the blender machine and fed into a vibrating sieving machine using 300 μm mesh which was called Fine CCP (F-CCP). A second portion, CCP were sieved through the previous mesh size without milling which was called Coarse-CCP (C-CCP). Their powder sizes were measured by an optical microscope and were used as the filler in NR composites. The average powder sizes of F-CCP and C-CCP were about 39 μm and 101 μm , respectively. And the morphology is shown in Figure 1.

Preparation of the composites

The formulation of the mixes was given in Table 1. The natural rubber used for the study was crepe rubber grade obtained from the local rubber companies. Coconut coir powders (CCP) were used as the filler. The composite compounds with different CCP loading were mixed using an internal mixer. The Sulphur and TMTD acted as a vulcanizing agent and were added during the milling step using a two-roll milling machine. The sheeted rubber compounds were kept for maturation at room temperature for 24 h until the mixture was hardened. After that, the rubber composite compounds were vulcanized in the compression-molding under 1

min preheating at 150 °C and compressed under a pressure of 75 kg/cm^2 (mold dimension: 160 x 160 x 2 mm^3).

Characterization

Mechanical properties

For the stress-strain behavior of composite samples, the Shimadzu Testing Machine (AG-X Plus 10kN model) was used. The tensile properties of the composites were tested according to ASTM D412-1998, at a crosshead speed of 500 mm/min at room temperature. The Hardness test on Shore-A Durometer according to ASTM D2240 was conducted using Bareiss Digi test II hardness tester. The specimen should be at least 6 mm thick and hardness reading was performed within three seconds during testing. Finally, the hardness was measured for five different positions and the average values were presented.

Physical properties

The swelling measurements of the composites were examined in the toluene. The specimen with a thickness of 2 mm was cut into a rectangular shape in the dimension of 25x25 mm. Then the composite samples were dried at 60°C for 24 h. The specimen was immersed in toluene for 48 h at 25°C. The swollen composite sample was taken out of the toluene and wiped with tissue paper to remove excess toluene. Then, the weight of the swollen samples was determined precisely. This data can be determined for the percentage of

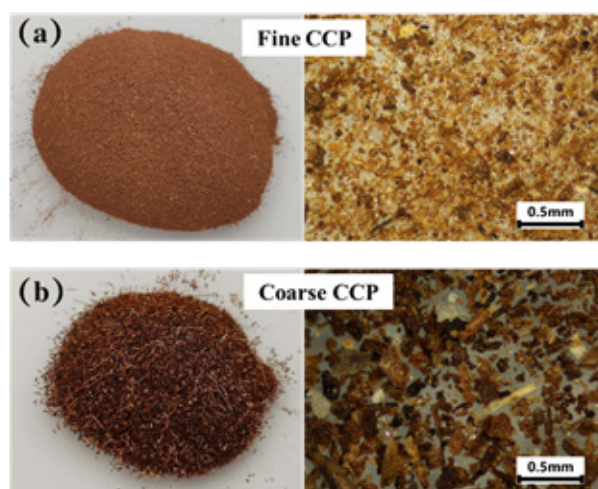


Figure 1. (a) CCP with milling by blender machine (F-CCP) and (b) CCP without milling (C-CCP) and both sieving through 300 μm sieve size

Table 1. Mixing formulations of composites

Ingredient	phra
Crepe rubber (NR)	100
ZnO	5
Stearic acid	2
Wingstay L	1
Ozone wax	1
Clay	100
White oil	1
CCPb	0, 25, 50, 75, 100
TMTDc	1
Sulfur	1.7

a Parts per hundred of rubber

b Coconut CoirPowders of both fine and coarse in particle sizes

c Tetramethylthiuram Disulfide

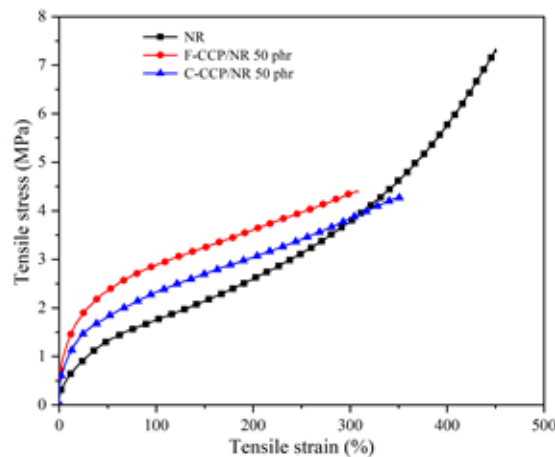


Figure 2. Stress-strain curves of NR, F-CCP/NR and C-CCP/NR composites with CCP content of 50 phr.

swelling and also rubber-filler interactions. The percentage of swelling can be calculated by;

$$\text{Swelling (\%)} = \frac{M - M_d}{M_d} \times 100 \quad (2)$$

Where M and Md are the wet weight after removing the excess toluene and dry weight, respectively.

Results and discussion

Figure 2 shows the tensile stress-strain curves in CCP/NR composites with 50 phr of F-CCP and C-CCP. The NR sample without CCP loading exhibits a typical

stress-strain behavior of vulcanized rubber showing the ultimate strain of around 440%. It is noted that all the CCP/NR composites have higher moduli than the NR sample. In the presence of 50 phr of C-CCP/NR composite, the ultimate strength and strain are reduced. Tensile strength was decreased by about 42% and strain at the break by 23%.

The CCP with the massive diameter causes deterioration in the properties due to the formation of voids at the CCP-matrix interface because of insufficient interfacial interaction (Parambath et al., 2019). Besides, the F-CCP/NR composite was observed that the tensile

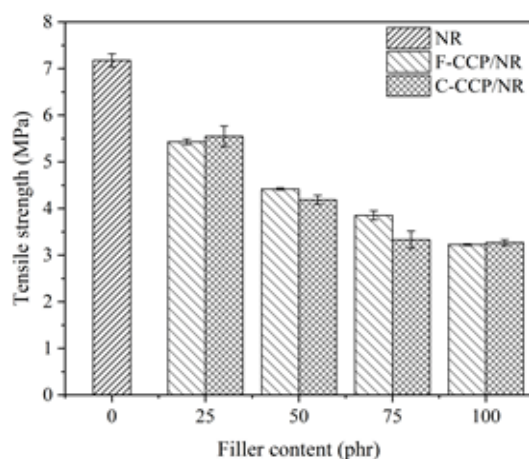


Figure 3. The effect of filler content on tensile strength of NR, F-CCP/NR and C-CCP/NR composites

strength can be improved. Although the value of elongation at break decreased from 341% to 311%, however, the F-CCP/NR composites retain the elastic properties of the pure NR

The effect of filler loading with different CCP sizes on tensile strength of CCP/NR composites is shown in Figure 3. As observed, the increase of CCP contents affected in the weaken tensile strength when compared with the NR compound. For the CCP/NR composites, the increase of CCP content inducing tensile strength decreased. It is found that the maximum value of tensile strength at 25 phr of filler content. This result implies that the CCP/NR composite at 25 phr uniform the dispersion of filler in NR compounds and might be the effect of better interfacial adhesion due to a large interfacial area of contact contributes to a higher tensile strength (Balan et al., 2017; Herrera-Franco et al., 2004). When the filler contents are applied above 25 phr, the tensile strength decreased due to weak interaction and bonding between the filler and NR matrix are responsible for the decline of tensile strength. The other reason might be due to agglomeration. And, therefore, the filler – filler interaction of the CCP filler also increases (Balan et al., 2017). This is consistent with the study of Leha et al. which presents the effect of filler from 10 to 40 %. The filler content at 25% showed the highest tensile strength. For more addition of the filler, it presented a decrease in mechanical properties (Noor Leha et al., 2014). For the comparison of the tensile strength between F-CCP/NR and C-CCP/NR composites, it was

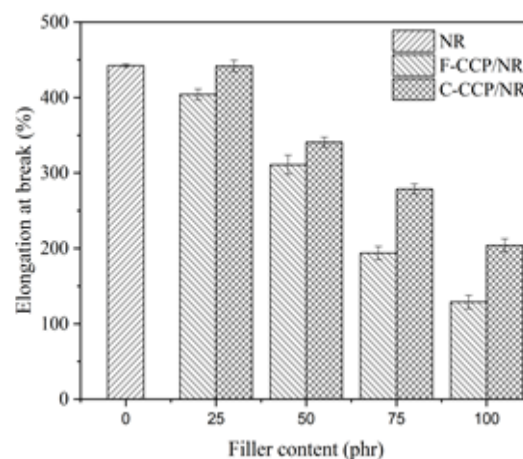


Figure 4. The effect of filler content on elongation at break of NR, F-CCP/NR and C-CCP/NR composites.

found that the filler content at 25 and 100 phr, C-CCP/NR exhibited the value which closes to the value of F-CCP. However, the filler contents of 50 and 75 phr exhibit the tensile strength of F-CCP/NR higher value than C-CCP/NR. This is due to the effect of the high specific surface area of filler sizes on the NR matrix (Sareena et al., 2012).

Figure 4 shows the elongation at break of the CCP/NR composites is decreased with increasing filler content due to the addition of filler which reduces the mobility and increases the brittleness of the composites (Islam et al., 2017).

The lower elongation at high filler content might be due to the void portion is filled up according to the adding of filler content. Besides, the increase of CCP filler content in the NR compounds stiffens and also hardens the NR compounds. It will reduce resilience and toughness that leads to lower elongation. The toughness behavior is showed in Figure5. Comparing the elongation at break at the different size of CCP loading, it is observed that F-CCP/NR composites provide the higher elongation than those of C-CCP/NR composites. This result implies that it is due to better interaction between F-CCP and NR matrix (Parambath et al., 2019; Wongsorat et al., 2014).

Figure 6. shows the effect of filler content on the modulus of elasticity of F-CCP/NR and C-CCP/NR composites. Fillers are known to enhance the modulus, exhibited that the modulus of the filler is higher than that of the NR matrix (0 phr) (Sareena et al., 2012). In

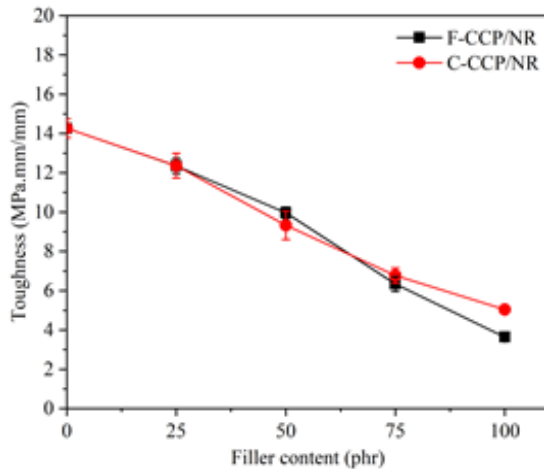


Figure 5. Effect of filler content on the toughness of F-CCP/NR and C-CCP/NR composites.

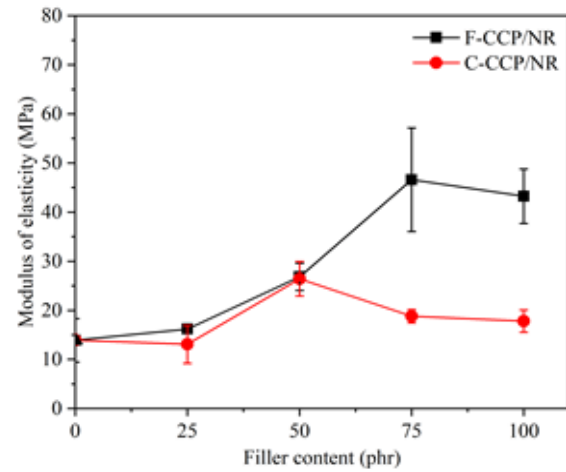


Figure 6. Effect of filler content on Modulus of elasticity of F-CCP/NR and C-CCP/NR composites.

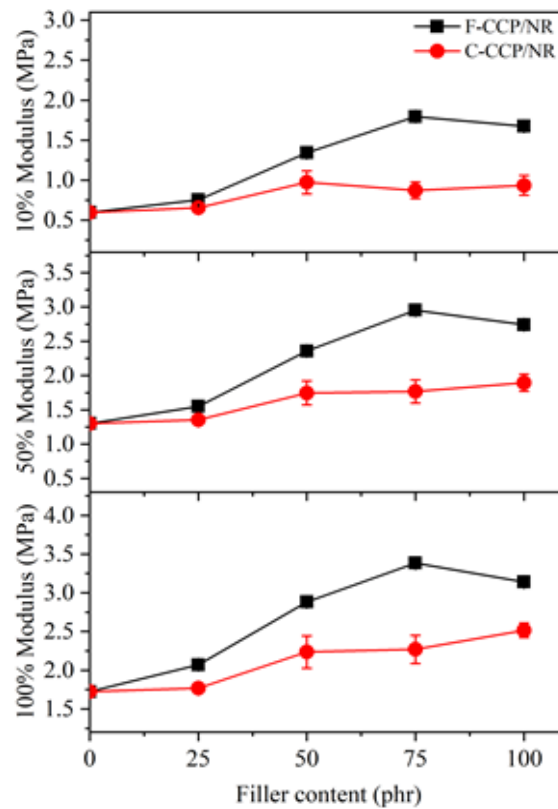


Figure 7. Moduli at (a) 10% strain, (b) 50% strain and (c) 100% strain of F-CCP and C-CCP filled NR composites

the case of F-CCP/NR, composites increase in the modulus with the filler content increasing almost linear up to 75 phr. While the modulus decreased when further increasing the filler content at 100 phr. For the C-CCP/NR composites, it exhibits an increase of modulus with the filler content up to 50 phr. Then an increasing filler content causes the modulus trend to decrease which is lower as compared with F-CCP/NR composites. This result implies that modulus of F-CCP/NR composites improved due to the homogeneous distribution

and their effective interaction with the matrix restrain the molecular movement (Parambath et al., 2019). The agglomeration of filler at a higher loading does not act a disturbing effect on the modulus due to it is measured at low strains which the stress concentration is not enough for initiation of crack.

The effect of filler content on the reinforcing efficiency of F-CCP and C-CCP, moduli at 10%, 50%, and 100% strains of the composites are observed in Figure 6. This variation is a measure of the stiffness of compos-

ites. It can be shown that 10%, 50%, and 100% strains increase slightly with increasing filler content. This reveals that the filler powder acts like rigid particulates since it has a higher modulus than the NR matrix (Saareena et al., 2012). The moduli at 10%, 50% and 100% strains of the composites containing F-CCP were higher than those containing C-CCP. This indicates that the smaller sized filler in F-CCP/NR composites performed the higher modulus values than those of larger sized filler in C-CCP/NR composites.

Figure 8. shows the hardness of CCP/NR composites with different sizes and filler loading of CCP. Hardness increases with the increase in filler content. The hardness of F-CCP/NR composites exhibits higher values than C-CCP filled composites. Hardness is a measure of the resistance to deformation. The incorporation of fillers into an NR matrix reduces the elasticity of rubber. All fillers used are non-deformable solids and the addition of more rigid particles leads to increased rigidity and stiffness of the material.

The CCP/NR composites with different sizes of filler content characterized to examine swelling in toluene at room temperature for 48 h are presented in Figure 9. The highest swelling value is NR composite at 197% while the tendency swelling decreases with increasing of the filler loading in the NR composites. This implies that the filler loading in NR blends that restrict the molecular movement of the rubber.

This then made it more difficult for the toluene to penetrate through the rubber, thus, decreasing the swelling percentage. However, the swelling percentage decreased up to 50 phr in solvent uptake (%) at equilibrium swelling of CCP filled NR can be additionally described in the increase in contact surface area between polymer and filler.

At lower loading, the dominating effect is polymer-filler networks, whereas, at higher loading, the filler-filler networks will govern in the composites (Swapna et al., 2016). The result was related to the increase in tensile modulus, hardness, and also tensile strength for CCP filled NR composite.

Conclusion

In summary, this report presented the effect of particle sizes of fine and coarse coconut coir powders on the mechanical properties of NR and CCP/NR composites. All CCP/NR composites were prepared with 25, 50, 75, and 100 phr of CCP filler content. The increase in CCP content has decreased the tensile strength, elongation at break and toughness, whereas increasing the modulus of elasticity of CCP/NR composites. The result reveals that the CCP/NR composite of 25 phr filler loading exhibits better mechanical properties. F-CCP filler exhibits better mechanical properties and hardness as compared to C-CCP filler. The swelling values of CCP/NR composites in the toluene solution trend to decrease

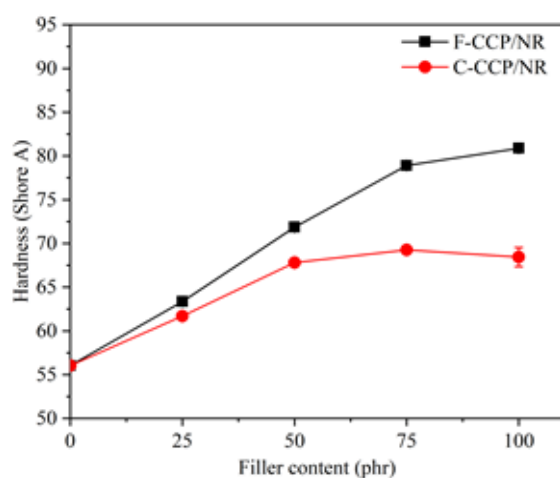


Figure 8. Variation of hardness with filler content for CCP/NR composites

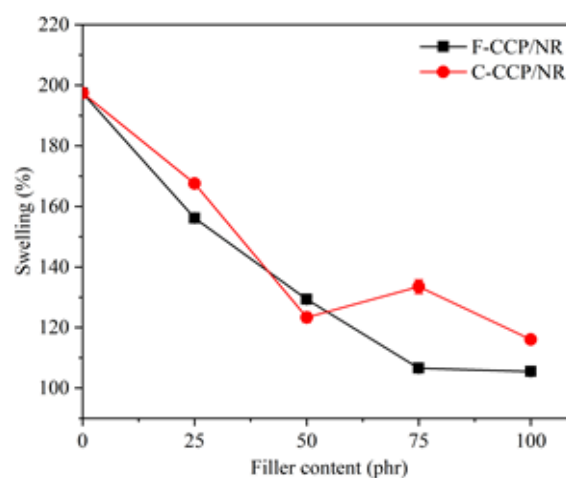


Figure 9. The effect of CCP loading on the swelling percentage of NR composites

with increasing filler loading in the NR composites. These results indicate that CCP/NR composites can be developed and apply for rubber mat.

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References

- Balan, A. K., Mottakkunnu Parambil, S., Vakyath, S., Thulissery Velayudhan, J., Naduparambath, S., & Etathil, P. (2017). Coconut shell powder reinforced thermoplastic polyurethane/natural rubber blend-composites: effect of silane coupling agents on the mechanical and thermal properties of the composites. *Journal of Materials Science*, 52(11), 6712-6725. doi:10.1007/s10853-017-0907-y
- Faruk, O., Bledzki, A. K., Fink, H.-P., & Sain, M. (2012). Biocomposites reinforced with natural fibers: 2000–2010. *Progress in Polymer Science*, 37(11), 1552-1596. doi:https://doi.org/10.1016/j.progpolymsci.2012.04.003
- Geethamma, V. G., Thomas Mathew, K., Lakshminarayanan, R., & Thomas, S. (1998). Composite of short coir fibres and natural rubber: effect of chemical modification, loading and orientation of fibre. *Polymer*, 39(6), 1483-1491. doi:https://doi.org/10.1016/S0032-3861(97)00422-9
- Herrera-Franco, P. J., & Valadez-González, A. (2004). Mechanical properties of continuous natural fibre-reinforced polymer composites. *Composites Part A: Applied Science and Manufacturing*, 35(3), 339-345. doi:https://doi.org/10.1016/j.compositesa.2003.09.012
- Islam, M., Das, S., Saha, J., Paul, D., Islam, M., Rahman, M., & Khan, M. (2017). Effect of Coconut Shell Powder as Filler on the Mechanical Properties of Coir-polyester Composites. *Chemical and Materials Engineering*, 5, 75-82. doi:10.13189/cme.2017.050401
- Ismail, H., Edyham, M. R., & Wirjosentono, B. (2002). Bamboo fibre filled natural rubber composites: the effects of filler loading and bonding agent. *Polymer Testing*, 21(2), 139-144. doi:https://doi.org/10.1016/S0142-9418(01)00060-5
- Kaewduang, M., Chaichana, E., Jongsomjit, B., & Jaturapiree, A. (2015). Use of coir-filled LLDPE as a reinforcement for natural rubber composite. Paper presented at the Key Engineering Materials.
- Luz, S. M., Gonçalves, A. R., & Del'Arco, A. P. (2007). Mechanical behavior and microstructural analysis of sugarcane bagasse fibers reinforced polypropylene composites. *Composites Part A: Applied Science and Manufacturing*, 38(6), 1455-1461. doi:https://doi.org/10.1016/j.compositesa.2007.01.014
- Macedo, J. d. S., Costa, M. F., Tavares, M. I. B., & Thiré, R. M. S. M. (2010). Preparation and characterization of composites based on polyhydroxybutyrate and waste powder from coconut fibers processing. *Polymer Engineering & Science*, 50(7), 1466-1475. doi:10.1002/pen.21669
- Monteiro, S. N., Terrones, L. A. H., & D'Almeida, J. R. M. (2008). Mechanical performance of coir fiber/polyester composites. *Polymer Testing*, 27(5), 591-595. doi:10.1016/j.polymertesting.2008.03.003
- Noor Leha, A. R., & Nordin, N. A. (2014). Effect of Filler Compositions on the Mechanical Properties of Bamboo Filled Polyester Composite. *Advanced Materials Research*, 879, 90-95. doi:10.4028/www.scientific.net/AMR.879.90
- Panthapulakkal, S., Zereshkian, A., & Sain, M. (2006). Preparation and characterization of wheat straw fibers for reinforcing application in injection molded thermoplastic composites. *Bioresource Technology*, 97(2), 265-272. doi:https://doi.org/10.1016/j.biortech.2005.02.043
- Parambath Kanoth, B., Thomas, T., Joseph, J. M., & Narayanankutty, S. K. (2019). Restructuring of coir to microfibers for enhanced reinforcement in natural rubber. *Polymer Composites*, 40(1), 414-423. doi:10.1002/pc.24667
- Sareena, C., Ramesan, M. T., & Purushothaman, E. (2012). Utilization of coconut shell powder as a novel filler in natural rubber. *Journal of Reinforced Plastics and Composites*, 31, 533-547. doi:10.1177/0731684412439116

- Sarki, J., Hassan, S. B., Aigbodion, V. S., & Ogheneweta, J. E. (2011). Potential of using coconut shell particle fillers in eco-composite materials. *Journal of Alloys and Compounds*, 509(5), 2381-2385. doi:<https://doi.org/10.1016/j.jallcom.2010.11.025>
- Swapna, V. P., Stephen, R., Greeshma, T., Sharan Dev, C., & Sreekala, M. S. (2016). Mechanical and swelling behavior of green nanocomposites of natural rubber latex and tubular shaped halloysite nano clay. *Polymer Composites*, 37(2), 602-611. doi:10.1002/pc.23217
- Wongsorat, W., Suppakarn, N., & Jarukumjorn, K. (2014). Effects of compatibilizer type and fiber loading on mechanical properties and cure characteristics of sisal fiber/natural rubber composites. *Journal of Composite Materials*, 48(19), 2401-2411. doi:10.1177/0021998313498790

Investigation of Physical and Mechanical Properties of Colored TiO₂ Thin Films deposited by RF Magnetron Sputtering

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Abstract

The colored titanium dioxide (TiO₂) thin films were prepared by RF magnetron sputtering technique using purity TiO₂ target. In this study, the deposition time for sputtering process was varied from 60, 75, 90, 105 and 120 minutes by using the fixed appropriate sputtering power at 100 W. The working pressure of argon gas was kept constant at 6.0x10⁻¹ Pa. As a consequence, TiO₂ thin films were deposited on both glass and stainless steel substrates for color's film observation. To study the physical and mechanical properties of colored TiO₂ thin films, optical transmission, surface morphology and structure were investigated by using UV-Vis Spectrophotometer, FESEM and XRD, thin film thickness and adhesion were measured by calotester and micro scratch tester, respectively. It was found that the colored TiO₂ thin films were clearly observed on both substrates. The optical transmission for TiO₂ films showed highly transmission in the visible regions and surface morphologies showed nano-scale grain size and smooth surface. The structure of all TiO₂ thin films exhibited amorphous structure. For these results, thicknesses of colored TiO₂ thin films are approximately 70 nm to 150 nm. In addition, the adhesion of colored films performed a good adhesion by increasing deposition time.

Keyword: TiO₂ thin film, Colored thin film, RF magnetron sputtering

Introduction

Titanium dioxide (TiO₂) thin film is attractive and utilizing material for many applications because of their interesting physical, chemical, optical and electrical properties. Many applications of TiO₂ thin film are extensively such as photovoltaic materials (Timoumi, Alamri and Alamri, 2018), photocatalytic properties (Zahedi et al., 2015), gas sensor (Salman, Shihab, and Elttayef, 2019), hydrophilic and hydrophobic properties (Xiong et al., 2015; Bharti, Kumar and Kumar, 2016; Gao et al., 2015). The physical and chemical techniques have been applied to fabricate the TiO₂ thin films such as spray (Momeni et al., 2015), sol-gel (Jiang, et al., 2019; Meher and Balakrishnan, 2014), pulse laser (Zhang et al., 2015; Ishii et al., 2015), chemical vapor deposition (Astinchap and Laelabadi, 2019) and sputtering (Guillén and Herrero, 2017; Nezar et al., 2017). However, for enhanc-

ing thin film uniformity and adhesion on a substrate, the magnetron sputtering is a more favorable technique for many types of coating materials such as oxides, nitrides, carbides, fluorides and arsenides than that of the wet process. One of the most attractive thin films is the variation of colors, especially gold-like color. Regularly, many composite thin films including metal oxides as well as nitrides such as TiN, ZnO, ZrN, AlTiN and TiO₂ have their specific colors for decorative and hard coatings (Neugebohrn et al., 2019; Niyomsoan et al., 2002; Panjan et al., 2014). In this work, the colored TiO₂ films were fabricated by radio frequency (RF) magnetron sputtering on glass and stainless steel (SS) substrates for color change monitoring in order to investigate the adhesion of colored thin films. The deposition time were varied from 60 - 120 minutes with constant RF power and argon (Ar) gas pressure during deposition process.

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For observing the adhesion of thin film, one of the outstanding mechanical instruments is the scratch tester which causes surface failure to indicate the adhesion and scratch resistance of the films (Mercier et al., 2017; Zivica et al., 2012). The optical properties of colored films were characterized by UV-Vis Spectrophotometer, the morphologies were characterized by Field Emission Scanning Electron Microscope (FESEM). Thin film structures were analyzed by X-ray diffractometer (XRD). For mechanical testing, the thicknesses of TiO_2 films were measured by calotest technique and thin film adhesion and scratch resistance were observed by micro scratch tester.

Experiment

1. Thin film fabrication

The various colored TiO_2 thin films were fabricated by RF magnetron sputtering technique using 99.95% purity TiO_2 ceramic target with thickness and diameter of 6 mm and 50.8 mm, respectively. TiO_2 films were deposited onto 1 mm thick of slide glass (2.54 cm x 7.62 cm) and 2 mm thick of stainless steel (3 cm x 3 cm) substrates. Prior to deposition, glass and stainless steel substrates were cleaned ultrasonically in acetone, methanol and deionized water for 15 minutes and then dried before loading into the deposition chamber. The distance between the target and substrate holder was fixed at 10 cm. Base pressure in the chamber was approximately 2.0×10^{-4} Pa at starting the sputtering process. Then, argon sputtering gas was feed into the chamber with the controlled working pressure at 6.0×10^{-1} Pa and RF power were kept constant and 100 W. In this study, TiO_2 thin film was deposited at room temperature and the substrate holder was continuous-

ly rotated by varying the deposition time from 60, 75, 90, 105 and 120 minutes. For thin film characterization, the optical transmission, surface morphology, structure, thickness and the adhesion were investigated.

2. Thin film characterization

Optical properties of the colored TiO_2 thin films deposited on glass substrate were analyzed by UV-Vis Spectrophotometer (UV-1700, SHIMADZU). Surface morphologies of the colored TiO_2 thin films were observed by Field Emission Scanning Electron Microscope operated at 5 kV (FESEM, JEOL JSM-6340F). The structures were characterized by X-ray diffractometer (XRD, Rigaku SmartLab). Besides, thicknesses of the TiO_2 thin films were characterized by calotester (Calotest compact CATc, Anton Paar). In addition, the adhesion and scratch resistance of the colored TiO_2 films were investigated by using Micro Scratch Tester with Rockwell diamond stylus indenter (MST3, Anton Paar).

Results and discussion

TiO_2 thin films grown on glass and stainless steel substrates can be observed with the observers' naked eyes in Figure 1. When the deposition time increased from 60-120 minutes, the colors were varied and adhered on both glass and stainless steel substrates. TiO_2 thin films grown on glass substrate, the colored were slightly changed from purple, blue, green, orange and pink. In case of stainless steel substrates, the colored were quite different from the colors on glass substrate as blue, green, gold, rose gold and pink because of the reflection of substrate material. It can be obviously seen that the different substrates can lead to slightly different colored films.

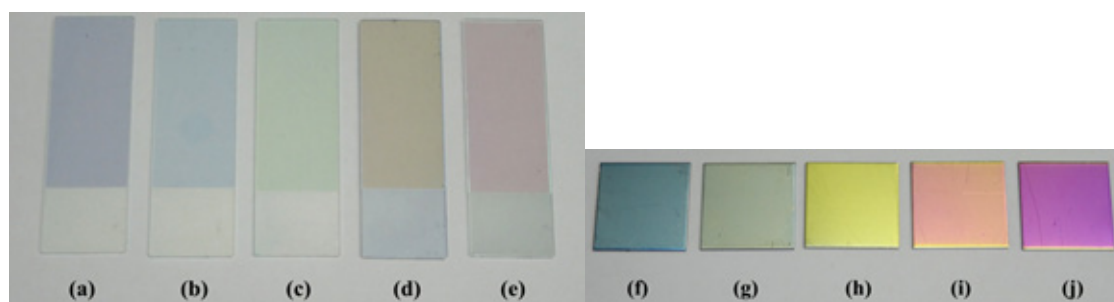


Figure 1. The colored TiO_2 thin films grown on glass and stainless steel substrates by increasing deposition time, (a,f) 60 minutes, (b,g) 75 minutes, (c,h) 90 minutes, (d,i) 105 minutes and (e,j) 120 minutes.

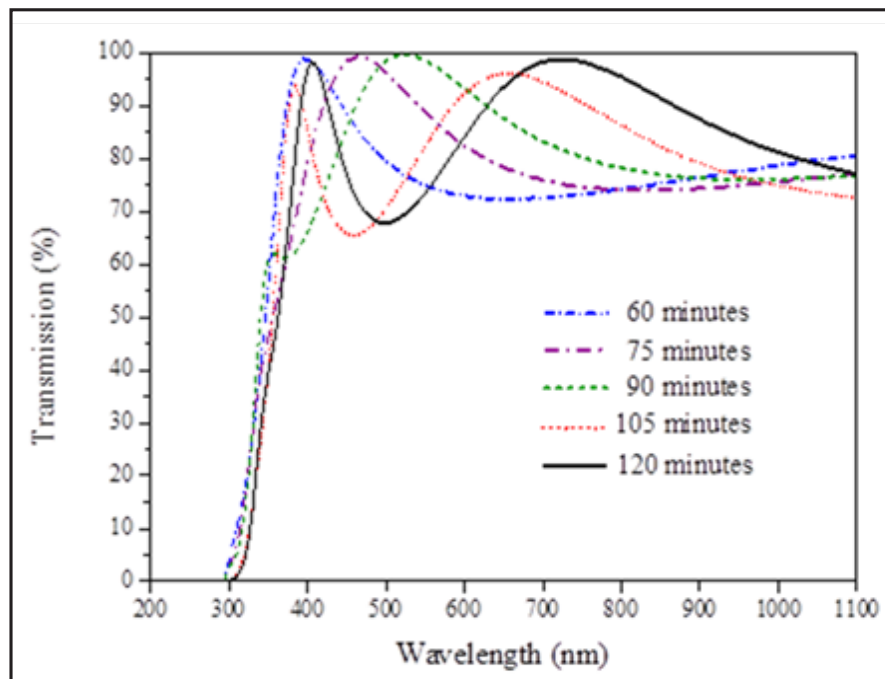


Figure 2. The optical transmission of TiO_2 thin films by varying deposition time from 60-120 minutes.

1. Optical transmissions

Optical transmission spectra of different deposition time of TiO_2 thin films grown on glass substrates was measured as the function of wavelengths ranging between 300 nm and 1100 nm by UV-Vis Spectrophotometer as seen in Figure 2. In order to increase deposition time from 60-120 minutes with constant sputtering power at 100 W, TiO_2 thin films obtained highly transmission around 70-98% with a sharply absorption edge around 300-320 nm. The transparency levels of the films for 105 and 120 minutes were slightly lower and the absorption edges were slightly higher. It can be indicated that the increase of deposition time can cause increasing thin film thickness which related with band gap energy. In addition, all TiO_2 thin films had high transmission in the visible range (400-700 nm) which could be easily observed by naked eyes. As a result, the interference layer of thin film and the substrate is also depended on thin film thickness.

2. Surface morphologies

The results of different deposition time on surface morphologies of the TiO_2 thin films grown on glass substrate were observed by FESEM as shown in

Figure 3. Surface morphologies of all TiO_2 films were comparatively nano-scale particle size and smooth surface. For the deposition time at 60 minute, the surface morphologies were rather smaller and denser particles than those of other conditions. In case of increasing deposition time, the morphologies had rather bigger and rougher than the less time deposition. It can be suggested that the sputtered particles had more time to arrange themselves and accumulate on the substrates.

3. Thin film structures

The structure of TiO_2 thin films by varying deposition time were characterized by x-ray diffractometer with grating incident at 1θ between incident x-ray and substrate. The x-ray diffraction patterns of TiO_2 thin film by increasing deposition time can be seen in Figure 4. All TiO_2 thin films compared with a bare glass substrate showed amorphous structure even though the deposition time was increased. These results could be explained that TiO_2 thin films deposited at room temperature have no activated energy by thermal treatment from the substrate to rearrange and enhance the crystalline structure.

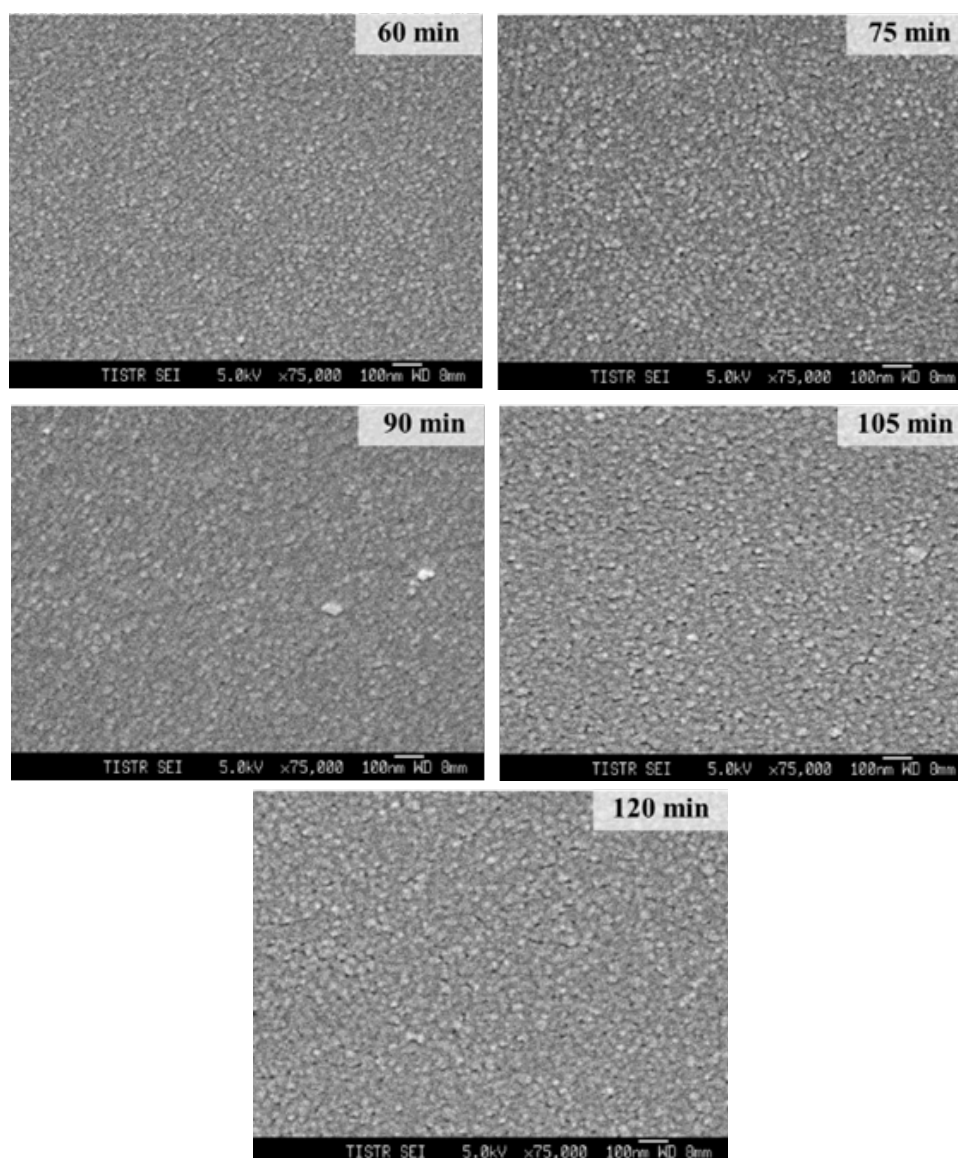


Figure 3. Surface morphologies of TiO_2 thin films by varying deposition time from 60-120 minutes.

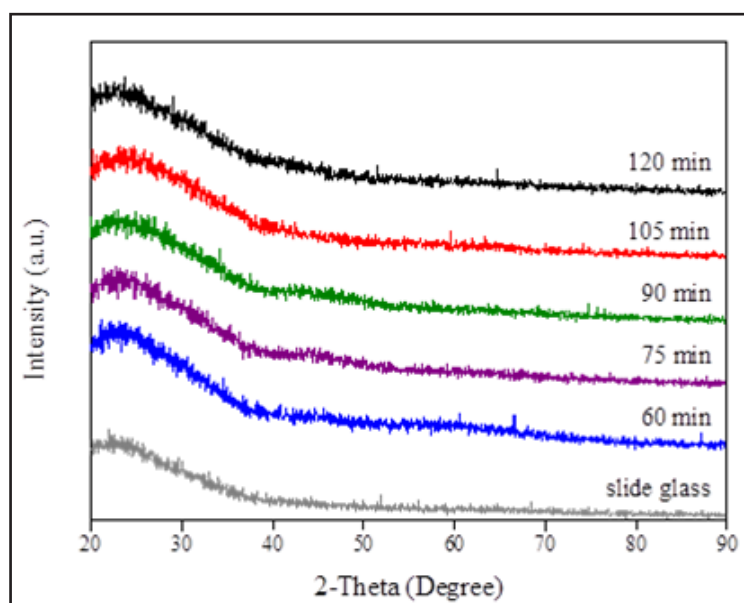


Figure 4. Structure of TiO_2 thin films by varying deposition time from 60-120 minutes.

4. Thin film thicknesses

The thickness of various colored TiO_2 thin films grown on glass substrate were measured by calotester. In this method, the sample was fixed at the stage and a rotating steel ball with a 20 mm diameter rotated on a thin film surface. Thus, the difference between abrasive layer of TiO_2 thin film and glass substrate is the thickness of the film. For various colored TiO_2 thin film, the thicknesses were increased by increasing the deposition time. When the deposition time increased from 60 – 120 minutes, the thicknesses of TiO_2 thin films are absolutely increased from 70.1 – 151.2 nm. TiO_2 thin film gained the average deposition rate around 1.22 nm/min (The deposition rate was defined by a ratio between thickness to deposition time) with the standard deviation of 0.03. Thicknesses and deposition rates of TiO_2 thin films by varying deposition time from 60-120 minutes can be seen in Table 1 and Figure 5.

5. Thin film adhesions

The adhesion of the colored TiO_2 thin films deposited on stainless steel was measured by micro scratch tester with a normal force from 0.01 N to 2.00 N. However, thin film coating quality is related to the adhesion characteristics between thin film and substrate. The surface failure behavior of each sample was observed under a microscope scratching with a progressive load along the scratch distance of 2 mm. Three different types of critical load (L_c) were indicated along the scratch trace; a start of the scratch (L_{c1}), delamination (L_{c2}) and complete delamination (L_{c3}) as shown in Table 2. Figure 6 shows the optical photographs of the scratch trace of increasing deposition from 60-120 minutes. The scratch testing results of TiO_2 thin films deposited from 60-120 minutes had the complete delamination at critical load of approximately 1.09 N - 1.80 N, respectively. As a result, colored TiO_2 thin films per-

Table 1. Thicknesses and deposition rates of TiO_2 thin films by varying deposition time from 60-120 minutes.

Deposition time (minutes)	Deposition rate (nm/min)	Thickness (nm)
60	1.17	70.1
75	1.21	91.0
90	1.24	111.3
105	1.20	126.0
120	1.26	151.2

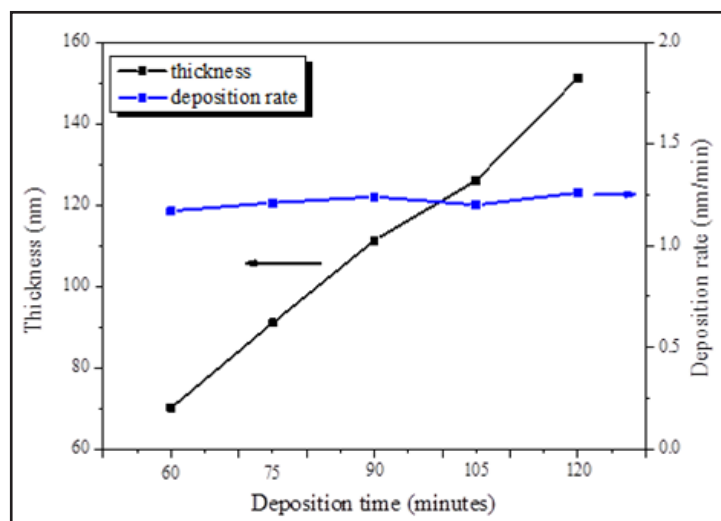


Figure 5. Thicknesses and deposition rates of TiO_2 thin films by varying deposition time from 60-120 minutes.

formed good adhesion on the substrate because of preparing by PVD technique even though the structures of the film had amorphous structure. Moreover, the maximum deposition time as well as maximum thickness exhibits the longer distance of delamination than those of thinner thickness.

Conclusions

The physical and mechanical properties of colored TiO_2 thin films prepared by RF magnetron sputtering technique were studied on both physical and mechanical properties such as the transmission, morphology, structure, thickness and the adhesion of the films. The

thicknesses of the films were increased from 70.1-151 nm with increasing deposition time at 60-120 minutes with approximately constant deposition rate. Then, the colors of thin film can also be change by the thickness and the interference of light on substrates. The colored TiO_2 thin films have high transmission around 70-98% at the visible range region. Then, the surface morphologies exhibit smooth surface with nano-scale particle size and then the structure of colored TiO_2 thin films show amorphous structure due to no thermal treatment during thin film deposition. Moreover, scratch testing results show good adhesion between various TiO_2 thin films and the substrate.

Table 2. The critical load of scratch testing by varying deposition time from 60-120 minutes.

Deposition time (minutes)	Lc1 (N)	Lc2 (N)	Lc3 (N)
60	0.20	0.50	1.09
75	0.21	0.64	1.15
90	0.21	0.69	1.34
105	0.23	0.97	1.44
120	0.27	1.29	1.80

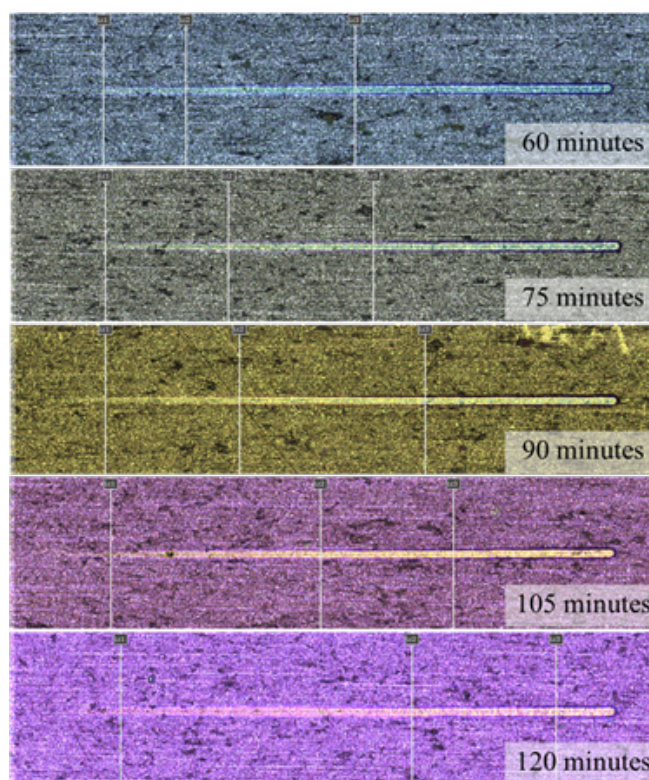


Figure 6. The scratch traces with critical loads of TiO_2 thin films by varying deposition time from 60-120 minutes.

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References

- Astinchap, B. and Laelabadi, K. G. (2019). Effects of substrate temperature and precursor amount on optical properties and microstructure of CVD deposited amorphous TiO₂ thin films. *Journal of Physics and Chemistry of Solids*, 129, 217-226.
- Bharti, B., Kumar, S. and Kumar, R. (2016). Superhydrophilic TiO₂ thin film by nanometer scale surface roughness and dangling bonds. *Applied Surface Science*, 364, 51-60.
- Gao, Z., Zhai, X., Liu, F., Zhang, M., Zang, D. and Wang, C. (2015). Fabrication of TiO₂/EP superhydrophobic thin film on filter paper surface. *Carbohydrate Polymers*, 128, 24-31.
- Guillén, C. and Herrero, J. (2017). TiO₂ coatings obtained by reactive sputtering at room temperature: Physical properties as a function of the sputtering pressure and film thickness. *Thin Solid Films*, 636, 193-199.
- Ishii, A., Nakamura, Y., Oikawa, I., Kamegawa, A. and Takamura, H. (2015). Low-temperature preparation of high-n TiO₂ thin film on glass by pulsed laser deposition. *Applied Surface Science*, 347, 528-534.
- Jiang, Y., Shi, K., Tang, H. and Wang, Y. (2019). Enhanced wettability and wear resistance on TiO₂/PDA thin films prepared by sol-gel dip coating. *Surface and Coatings Technology*, 375, 334-340.
- Meher, S.R. and Balakrishnan, L. (2014). Sol-gel derived nanocrystalline TiO₂ thin films: A promising candidate for self-cleaning smart window applications. *Materials Science in Semiconductor Processing*, 26, 251-258.
- Mercier, D., Mandrillon, V., Parry, G., Verdier, M., Estevez, R., Bréchet, Y. and Maindron, T. (2017). Investigation of the fracture of very thin amorphous alumina film during spherical nanoindentation. *Thin Solid Films*, 638, 34-47.
- Momeni, M., Golestani-Fard, F., Saghaian, H., Barati, N. and Khanahmadi, A. (2015). Development of visible light activated TiO₂ thin films on stainless steel via sol spraying with emphasis on microstructural evolution and photocatalytic activity. *Applied Surface Science*, 357, part B, 1902-1910.
- Neugebohrn, N., Gehrke, K., Brucke, K., Götz, M. and Vehse, M. (2019). Multifunctional metal oxide electrodes: Colour for thin film solar cells. *Thin Solid Films*, 685, 131-135.
- Nezar, S., Saoula, N., Sali, S., Faiz, M., Mekki, M., Laoufi, N. A. and Tabet, N. (2017). Properties of TiO₂ thin films deposited by rf reactive magnetron sputtering on biased substrates. *Applied Surface Science*, 395, 172-179.
- Niyomsoan, S., Grant, W., Olson, D. L. and Mishra, B. (2002). Variation of color in titanium and zirconium nitride decorative thin films. *Thin Solid Films*, 415, 187-194.
- Panjan, M., Klanjšek Gunde, M., Panjan, P. and Čekada, M. (2014). Designing the color of AlTiN hard coating through interference effect. *Surface and Coatings Technology*, 254, 65-72.
- Salman, S. H., Shihab, A. A. and Elttayef, A. – HK. (2019). Design and construction of Nanostructure TiO₂ thin film gas sensor prepared by R.F magnetron sputtering technique. *Energy Procedia*, 157, 283-289.
- Timoumi, A., Alamri S. N. and Alamri, H. (2018). The development of TiO₂-graphene oxide nano composite thin films for solar Cells. *Results in Physics*, 11, 46-51.
- Xiong, Y., Lai, M., Li, J., Yong, H., Qian, H., Xu, C., Zhong, K. and Xiao, S. (2015). Facile synthesis of ultra-smooth and transparent TiO₂ thin films with superhydrophilicity. *Surface and Coatings technology*, 265, 78-82.
- Zahedi, F., Behpour, M., Ghoreish, S. M. and Khalilia, H. (2015). Photocatalytic degradation of paraquat herbicide in the presence TiO₂ nanostructure thin films under visible and sun light irradiation using continuous flow photoreactor. *Solar Energy*, 120, 287-295.

- Zhang, Z., Wong, L. M., Zhang, Z., Wu, Z., Wang, S., Chi, D., Hong, R. and Yang, W. (2015). Pulse laser deposition of epitaxial TiO₂ thin films for high-performance ultraviolet photodetectors. *Applied Surface Science*, 355, 398–402.
- Zivica, F., Babic, M., Adamovic, D., Mitrovic, S., Todorovi, P., Favaro, G. and Pantic, M. (2012). Influence of the surface roughness on adhesion of chrome coatings on alloy tool steel X165CrMoV12. *Journal of the Balkan Tribological Association*, 18(2), 228–237.

The role of wild pollinators enhancing the sustainable productivity and quality of arabica coffee in agroforestry

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Abstract

Crop, coffee, productivity and quality, in this study, was conducted at Ban Khun Lao, Chiang Rai Province and investigated the impact of pollination services. The different pollinations were compared between autogamy, wind pollination, and open pollination on various parameters following: fertilisation ratio, fruit set, cherry weight, ripening uniformity. Among these, open pollination was recorded at the significantly highest results. Data on weight of green beans and total yield exhibited clearly that open pollination were heavier than on wind pollination and autogamy. In addition, the taste and aroma of given coffee from open pollination were honey, caramelised and jasmine which navigated mostly to the sweet and floral. These results indicated that the community and diversity of insect pollinators had the dramatic effectiveness toward the productivity and quality of arabica coffee in organic-agroforestry.

Keywords: Wild pollinators, Arabica coffee, Agroforestry, Productivity, Cup quality

Introduction

Agroforestry is the land-use system which applied together with technology and land management in perennials area aimed for agricultural production. In agroforestry system, particularly, can be defined as a dynamic, ecologically based, natural resource management system that benefit to human, notably pollination services (Priess et al., 2007). Pollination service is the most important for both natural and agricultural system due to most of flowering plants depend on insect pollinators for crop production. (Bentrup et al., 2019). In our previous investigation, we studied on insect diversity and density of insect fauna in agroforestry area, coffee farming among natural plants, at Ban Khun Lao, Wieng Pa Pao district, Chiang Rai Province, northern Thailand, which leads to present study “The role of wild pollina-

tors enhancing the sustainable productivity and quality of arabica coffee in agroforestry”. *Coffea arabica*, is dominant variety of coffee in northern Thailand. The previous study indicated that coffee flower pollination was performed by a number of native insect species. (Wissarut et al., 2017). These species contained various of insect orders which were confederate in action. Even though, wind pollination and autogamy were usually occurred; there were various evidence that quality and quantity of arabica coffee bean were enhanced by insect pollinators (Ngo et al., 2011; Smith, 2010). The number of cherries was increased significantly in the area where has higher density and diversity of insect fauna. High diversity and density pollinator. High diversity and density of pollinator service increased effectiveness of pollination by enormous number of pollens deposited on

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stigmas and the chance of cross pollination. In addition, ripening was more uniformity when flowers were pollinated from insect pollinators for example bees, and stingless bees (Corbet et al., 2015; Karanja et al., 2013; Klein et al., 2003; Vergara & Badano, 2009)

Not only in productivity, coffee cup quality was also influenced by the many inputs especially the pollination services from insect. Previous reports revealed that taste and aroma of processed coffee were enhanced by the pollination distinctively. (Karanja et al., 2013; Ricketts et al., 2004; Roubik, 2002) Mostly, the higher quality of *C. arabica* commonly grown in north of Thailand were located where national park nearby. Therefore, organic plantation method is probably introduced and recognised for growers allowing not any effects to the natural fauna and flora.

Materials and methods

The experiment was carried out in flowering season of April, 2016 in arabica coffee plantation where organic practices held at 1,200 to 1,300 metres above sea level at Ban Khun Lao village Wieng Pa Pao district, Chiang Rai province, north of Thailand. The temperature ranged between 13.23 ± 2.01 to 27.45 ± 1.75 °C and relative humidity was between 78.41 ± 3.12 and $86.73 \pm 2.21\%$. The 5 to 6 years old coffee plants without any applied chemicals, were selected from 3 different sites for replication. Branches on selected plants with six clusters of flower pods were marked for experiment. There were 3 treatments: self pollination or autogamy (control treatment), wind pollination (none of insect pollinating service), and open pollination (insect pollinating services).

Number of flower pods on selected branches were counted prior applied experimental treatments, including (1) self pollination or autogamy (2) wind pollination and (3) open pollination or insects' pollinator service, which were considered as control treatment, insects' pollination services and insects' pollination services. Firstly, self pollination or autogamy, the pods were covered with gauze to protect them from other factors, while the second treatment-wind pollination, the selected branches with six clusters of flowers were

enclosed in nylon mesh bag until the pollination completed. The latter treatment, the selected branches were leaved openly to attract the pollinators.

After 3 days, the distinctly enlarged receptacles were counted and the fertilisation ratio were calculated using the following equation : (Number of enlarged receptacles/ total number of flower pods) x100. The treatments then were left for harvesting.

At harvesting season, the total number of cherries and ripe fruits were counted, then ripening uniformity was calculated using (Number of ripe cherries/ total number of cherries) x100. Fully ripe cherries were picked from each treatment were weighed and measured for quantifying. Cherries, then, were taken into dried processing within 10 hours to prevent the deterioration that might affect to quality cup. The 100 green beans and total yield per shrub were weighted.

The none defected coffee green beans from each 3 different pollination methods were taken into light roasting process, hot-air roaster with internal temperature of 180°C – 205°C until the beans popped or cracked and expanded in size to serve cupping process. The light roasting was held for the cupping study to retain more the origin flavours and unique elements, reflecting the natural qualities of the coffee and also offered the multilayered complexity, revealing traces of sweetness, fruit tanginess, or even subtle floral aroma. The coffee cupping was conducted at the laboratory of the coffee factory of Green Net Co., Ltd using the method by SCAA to clarify the characteristics of the given coffee from the experiment. The quality scale ranged following the table below. Moreover, the specific descriptors of coffee experiences were navigated using the Coffee Flavour Wheel of SCAA.

Results and Discussion

The 3 different pollination methods from the previous flowering season in 2016 were significantly different from all aspects. The open pollination with the services of various insect pollinators of organic coffee agroforestry showed the dramatic highest of the successful fertilisation with 98.75 % followed by wind pollination showing at 64.23%. In contrast, the lowest

Table 1 Standard quality scale from SCAA Cupping Form.

Scale	Description
6.00 - 6.75	Good
7.00 - 7.75	Very good
8.00 - 8.75	Excellent
9.00 - 9.75	Outstanding

Table 2 Pre-harvest productivity of arabica coffee given from 3 different pollination method.

Pollination methods	Fertilisation ratio (%)	Fruit set (%)	Berry weight (g)	Ripening uniformity (%)
Autogamy	23.71±1.23	4.2±0.52	1.61±0.04	8.38±0.12
Wind pollination	64.23±2.01	11.3±0.42	2.13±0.05	34.84±0.24
Open pollination	98.75±1.34	75.43±0.65	2.14±0.07	88.26±0.51
MEAN	81.79	30.31	1.96	43.83
F-test	**	**	**	**

Table 3 Yield and quantity of coffee green bean given from 3 different pollination method

Pollination methods	Weight of 100 green beans (g)	Total yield (green bean) per shrub (kg)
Autogamy	14.95±2.2	0.05±0.3
Wind pollination	17.77±2.3	0.11±0.02
Open pollination	18.25±1.8	0.25±0.02
MEAN	16.65	0.13
Prop entry	**	**

successful fertilisation was observed from the autogamy showing at 23.71%. Similarly, the highest percentage of fruit set was observed from the open pollination followed by wind pollination and autogamy showing at 75.43%, 11.30%, and 4.20%, respectively. Noticeably, cherry weights per fruit from the open pollination and wind pollination were not significantly different at 2.14 g and 2.13g. While the autogamy gave the lowest cherry weight at 1.61 g. Moreover, the ripening uniformity from these pollination methods was significantly different.

The open pollination remained the good productivity of ripening uniformity at 88.26% followed by wind pollination and autogamy showing at 34.84g, and 8.38g. (Table 2)

Weight of 100 green beans from 3 different pollination methods was clarified that open pollination gave the highest quantity than the 2 others. Consequently, the total yield of green bean observed from the open pollination was better than wind pollination and autogamy, distinctively. (Table 3)

The fertilisation ratio held from the enlarged receptacles due to the fact that after the incident of fertilisation, the ovary within the flower suddenly developed. This current results have shown that fertilisation ratio of coffee flower given from open pollination is approximately four times greater than on autogamy which are similar to the other studies. (Karanja et al., 2013) Moreover, the consequence of open pollination had distinctively shown the difference to other pollination methods. The amount of fruit-set from open-pollinated flower was the greatest among three pollination method. Correspondingly, the higher coffee yields given from open pollination have been reported in previous studies. The berry weight from open pollination was heavier than on autogamy and wind pollination which were bagged with fine-mesh. These translated to higher yielding coffee (Karanja et al., 2013)

With the SCAA coffee cupping form, there were similar results from the different pollination methods.

The total scores were slightly different ranging from 66.75, 65.75, and 64.75 from the open pollination, wind pollination and autogamy, respectively. The 2 variables were discarded out from the consideration for instance uniformity and clean cup, because only non-defected green beans had been selected prior to the roasting process. The sweetness and body of coffee were similar among the 3 pollination methods. The acidity from autogamy was merely higher than the others which represented a bit sourer. The autogamy also lost some balance compared to the others. Importantly, the highest scores of the fragrance or aroma of coffee were obtained from the open pollination followed by wind pollination and autogamy. (Figure 1)

According to the Coffee Flavour Wheel from SCAA, the taste and aroma of given coffee from open pollination were honey, caramelised and jasmine which navigated mostly to the sweet and floral. The wind pollination in contrast gave the green/ vegetative

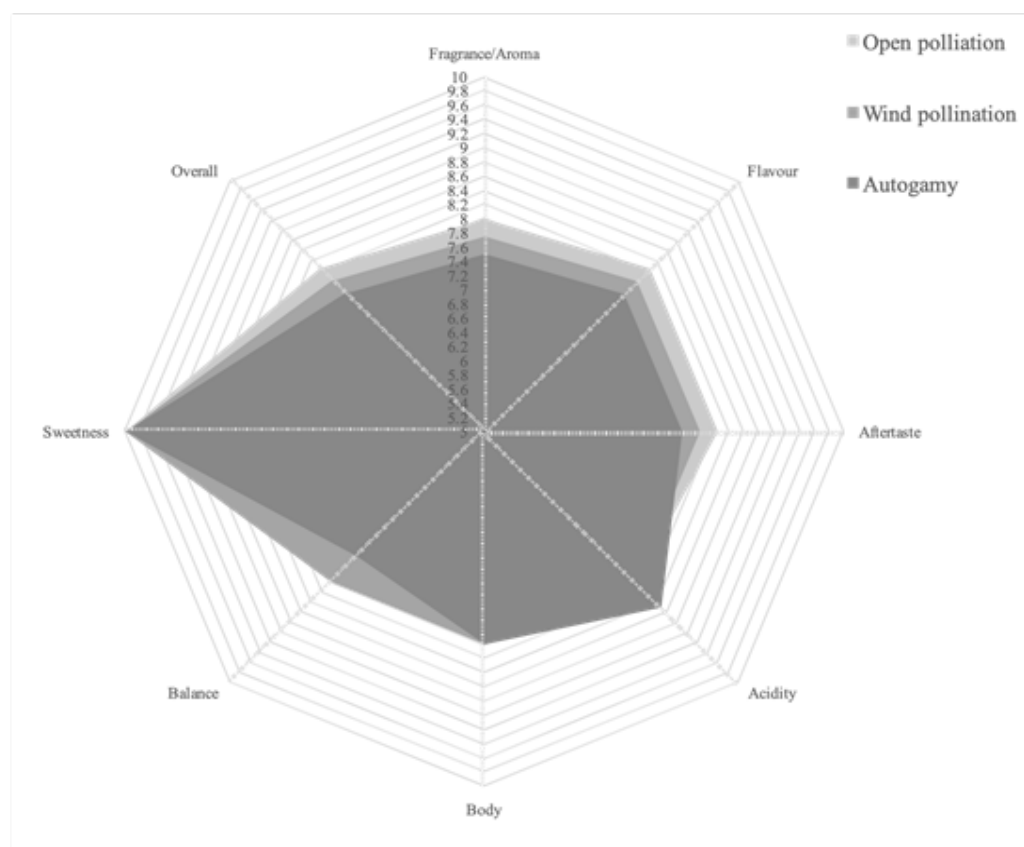


Figure 1. The quality cup of coffee from 3 different pollination methods. The variables compared were following: sweetness, overall, fragrance/aroma, flavour, aftertaste, acidity, body, and balance and were considered with the method by SCAA.

sense from fresh and herb-like aroma. Interestingly, the aromas navigated on flavour wheel were varied for instance brown sugar, nutty, and cereal.

This recent study has shown that the wild pollinators caused the cross pollination of coffee flowers were not influenced only on the enhancement of fertilisation, fruit set, and berry weight, but also played a significant role to cup quality. This phenomenon was described as the presence of pollinators affected to the quality of flower fertilisation, avoiding the misshapen fruits, and defected green bean. Without pollinators, the coffee flowers were led to self pollination or autogamy which inferior biological qualities associated with the pleasant aroma of cup quality. (Karanja et al., 2011; Karanja et al., 2014; Krishnan et al., 2012; Roubik, 2002)

Conclusion

Ecological service available from pollinating insect community as can be seen from open pollination was significant influenced to the productivity of arabica coffee especially in pre-harvest stage. The quality of fertilisation, fruit set, cherry weight, and ripening uniformity given from open pollination were superior than

on wind pollination and self pollination or autogamy. These beneficial productivities initiate to the lower cost in hand-picking harvest, less time consuming of green bean grading. Importantly, the presence of wild pollinators built up the uniqueness of cup quality. As can be seen from the Coffee Flavour Wheel, the light roasted coffee given from open pollination indicated to wild flowers scent. Therefore, keeping abundance of wild pollinators where the organic agriculture is held for coffee plantation could lead the potential of arabica coffee production.

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References

- Bentrup, G., Hopwood, J., Adamson, N., & Vaughan, M. (2019). Temperate Agroforestry Systems and Insect Pollinators: A Review. *Forests*, 10, 981. doi:10.3390/f10110981
- Corbet, S., Williams, I., & Osborne, J. (2015). Bees and the Pollination of Crops and Wild Flowers in the European Community. *Bee World*, 72, 47-59. doi:10.1080/0005772X.1991.11099079
- Karanja, R., Gikungu, M., Njoroge, G., Newton, L. E., & Kihoro, J. M. (2011). Comparison of Bee Pollinators of Coffee in Organic and Conventional Farms. *Asian Journal of Agricultural Sciences*, 3(6), 469-474.
- Karanja, R., Njoroge, G., Gikungu, M., & Newton, L. E. (2014). Pollination Efficiency of Bee Species Pollinating Coffea Arabica in Kiambu County Kenya. *Current Research Journal of Biological Sciences*, 6, 179-182. doi:10.19026/crjbs.6.5190
- Karanja, R., Njoroge, G., Kihoro, J., Gikungu, M., & Newton, L. E. (2013). The Role of Bee Pollinators in Improving Berry Weight and Coffee Cup Quality. *Asian Journal of Agricultural Sciences*, 5, 52-55. doi:10.19026/ajas.5.4841
- Klein, A. M., Dewenter, I. S., & Tschardtke, T. (2003). Bee pollination and fruit set of Coffea arabica and C. canephora (Rubiaceae). *American Journal of Botany*, 90(1), 153-157.
- Krishnan, S., Kushalappa, C. G., Shaanker, R. U., & Ghazoul, J. (2012). Status of pollinators and their efficiency in coffee fruit set in a fragmented landscape mosaic in South India. *Basic and Applied Ecology*, 13(3), 277-285. doi:http://dx.doi.org/10.1016/j.baae.2012.03.007

- Ngo, H. T., Mojica, A. C., & Packer, L. (2011). Coffee plant – pollinator interactions: a review. *Canadian Journal of Zoology*, 89(8), 647-660. doi:10.1139/z11-028
- Priess, J., Mimler, M., Klein, A., Schwarze, S., Tschardtke, T., & Steffan-Dewenter, I. (2007). Linking deforestation scenarios to pollination services and economic returns in coffee agroforestry systems. *Ecological applications* : a publication of the Ecological Society of America, 17, 407-417. doi:10.1890/05-1795
- Ricketts, T., Daily, G., Ehrlich, P., & Michener, C. (2004). Economic value of forest to coffee production. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 12579-12582. doi:10.1073/pnas.0405147101
- Roubik, D. W. (2002). The value of bees to the coffee harvest. *Nature*, 417, 708.
- Smith, D. J. (2010). *Agroforestry: Reconciling Production with Protection of the Environment*. The Organic Research Centre, 5.
- Vergara, C. H., & Badano, E. I. (2009). Pollinator diversity increases fruit production in Mexican coffee plantations: The importance of rustic management systems. *Agriculture, Ecosystems & Environment*, 129(1–3), 117-123. doi:http://dx.doi.org/10.1016/j.agee.2008.08.001
- Wissarut, S., Phawini, K., Dumrongpon, D., & Duangthip, K. (2017, August 22nd-24th). Diversity of insect fauna and pollinators in organic arabica coffee plantation at Ban Khun Lao, Chiang Rai province, the north of Thailand. Paper presented at the ASEAN+6 Organic Agriculture Forum 2017, Balios Resort Khaoyai, Nakhon Ratchasima, Thailand.