DEVELOPMENT OF AN ORGANOPHOSPHATE AND CARBAMATE PESTICIDE TEST KIT BY USING A MAGNETIC PARTICLE COATED WITH ESTERASE ENZYME FROM HONEY BEE HEADS

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MASTER OF SCIENCE
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MASTER OF SCIENCE
IN HEALTH SCIENCES RESEARCH

Examination Committee :

Ting Xu
....Chairman
(Prof. Dr. Ting Xu)

.....Member
(Asst. Prof. Dr. Surat Hongsibsong)

Sawaeng
.....Member
(Dr. Sawaeng Kawichai)

Advisory Committee :

.....Advisor
(Asst. Prof. Dr. Surat Hongsibsong)

Sawaeng
.....Co-advisor
(Dr. Sawaeng Kawichai)

29 March 2024

Dear Asst. Prof. Dr. Surat Hongsibsong, Family, and Friends,
I want to express my sincere thanks for your incredible support and understanding. Your help has been instrumental in making me strong and successful.

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Udomsap Jaitham

หัวข้อปริญญานิพนธ์	การพัฒนาชุดทดสอบสารกำจัดศัตรูพืชกลุ่มออร์กาโนฟอสเฟตและคาร์บาเมตโดยใช้อนุภาคแม่เหล็กที่เคลือบด้วยเอนไซม์เอสเตอเรสจากหัวผึ้ง
ผู้เขียน	นาย อุดมทรัพย์ ใจธรรม
ปริญญา	วิทยาศาสตรมหาบัณฑิต (การวิจัยวิทยาศาสตร์สุขภาพ)
คณะกรรมการที่ปรึกษา	ผศ.ดร. สุรัตน์ หงษ์สิบสอง อาจารย์ที่ปรึกษาหลัก ดร. แสวง กาวิชัย อาจารย์ที่ปรึกษาร่วม

บทคัดย่อ

ออร์กาโนฟอสเฟต (OPs) และคาร์บาเมต (CMs) เป็นตัวแทนของกลุ่มสารกำจัดศัตรูพืชที่สำคัญที่ใช้กันอย่างแพร่หลายในการเกษตรของไทย สาธารณสุข และการควบคุมศัตรูพืช ในประเทศไทย การพึ่งพาสารกำจัดศัตรูพืชเหล่านี้ในการจัดการศัตรูพืช รวมถึงแมลง วัชพืช และเชื้อรา มีความสำคัญมาก สารประกอบเหล่านี้ออกฤทธิ์โดยการยับยั้งอะเซทิลโคลีนเอสเทอเรส ซึ่งเป็นเอนไซม์สำคัญในการสลายอะซิติลโคลีน อย่างไรก็ตาม วิธีการวิเคราะห์แบบเดิมๆ สำหรับการตรวจจับ OP และ CM มักจะมีค่าใช้จ่ายสูง โดยต้องใช้อุปกรณ์เฉพาะทางและนักวิเคราะห์ที่มีทักษะ สิ่งนี้ตอกย้ำความจำเป็นเร่งด่วนสำหรับเทคนิคการตรวจจับที่คุ้มค่า รวดเร็ว และใช้งานง่าย

เพื่อตอบสนองต่อความจำเป็นนี้ การศึกษาของเรานำเสนอวิธีการที่เป็นนวัตกรรมสำหรับการตรวจจับ OP และ CM ในประเทศไทย วิธีการนี้ใช้ประโยชน์จากอนุภาคแม่เหล็กที่เคลือบด้วยเอนไซม์เอสเทอเรสที่สกัดจากหัวผึ้ง รวมกับวิธีวัดสี ขีดจำกัดการตรวจจับ (LOD) ที่ได้รับสำหรับ OP โดยเฉพาะไดคลอวอส และ CM เช่น ฟีโนบูคาร์บ นั้นต่ำอย่างน่าประทับใจที่ 0.002 มก./ลิตร การวิเคราะห์เปรียบเทียบกับการศึกษาก่อนหน้านี้แสดงให้เห็นถึง LOD ที่เหนือกว่าของวิธีที่เราพัฒนาขึ้น นอกจากนี้ เมื่อเปรียบเทียบกับแก๊สโครมาโตกราฟีด้วยการตรวจจับเปลวไฟโฟโตเมตริก (GC-FPD)

วิธีการของเราแสดงให้เห็นประสิทธิภาพที่เทียบเคียงได้ในการตรวจจับ OP และ CM ที่ตกค้างในผักและผลไม้

เทคนิคที่ก้าวล้ำนี้นำเสนอชุดทดสอบที่คุ้มค่าสำหรับการตรวจจับ OP และ CM ในประเทศไทย โดยมีศักยภาพในการลดค่าใช้จ่ายและทำให้กระบวนการตรวจสอบง่ายขึ้น ความสามารถในการตรวจจับสารตกค้างในผักและผลไม้เน้นการใช้งานจริงสำหรับการใช้งานทางการเกษตร ซึ่งถือเป็นก้าวสำคัญในการติดตามตรวจสอบสารกำจัดศัตรูพืชตกค้างในประเทศที่เข้าถึงได้ง่ายและมีประสิทธิภาพมากขึ้น แม้จะมีความท้าทายที่เกี่ยวข้องกับการใช้สารกำจัดศัตรูพืช แต่วิธีการของเรายังคงสัญญาว่าจะเพิ่มความปลอดภัยและประสิทธิภาพของการจัดการสารกำจัดศัตรูพืชในประเทศไทย

Thesis Title	Development of an Organophosphate and Carbamate Pesticide Test Kit by Using a Magnetic Particle Coated with Esterase Enzyme from Honey Bee Heads
Author	Mr. Udomsap Jaitham
Degree	Master of Science (Health Sciences Research)
Advisory Committee	Asst. Prof. Dr. Surat Hongsibsong — Advisor Dr. Sawaeng Kawichai — Co-advisor

ABSTRACT

Organophosphates (OPs) and carbamates (CMs) represent pivotal pesticide classes extensively employed in Thai agriculture, public health, and pest control. In Thailand, the reliance on these pesticides to manage pests, including insects, weeds, and fungi, is substantial. These compounds exert their effects by inhibiting acetylcholinesterase, a critical enzyme for acetylcholine breakdown. However, the conventional analytical methods for detecting OPs and CMs are often cost-prohibitive, demanding specialized equipment and skilled analysts. This underscores the urgent necessity for cost-effective, rapid, and user-friendly detection techniques.

In response to this imperative, our study presents an innovative method for OPs and CMs detection in Thailand. This method leverages magnetic particles coated with esterase enzyme extracted from honey bee heads, combined with a colorimetric approach. The achieved limits of detection (LODs) for OPs, notably Dichlorvos, and CMs, such as Fenobucarb, were impressively low at 0.002 mg/L. Comparative analyses with preceding studies demonstrated the superior LODs of our developed method. Furthermore, when benchmarked against Gas Chromatography with Flame Photometric Detection

(GC-FPD), our method exhibited comparable efficacy in detecting OPs and CMs residues in fruits and vegetables.

This groundbreaking technique proposes a cost-effective test kit for OPs and CMs detection in Thailand, with the potential to reduce expenses and simplify verification processes. Its capability to detect residues in fruits and vegetables highlights its practicality for agricultural applications, representing a noteworthy stride towards more accessible and efficient pesticide residue monitoring in the country. Despite the challenges associated with pesticide use, our method holds promise for enhancing the safety and efficiency of pesticide management in Thailand.

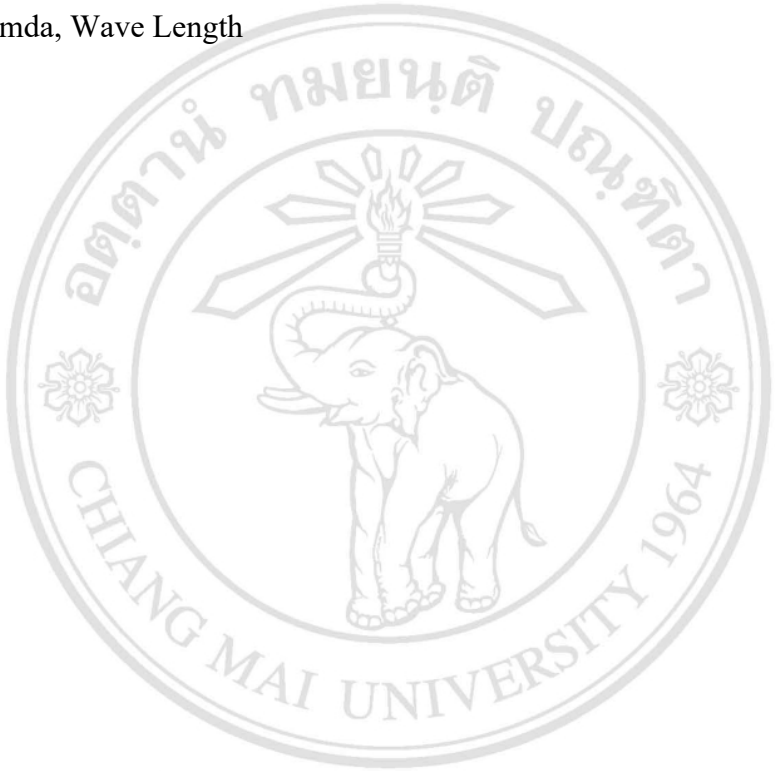
CONTENTS

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ANA	Alpha naphthyl acetate
CMs	Carbamate groups
GC	Gas chromatography techniques
GC/MS	Gas chromatography coupled with mass spectrometry
HPLC	High-performance liquid chromatography
IC_{50}	50% Inhibitory Concentration
IC_{10}	10% Inhibitory Concentration
LC-MS/MS	Liquid chromatography coupled with mass spectrometry
LOD	Limits of detection
MNPs	Magnetic nanoparticles
MRLs	Maximum residue limits for pesticides in food
Ops	Organophosphate groups
pH	Potential of Hydrogen ion

LIST OF SYMBOLS

α	Alpha, Substrate Coefficient
°C	degree Celsius
λ	Lamda, Wave Length



CHAPTER 1

Introduction

1.1 Statement of the problem

Over the last few decades, Thailand's agricultural sector has grown and diversified, and one of the hallmarks of this growth has been the increased use of heavy machinery, hybrid seeds, and synthetic pesticides. These transformations in agricultural practices have presented new challenges, particularly concerning the health and safety of agricultural workers and the environmental impact of intensive pesticide use. In order to maintain good agricultural yields and manage pests like weeds, fungus, and insects, Thailand, like many other countries, mostly relies on pesticides [1]. Thailand continues to increase its annual import of pesticides, with commonly imported insecticide classes including organophosphate (OPs; e.g., chlorpyrifos) and carbamate (CMs; e.g., fenobucarb, methomyl) [2]. As a result of widespread use, there is a release of residues from arbitrary harmful pesticides, which represents a significant risk to the environment and human health [3]. There are many ways that OPs and CMs can enter the human body, including through the respiratory tract, gastrointestinal tract, skin, eyes, and wounds. When OPs or CMs enter the body, they are distributed in various tissues or organs through the blood circulation system, causing toxic reactions [4], inhibiting acetylcholinesterase (AChE), which is responsible for many biochemical reactions. For example, OPs, which are carcinogenic, pose risks to the endocrine system, metabolism, nervous system, liver disorders, psychiatric symptoms, and neuritis [5], while CMs affect diarrhea, respiratory disorders, the nervous system, carcinogens, and reproductive toxicity [6]. The effect of the contamination of OPs and CMs in production agriculture is food safety. Over the past several years, food safety incidents have occurred frequently, affecting public health and social well-being and causing severe economic losses in the food industry [7]. Food safety is recognized by the World Health Organization and member countries as an important

public health function [8] and is one of the most important public problems around the world [9]. Modern analytical techniques are widely used in the food industry to ensure the quality and safety of all types of food, from farmland to dining tables [10].

Currently, food safety testing techniques generally include confirmed quantitative tests, rapid screening test techniques, gas chromatography techniques (GC) [11], high-performance liquid chromatography (HPLC) [12], gas chromatography coupled with mass spectrometry (GC/MS) [13], and liquid chromatography coupled with mass spectrometry (LC-MS/MS) [14]. These methods are quite expensive and need highly experienced personnel, pricey purification processes, and specialised primary equipment, despite the fact that they are very effective and can detect very low amounts. [15]. New technologies based on biological detection systems have surfaced during the last few decades. Of these methods, biosensors have demonstrated promising futures since they are easy to use and less expensive than traditional methods. For the goal of identifying OPs and CMs, biosensors based on acetylcholinesterase (AChE) inhibition have been thoroughly investigated [16].

This research endeavors to address this need by proposing the "Development of Organophosphate and Carbamate Pesticide Test Kit by Using Magnetic Particle Coated with Esterase Enzyme from Honeybee Heads." Honeybees are indeed excellent indicators for detecting environmental pesticide contamination due to their foraging behavior and sensitivity to chemical pollutants [17]. However, compared to other biological species (vertebrates or invertebrates), few studies have used bees as biological indicators [18]. Acetylcholinesterase (AChE) from honey bee is a biological indicator to evaluate the effects of organophosphate and carbamate [19] and then use the responses of other biological indicators after exposure to pesticides [20]. Enzymes from honey bee are highly sensitive and selective to specific substrates. By one of the mechanisms of action, a signal is generated that can be detected after transfer and allows for the measurement of the amount of contaminants present in the sample [21]. In particular, OPs and CMs pesticides can be detected using acetylcholinesterase (AChE). A decrease in the activity of this enzyme is useful in monitoring the presence of OPs and CMs in the sample because the more inhibitors there are, the less AChE activity will be [22].

In recent years, novel biological detection systems have emerged, with biosensors taking center stage for their simplicity, cost-effectiveness, and efficiency compared to traditional methods. Biosensors that rely on the inhibition of acetylcholinesterase (AChE) have been particularly well-received for detecting OPs and CMs pesticides [16]. Within the realm of enzyme Zahirinejad, nanoparticles have become increasingly attractive as precursors due to their large surface-to-volume ratio, allowing for nanoscale reactions, increased sensitivity, and the stabilization of three-dimensional structures [23]. Magnetic nanoparticles (MNPs) have garnered special attention because they can be adorned with various target molecules and are easily recoverable using external magnetic fields, thereby preventing enzyme loss post-detection [24]. Unlike traditional techniques such as electrophoresis or chromatography, MNPs do not require sample pre-treatment, ensuring high operational speed and accuracy [25].

This research presents a comprehensive exploration into the development and optimization of a test kit for detecting organophosphate and carbamate pesticides. It involves fixing acetylcholinesterase on magnetic microbeads using a method based on nickel-histidine affinity and proposes an innovative approach for traditional honeybee acetylcholinesterase fixation through covalent coupling on magnetic particle. This is followed by colorimetric detection, offering high accuracy in visually detecting target analytes without the need for complex equipment [26–27]. The research also evaluates the specificity, practicality, and stability of the developed color sensors. These colorimetric sensors offer rapid detection, high sensitivity, and low cost for the detection of OPs and CMs residues, making them a valuable tool for enhancing food safety in agricultural products.

1.2 Research objectives

The aims of this research are as follows:

1.2.1 To develop a test kit for detecting organophosphate and carbamate pesticides using magnetic particles coated with esterase enzymes extracted from honeybee heads.

CHAPTER 2

Research Articles

2.1) Published paper

2.1.1) Author's Name Udomsap Jaitham, Sumed Yadoung, Anurak Wongta, Bajaree Chuttong, Khanchai Danmek, and Surat Hongsibsong , "Publication Title"Color Test Kit for Detecting Organophosphate and Carbamate Pesticides by Using Esterase Extract from Honey Bees, Journal Philippine Journal of Science, Vol.152 Issue: 3 Date: June 2023, Page No. 1245–1252 DOI: https://doi.org/10.56899/152.03.39

Research Objectives, Hypotheses, and Significant Findings: Developing a color test kit for detecting organophosphate and carbamate pesticides using esterase extract from honey bees significantly advances environmental and agricultural safety. This innovative approach leverages the natural enzymatic properties of esterase from honey bees to detect the presence of harmful pesticides in the environment. The hypothesis behind this research is that the esterase enzyme, when coated onto magnetic particles, can specifically bind to organophosphate and carbamate pesticides, leading to a color change that is indicative of their presence. This method offers a non-destructive, rapid, and cost-effective way to monitor pesticide contamination in water sources, soil, and other environmental samples.

2.1.1.1) Research Objectives:

1) **To develop a magnetic particle-coated esterase enzyme from honey bees:** This involves isolating the esterase enzyme from honey bee heads and coating it with magnetic particles. The enzyme's ability to hydrolyze organophosphates and carbamates is exploited to detect these pesticides.

2) **To validate the sensitivity and specificity of the test kit:** This involves testing the kit's ability to accurately detect organophosphate and carbamate pesticides in various environmental samples,

including water, soil, and air, while minimizing false positives and negatives.

3) **To assess the applicability of the test kit in different environmental conditions:** This includes testing the kit's performance at various pH levels, temperatures, and contaminants that might interfere with the enzyme's activity.

2.1.1.2) Significant Findings:

1) **Development of a functional magnetic particle-coated esterase enzyme:** The research successfully developed a magnetic particle-coated esterase enzyme that can specifically bind to organophosphate and carbamate pesticides. This breakthrough was achieved by optimizing the coating process to ensure maximum enzyme activity and specificity.

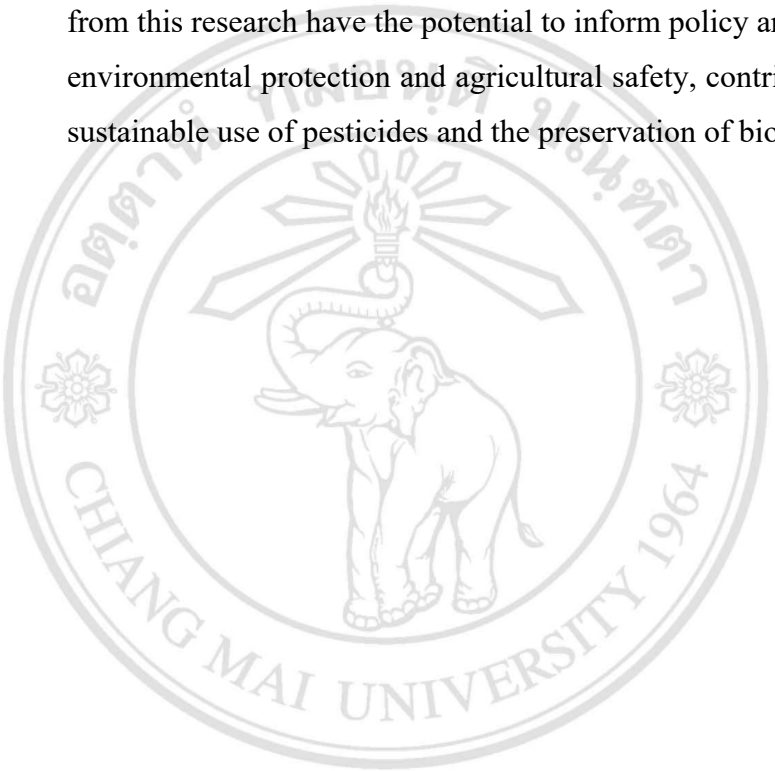
2) **High sensitivity and specificity of the test kit:** The test kit demonstrated high sensitivity and specificity in detecting organophosphate and carbamate pesticides in environmental samples. This was validated through rigorous testing against known standards and controls.

3) **Applicability across different environmental conditions:** The test kit showed good performance across a range of pH levels, temperatures, and in the presence of other contaminants. This indicates its broad applicability in various environmental monitoring scenarios.

4) **Cost-effectiveness and ease of use:** The magnetic particle-coated esterase enzyme test kit was found to be cost-effective and easy to use, making it a practical tool for environmental and agricultural safety monitoring.

2.1.1.3) Conclusion:

1) The development of a color test kit for detecting organophosphate and carbamate pesticides using a magnetic particle coated with esterase enzyme from honey bees represents a significant advancement in environmental monitoring. This innovative approach not only offers a non-destructive method for pesticide detection but also leverages the natural enzymatic properties of honey bees to address a critical environmental and health concern. The findings from this research have the potential to inform policy and practice in environmental protection and agricultural safety, contributing to the sustainable use of pesticides and the preservation of biodiversity.



CHAPTER 3

Conclusion

In summary, this study successfully employed enzymes extracted from honeybee heads and naphthyl acetate to develop a robust color test method for detecting pesticides, particularly organophosphates (OPs) and carbamates (CMs), which share similar modes of action. The determined limit of detection for chlorpyrifos ranged between 0.04 and 0.36 mg/L, while the IC50 values of CMs such as carbaryl, ethiofencarb, oxyamyl, and fenobucarb varied from 0.01 to 0.37 mg/L, respectively. The validation study outcomes offer a promising avenue for accurately identifying pesticide-contaminated fruits and vegetables using esterase enzymes, providing both simplicity in implementation and significant benefits for human health. Furthermore, the potential applicability of this detection method in agricultural settings warrants further investigation in future studies.

Previous research successfully detected organophosphates (OPs) and carbamates (CMs) pesticides, leading to the further development of synthesized magnetic nanoparticles (MNPs) coated with esterase enzymes extracted from honeybee heads for pesticide detection. The MNPs were synthesized via the co-precipitation method, showcasing their distinctive magnetic properties. Structural analysis using FTIR spectroscopy confirmed the presence of metal-oxygen bonds and specific chemical components in the synthesized MNPs. Additionally, the integration of enzymes onto MNPs and subsequent inhibition studies shed light on the efficacy of enzyme coating and the successful integration of metal oxides.

Furthermore, our exploration into enzymatic activity from honeybee heads revealed species-specific variations in sensitivity to organophosphate and carbamate pesticides, underscoring the necessity of considering such factors in pesticide detection applications employing honeybee enzymes.

The utilization of Response Surface Methods for optimizing enzymatic activity underscored the significance of pH, percentage of methanol, and temperature, with

optimal conditions achieved at pH 7, 5% methanol, and temperatures ranging between 25 to 30°C.

Lastly, the inhibition of extracted esterase by pesticides (OPs and CMs) provided comprehensive insights into the correlation between pesticide concentrations and enzyme activity inhibition. The resulting IC50 values further elucidated the inhibitory effects of varying doses of organophosphates and carbamates, highlighting their potency in impacting enzymatic activities.

In conclusion, these findings significantly contribute to the understanding of magnetic nanoparticle synthesis, enzyme behavior, and the effects of pesticides on enzymatic activities, offering valuable insights for applications in nanotechnology and environmental monitoring.

References

[1] Panuwet, P., Siriwong, W., Prapamontol, T., Ryan, P. B., Fiedler, N., Robson, M. G., & Barr, D. B. (2012). Agricultural pesticide management in Thailand: status and population health risk. *Environmental science & policy*, *17*, 72-81.

[2] Laohaudomchok, W., Nankongnab, N., Siriruttanapruk, S., Klaimala, P., Lianchamroon, W., Ousap, P., ... & Woskie, S. (2020). Pesticide use in Thailand: Current situation, health risks, and gaps in research and policy. *Human and Ecological Risk Assessment: An International Journal*, *27*(5), 1147-1169.

[3] Pehkonen, S. O., & Zhang, Q. (2002). The degradation of organophosphorus pesticides in natural waters: a critical review. *Critical reviews in environmental science and technology*, *32*(1), 17-72.

[4] González-Alzaga, B., Lacasaña, M., Aguilar-Garduño, C., Rodríguez-Barranco, M., Ballester, F., Rebagliato, M., & Hernández, A. F. (2014). A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. *Toxicology letters*, *230*(2), 104-121.

[5] Pope, C., Karanth, S., & Liu, J. (2005). Pharmacology and toxicology of cholinesterase inhibitors: uses and misuses of a common mechanism of action. *Environmental toxicology and pharmacology*, *19*(3), 433-446.

[6] Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Grewal, A. S., ... & Kaushal, J. (2021). An extensive review on the consequences of chemical pesticides on human health and environment. *Journal of cleaner production*, *283*, 124657.

[7] Aung, M. M., & Chang, Y. S. (2014). Traceability in a food supply chain: Safety and quality perspectives. *Food control*, *39*, 172-184.

[8] Kirk, M. D., Pires, S. M., Black, R. E., Caipo, M., Crump, J. A., Devleesschauwer, B., ... & Angulo, F. J. (2015). World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. *PLoS medicine*, *12*(12), e1001921.

[9] Cheng, W., Tang, X., Zhang, Y., Wu, D., & Yang, W. (2021). Applications of metal-organic framework (MOF)-based sensors for food safety: Enhancing mechanisms and recent advances. *Trends in Food Science & Technology*, *112*, 268-282.

[10] Gallo, M., & Ferranti, P. (2016). The evolution of analytical chemistry methods in foodomics. *Journal of Chromatography A*, *1428*, 3-15.

[11] Miao, Q., Kong, W., Yang, S., & Yang, M. (2013). Rapid analysis of multi-pesticide residues in lotus seeds by a modified QuEChERS-based extraction and GC–ECD. *Chemosphere*, *91*(7), 955-962.

[12] Lu, S., Wu, D., Li, G., Lv, Z., Gong, P., Xia, L., ... & Wu, Y. (2017). Facile and sensitive determination of N-nitrosamines in food samples by high-performance liquid chromatography via combining fluorescent labeling with dispersive liquid-liquid microextraction. *Food chemistry*, *234*, 408-415.

[13] Osaili, T. M., Al Sallagi, M. S., Dhanasekaran, D. K., Odeh, W. A. B., Al Ali, H. J., Al Ali, A. A., ... & Holley, R. (2022). Pesticide residues in fresh vegetables imported into the United Arab Emirates. *Food Control*, *133*, 108663.

[14] Pasar, R., Ms, P., & P, N. (2022). Simultaneous determination of dimethoate and its metabolite omethoate in curry leaf using LC-MS/MS and risk assessment. *Journal of Separation Science*, *45*(11), 1831-1838.

[15] Istamboulie, G., Cortina-Puig, M., Marty, J. L., & Noguer, T. (2009). The use of Artificial Neural Networks for the selective detection of two organophosphat insecticides: Chlorpyrifos and chlorfenvinfos. *Talanta*, *79*(2), 507-511.

[16] Istamboulie, G., Andreescu, S., Marty, J. L., & Noguer, T. (2007). Highly sensitive detection of organophosphorus insecticides using magnetic microbeads and genetically engineered acetylcholinesterase. *Biosensors and Bioelectronics*, *23*(4), 506-512.

[17] Barišić, D., Lazarić, K., Lulić, S., Vertačnik, A., Dražić, M., & Kezić, N. (1994). 40K, 134Cs and 137Cs in pollen, honey and soil surface layer in Croatia. *Apidologie*, *25*(6), 585-595.

[18] Van der Oost, R., Beyer, J., & Vermeulen, N. P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental toxicology and pharmacology*, *13*(2), 57-149.

[19] Attencia, V. M., Ruvolo-Takasusuki, M. C. C., & De Toledo, V. D. A. A. (2005). Esterase activity in Apis mellifera after exposure to organophosphate insecticides (Hymenoptera: Apidae). *Sociobiology*, *45*(3), 587-595.

[20] Badiou, A., Meled, M., & Belzunces, L. P. (2008). Honeybee Apis mellifera acetylcholinesterasea biomarker to detect detect deltamethrin exposure. *Ecotoxicology and Environmental Safety*, *69*(2), 246-253.

[21] Aragay, G., Pino, F., & Merkoci, A. (2012). Nanomaterials for sensing and destroying pesticides. *Chemical reviews*, *112*(10), 5317-5338.

[22] Pundir, C. S., & Chauhan, N. (2012). Acetylcholinesterase inhibition-based biosensors for pesticide determination: A review. *Analytical Biochemistry, 492*(1), 19-31.

[23] Stanciu, L., Won, Y. H., Ganesana, M., & Andreescu, S. (2009). Magnetic particle-based hybrid platforms for bioanalytical sensors. *Sensors*, *9*(4), 2976-2999.

[24] Zahirinejad, S., Hemmati, R., Homaei, A., Dinari, A., Hosseinkhani, S., Mohammadi, S., & Vianello, F. (2021). Nano-organic supports for enzyme immobilization: scopes and perspectives. *Colloids and Surfaces B: Biointerfaces*, *204*, 111774.

[25] Gao, X. J., Fan, X. J., Chen, X. P., & Ge, Z. Q. (2018). Immobilized β-lactamase on Fe 3 O 4 magnetic nanoparticles for degradation of β-lactam antibiotics in wastewater. *International Journal of Environmental Science and Technology*, *15*, 2203-2212.

[26] Jaitham, U., Yadoung, S., Wongta, A., Chuttong, B., Danmek, K., & Hongsibsong, S. (2023). Color Test Kit for Detecting Organophosphate and Carbamate Pesticides by Using Esterase Extract from Honey Bees. *Philippine Journal of Science*, *152*(3).

[27] Chen, J., Zhang, Y. L., Wu, Y., & Zhang, J. (2013). The Basic Study of Plant Esterase in Pesticide Residue Examination. *Advanced Materials Research*, *726*, 950-957.

[28] RĂCUCIU, M., CREANGĂ, D., Airinei, A., & BĂDESCU, V. (2008). Synthesis method influence on water based magnetic fluid properties. *Journal of optoelectronics and advanced materials*, *10*(3), 635-638.

[29] Meng, X. Y., Li, Y. S., Zhou, Y., Zhang, Y. Y., Yang, L., Qiao, B., ... & Wang, X. R. (2015). An enzyme-linked immunosorbent assay for detection of pyrene and related polycyclic aromatic hydrocarbons. *Analytical biochemistry*, *473*, 1-6.

[30] Sapbamrer, R., & Hongsibsong, S. (2014). Organophosphorus pesticide residues in vegetables from farms, markets, and a supermarket around Kwan Phayao Lake of Northern Thailand. *Archives of environmental contamination and toxicology*, *67*, 60-67.

CURRICULUM VITAE

Author's Name Mr. Udomsap Jaitham

Education 2016: B.S. (Chemistry), Faculty of Science and Technology, Chiang Mai Rajabhat, Chiang Mai, Thailand.

Published paper Author's Name: **Udomsap Jaitham**, Sumed Yadoung, Anurak Wongta, Bajaree Chuttong, Khanchai Danmek, and Surat Hongsibsong. Publication Title: "Color Test Kit for Detecting Organophosphate and Carbamate Pesticides by Using Esterase Extract from Honey Bees" Journal: Philippine Journal of Science. Volume: 152, Issue: 3, Page Range: 1245–1252, Date: June 2023, ISSN: 0031 – 7683, DOI: https://doi.org/10.56899/152.03.39.

Authors: Anurak Wongta, Surat Hongsibsong, Priyanshi Anand, **Udomsap Jaitham**, Nootchakarn Sawarng, Wasin Wongwilai, and Pongsathorn Dhumtanom. Publication Title: "Developing an In-House Colorimetric Method for Detecting Organophosphate and Carbamate Residue Using Cricket Cholinesterase." Journal: Chemical and Biological Technologies in Agriculture. Volume: 2023, 10:65, DOI: https://doi.org/10.1186/s40538-023-00442-3.

Presentation Poster Presentation at The 35th Annual Meeting of the Thai Society for Biotechnology and International Conference (TSB 2023) on Sustainable Development through Bio-Circular Green (BCG) Economy Model, scheduled for 26-29 November 2023 at the Greenery Resort - Khao Yai. In title: "Preparation of Esterase Enzyme from Honey Bee Heads and Its Application for Detecting Organophosphate and Carbamate Insecticides"

Presentation at the 17th International Warsaw Invention Show (IWIS) on "Transforming Agricultural Waste into Advanced Bio-Based Materials Through a Sustainable Green Process - Nanocellulose," organized by Warsaw University of Technology, Poland.

Experience

January 2024 - Present: Research Assistant at Chiang Mai University's Research Institute for Health Sciences, Environmental and Occupational Health Research Unit.

May - October 2023, Student Exchange at the Institute of Environmental Engineering, National Sun Yat-sen University in Taiwan. During this period, I conducted research on the dissolution of cellulose and lignin from biomass materials and the synthesis of bioplastics using Deep Eutectic Solvent (DES). My supervisor for this research project was Assistant Professor Dr. Ken-Lin Chang.