# THE SIZE EFFECT OF OIL EMULSION ON THE INHIBITION OF CUCUMBER DOWNY MILDEW

Kai-Fen Tu[1], Jenn-Wen Huang[2,3], Chih-Huang Weng[4], Yao-Tung Lin[1, 2*]

[1]Department of Soil and Environmental Sciences, National Chung Hsing University, 145 Xingda Rd., South Dist., Taichung City 402, Taiwan (R.O.C.)

[2]Innovation and Development Center of Sustainable Agriculture, National Chung Hsing University, 145 Xingda Rd., South Dist., Taichung City 402, Taiwan (R.O.C.)

[3]Department of Plant Pathology, National Chung Hsing University, 145 Xingda Rd., South Dist., Taichung City 402, Taiwan (R.O.C.)

[4]Department of Civil and Ecological Engineering, I-Shou University, No.1, Sec. 1, Syuecheng Rd., Dashu District, Kaohsiung City 840, Taiwan (R.O.C.)

## Abstract

*Cucumber is one of the most important agricultural products in many countries. Presently, the annual global cucumber production was approximately 71 million tonnes. Nonetheless, downy mildew, a common disease caused by Pseudoperonospora cubensis (PC), has led to a devastating impact on the quality of cucumber. The long-term usage of fungicide leads the occurrence of fungicide resistance and decreases host resistance. Emulsions synthized by oil could be an alternative for plant antifungal protectants. So far, no literature reported the effect of droplet size of emulsion on plant diseases. In this research, we synthesized environmental friendly antifungal micro/nano-emulsions using six types of oil (neem oil, moringa oil, soybean oil, cinnamon oil, clove oil, and camellia oil) and evaluated their antifungal activity of the emulsions upon the cucumber downy mildew. The key parameters affecting process of synthesizing droplet size of micro/nano-sized emulsions, including temperature, homogenization speed, oil to surfactant ratio, water ratio and surfactant ratio were evaluated. Then, the antifungal activity against cucumber downy mildew of these emulsions were tested via different oil types, oil concentrations (0.0125% - 0.1%), and droplet size of emulsions (100 - 1,000 nm). Results showed that 0.1% of moringa-type, cinnamon-type and clove-type emulsion had better antifungal efficacy compared to neem-type, soybean-type, and camellia-type. As decreasing the oil concentration from 0.1% to 0.025%, the disease incidence of moringa-type and cinnamon-type emulsion did not show significant changes, by contrast, the clove-type emulsion increased with decreasing oil concentration. The droplet size of cinnamon-type emulsion synthesized by 0.025% cinnamon oil increased from around 114 to 1,209 nm with the disease incidence increased from 6.67% to 30%, which could be attributed to collision of oil droplets and sporangia The findings suggest that cinnamon oil emulsion is an excellent antifungal agent and can be used for protecting cucumber from the incidence of downy mildew.*

**Keywords:** *cucumber, downy mildew, Pseudoperonospora cubensis (PC), emulsion, size, cinnamon oil*

## 1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) which produces tender edible fruits is one of the economically crops in the world (Fan et al., 2019, Luan et al., 2019). The annual global cucumber production was approximately 71 million tonnes (FAO, 2018). It is also an important vegetable in Taiwan. Its sales value continues to raise and reached 12.7 billion in 2019 (Councilofagriculture, 2019). However, plant diseases caused by pathogenic fungi lead to seriously impact on crops along with decreased production and quality (Luan et al., 2019, Li et al., 2020). Of them, cucumber downy mildew caused by the oomycete *Pseudoperonospora cubensis* (Berk. Et Curt.) is a devastating disease that appears in around 80 countries and over 50% cucumber yield can be decreased (Fan et al., 2019, Luan et al., 2019, Salas et al., 2019). To protect crops, fungicides are widely used which also leads to a large usage amount increase (Zhang et al., 2019c). Currently, the numerous and improper use of fungicides certainly cause to develop resistance (Zhang et al., 2019b). Some researches also mentioned that potential safety hazards

and environmental pollution such as soil and groundwater contamination. In addition, dizziness, sleepiness, loss of appetite, liver damage, fetal malformation, a series of acute and chronic poisoning symptoms are confirmed (Zhang et al., 2019a, Zhang et al., 2019c). Therefore, an environmental friendly plant antifungal protectants is necessary.

Emulsion have been widely utilized in various fields such as agriculture, food preparation, medicine, cosmetic and aroma applications and contamination remediation. It can be regarded as antimicrobial agent, drug delivery carrier, food additive and plant protectant (Moghimi et al., 2016, Swathy et al., 2018). For agricultural use, emulsion was applied to encapsulate high toxic active compounds such as chlorpyrifos and parathion. Nowadays, it tends to encapsulate environmental friendly compounds such as plant extracts and plant oils (Wang, 2007). Eight types of cooking oil (corn oil, sesame oil, peanut oil, sunflower oil, safflower oil, canola oil, grape seed oil and soybean oil) were test the tomato powdery mildew. Results showed that all of the oils can inhibit tomato powdery mildew after emulsification. Among these, sunflower oil emulsion could lead to the inhibition on mycelial growth and conidia germination (Ko et al., 2003). *Cymbopogon citratus*, *Cymbopogon martini*, and *Pelargonium graveolens* are emulsified and test the efficacy of controlling seed borne fungi of rice such as *Curvularia lunata*, *Fusarium moniliforme*, *Bipolaris oryzae*, and *Sarocladium oryzae*. This research indicated that the inhibition on the mycelial growth increased along with the increase of oil concentration. *C. citratus* oil emulsion at 30% oil concentration could reach 100% inhibition (Naveenkumar et al., 2017). Thymol nanoemulsion showed *in vitro* growth inhibition of *Xanthomonas axonopodis* and the seed treatment and foliar application could significantly lowered the disease severity (33.3-3.3%) (Kumari et al., 2018). Therefore, emulsions synthized by oil could be regarded as an alternative for plant antifungal protectants. However, lots studies reported oil emulsion can protect plant disease attributed to the antimicrobial compound of the oils or the oil concentration. No literature evaluated the effect of droplet size of emulsion on any plant diseases. The objectives of the research are to synthesized environmental friendly antifungal micro/nano-emulsions using six types of oil (neem oil, moringa oil, soybean oil, cinnamon oil, clove oil, and camellia oil) and evaluated their antifungal activity of cucumber downy mildew. The key parameters affecting process of synthesizing droplet size of micro/nano-sized emulsions, including hydrophilic–lipophilic balance, temperature, homogenization speed, and oil to surfactant ratio were evaluated. Then, the antifungal activity against cucumber downy mildew of these emulsions were tested via different oil types, oil concentrations (0.0125% - 0.1%), and droplet size of emulsions (100 - 1,000 nm).

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The oils such as neem oil, moringa oil, soybean oil, cinnamon oil, clove oil, and camellia oil were seen as oil phase purchased form local market. Span 80 (1338-43-8, Sigma-Aldrich, Taiwan) and Tween 80 (9005-65-6, Sigma-Aldrich, Taiwan) was used as surfactant. This research investigated the oil droplet sizes of emulsions by adjustment the parameters of operating temperature, oil to surfactant ratio, water ratio and surfactant ratio and homogenization speed.

### *2.2. Preparation of emulsions*

The soybean oil was selected to test the inferences on droplet size. The emulsions were homogenized (Ultra Turrax T25, IKA, Germany) with soybean oil, DI water and surfactants by consideration the parameters of hydrophilic–lipophilic balance, temperature, homogenization speed, and oil to surfactant ratio.

#### *2.2.1. The operating temperatures*

The emulsions were prepared at five different operating temperatures (25, 30, 40, 50, 60, 70, 80℃) by the water bath (SB-1200, EYELA, Japan) and were measured the droplet sizes after preparation.

*2.2.2. The oil to surfactant ratio, water ratio and surfactant ratio*

Emulsion is composed of oil, DI water and surfactant so the ratio between them has the influence on the droplet size of emulsion. To study the effect of the oil to surfactant ratio on emulsions, emulsion formed with different oil to surfactant ratios, 5:1, 5:3, 1:1, 1:3 and 1:5, were prepared. Besides this, the DI water ratio (82, 86, 90, 94 and 98%) and surfactant ratio (1%, 3%, 5%, 7% and 9%) were also researched.

*2.2.3. The homogenization speeds*

To investigate the effects of homogenization speeds (3000, 5000, 7000, 9000, 11000, 13000 and 16000 rpm) on emulsion droplet size, these speeds were set for 10 minutes to make sure the impact on the droplet sizes.

*2.3. Droplet size analysis of emulsions*

The droplet size of these emulsions were measured using dynamic light scattering (nano ZS, Malvern, United Kingdom). Measurements were performed after an initial 60 s period of temperature equilibrium with each record the average of 10 runs each lasting 10 s in duration. Instrument was set 173˚ scatter angle. Results are reported as the average of 6 measurements. All samples were measured and analyzed with Malvern Zetasizer Software v7.11.

*2.4. Efficacy of emulsions on cucumber downy mildew*

*2.4.1 Crop and strain source, culture and observation*

The fungal strain *Pseudoperonospora cubensis* (*P. cubensis*) PC-001 was provided by Council of Agriculture, Executive Yuan, Taiwan. Cucumber seeds (*Cucumis sativus L.*, cv. Xiu-Yan) were purchased from known-you seed co., ltd. and planted into the potting soil in the greenhouse for 3-4 weeks. The second leaf counted from bottom to top was cut and soaked into the bleach water (Clorox®) which was diluted 8-fold for 10 seconds. Furthermore, the leaf was moved into sterile water 2 times for cleaning the bleach water and then moved into the glass dish with the back onward. For keeping moist, the glass dish contained a moist film paper and the petiole was covered with moist sterile cotton. *P. cubensis* was inoculated in the leaf at 20℃ for 7 days.

*2.4.2 Antifungal activity of emulsions on cucumber downy mildew*

The dropping method was applied to inoculate *P. cubensis*. *P. cubensis* was washed away the leaf by sterile water and dropped into the leaf back with appropriate concentration. The disease incidence was calculated after 7 days. Disease incidence (%) = (No. of disease lesions showed up)/(Total number of inoculation per leaf) × 100

(a) Disease incidence for cucumber detached leaves

The six types of oil (neem oil, moringa oil, soybean oil, cinnamon oil, clove oil, and camellia oil) were used to prepare different droplet size of emulsions. The emulsions with the various droplet sizes were dropped on the cucumber detached leaves by dropping method. Each detached leaf has 10 droplets of emulsions and then dropped the sporangia with the concentration of 20 sporangia/10 μl of *Pseudoperonospora cubensis* PC-001 at 20°C with lighting 12 hours a day for 7 days. The control treatment was sterile water and each detached leaf was a replicate for a triplicate experiment. Then, the cinnamon-type emulsion was chosen to test the efficacy of different oil concentration. After manufactured these emulsions, the emulsions were diluted from 0.025%-0.1% and dropped on the cucumber detached leaves by dropping method.

(b) Disease severity for cucumber plant

For spray inoculation, sporangia (1 mL, $5 \times 10^4$ sporangia/ ml) and 0.025% cinnamon-type emulsions were individually sprayed on the first, second and third leaf counted from bottom to top by a mini-sprayer. The inoculated plants were bagged for 2 days to keep the moisture in a greenhouse. Downy mildew severity was calculated 1 day before inoculation. Disease severity was calculated using the following scale: 0, no lesions; 1, lesion areas 1-5%; 2, lesion areas 6-25%; 3, lesion areas 26-50%; 4, lesion areas 50-75%; and 5, lesion areas 76-100%. Disease severity was determined by the following

formula: Disease severity (%) = [Σ (disease index × number of infected leaves)/(total number of leaves)× 5] × 100 (Ishii et al., 2001, Michereff et al., 2009).

(c) Effect of emulsions on zoospores released from sporangia

The cinnamon-type emulsion was mixed with the sporangia with the same volume of *Pseudoperonospora cubensis* PC-001. The sample was away from the light and put into an incubator under 20°C for 2 hours. After 2 hours, the sample dropped on the slide glass and put into a petri dish contained a L-type stir rod on the top of moist filter paper. The control treatment was sterile water and each slide glass was a replicate for triplicate experiment.

## 3. RESULTS

### *3.1 Effect of operating parameters on droplet size of emulsions*

#### *3.1.1 The operating temperatures*

The hydrophilicity of surfactant decreases by the increase of temperature that causes more oils to dissolve in the water and the O/W emulsion converts to the W/O emulsion when it cools down (Hwung, 1998). It's mentioned that the oil droplet was decrease during the process (Friberg et al., 2011). However, the result shows that the droplet size didn't vary obviously regardless of the temperature (**Figure 1**). It may be caused by the low oil to surfactant (1:5) and low oil concentration (0.6%). Therefore, the emulsion is stable and the oil droplet was easy to distribute.

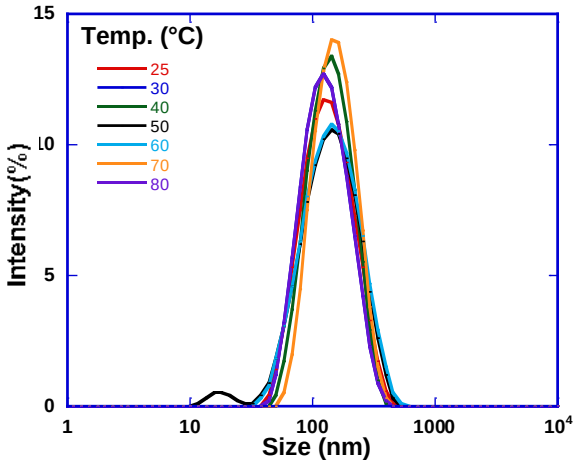



**Figure 1:** The various droplet sizes of emulsions under different temperature.

Experimental conditions: soybean oil: 0.6 g, span 80: 2.05 g, tween 80:0.95 g, DI water:96.4 g, speed:13000 rpm for 10 min.

#### *3.1.2 The oil to surfactant ratio, water ratio and surfactant ratio*

Because the droplet sizes of emulsions are influenced by the composition of oil, DI water and surfactant, the ratio between them was investigated. The oil to surfactant ratio affected the droplet size of oil in the emulsion (Lin, 2011) so the effect of oil to surfactant ratio on emulsions was tested. The result shows that the droplet size of emulsion decreases by the increase in oil to surfactant ratio which caused by the encapsulation of surfactant (**Figure 2**). In this study, the oil to surfactant ratio of 1:1 was investigated the influences of water ratio from 82 to 98%. Previous study shows that the water ratio impacts the oil dispersity in the emulsion (Lin, 2011). After increasing the water ratio, the space for oil to disperse increases, causing the result of oil droplet collision decreases. Therefore, the droplet size of oil in the

emulsion decreased with the increase of the water ratio (**Figure 3**). Besides, the surfactant ratio was also researched and explored the critical micelle concentration (CMC). If surfactants keep adding into a solution contained two immiscible liquids, the droplet size of the emulsion won't increase when the concentration of surfactant is higher than the CMC (Ou, 1992). The result shows the droplet size of emulsions were all around 200 nm when the surfactant concentrations were higher than 3%. However, the droplet size of emulsion was bigger in 1% surfactant concentration (**Figure 4**). As the result shown, the surfactant was not enough to encapsulate the oil when the surfactant concentration was below its CMC. Nevertheless, the droplet size of emulsions kept 200 nm when the surfactant concentration reached 3%. It means that 3% surfactant is the CMC of this emulsion.

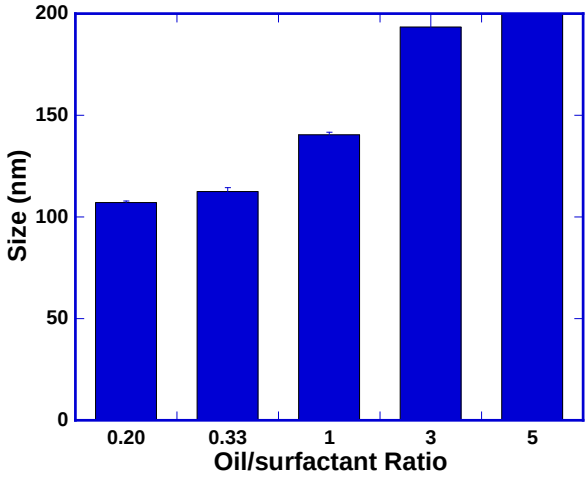



**Figure 2:** The droplet size of emulsion with different ratio of oil to surfactant.

Experimental conditions: temperature: 25℃, oil: 15-0.6 g, span 80: 2.05 g, tween 80: 0.95 g, and DI water: 82-96.4 g, speed: 13000 rpm for 10 min

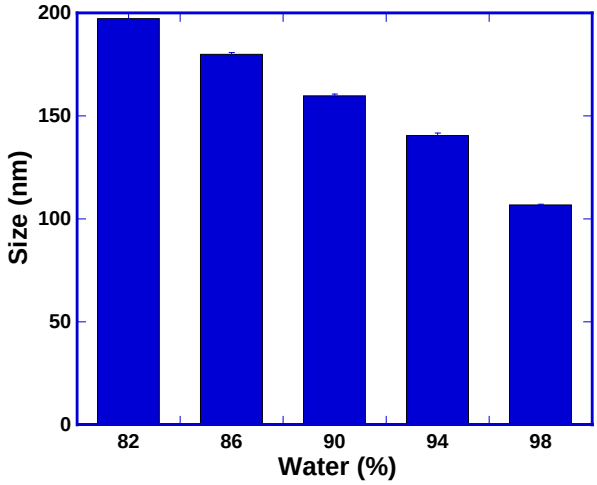



**Figure 3:** The droplet size of emulsion with different water ratio.

Experimental conditions: temperature: 25℃, oil: 1-9 g, span 80: 0.68-6.15 g, tween 80: 0.32-2.85 g, and DI water: 82-98 g, ratio of oil to surfactant:1 speed: 13000 rpm for 10 min

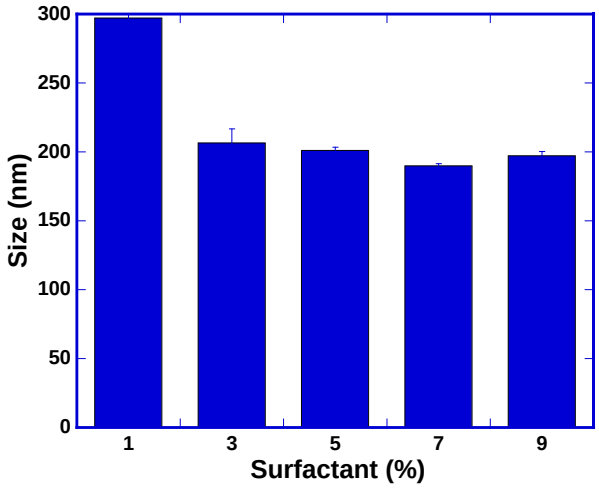



**Figure 4:** The droplet size of emulsion with different surfactant ratio.

Experimental conditions: temperature: 25°C, oil: 9 g, span 80: 0.68-6.15 g, tween 80: 0.32-2.85 g, and DI water: 90-82 g, ratio of oil to surfactant:1 speed: 13000 rpm for 10 min

*3.1.3 The homogenization speeds*

The result shows when the homogenization speed increased, the droplet size of emulsion decreases (**Figure 5**). The reason is that the energy provided by the system increased and the capability to disperse the oil increased. Therefore, the droplet size of emulsion decreased when increasing the homogenization speeds.

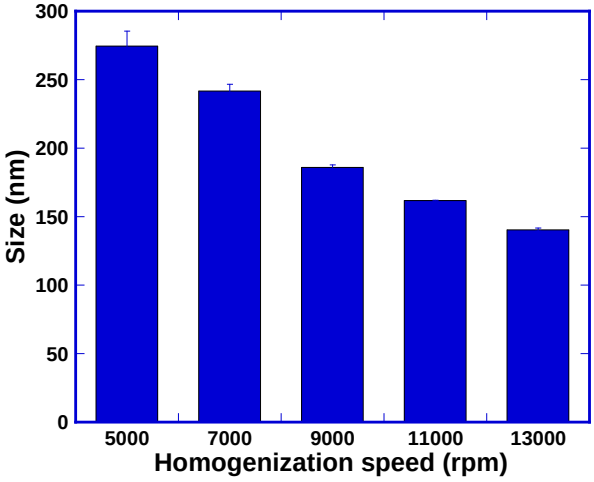



**Figure 5:** The droplet size of emulsion with different homogenization speed.

Experimental conditions: temperature: 25°C, oil: 3 g, span 80: 2.05 g, tween 80: 0.95 g, and DI water: 94 g, ratio of oil to surfactant:1 speed: 5000-13000 rpm for 10 min

*3.2 Antifungal activity of emulsions on cucumber downy mildew*

*3.2.1 Disease incidence for cucumber detached leaves*

After investigating the parameters of the droplet size of emulsions, the different droplet size of emulsions was manufactured by these parameters and applied to control cucumber downy mildew. Among these tested oil, neem-type emulsions with various droplet sizes didn't have various effects (data not shown).

Camellia oil and soybean oil had the efficacy at 0.1% oil concentration. Clove oil, cinnamon oil and moringa oil showed high efficacy at 0.1%. The results are shown below.

The camellia-type and soybean-type emulsions with various droplet sizes were applied to control the *P. cubensis* PC-001 on cucumber detached leaves and calculated the disease incidence (**Figure 6** (a)) and (**Figure 6** (b)). Both types of oil revealed various droplet size of emulsions had various effects. In camellia-type emulsions, the leaves treated with 1103 nm had the lowest disease incidence (7%) by the bigger droplet size. The 136 and 513 nm had the highest disease incidences (93% and 97%) compared to others. Similar to soybean-type emulsions, the various droplet size of emulsions also had various effects. The leaves treated with 137 nm had the lowest disease incidence (57%) by the smaller droplet size. However, the 291, 585 and 2452 nm had the highest disease incidences (97%, 100% and 97%) compared to others. Both types of emulsion indicated that the disease incidences didn't have a trend with the increasing droplet size. As to clove-type, cinnamon-type and moringa-type emulsions were shown in **Figure 6** (c)-(e). In these results, the various droplet size of emulsions didn't have various effects. The similar disease incidence with different droplet size may be caused by the high concentration. Therefore, cinnamon-type emulsion was selected to test the efficacy of different oil concentration.

As the clove-type, cinnamon-type and moringa-type emulsions didn't show the effect on droplet size, the suitable oil concentration were tested by cinnamon-type emulsions. It indicated that the cinnamon-type emulsion could have effect above 0.025% oil concentration and the disease incidences were less than 3% (**Figure 7**). Therefore, the clove-type, cinnamon-type and moringa-type emulsions were tested at 0.025% to evaluate the size effect.

The clove-type, cinnamon-type and moringa-type emulsions were adjusted to 0.025% to confirm the size effect (**Figure 8** (a)-(c)). The size effect was revealed at 0.025% oil concentration. For clove-type emulsion, the disease incidences increased after decreasing the concentration. The smaller droplet size (152 nm) had the lower disease incidence (70%). However, other droplet sizes didn't show the efficacy (90-100%). Compared to clove-type emulsions, cinnamon-type and moringa-type emulsions had lower disease incidences (7-30% and 3-30%). Besides, both types of emulsion shown the trend that the efficacy decrease with the increase of droplet size.

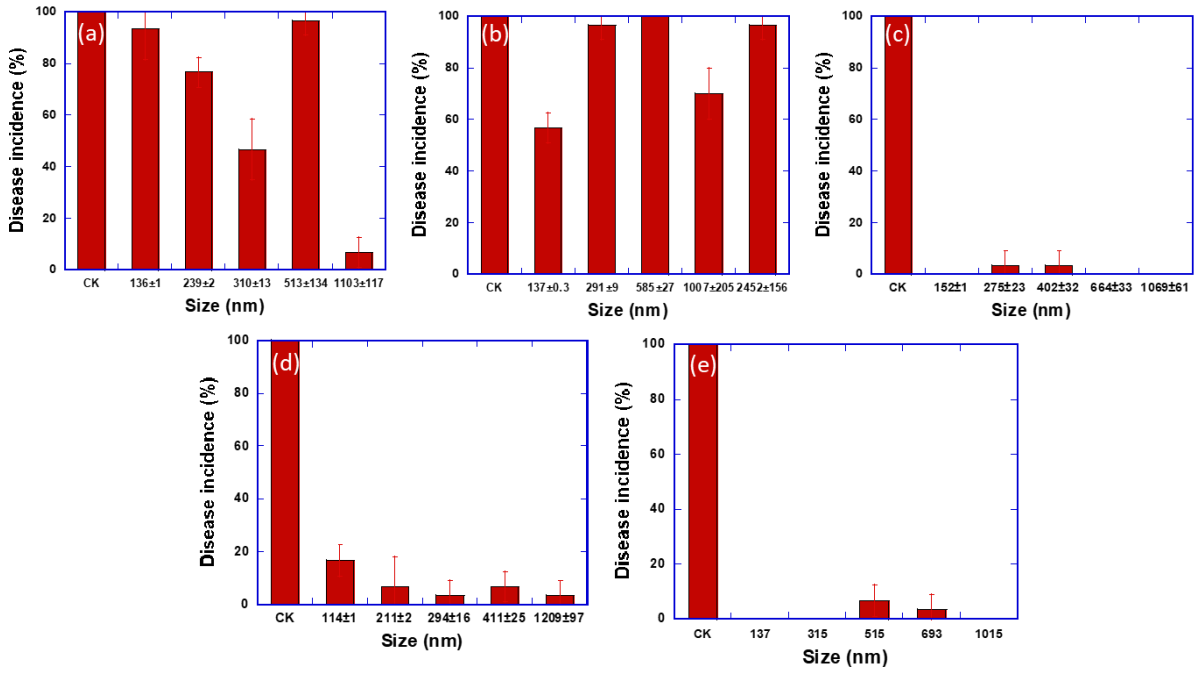



**Figure 6:** Efficacy of various droplet sizes of 0.1% oil emulsions on control of downy mildew of cucumber detached leaves. (a) camellia-type (b) soybean-type (c) clove-type (d) cinnamon-type (e) moringa-type

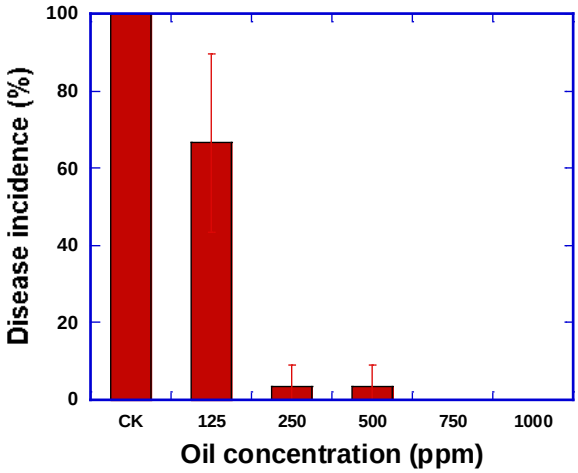



**Figure 7:** Efficacy of various oil concentration of cinnamon-type emulsions on control of downy mildew of cucumber detached leaves.

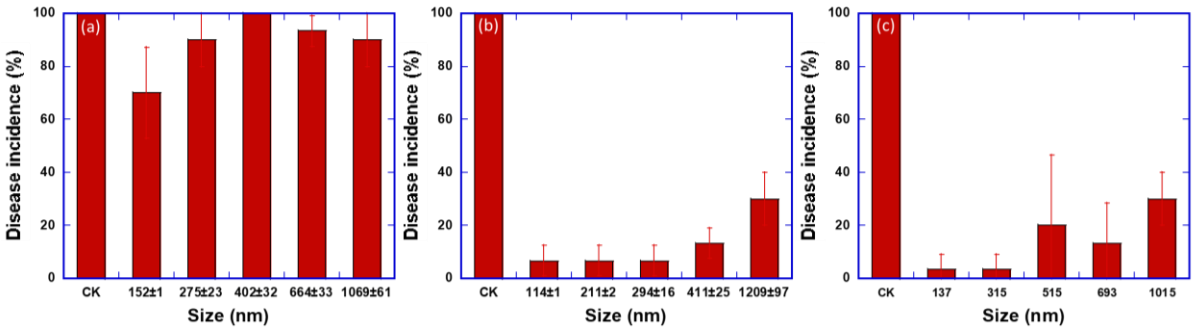



**Figure 8:** Efficacy of various droplet sizes of 0.1% oil emulsions on control of downy mildew of cucumber detached leaves. (a) clove-type (b) cinnamon-type (c) moringa-type

*3.2.2 Disease severity for cucumber plant*

The cinnamon-type emulsions were selected to conduct the greenhouse experiment. As the result shown, the smaller droplet size (114 nm) had the lower disease severity (33%) and the leave didn't have obvious symptom. It indicated the cinnamon-type emulsions could have effect on greenhouse experiment (**Figure 9**).

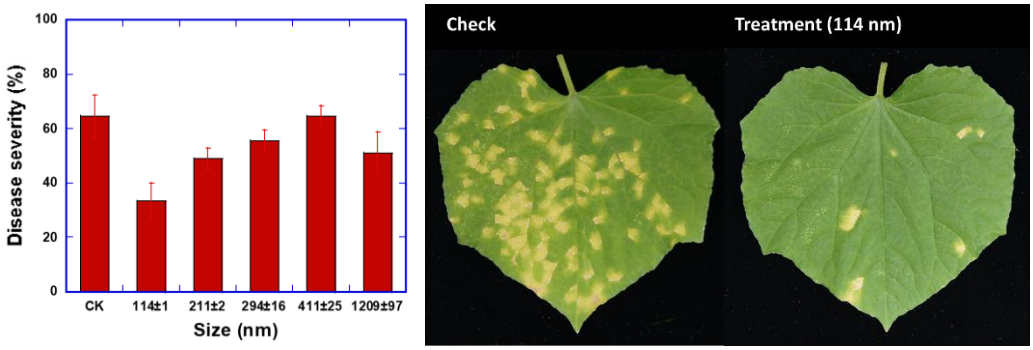



**Figure 9:** Efficacy of various droplet sizes of 0.025% cinnamon-type emulsions 24 h before inoculation for controlling downy mildew in the greenhouse.

*3.2.3 Effect of emulsions on zoospores released from sporangia*

The 114 nm cinnamon-type emulsions were selected to evaluated the zoospores released from sporangia. As the result shown, the cytoplasm of *P. cubensis* differentiated into zoospores in the control treatment (**Figure 10** (a)). However, the cytoplasm of *P. cubensis* didn't differentiate into zoospores under the treatment with smaller droplet size (114 nm). It showed that the sporangia germination was damaged while the release of zoospore was inhibited (5.33%). As the result, *Pseudoperonospora cubensis* PC-001 can be controlled by emulsions.

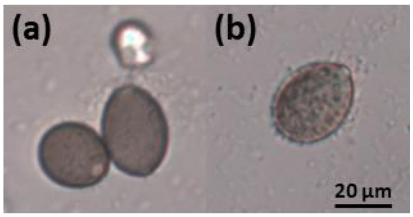



**Figure 10:** Effect of 114 nm cinnamon-type emulsions on zoospores released from sporangia of *P. cubensis* PC-001. (a) Control treatment; (b) treated with 114 nm cinnamon-type emulsion.

## 4. DISCUSSION

The different oil droplet size emulsion could synthized by the adjustment of emulsion parameters such as temperature, homogenization speed, oil to surfactant ratio, water ratio and surfactant ratio. In this research, different temperature didn't be related to the droplet size (**Figure 1**). It may be related to the low oil to surfactant (1:5) and low oil concentration (0.6%), which make the oil droplet much easier to dispersive. With the increase the oil to surfactant ratio, the droplet size increased (**Figure 2**). It indicated that the suitable ratio could lead to the encapsulation of oil droplet. The increase of water ratio leads to the decrease of droplet size (**Figure 3**). The droplet could scatter in the water phase without collision. Furthermore, the critical micelle concentration (CMC) could also find in this research (**Figure 4**). The droplet size didn't have obvious change above 3% surfactants concentration and keep around 200 nm, which indicated that 3% surfactants concentration could encapsulate oil droplet efficient. Therefore, the CMC was reached. As the homogenization speed increased, the droplet size decrease (**Figure 5**). Majeed et al. (2016) used high pressure homogenizer to prepare the emulsions and indicated that homogenization pressure increased along with the droplet size decreased. Besides, the increase of homogenization speed also improved the decrease of droplet size in other study (Otoni et al., 2014). Both researches confirmed the input energy rise can lead to smaller droplet size. It also can find in this research.

Six types of oil (neem oil, moringa oil, soybean oil, cinnamon oil, clove oil, and camellia oil) were used to synthize emulsion and control the cucumber downy mildew. As the result shown, 0.1% of moringa-type, cinnamon-type and clove-type emulsion had better antifungal efficacy compared to neem-type, soybean-type, and camellia-type (**Figure 6**). Neem-type emulsion didn't have efficacy to control cucumber downy mildew. Besides, lots researches investigated the insecticidal activity of neem oil (Camarda et al., 2018, Constanski et al., 2016, Formentini et al., 2016, Kala et al., 2019, Kolani et al., 2016, Kumar et al., 2019, Ngom et al., 2018). It indicated that neem oil has high efficacy in pest disease rather than fungal disease. Both soybean oil and camellia oil are the often seen cooking oil. Nevertheless, both of them do not have high efficacy on controlling cucumber downy mildew. These also revealed that the potential of cooking oil in plant fungal disease control. Decreasing the concentration from 0.1% to 0.025%, moringa-type and cinnamon-type emulsion still have significant effect (**Figure 8**). However, the disease incidence of clove-type emulsion increase obviously with decreasing oil concentration. It also revealed the size effect at 0.025% oil concentration. With the droplet size decreased, the disease incidence of moringa-type and cinnamon-type decreased (**Figure 9**). *Moringa oleifera* is called miracle tree nowadays because the effect on anticancer and skin inflammation and hyperproliferation (Cretella et al., 2020, Elsayed et al., 2016). In this research, the moringa-type emulsions revealed the high efficacy

on cucumber downy mildew. It reveals the potential of moringa oil in plant disease control. And the emulsification with smaller droplet size could improve the antifungal efficacy. Clove oil and cinnamon oil contain natural antimicrobial component eugenol and cinnamaldehyde, individually and therefore, be regarded as antimicrobial agents (Chuesiang et al., 2019, de Meneses et al., 2019, Franklyne et al., 2019, Majeed et al., 2016, Otoni et al., 2014, Park et al., 2018, Pongsumpun et al., 2020). Even both of them could control cucumber downy mildew at 0.1% oil concentration. However, when the oil concentration was decreased from 0.1% to 0.025%, the efficacy of clove-type emulsion decreased. As to cinnamon-type emulsion, it still revealed high efficacy, which indicated cinnamon-type emulsion was more suitable to be used as plant protectant. The disease severity was also evaluated by the cinnamon-type emulsion. As the result shows, the smaller droplet size also keeps the better efficacy. And the disease severity increased with the increase of droplet size.

The mechanism could be attributed to collision of oil droplets and sporangia. Therefore, the smaller droplet size showed better efficacy to control downy mildew. In addition, the microscopic observations showed that the sporangia germination was damaged while the release of zoospore was inhibited (5.33%) (**Figure 10**), confirming that the antifungal efficacy was related to the release of zoospore. Some researches also indicated the diluted emulsion could form a slight film to protect the germination of pathogenic microorganisms after emulsification. It also could reduce the opportunity of second transmission (Wang et al., 2009, Lin et al., 2004, Huang et al., 2018, Hsieh and Huang, 2009). Therefore, the result suggest that cinnamon-type emulsion is suitable to be an antifungal agent to control cucumber downy mildew

## 5. CONCLUSIONS

The emulsion synthesized parameters such as temperature, homogenization speed, oil to surfactant ratio, water ratio and surfactant ratio can reach different droplet size. Therefore, the oil emulsion could be a plant protectant with the specific droplet size. Compared with the oil types, the neem oil emulsion had the lowest efficacy to control the disease, following with soybean oil emulsion and camellia oil emulsion. The moringa-type, cinnamon-type and clove-type emulsion had better efficacy with 0.1% oil concentration. Besides, the cinnamon-type and moringa-type emulsion with various particles have better efficacy to control cucumber downy mildew. The droplet sizes increased with increasing the disease incidence at 0.025% oil concentration. From the optical microscope, the sporangia treated with cinnamon-type emulsion couldn't differentiate the cytoplasm. As the result above, the emulsions can use to control cucumber downy mildew and replace the use of traditional pesticides. Achieving the goal of decreasing the hazard of traditional pesticides contaminated the soil and groundwater.

## ACKNOWLEDGMENTS

This study was supported by the Ministry of Science and Technology of Taiwan (Grant No. NSC 105-2221-E-005 -005 -MY3, 106-2218-E-005 -011 -MY2, 106-3114-E-005 -003, 107-2218-E-005-007, 108-2221-E-005 -048 -MY3, 108-2218-E-005 -004), Industrial Development Bureau, Ministry of Economic Affairs of Taiwan (101-EC-17-A-A21-S1-229), National Synchrotron Radiation Research Center (NSRRC-2015-2-087-1), Innovation and Development Center of Sustainable Agriculture from the Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

**REFERENCES**

1. CAMARDA, A., PUGLIESE, N., BEVILACQUA, A., CIRCELLA, E., GRADONI, L., GEORGE, D., SPARAGANO, O. & GIANGASPERO, A. 2018. Efficacy of a novel neem oil formulation (RPO3 (TM)) to control the poultry red mite Dermanyssus gallinae. *Medical and Veterinary Entomology,* 32, 290-297.
2. CHUESIANG, P., SIRIPATRAWAN, U., SANGUANDEEKUL, R., YANG, J. S., MCCLEMENTS, D. J. & MCLANDSBOROUGH, L. 2019. Antimicrobial activity and chemical stability of cinnamon oil in oil-in-water nanoemulsions fabricated using the phase inversion temperature method. *Lwt-Food Science and Technology,* 110, 190-196.
3. CONSTANSKI, K. C., ZORZETTI, J., SANTORO, P. H., HOSHINO, A. T. & NEVES, P. 2016. Inert powders alone or in combination with neem oil for controlling Spodoptera eridania and Spodoptera frugiperda (Lepidoptera: Noctuidae) larvae. *Semina-Ciencias Agrarias,* 37, 1801-1810.
4. COUNCILOFAGRICULTURE 2019. 2019 Statistical Annual Report.
5. CRETELLA, A. B. M., SOLEY, B. D., PAWLOSKI, P. L., RUZISKA, R. M., SCHARF, D. R., ASCARI, J., CABRINI, D. A. & OTUKI, M. F. 2020. Expanding the anti-inflammatory potential of Moringa oleifera: topical effect of seed oil on skin inflammation and hyperproliferation. *Journal of Ethnopharmacology,* 254.
6. DE MENESES, A. C., SAYER, C., PUTON, B. M. S., CANSIAN, R. L., ARA?JO, P. H. H. & DE OLIVEIRA, D. 2019. Production of clove oil nanoemulsion with rapid and enhanced antimicrobial activity against gram-positive and gram-negative bacteria. *Journal of Food Process Engineering,* 42.
7. ELSAYED, E. A., SHARAF-ELDIN, M. A., EL-ENSHASY, H. A. & WADAAN, M. 2016. In vitro Assessment of Anticancer Properties of Moringa peregrina Essential Seed Oil on Different Cell Lines. *Pakistan Journal of Zoology,* 48, 853-859.
8. FAN, Y. T., CHUNG, K. R. & HUANG, J. W. 2019. Fungichromin Production by Streptomyces padanus PMS-702 for Controlling Cucumber Downy Mildew. *Plant Pathology Journal,* 35, 341-350.
9. FAO 2018. FAOSTAT.
10. FORMENTINI, M. A., ALVES, L. F. A. & SCHAPOVALOFF, M. E. 2016. Insecticidal activity of neem oil against Gyropsylla spegazziniana (Hemiptera: Psyllidae) nymphs on Paraguay tea seedlings. *Brazilian Journal of Biology,* 76, 951-954.
11. FRANKLYNE, J. S., EBENAZER, L. A., MUKHERJEE, A. & NATARAJAN, C. 2019. Cinnamon and clove oil nanoemulsions: novel therapeutic options against vancomycin intermediate susceptible Staphylococcus aureus. *Applied Nanoscience,* 9.
12. FRIBERG, S. E., CORKERY, R. W. & BLUTE, I. A. 2011. Phase Inversion Temperature (PIT) Emulsification Process. *Journal of Chemical & Engineering Data,* 56, 4282-4290.
13. HSIEH, T. F. & HUANG, H. Z. 2009. Disease control and plant health management. *2009 Conference on Flower Health Management.*
14. HUANG, J. W., Q., X. Q. & SHEN, Y. M. 2018. Development and application of environmentally friendly plant proctants. *Conference of Organic and Environmental Friendly Farming*
15. HWUNG, S. J. 1998. *Theory and Application of the Surfactant-Emulsifying Essential Oil.* Master degree, Da Yeh University.

16. ISHII, H., FRAAIJE, B. A., SUGIYAMA, T., NOGUCHI, K., NISHIMURA, K., TAKEDA, T., AMANO, T. & HOLLOMON, D. W. 2001. Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. *Phytopathology*, 91, 1166-71.

17. KALA, S., NAIK, S. N., PATANJALI, P. K. & SOGAN, N. 2019. Neem oil water dispersible tablet as effective larvicide, ovicide and oviposition deterrent against Anopheles culicifacies. *South African Journal of Botany*, 123, 387-392.

18. KO, W. H., WANG, S. Y., HSIEH, T. F. & ANN, P. J. 2003. Effects of Sunflower Oil on Tomato Powdery Mildew Caused by Oidium neolycopersici. *Journal of Phytopathology*, 151, 144-148.

19. KOLANI, L., SANDA, K., AGBOKA, K., MAWUSSI, G., KOBA, K. & DJOUAKA, R. 2016. Investigation of Insecticidal Activity of Blend of Essential Oil of Cymbopogon schoenanthus and neem oil on Plutella xylostella (Lepidoptera: Plutellidae). *Journal of Essential Oil Bearing Plants*, 19, 1478-1486.

20. KUMAR, R., KRANTHI, S., NAGRARE, V. S., MONGA, D., KRANTHI, K. R., RAO, N. & SINGH, A. 2019. Insecticidal activity of botanical oils and other neem-based derivatives against whitefly, Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae) on cotton. *International Journal of Tropical Insect Science*, 39, 203-210.

21. KUMARI, S., R V, K., CHOUDHARY, R. C., SHARMA, S. S., PAL, A., RALIYA, R., BISWAS, P. & SAHARAN, V. 2018. Thymol nanoemulsion exhibits potential antibacterial activity against bacterial pustule disease and growth promotory effect on soybean. *Scientific Reports*, 8.

22. LI, Y. T., LIN, J., YAO, W. Q., GAO, G. L., JING, D. W. & WU, Y. 2020. Discovery of a new fungicide by screening triazole sulfonylhydrazone derivatives and its downy mildew inhibition in cucumber. *Journal of Heterocyclic Chemistry*, 57, 2128-2138.

23. LIN, C.-L. 2011. Transport Assessment of Applying Nano-scale Zero-Valent Iron Emulsion in Groundwater.

24. LIN, J. T., AN, B. J., ZHANG, Q. A., LO, Z. C. & HSIEH, T. F. 2004. *Non-Pesticide Control of Crop Diseases* Council of Agriculture, Executive Yuan, Taiwan.

25. LUAN, Q. Q., CHEN, C. H., LIU, M. Y., LI, Q., WANG, L. N. & REN, Z. 2019. CsWRKY50 mediates defense responses to Pseudoperonospora cubensis infection in Cucumis sativus. *Plant Science*, 279, 59-69.

26. MAJEED, H., LIU, F., HATEGEKIMANA, J., SHARIF, H. R., QI, J., ALI, B., BIAN, Y. Y., MA, J. G., YOKOYAMA, W. & ZHONG, F. 2016. Bactericidal action mechanism of negatively charged food grade clove oil nanoemulsions. *Food Chemistry*, 197, 75-83.

27. MICHEREFF, S. J., NORONHA, M. A., LIMA, G. S., ALBERT, Í. C., MELO, E. A. & GUSMóO, L. O. 2009. Diagrammatic scale to assess downy mildew severity in melon. *Horticultura Brasileira*, 27, 76-79.

28. MOGHIMI, R., ALIAHMADI, A., MCCLEMENTS, D. J. & RAFATI, H. 2016. Investigations of the effectiveness of nanoemulsions from sage oil as antibacterial agents on some food borne pathogens. *Lwt-Food Science and Technology*, 71, 69-76.

29. NAVEENKUMAR, R., MUTHUKUMAR, A., SANGEETHA, G. & MOHANAPRIYA, R. 2017. Developing eco-friendly biofungicide for the management of major seed borne diseases of rice and assessing their physical stability and storage life. *Comptes Rendus Biologies*, 340, 214-225.

30. NGOM, S., PEREZ, R. C., MBOW, M. A., FALL, R., NIASSY, S., COSOVEANU, A., DIOP, S. M., NDIAYE, E. B., DIOP, M. & LOGNAY, G. 2018. Larvicidal activity of Neem oil and three plant essential oils from Senegal against Chrysodeixis chalcites (Esper, 1789). *Asian Pacific Journal of Tropical Biomedicine*, 8, 67-72.

31. OTONI, C. G., DE MOURA, M. R., AOUADA, F. A., CAMILLOTO, G. P., CRUZ, R. S., LOREVICE, M. V., SOARES, N. & MATTOSO, L. H. C. 2014. Antimicrobial and physical-mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films. *Food Hydrocolloids*, 41, 188-194.

32. OU, C. C. 1992. *Emulsification technology practice*, Fu Han.

33. PARK, J. B., KANG, J. H. & BIN SONG, K. 2018. Antibacterial activities of a cinnamon essential oil with cetylpyridinium chloride emulsion against Escherichia coli O157:H7 and Salmonella Typhimurium in basil leaves. *Food Science and Biotechnology*, 27, 47-55.

34. PONGSUMPUN, P., IWAMOTO, S. & SIRIPATRAWAN, U. 2020. Response surface methodology for optimization of cinnamon essential oil nanoemulsion with improved stability and antifungal activity. *Ultrasonics Sonochemistry*, 60.

35. SALAS, S. E., SHEPHERD, C. P., NGUGI, H. K. & GENET, J. L. 2019. Disease Control Attributes of Oxathiapiprolin Fungicides for Management of Cucurbit Downy Mildew. *Plant Disease*, 103, 2812-2820.

36. SWATHY, J. S., PRABHAKAR, M., THOMAS, J., MUKHERJEE, A. & CHANDRASEKARAN, N. 2018. Antimicrobial potency of high-energy emulsified black pepper oil nanoemulsion against aquaculture pathogen. *Aquaculture*, 491, 210-220.

37. WANG, H. L., HSIEH, T. F. & CHUANG, Y. Y. 2009. Non-pesticide control of plant diseases and pest diseases. *Science Development*, 443.

38. WANG, L. J. 2007. *Preparation and Efficacy Test of Pesticide Microemulsion.* Anhui University.

39. ZHANG, F., XIN, M., YU, S. Q., LIU, D., ZHOU, X. Y. & QIN, Z. W. 2019a. Expression and Functional Analysis of the Propamocarb-Related Gene CsMCF in Cucumber. *Frontiers in Plant Science*, 10.

40. ZHANG, P. F., GUAN, A. Y., XIA, X. L., SUN, X. F., WEI, S. Y., YANG, J. L., WANG, J. F., LI, Z. N., LAN, J. & LIU, C. L. 2019b. Design, Synthesis, and Structure-Activity Relationship of New Arylpyrazole Pyrimidine Ether Derivatives as Fungicides. *Journal of Agricultural and Food Chemistry*, 67, 11893-11900.

41. ZHANG, R., PAN, Z. H., WANG, X. Y., SHEN, M. L., ZHOU, J. J., FU, Z. W. & JIN, Y. X. 2019c. Short-term propamocarb exposure induces hepatic metabolism disorder associated with gut microbiota dysbiosis in adult male zebrafish. *Acta Biochimica Et Biophysica Sinica*, 51, 88-96.