INVESTIGATION ON EFFICIENCY OF NANOPARTICLE AND EXTRACTS FROM PLANTS, AND AGRICULTURAL RESIDUE FOR INHIBITING *Aspergillus sp*. FROM PARA RUBBER IN PDA MEDIA

Sujittrawan Prachanban[1], Waraporn Kosanlavit[2], Tharathip Rattana[3], Napat Noinumsai[4]

Graduated School[1], Faculty of Science and Technology[2,3,4], Nakhon Ratchasima Rajabhat University, Thailand.

Email: sujittrawan38@gmail.com[1], waraporn.k@nrru.ac.th[2]

Received: April 23, 2022; **Revised**: May 5, 2022; **Accepted**: May 30, 2022

Abstract

The purposes of the investigation on nanoparticle efficiency of extracts from plants, vegetables, herbs and agricultural residue for inhibiting Aspergillus sp. in rubber in Pa Yup Nai Sub-district, Wang Chan District, Rayong Province were to study the efficiency of nanoparticles extracted from plants, vegetables, herbs and agricultural residue for inhibiting Aspergillus sp. in PDA medium. The research was conducted by isolating the fungus in the PDA medium by testing the inhibition zone for 24 and 48 hours of the 10 treatments. Zinc oxide nanoparticles were found to have the highest inhibitory effect on Aspergillus sp., followed by betel nut extracts and the controlled extract which was the least effective. The efficiency decreased as the duration increased from 24 hours to 48 hours. However, the aforementioned test can provide an alternative for rubber cup manufacturers according to their needs and problems.

Keywords: fungal inhibition, *Aspergillus sp.*, nanoparticles, extract

Introduction

Thailand is the world's number 1 exporter of rubber which generates income of more than 400 billion baht per year. Most of the rubber is in the form of primarily processed raw materials such as smoked rubber sheets, block rubber and concentrated rubber (Ionic Co., Ltd., 2018). However, rubber farmers who produce raw rubber sheets still encounter fungi problems on the rubber sheets.

As a result, rubber sheet farmers are affected by market prices, resulting in poor quality of rubber sheets and low selling prices. Fungi on rubber sheets can be caused by a number of reasons, such as the rubber sheet itself, high humidity that facilitates the growth of fungi, rubber sheet production processes from latex tapping, transportation, as well as equipment used in production which is contaminated with fungi. It was also found that the composition of the rubber has nutrients that the fungi can use as medium. The fungi found on rubber sheets include Aspergillus, Penicillium, Fusarium, Paecilomyces and Trichoderma (PongSawat, 2012).

Fungal control and prevention can be done by using paranitrophenol in the rubber sheet production process. However, it has been found that paranitrophenol is a harmful substance to people and is also a carcinogen, so it had to be stopped (Saowaluk, 2015). Nano zinc oxide and nano-carbon is a nanotechnology that has the ability to prevent fungi and bacteria that cause many plant diseases. Herbal plants such as mangosteen peel, pineapple, betel nut, chromolaena odorata leaves and Senna alata also have the ability to inhibit fungi.

Therefore, this thesis focused on the inhibition of Aspergillus sp. in rubber cups with zinc oxide nanoparticles, carbon nanoparticles and extracts from plants, vegetables, herbs and agricultural residue in order to increase the value of rubber sheets and agricultural residue.

Research Objectives

To investigate the efficiency of nanoparticles extracted from plants, vegetables, herbs and agricultural residue for inhibiting Aspergillus sp. in PDA medium.

Literature Review

Importance of rubber to the economy and society, rubber is another plant that is important to the economy of Thailand. It has been found that there are approximately 1 million rubber-related farmers and businesses or no less than 6 million people. Thailand is the world's number 1 exporter of rubber and rubber products since 1991. In 2018, Thailand produced 4.85 million tons of rubber, and 4.30 million tons of which (88 percent of the total production) were exported and 631,635 tons (13% of the total production) were for domestic use, generating more than 500 billion baht per year. However, most of the exported rubber is in the form of primarily processed raw materials which have low added

value, such as smoked rubber sheets, rubber blocks, and latex, resulting in less impact on income generation for the country and farmers. If this issue has been addressed and improved, it will benefit the country and the rubber farmers enormously. Rubber is still one of the economic crops that are necessary to promote careers and improvement opportunities. In addition, rubber is an important economic crop for the economy of the South and Thailand. Latex which is a product obtained from the food trunk tube in the bark of the rubber tree can be used as a raw material for making various rubber products for a wide range of industrial uses, from heavy industries such as tire production to household appliances The latex obtained from the rubber tree has some properties that synthetic rubber does not. Therefore, rubber is important to Thailand for the above reasons (Agricultural Research Development Agency, 2021).

Research Methodology

1. Preparation of nanoparticle solutions, extraction and bio-fermented water

1.1 Preparation of nanoparticle solutions

Zinc oxide nanoparticles (Treatment 2) were prepared at a concentration of 50 mg/l by weighing 50 mg of zinc oxide nanoparticles, adding distilled water to a volume of 1 liter and shaking well. The solution has to be shaken well before use (Waraporn Kosanlavit, 2013).

Carbon nanoparticles (Treatment 3) were prepared at a concentration of 50 mg/l by weighing 50 mg of carbon nanoparticles, adding distilled water to a volume of 1 liter and shaking well. The solution has to be shaken well before use (Waraporn Kosanlavit, 2013).

1.2 Preparation of extracts from plants, vegetables and herbs

Extracts were obtained from 4 plants, vegetables and herbs, namely morning glory, betel nut, Chromolaena odorata leaves and Senna alata. They were separately washed, chopped and dried in the shade at room temperature. When dry, each plant was extracted in 95 percent ethanol by soaking in methanol at room temperature. The extraction was performed 3 times on each plant, once every 7 days. Each extract was evaporated by a rotary vvaporator with a rotation setting of 150 rpm at 40 °C. Coarsely concentrated extracts of each plant were obtained, including morning glory extract (Treatment 4), betel

nut extract (Treatment 5), Chromolaena odorata extract (Treatment 6) and Senna alata extract (Treatment 7).

1.3 Preparation of bio-fermented water from agricultural residue

The preparation of bio-fermented water from agricultural residue included 3 recipes, namely betel nut peel, mangosteen peel, and pineapple residue.

Bio-fermented water from betel nut bark was repaired by cracking betel nuts into small pieces for 5 kg, then putting it into a 22-liter bucket, adding 5 kg of molasses, adding the PD. 2 solution (dissolve 1 sachet of PD. 2 powder in 5 liters of water and leave it for 5 minutes) into the bucket and mixing well. The lid was closed loosely to allow air circulation. The bio water was ferment for at least 7-14 days and then filtered until the remaining water was the leavening agent. Use 2 tablespoons of leavening agent per 20 liters of water (Department of Land Development, 2021).

Bio-fermented water from mangosteen peel was prepared by chopping mangosteen peel into small pieces for 5 kg, then putting it into a 22-liter bucket, adding 5 kg of molasses, adding the PD. 2 solution (dissolve 1 sachet of PD. 2 powder in 5 liters of water and leave it for 5 minutes) into the bucket and mixing well. The lid was closed loosely to allow air circulation. The bio water was ferment for at least 7-14 days and then filtered until the remaining water was the leavening agent. Use 2 tablespoons of leavening agent per 20 liters of water (Department of Land Development, 2021).

Bio-fermented water from pine apple residue was prepared by chopping pine apple shell and core and into small pieces for 5 kg, then putting it into a 22-liter bucket, adding 5 kg of molasses, adding the PD. 2 solution (dissolve 1 sachet of PD. 2 powder in 5 liters of water and leave it for 5 minutes) into the bucket and mixing well. The lid was closed loosely to allow air circulation. The bio water was ferment for at least 7-14 days and then filtered until the remaining water was the leavening agent. Use 2 tablespoons of leavening agent per 20 liters of water (Department of Land Development, 2021).

2. Fungi isolation from rubber cups

The rubber cup with natural fungi was isolated in the laboratory with an aseptic technique. The rubber cup was soaked in 10% Clorox to clean and kill unwanted bacteria for 3-5 minutes and then rinsed 3 times with distilled water. The rubber cup was placed on a potato dextrose agar (PDA) and incubated at room temperature for 4 days. Characteristics were studied under a microscope, and each fungus was isolated for purification.

In the investigation on nanoparticle efficiency of extracts from plants, vegetables, herbs and agricultural residue for inhibiting Aspergillus sp. in the PDA on a petri dish, the fungal culture medium was prepared using a double layer culture method by pouring a thin layer of 15 ml of PDA on the Petri dish and leave to dry (approximately 2-3 hours). Then, the micropipette was used to take 1ml of fungal suspension cells to mix it on the PDA container which was heated to approximately 45°C for 20 ml. Then 5 ml of the content was poured on the petri dish to cover the bottom layer and left to dry. Then, the disc diffusion method was performed.

The inhibition test was performed by using the disc diffusion method (Department of Agriculture, 2013) by using the micropipette to suck each of the 10 treatments (1 control + 9 experimental substances), namely:

Treatment 1: Control (Distilled water steamed and disinfected)

Treatment 2: Zinc oxide nanoparticle solution

Treatment 3: Carbon nanoparticle solution

Treatment 4: Extracts from morning glory

Treatment 5: Extracts from betel nuts

Treatment 6: Extracts from Chromolaena odorata leaves

Treatment 7: Extracts from Senna alata

Treatment 8: Fermented water from betel nuts

Treatment 9: Fermented water from mangosteen peel

Treatment 10: Fermented water from pine apple residue

7 microlitre of each treatment was dropped onto the No.1 paper disc with 5 mL diameter paper disc which was sterilised. The sterilised tweezer was used to pick up the paper disc and drop it onto 4 points per PDA petri dish with Aspergillus sp. Each type of fungi was isolated to mix with each treatment.

Treatment 1: Control (Distilled water steamed and disinfected): T1R1, T1R2, T1R3, and T1R4

Treatment 2: Zinc oxide nanoparticle solution : T2R1, T2R2, T2R3, and T2R4

Treatment 3: Carbon nanoparticle solution: T3R1, T3R2, T3R3, and T3R4

Treatment 4: Extracts from morning glory : T4R1, T4R2, T4R3, and T4R4

Treatment 5: Extracts from betel nuts : T5R1, T5R2, T5R3, and T5R4

Treatment 6: Extracts from Chromolaena odorata leaves : T6R1, T6R2, T6R3, and T6R4

Treatment 7: Extracts from Senna alata : T7R1, T7R2, T7R3, and T7R4

Treatment 8: Fermented water from betel nuts : T8R1, T8R2, T8R3, and T8R4

Treatment 9: Fermented water from mangosteen peel : T9R1, T9R2, T9R3, and T9R4

Treatment 10: Fermented water from pine apple residue : T10R1, T10R2, T10R3, and T10R4

The petri dish was incubated at room temperature for 3 days. Results were recorded after 24 and 48 hours by measuring the width of the clear zone or inhibition zone using a vernier caliper in mm.

Results

Results of investigation on nanoparticle efficiency of extracts from plants, vegetables, herbs and agricultural residue for inhibiting *Aspergillus sp*. from isolation in the PDA medium

Table 1 Results of investigation on nanoparticle efficiency of extracts from plants, vegetables, herbs and agricultural residue for inhibiting Aspergillus sp. from isolation in the PDA medium after 24 and 48 hours

Item	Inhibition Zone (mm.)					
	TiR1	TiR2	TiR 3	TiR4	Mean	±SD
After 24 hours						
T1: Control	0.00	0.00	0.00	0.00	0.00	±0.00
T2: Zinc oxide nanoparticle solution	32.00	28.00	30.00	31.00	30.25	±1.48
T3: Carbon nanoparticle solution	18.00	14.00	17.00	11.00	15.00	±2.74
T4: Extracts from morning glory	9.00	9.00	13.00	11.00	10.50	±1.66
T5: Extracts from betel nuts	25.00	27.00	28.00	19.00	24.75	±3.49
T6: Extracts from Chromolaena odorata leaves	17.00	15.00	18.00	19.00	17.25	±1.48
T7: Extracts from Senna alata	7.00	9.00	0.00	9.00	6.25	±3.70
T8: Fermented water from betel nuts	22.00	21.00	25.00	19.00	21.75	±2.17
T9: Fermented water from mangosteen peel	15.00	17.00	11.00	13.00	14.00	±2.24
T10: Fermented water from pine apple residue	21.00	19.00	17.00	25.00	20.50	±2.96
Results after 48 hours						
T1: Control	0.00	0.00	0.00	0.00	0.00	±0.00

T2: Zinc oxide nanoparticle solution	23.00	24.00	25.00	23.00	23.75	±0.83
T3: Carbon nanoparticle solution	12.00	11.00	11.00	9.00	10.75	±1.09
T4: Extracts from morning glory	6.00	7.00	9.00	6.00	7.00	±1.22
T5: Extracts from betel nuts	16.00	15.00	14.00	15.00	15.00	±0.71
T6: Extracts from Chromolaena odorata leaves	11.00	9.00	13.00	11.00	11.00	±1.41
T7: Extracts from Senna alata	0.00	6.00	0.00	7.00	3.25	±3.27
T8: Fermented water from betel nuts	16.00	15.00	15.00	17.00	15.75	±0.83
T9: Fermented water from mangosteen peel	9.00	8.00	9.00	7.00	8.25	±0.83
T10: Fermented water from pine apple residue	17.00	15.00	8.00	19.00	14.75	±4.15

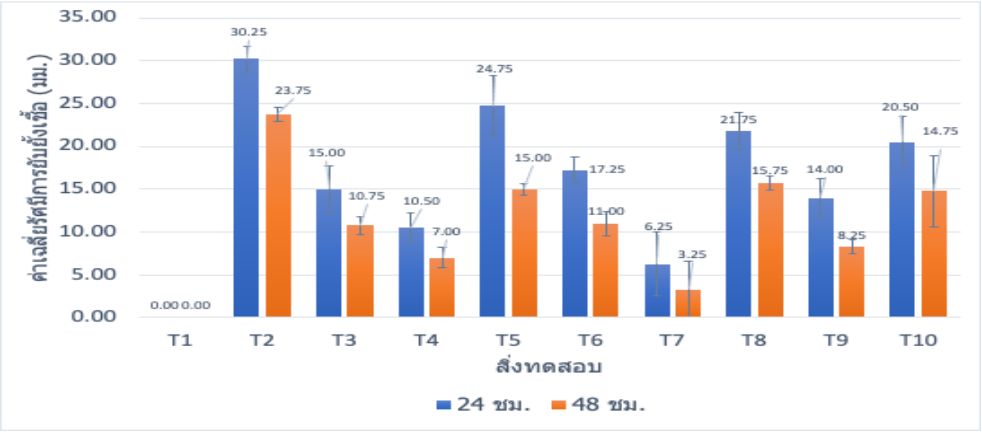

Figure 2 Mean of inhibition of *Aspergillus sp.* from isolation in the PDA medium after 24 and 48 hours

Discussion

The investigation on nanoparticle efficiency of extracts from plants, vegetables, herbs and agricultural residue for inhibiting Aspergillus sp. from isolation in the PDA medium revealed that zinc oxide nanoparticles had the highest Aspergillus sp. inhibition efficiency because zinc oxide nanoparticles were a substance containing the inhibitory property and could kill both bacteria and fungi well, followed by extracts from betel nuts, fermented water from betel nuts because betel nuts contained substances that caused a astringent taste. According to the study, vegetables and herbs with astringent flavour contained tannins which could inhibit and kill bacteria and fungi. Fermented water from pine apple residue, extracts from Chromolaena odorata leaves, fermented water from mangosteen peel, carbon nanoparticle solution, extracts from morning glory, extracts from Senna alata had Aspergillus sp subsequent inhibitory efficiency, respectively. Fermented water was acidic which could inhibit fungi. The Aspergillus sp. inhibition efficacy in the PDA decreased as the duration increased from 24 h to 48 h due to increased fungal growth and decreased volume and efficiency as a result of evaporation and oxidation of some substances (Dmitrily, 2009; Masciangioli, 2003; Songpol, 2011).

Conclusion

In the investigation on nanoparticle efficiency of extracts from plants, vegetables, herbs and agricultural residue for inhibiting Aspergillus sp. from isolation in the PDA medium, in terms of the Aspergillus sp. inhibition efficacy in the PDA medium by testing the inhibition zone after 24 and 48 hours with 10 treatments, it can be concluded that zinc oxide nanoparticles had the highest Aspergillus sp. inhibition efficiency, followed by extracts from betel nuts, fermented water from betel nuts, fermented water from pine apple residue, extracts from Chromolaena odorata leaves, fermented water from mangosteen peel, carbon nanoparticle solution, extracts from morning glory and extracts from Senna alata, respectively. The control treatment had the lowest Aspergillus sp. inhibition efficiency in the PDA medium. It can be further concluded that the Aspergillus sp. inhibition efficacy in the PDA medium decreased as the duration increased from 24 h to 48 hours.

References

Agricultural Research Development Agency. (2021). Rubber tree. Access on www.arda.or.th.

Department of Land Development. (2021). Bio-fermented water. Access on www.ldd.go.th.

Dmitriy Obolskiy, et al. (2009). Garcinia mangostana L.: A Phytochemical and Pharmacological Review. Phytotherapy Research. 23, 1047-1065.

Ionic Co., Ltd. (2018). Para Rubber. Access on www:ionic.co.th.

Masciangioli T. and Zhang W.X. (2003). Environmental technologies at the Nanoscale. Environmental Science & Technology, March 1, 102-108.

Pongsawat Premphet* Komgrit Leksakul Tawatchai Sririn Patcharin Choomkaew. (2012). Analysis of Fungi Occurred and Assessment of Carbon Footprint in Smoked Rubber Process. Industrial Engineering Conference. Petchaburi, Thailand.

Saowaluk Pongpaijit et al. (2015) Selection of microorganisms capable of controlling fungi. contaminated on the rubber sheet. Faculty of Science Prince of Songkla University: Songkhla.

Songpol Chivapat, et al. (2011). Chronic Toxicity Study of Garcinia mangostana Linn. Pericarp Extract. The Thai Journal of Veterinary Medicine. 41(1), 45-53.

Waraporn Kosanlavit. (2013). The Efficiency of nZnO for Remediation of Trinitrotoluene Contaminated Water. International Journal of Chemical Engineering and Applications, Vol. 6, No. 2, April 2015