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Efficacy Evaluation of Four Fungicides on a Water Agar Platform Smeared with Rust Fungal spores

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Abstract

Sun, E. J. and Lei, I. S. (2022). Efficacy evaluation of four fungicides on a water agar platform smeared with rust fungal spores. Taiwan Pestic. Sci. 13: 19-34.

The study is to develop a water agar platform method for quickly evaluating the efficacy of four fungicides applied to obligate rust fungi on crops and trees. This method uses a 9 cm sterile Petri dish filled with a thin layer of 3% water agar. A rectangular platform (6 × 1 cm) connected with five 3 cm dilution lines was designed for the water agar plate. The platform works as an *in vitro* field where test fungal spores are mixed with four fungicides. After approximately 10 minutes of mixing, five streaks are made with an aseptic loop to tow the treated spores outside the platform along the dilution lines to their ends. Four rust uredospores collected on-site from diseased leaves, including corn rust, grape rust, fig rust, and frangipani rust, were assayed against four common fungicides in Taiwan. Our results showed that mancozeb (80% WP) is the best agent to control corn rust, while chlorothalonil (75% WP) was the best for controlling grape, fig, and frangipani rusts. Bordeaux mixture (6:4) also had good efficacy for fig rust. The inhibition of germination and the morphological modification of spores inside and outside the platform can also be observed and compared with each other. If necessary the microscopic observation can be extended for two more days. Our results showed that chlorothalonil completely and permanently inhibited spore germination within and outside the platform, suggesting that chlorothalonil is a therapeutic fungicide. Conversely, the Bordeaux mixture experiments showed that uredospore germination rates outside the platform were higher than within the platform, indicating that it is only an inhibitive chemical for the rust uredospore. Our assessment of the morphological modification of spores showed that mancozeb at a 500X dilution causes germ tube and

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mycelia shapes of fig rust to be prominently different from normal blanks. The germ tubes are shortened, with stunted ends, while some mycelia show a slimmer shape. However, some of the germ tubes and mycelia outside the platform area recovered to near normal, indicating that mancozeb plays only an inhibition function. The practical information obtained from this method is quite useful for farmers, tree owners, plant doctors, or tree doctors when they want to select a fungicide to reduce the severity of a disease, and to avoid the inappropriate use of non-effective or resistance-inducing fungicides. Due to the low cost and simplicity of operation, this water agar platform method is especially suitable for plant medicine students or researchers who prescribe fungicides and conduct fungicidal screenings in the fields.

Key words: water agar platform, fungicidal screening, germination inhibition, morphological modification, corn rust, grape rust, fig rust, frangipani rust

Introduction

Since 2011, the master's program in plant medicine at National Taiwan University (NTU) had been established with the mission of training plant doctors and tree doctors in Taiwan. In addition to NTU, there are three other universities setting up similar plant medical programs or departments with the same goals around the same years. These are the Department of Plant Medicine of National Pintung University of Science and Technology, Master's Program for Plant Medicine and Good Agricultural Practice of National Chung Hsing University, and Department of Plant Medicine of National Chiayi University. Some of the graduate students from these programs and departments are currently working as intern plant doctors in about 50 counties or cities in Taiwan, all with the same great mission of diagnosing plant diseases or pests and giving prescriptions or control recommendations to serviced farmers or tree owners.

However, because there are numerous species of insects and pathogens affecting or ravaging crops or trees in subtropical and tropical Taiwan, the availability of insecticides and fungicides to effectively and safely combat harmful targets are usually insufficient for the on-site plant doctor or tree doctor. Basically, the "control rates" of registered insecticides or fungicides against related targets has not been clearly reported by scientists in Taiwan. Pesticide companies have usually been reluctant to release "control rate" information of their registered insecticides or fungicides. Moreover, insecticide- or fungicide-resistant insects or pathogens are now prevailing due to often repeated or long-timed uses of the same groups of chemicals. For these reasons we have tested some practical assay methods for quickly evaluating the "efficacy" of any registered insecticide or fungicide against target pests. The first is the water agar platform method for quickly evaluating the potential fungicides against obligate rust fungi on crops or trees.

There were many bactericidal and fungicidal assay methods reported in the past, as reviewed by Balouiri *et al* [1]. in 2016. The common bioassay methods include disk-diffusion, well diffusion, broth or agar dilution, and poison food techniques. Some other specific methods were also reported by other scientists. For instance, Duan *et al*. in 2018 used a microtiter plate method for screening fungicides for controlling grape anthracnose fungi [4]. Also, Chang in our laboratory used some special methods, including the "Y-plate poison food method" and "3-way ring-plate method," for fungicidal screening [2]. These complicated techniques usually require specific equipment or materials and take long time to operate. Here we have developed a new simple method to do the fungicidal assay job.

Materials and methods

Investigation and collection of rust disease materials from the field

Four rust fungi on crops or trees were diagnosed and collected from fields around Taipei and Central Taiwan. These are the corn rust, grape rust, fig rust, and frangipani rust. Hosts, scientific names, symptoms, and estimated loss rates based on diseased leaf area (%) are listed in (Table 1).

Diseased leaves were collected in PE plastic bags. Their uredospores were observed and identified under a light microscope. Spore morphology was compared with descriptions in the literature [3,5,6,8]. The study was carried out in the warm seasons of 2020 and 2021; therefore, only uredospores

Table 1. Biological information and estimated diseased leaf area % of four rust fungi in this study

Host	Disease name	Scientific name	Symptoms	Estimated diseased leaf area (%)
Corn	southern rust	*Puccinia polysora* Underw	uredia pustules yellowish brown	10-30
Grape	grape rust	*Phakopsora ampelopsidis*	uredia pustules yellowish brown	30-50
Fig	fig rust	*Cerotelium fici*	uredia pustules yellowish	30-50
Frangipani	frangipani rust	*Coleosporium plumeriae*	orange pustules	20-40

were found on leaf samples. A preliminary germination test was conducted on 3% water agar for each species. For example, the uredospores of fig rust usually germinate within 1-2 h, with rapid extension of curved mycelia on an agar surface. All three of the other species also germinate mostly within a few hours. Germination time information is useful for designing further fungicidal experiments on the water agar platform.

Preparation of water agar platform for fungicidal experiments

After sterilization, 8-10 mL of 3% water agar is poured onto sterile 9 cm PE Petri dishes to make a thin, solid medium. On the back of each PE Petri dish, a rectangular platform is outlined with a blue marking pen, using a plastic template as shown in (Fig. 1). The platform is 6 cm long and 1 cm wide with five connected dilution streak lines as illustrated in Fig. 1, that are each 3 cm long and 1 cm from adjacent lines. The whole 3% water agar platform as shown in Fig. 1 was kept sterile and used for further assay.

Experimental procedure for determining fungicidal efficacy on the water agar platform

Uredospores of each rust were collected from diseased leaves, observed and identified under a light microscope, and an aseptic plastic pipette with 1-2 mL of sterile water was used to scrub the rust sori to collect uredospores into a sterile Petri dish. Spore concentration was then counted and adjusted to approximately 2×10^4/mL. An aliquot of 0.05 mL of the spore suspension (or five loops from a common

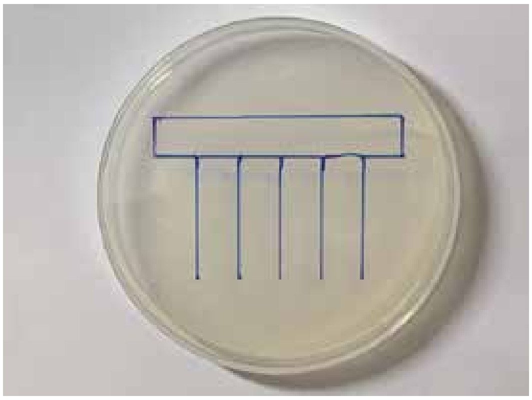

Fig. 1. The 3% water agar platform for fungicidal assay in this study. On the left is the plastic template for drawing the blue lines on the back of 9 cm Petri dish. On the right is a plate with a ready platform and five 3 cm long dilution lines.

inoculation loop) was dispensed evenly on the water agar platform. An aseptic inoculation loop was immediately used to smear the platform surface to evenly distribute the spores.

Candidate fungicides or agents used in this study included (1) mancozeb (80% wettable powder, Tocelo Chem., Co.), (2) chlorothalonil (75% wettable powder, Sinon Co.), (3) Bordeaux mixture (6:4 type, added with 0.5% surfactant Tween 20), (4) difenoconazole + propiconazole (50% emulsified concentrate, Syngenta Co.), and (5) narrow range oil (99% oil, Hanyu Energy Co.). The suspension or solution of candidate fungicides or agents at not only one dilution were prepared before the assay. Since BAPHIQ (Bureau of Animal and Plant Health Inspection and Quarantine, COA, Taiwan) has published the optimal concentration of each fungicide or agent, the published concentrations and/or the near ones were principally chosen for testing. After the platform surface was dried of free water, an aliquot (0.05 mL) of the newly prepared fungicide or agent was dispensed or pipetted onto the platform by the same procedure. An aseptic inoculation loop was also immediately used to smear the platform surface to evenly mix the fungicide with rust spores. To investigate the fungicidal mechanism or effects, after about ten minutes of reaction an aseptic inoculation loop was used to make five continuous streaks, each starting from the center of the platform and extending to the end of the dilution line as shown in Fig. 1. Platforms with only the spore suspension but without fungicides or agents were used as blank controls. All inoculated and treated water agar plates were incubated in an incubator at a temperature of 28°C and a light period of 12 h per day. Each treatment was repeated with three plates, and the experiment was repeated at least once.

Since most rust uredospores germinate within a few hours (usually within 1-3 hr), spore germination rates on each assay plate can be directly observed under a light microscope and counted normally for a total of 100 spores per platform within a day. When the germ tube is longer than the length of the spore, it is recorded as germinated. The germination inhibition rate was calculated by the equation:

Germination inhibition rate (%) =

(Control germination rate − treatment germination rate) ÷ Control germination rate × 100%

Observation of the inhibitive or therapeutic effects of test fungicides against rust fungi

To further investigate fungicidal effects or mechanisms, the germination rates of spores within the platform and along the dilution streak areas were observed and compared with each other. Theoretically, the dispensed 0.05 mL of a fungicide mixed with spores on the surface of the 3% water agar plate on the platform will be mostly absorbed by the spores and solid agar. The fungicide concentration outside the platform areas should be very low, as we used the 3% agar, which is harder than the normal 2%. Although diffusion will occur, fungicide concentration is proportionally reduced along the dilution line to a negligible concentration at the distance of 1-2 cm. A preliminary test with cotton blue in lactophenol and Congo red (0.5%) showed that the diffusions of both dyes outside the platform per day were about 3.5 and 3.2 mm, respectively. Therefore, if the spore germination rates within the platform areas are equal to those 1-cm away from the platform, it means that the fungicidal effects are irreversible. As a practical matter, when germination rates within and far away from the platforms are both zero, the deaths of both spore sets indicate that the fungicide has a lethal effect. Conversely, if spores recover their germination ability after being moved or towed away from the fungicide-treated platform, it means that the fungicide treatment showed only a temporary inhibitive function.

In order to further confirm the lethal effect of a fungicide against the target uredospores, the microscopic observation was extended for two more days. Those showed deformed and without any germination activity were reasonably judged as dead. A trypan staining method [7] can also be used to prove whether the cell or mycelium of the target fungus is dead or still alive.

Observations of morphological modifications of spores or mycelia after exposure to a fungicide or agent within or far away from the platform can be further conducted by the same manner.

Results

Determination of fungicidal efficacy against four rust fungi on the water agar platform

The study of the germination inhibition efficacy of four fungicides against corn rust uredospores was conducted in the warm seasons of 2020 and 2021. Diseased leaves were collected from Yun-Lin County. Four fungicides at not only one dilution were applied on uredospores within the water agar platform. Germination rates were observed after 18-24 h. The principal results are shown in (Table 2), indicating that mancozeb (80% WP) is the best agent to fight corn rust uredospores, while difenoconazole + propiconazole (50%) showed 77% efficacy toward this pathogen.

The assay on germination inhibition efficacy of four fungicides against grape rust uredospores was also conducted in warm seasons of 2020 and 2021. Diseased leaves were collected from a community yard around NTU. The same four fungicides were applied to spores within the water agar platform. Germination rates were observed after 18-24 h, and the principal results are expressed in (Table 3). They indicate that both mancozeb (80% WP) and chlorothalonil (75% WP) exhibit excellent control rates toward grape uredospores, while difenoconazole + propiconazole (50%) did not control this pathogen.

Experiments on germination inhibition rates of four fungicides against fig rust uredospores were also conducted in the warm seasons of 2020 and 2021. Diseased leaves were collected from potted trees grown by our lab. The same four fungicides were applied to uredospores within the water agar platform. Germination rates were observed after 18-24 h,

Table 2. Germination inhibition rates of four fungicides against corn rust uredospores on 3% water agar platforms observed after 18-24 h

Fungicide	Dilution (X)	Germination rate (%)[1)]	Inhibition rate (%)
Ck	-	29.0 a	
Mancozeb (80% WP)	400	2.7 c	91
Chlorothalonil (75% WP)	500	13.7 bc	53
Bordeaux mixture (6:4)	100	22.3 ab	23
Difenoconazole + Propiconazole (50% EC)	5,000	6.7 c	77

[1)] Data are means of three replicates. Those with the same letter are not significantly different (P = 0.05) according to Fisher's protected least significant difference test

Table 3. Germination inhibition rates of four fungicides against grape rust uredospores on 3% water agar platform observed after 18-24 h

Fungicide	Dilution (X)	Germination rate (%)[1)]	Inhibition rate (%)
Ck	-	52.70 a	
Mancozeb (80% WP)	400	0.67 d	99
Chlorothalonil (75% WP)	500	0.33 d	99
Bordeaux mixture (6:4)	100	20.00 c	62
Difenoconazole + Propiconazole (50% EC)	5,000	36.30 b	31

[1)] Data are means of three replicates. Those with the same letter are not significantly different (P = 0.05) according to Fisher's protected least significant difference test with principal results shown in (Table 4), indicating that both chlorothalonil (75% WP) and Bordeaux mixture (6:4) are excellent at overcoming fig rust, while difenoconazole + propiconazole (50%) did not affect this fungus.

Research on the germination inhibition efficacy of three fungicides against frangipani rust uredospores was conducted in the warm seasons of 2020 and 2021. Diseased leaves were collected from the NTU campus. Three fungicides were applied to uredospores within the water agar platform. Germination rates were observed after 18-24 h, with the principal results in (Table 5). They indicate that chlorothalonil (75% WP) is the best agent for overcoming frangipani rust, while mancozeb (80% WP) did not affect this fungus.

Observation of the fungicidal mechanisms of test fungicides against rust fungi

Experiments were conducted on fig rust uredospores in October, 2021, with three fungicides, including mancozeb (80% WP), chlorothalonil (75% WP), and Bordeaux mixture

Table 4. Germination inhibition rates of four fungicides against fig rust uredospores on the 3% water agar platform observed after 18-24 h

Fungicide	Dilution (X)	Germination rate (%)[1)]	Inhibition rate (%)
Ck	-	53.70 a	
Mancozeb (80% WP)	400	4.70 b	91
Chlorothalonil (75% WP)	500	0.33 b	99
Bordeaux mixture (6:4)	100	1.70 b	97
Difenoconazole + Propiconazole (50% EC)	5,000	52.00 a	3.2

1) Data are means of three replicates. Those with the same letter are not significantly different (P = 0.05) according to Fisher's protected least significant difference test

Table 5. Germination inhibition rates of three fungicides against frangipani rust uredospores on 3% water agar platform observed after 18-24 h

Fungicide	Dilution (X)	Germination rate (%)[1)]	Inhibition rate (%)
Ck	-	5.3 a	
Mancozeb (80% WP)	400	5.7 a	0
Chlorothalonil (75% WP)	500	0.0 b	100
Narrow range oil (99% oil)	200	2.7 ab	49

1) Data are means of three replicates. Those with the same letter are not significantly different (P = 0.05) according to Fisher's protected least significant difference test

(6:4), in various concentrations. Normal uredospores without fungicide usually germinate within 1-2 hours, and the germ tube extends straight to a distance of about 50 μm and then usually turns its direction and grows into a curved mycelium as in (Fig. 2).

When fungicide is applied to the uredospores of fig rust on the water agar platform, germination is usually inhibited and, in some instances, its modified morphology is observable under a microscope. The inhibition of germination and morphological change in spores outside the platform can also be observed and compared to those within the platform. The results of the germination rates of fig rust spores within and outside the platform are shown in (Table 6).

Chlorothalonil experiment results showed that uredospores were completely and permanently inhibited by this fungicide at dilutions of

Fig. 2. Normal germination morphology of fig rust uredospores on 3% water agar platform after 18 hours incubation at 28°C (scale bar = 20 μm).

Table 6. Germination inhibition rates of fig rust uredospores along dilution streaks after mixing with three fungicides within the water agar platform for 10 minutes and incubated for 24 h

Fungicide	Dilution (X)	Germination rate within the platform (%) [1)]	Germination rate 1-2 cm outside the platform (%) [1)]
Ck	-	53	-
Chlorothalonil (75% WP)	500	0	0
	1,000	0	0
	2,000	0	0
Mancozeb (80% WP)	500	33	32
	2,000	35	37
Bordeaux mixture (6:4)	100	0	0
	500	0	18

[1)] Data are means of three replicates

500X, 1,000X, and 2,000X, on the 3% water agar platform and outside the platform as shown in (Fig. 3). Even after two more days they showed the same results. These results suggest that chlorothalonil (75%WP) at these dilutions exhibits a therapeutic effect against fig uredospores.

Conversely, uredospores treated with Bordeaux mixture (6:4) along dilution streaks have higher germination rates than within the platform. This indicates that the Bordeaux agent shows only an inhibitive effect on the fungus.

Observations of morphological changes on germinated rust spores after fungicide treatment on water agar platform

Fig rust was used for observing morphological modifications of germinated uredospores after treated with fungicides. Three fungicides, including mancozeb (80% WP), chlorothalonil (75% WP), and Bordeaux mixture (6:4), were applied to spores on the water agar platform. The morphology of germ tubes and mycelia within the platform and 1-2 cm away from the platform were observed and compared with each other. The normal morphology of the germ tubes is usually to extend straight for about 50 μm, then turn its direction and grow into a curved mycelium as in Fig. 2.

The mancozeb experiment showed that at the 500X dilution, the shape of the germ tube and its mycelia were prominently different from the blanks. The germ tubes were shortened and had stunted ends, while some mycelia showed a slender shape as in (Fig. 4). However, some of the germ tubes and the mycelia outside the platform area grow normally, as in (Fig. 5), indicating that mancozeb is only an inhibitive fungicide.

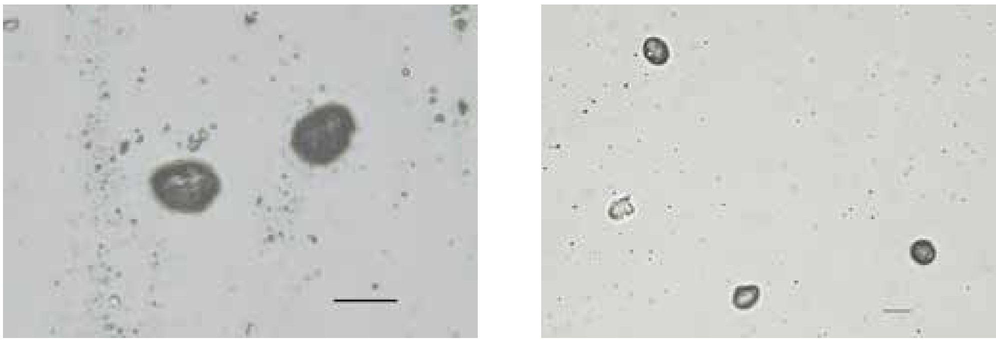

Fig. 3. Complete inhibition of fig rust uredospores 24 hours after mixing with chlorothalonil (75% WP) at 500X dilution on the 3% water agar platform. The two uredospores on the left within the platform were completely inhibited, while the three on the right that were 1-2 cm from the platform were also completely inhibited (scale bar = 20 μm).

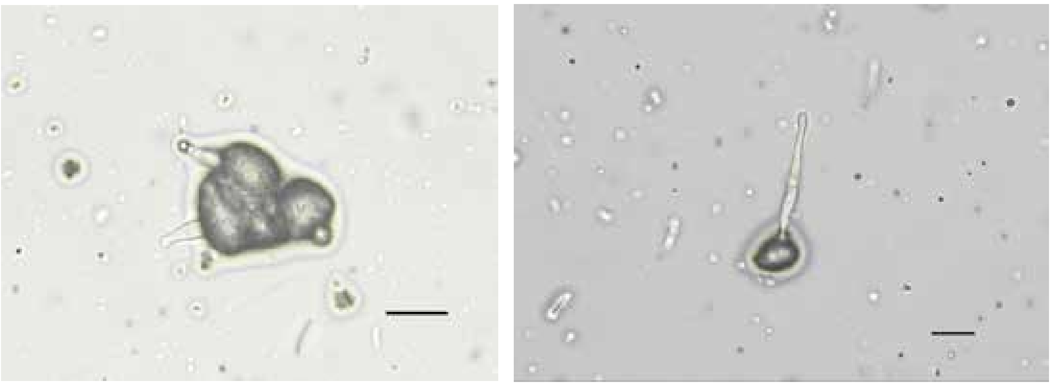

Fig. 4. Morphological modification of fig rust uredospores 24 hours after mixing with mancozeb (80% WP) at 500X dilution on a 3% water agar platform. Germ tubes of the uredospores (left) within the platform show stunted tips after fungicide treatment, while mycelia (right) are slimmer than normal blanks, as in Fig. 2 (scale bar = 20 μm).

Fig. 5. Germination morphology of fig rust uredospores 24 hours after mixing with mancozeb (80% WP) at 500X dilution on a 3% water agar platform. Mycelia from uredospores within the platform are slimmer (left), while the one (right) that was 1.5 cm away from the platform, shows a normal recovery shape (scale bar = 20 μm).

Discussion

In the past, scientists have tried their best to find new drugs or chemicals for combating pathogenic bacteria and fungi that cause various plant diseases, animal or human diseases. It usually takes at least ten years to discover a new chemical or drug to the market. Basically, it needs to go through a series of screening, testing, toxicity assay, environmental and residue safeness assessment, and to undergo the process for a permit or release to be approved by authorities. The first step in a new drug route, however, is a fast and comprehensive screening for antibacterial or antifungal activity from innumerable candidate chemicals. Many bactericidal and fungicidal assay methods have been reported in the past, as reviewed by Balouiri *et al* [1]. in 2016. Popular bioassay methods include disk-diffusion, well diffusion, broth or agar dilution, and poison food techniques. Some other specific methods have also been reviewed by Balouiri *et al* [1]. and other scientists. For instance, Duan *et al.* in 2018 used a microtiter plate method for screening fungicides for controlling grape anthracnose fungi [4]. Also, Chang in our laboratory used some special methods, including the "Y-plate poison food method" and "3-way ring-plate method," for fungicidal screening [2]. These complicated techniques usually require specific equipment or materials. Before adopting a fast screening method, scientists or decision-makers theoretically need an evaluation or comparison of its reproducibility, accuracy, convenience, and cost-availability.

In this study, we have developed a new, easy method for screening the antifungal efficacy of a chemical or drug. This method uses only a few Petri dishes filled with a thin layer of 3% water agar. A rectangular platform combined with five dilution lines (Fig. 1) was designed to enable people to easily assay *in vitro* between test microorganisms and chemicals. The preparation of test microorganisms is not difficult since they can be collected on-site from the diseased plant or tree leaves, fruits, or seeds where they have already grown and produced offspring. The interference by coexistent or contaminated microorganisms is not an important problem because usually the uredospores germinate very fast and the experiment takes only one day.

None of the four rust fungi involved in this study are culturable in artificial media. Because they are obligate parasites, the traditional poison food method is not available for testing the fungicidal activity of a candidate chemical. Usually only field spraying tests can be adopted to evaluate the fungicidal efficacy of a candidate chemical. Our method, therefore, offers an alternative simple approach for screening chemicals which may control rust fungi. Another important group of obligate plant parasite is the powdery mildew pathogen. Theoretically, our method is also suitable for testing the efficacy of fungicides against powdery mildew fungi.

Our study on the germination inhibition efficacy of four fungicides reveals that mancozeb (80% WP) is the best agent to fight against corn rust uredospores. For grape rust, both mancozeb (80%WP) and chlorothalonil (75% WP) show excellent control efficacy. For fig rust, however, both chlorothalonil (75% WP) and Bordeaux mixture (6:4) have the good efficacy. Chlorothalonil (75% WP) also shows the best results for frangipani rust.

This practical information is quite useful for farmers, tree owners, plant doctors, or tree doctors when they want to select a fungicide to reduce the severity of diseases.

In this water agar platform method, the rectangular platform has five connected dilution lines for observing differences in germination rates or fungal morphology between the platform and outside the platform. Results of germination rates of fig rust spores within the platform and outside the platform revealed that chlorothalonil at dilutions of 500X, 1,000X, and 2,000X, is a therapeutic fungicide, as none of the uredospores outside the platform recovered and germinated after being towed away from the platform where the fungicide was added. Conversely, Bordeaux mixture (6:4) is proven to be only an inhibitive fungicide, as within the dilution streak area they recovered and had higher germination rates than within the platform. Therefore, this method can be used to easily elucidate the antifungal or antibacterial mechanisms of a candidate chemical or drug. We will conduct more such interesting studies in the near future.

The morphological modifications of microorganisms by fungicides is also important information for plant protection scientists. In this study, the morphologies of fig rust including the germ tubes and rust mycelia inside the platform and outside of it were compared. Our results showed that mancozeb at a 500X dilution significantly changed the shapes of the germ tube and mycelia relative to normal ones. The other more interesting is that some of the towed spores recovered to grow normal germ tubes and mycelia. This further supports the result that mancozeb is only an inhibitive fungicide for the four rust fungi.

This water agar platform method is especially suitable for plant medicine students or researchers. Since control rate information for commercialized fungicides and bactericides against pathogens had previously not been clear, the selection and recommendation of antifungal or antibacterial agents were often very difficult. Using this method, practical efficacy information can be obtained easily. The evaluation and selection of an optimal fungicide is therefore workable using this method. Because the cost of this method is very low, it is affordable to any new learner or student.

Even the fungicidal resistance of the on-site pathogen may be evaluated by this simple method because the on-site pathogen can be collected and used on the agar platform. When different plant doctors in different counties test the same fungicide and one of them finds a significantly lower inhibition rate, it indicates that the pathogen in the latter case has developed resistance toward this fungicide. However, since this is a preliminary early detection of fungicidal resistance, other detail studies should be conducted in together to express the actual situation and the extent of resistance.

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於水瓊脂平台上塗佈銹病菌孢子進行 4 種殺菌劑之藥效評估

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摘要

孫岩章、李綺璇。2022。於水瓊脂平台上塗佈銹病菌孢子進行 4 種殺菌劑之藥效評估。臺灣農藥科學 13：19-34。

本研究設計一種可以快速評估 4 種殺菌劑對作物或樹木絕對寄生之銹病菌藥效的方法，本方法係使用 9 公分之無菌塑膠培養皿，先倒入 3%的水瓊脂製備成薄層平板，再於底面之一側畫出一個 6 × 1 公分之長方形平台塗佈區，由此平台區依每 1 公分的間隔向另一側共拉出 5 條各長 3 公分的稀釋線。此平台區作為藥劑與真菌孢子的作用場域，當約等量的孢子懸浮液與藥劑在平台區均勻混合約 10 分鐘之後，即以無菌接種環將孢子群自平台區沿著稀釋線拉曳出平台，直到其末端。依此方法已自田間病株採集 4 種銹病菌，包括玉米、葡萄、無花果及緬梔之銹病菌夏孢子，並選取 4 種常用殺菌劑與之在平台上進行藥效的測試。結果發現 4 種藥劑中防治玉米銹病菌最有效的是鋅錳乃浦，而對抗葡萄、無花果及緬梔之銹病菌則以四氯異苯腈最具高效，但波爾多液對無花果銹病菌的防治效果亦極佳。於上述防治率測試之同時，亦觀察平台內與平台外稀釋區各銹病菌孢子發芽率及形態學之差異性，必要時可延長觀察 2 日，結果發現四氯異苯腈可讓無花果銹病菌夏孢子之發芽完全抑制，且平台外的孢子皆無法恢復發芽能力，說明該藥具有「殺菌」之藥效。與此相反的是波爾多液，經拖曳至平台外時，即見增高之發芽率，說明該藥僅屬於「抑菌」藥劑。在夏孢子發芽的發芽管及菌絲顯微形態學之研究上，發現稀釋 500 倍的鋅錳乃浦可使平台內的孢子發芽管變短及呈鈍尖狀，另使菌絲變細、變短。當被拖曳至平台外時，即見有恢復為較正常的發芽形態，也可供說明該藥僅屬於「抑菌」藥劑。利用此一水瓊脂平台法所得的是田間病菌及殺菌劑藥效的實況資料，故可供農民、

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樹木業主、植物醫師、樹木醫師在選擇已登記之防治用藥時，避免用錯藥劑及避開易生抗藥性藥劑的有力參考。由於本方法具有低成本、操作容易之優點，故特別適合植物醫學系所學生及研究人員，可供作為田間殺菌劑處方選擇及藥劑篩選的廣泛應用。

關鍵詞： 水瓊脂平台、殺菌藥效評估、發芽抑制、形態學變化、玉米銹病、葡萄銹病、無花果銹病、緬梔銹病