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Inhibition Efficiency of Extracts from Leaves of Piper betle Linn., Piper Sarmentosum Roxb., and Andrographis Paniculata (Burm.f.) Nees on the Growth of Colletotrichum Gloeosporioides in Nam Dok Mai Mango (Mongifera indica Linn.)

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Abstract

The efficiency of betal leaf, wildbetal leafbush and kariyat extract for funyal inhibition of Colletotrichum gloeosporioides on Nam Dok Mai Mango was studied. The objective was to study the herbs that are suitable for inhibition of Colletotrichum gloeosporioides that cause Anthracnose disease on Nam Dok Mai Mango and the appropriate concentration to extend the shelf life of Nam Dok Mai Mango. Three concentrations of three herbs extracted were used at 10, 15 and 20 ppm. The lowest concentrations were tested for Anthracnose disease inhibition and shelf life of Nam Dok Mai Mango by Dilution Susceptibility Test, using concentration at 10, 15 and 20 ppm. Measure the growth of hyphol on day 7 in PDA. Antifungal efficacy Showed that betal leaf extract was 0.00±0.00 mm. in all 3 concentrations, the wildbetal leafbush and kariyat extract were 3.23±0.25, 0.67±0.29, 0.40±0.17, 6.17±0.29, 6.00±0.50 and 5.00±0.50 mm. respectively. Betal leaf can inhibit furgal 100 percent at all concentration insignificantly. The lowest concentration was 10 ppm. can inhibit fungal growth on Nam Dok Mai Mango 100 and 96 percent compared with Nam Dok Mai Mango without soaked and soaked with distilled water respectively.

Keywords: Betal Leaf, Wildbetal Leafbush, Kariyat, Extraction, Nam Dok Mai Mango.

Introduction

Mango is an economic fruit that is popular for both fresh and processed form. In addition to domestic consumption, it can be exported to other countries as well. The major foreign markets of Thailand include: Malaysia, Singapore, Japan and other countries (Office of Agricultural Economics, B.E. 2565) in the current market competition in mango

exports. The main issues that are important consist of, price and quality of products. One of the main product quality issues which most important is the occurrence of Anthracnose disease after harvest which damages the products, while transporting and distributing the lower quality of products it is resulting to lower prices as well, or may not be able to sell that product at all (Arunee, B.E. 2564). The situation of producing Nam Dok Mai mangoes for to export in B.E. 2562-2565 was to face the problem of severe weather fluctuations which were worse than the previous year, it was also damaged by unseasonable rains which continued for several days caused mango damage by fungi, overall in the production process, post-harvest storage, transportation and distribution which anthracnose caused by fungi colletotrichum gloeosporioides, which causes postharvest rot disease (Angsuma, B.E. 2562), postharvest disease control of mangoes is commonly used by benzimidazole chemicals, such as, benomyl which used to dip the fruit before loading into the container, chemicals can infiltrate into the products causing unsafe conditions for consumers and workers (Krongchit, B E. 2563), but currently it was reported that benzimidazole chemicals have decreased the effectiveness of the disease control, both in Thailand and in the state of Florida, USA. Because there are fungal strains that are resistant to antifungals, including Colletotrichum Gloeosporioides and Lasiodiplodia Theobromae (Spalding, 2020), and in the United States, the use of benomyl has been banned for all post-harvest produce (Jones, 1988). And many countries are aware of the problem of chemical residues in agricultural products that affects the health of consumers, changes in ecology, environment and microbial resistance. Nowadays, there are people who are interested in new alternative ways to control pathogenic fungi by taking advantage of natural products such as plant extracts to replace the use of chemicals (Wanasanan, B.E. 2561). From the research of Wanlapa and Dara (B.E. 2560) studied crude extracts from betal Leaves, wildbetal leafbush and buffalo leaves, it was found that extracts from betal leaves and wildbetal leafbush can inhibit mold Colletotrichum gloeosporioides. And the research of Siriwan Smithaporn (B.E. 2563) studied the extraction from kariyat and moringa leaves, it was found that kariyat extract can inhibit the fungi that cause anthracnose in chili peppers. Therefore, if there is a study of the utilization of medicinal plants such as betal leaf, wildbetal leafbush and kariyat for use in inhibiting fungi Colletotrichum gloeosporioides that grow in Nam Dok Mai mangoes inevitably, then it can further adds the benefits, value and can help reduce the use of chemicals, also help with extend the shelf life of Nam Dok Mai mangoes in another way too.

Research Objective

1. To study suitable herbs to inhibit fungi Colletotrichum gloeosporioides that caused Anthracnose disease in Nam Dok Mai mango.

2. To study the appropriate amount of extract to extend shelf life of Nam Dok Mai mangoes.

Scope of the Study

Study on the extraction of extracts from 3 types of herbs, namely; betal leaf, wildbetal leafbush and kariyat for use in inhibiting fungi Colletotrichum gloeosporioides that caused Anthracnose disease in Nam Dok Mai mango, to extend the longer shelf life of Nam Dok Mai mangoes.

Beneficial Expected

1. Know the right herbs to inhibit Colletotrichum gloeosporioides fungi.

2. To reduce the use of chemicals in the process of extending the shelf life of Nam Dok Mai mangoes.

3. Know the right amount to extend the shelf life of Nam Dok Mai mangoes.

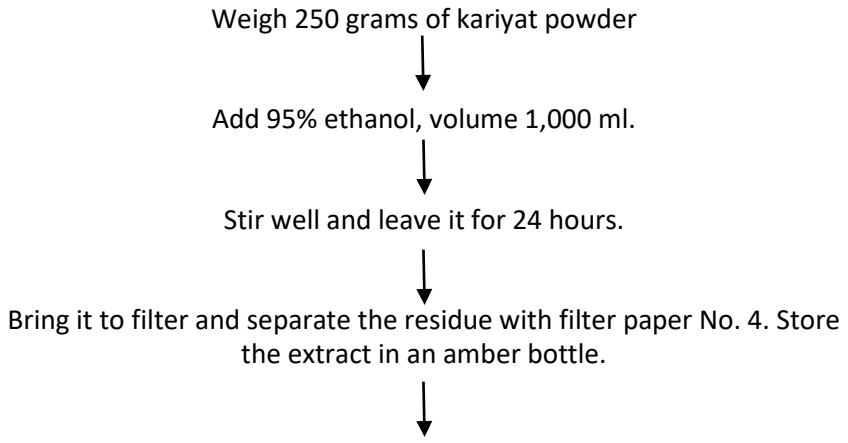
4. To be guidelines to use more beneficial from medicinal plants.

Research Method

Preparation of herbal extracts is to select herbs and bake them at 50 °C for 10 hours, till the herbs finely spun, weighed for 250 grams of herbal powder per 1,000 ml. of 95% ethanol, leaving it to soak at room temperature for 24 hours. After maturity, filter the residue with No. 4 filter paper, repeat it 2 times, and evaporate the filtered portion with a Rotary Evaporator at room temperature, store the extracted in a sealed amber bottle at 4 °C for further analysis, record the extracted weight to calculate and find out the percentage of it (Wichai, B.E. 2561).

Percentage of Extract = Extract weight / Plant sample weight X 100

Kariyat Extract Preparation



Bring kariyat residue to add 95% ethanol, volume 500 ml. Stir well and leave it for 24 hours.

↓

Bring it to filter and separate the residue with filter paper No. 4. Store the extract in an amber bottle.

↓

Separate the result extracted by a vacuum evaporator at 35 °C until no solvent is evaporated.

↓

Stored the extracted in a sealed amber bottle at 4 °C for further analysis.

↓

Bring the kariyat residue to dry, then weighed to calculate the extracted percentage.

Diagram 1. Process for preparation of kariyat extract. Source: Adapted from Wichai (B.E. 2561)

Preparation of betal leaf extract

Select betal leaves to clean then cut into smaller sizes

↓

Bake in a hot air oven at 60°C for 6 hours.

↓

Take the dried betal leaves and blend them thoroughly into powder.

↓

Weigh 250 grams of betal leaves powder.

↓

Add 95% ethanol, volume 1,000 ml.

↓

Stir well and leave it for 24 hours.

↓

Bring it to filter and separate the residue with filter paper No. 4. Store the extracted in an amber bottle.

↓

Bring betal leaves residue to add 95% ethanol, volume 500 ml. Stir well and leave it for 24 hours.

↓

Bring it to filter and separate the residue with filter paper No. 4. Store the extracted in an amber bottle.

↓

Separation of extract obtained by vacuum evaporation distiller at a temperature of 35 °C until no solvent evaporates.

↓

Stored the extracted in a sealed amber bottle at 4 °C for further analysis.

↓

Take the residue of betal leaves to dry, then weigh them to calculate the extracted percentage.

Diagram 2. Process of preparation of betal leaf extract. Source: Adapted from Wichai (B.E.2561)

Preparation of Wildbetal Leafbush extract

Collect the right wildbetal leafbush, clean and cut into small pieces.

↓

Bake in a hot air oven at 60 °C for 6 hours.

↓

Take the dried wildbetal leafbush and blend it thoroughly into a powder form.

↓

Weigh 250 grams of wildbetal leafbush powder.

↓

Add 95% ethanol, volume 1,000 ml.

↓

Stir well and leave it for 24 hours.

↓

Bring it to filter and separate the residue with filter paper No. 4, store the extracted in an amber bottle.

↓

Take wildbetal leafbush residue and add 95% ethanol, volume 500 ml. Stir well and leave it for 24 hours.

↓

Bring it to filter and separate the residue with filter paper No. 4, store the extracted in an amber bottle.

↓

Separate the extracted by a vacuum evaporator at 35 °C until no solvent is evaporated.

↓

Store the extracted in a sealed amber bottle at 4 °C for further analysis.

↓

Bring the wildbetal leafbush residue to dry and weigh it to calculate the extracted percentage.

Diagram 3. Process for preparing extracts from wildbetal leafbush.

Source: Adapted from Wichai (B.E. 2561)

Preparation Colletotrichum gloeosporioides fungus

C. gloeosporioides fungi is an anthracnose fungal strain of Nam Dok Mai mango.

↓

Bring the C. gloeosporioides fungus in order to cultivate the seedlings.

↓

By using a loop to heat to sterilize and cut the agar surface of the fungi by measuring 3 mm. in diameter

↓

Place the agar pieces in the middle of a petri dish.

↓

Incubated at 30 °C for 7 days.

↓

Observe and record the results.

Diagram 4. Preparation process of C. gloeosporioides fungus.

Source: Adapted from Tharathip (B.E. 2564)

Preparation of herbal extracts at various concentrations

Adjusted the herbs extracted to reduce the concentration level, distilled water was diluted in every 2 times of these extraction concentrations at 30, 20 and 10 ppm. distilled water was used as a control sample by adjusting the extract concentration volume as in Table 1.

Table 1. Adjustment of the volume concentration of herbal extracts.

Concentration Desired (ppm)	Volume Desired (ml)	Concentration used (ppm)	Concentration Volume used (ml)	Adjust the volume with the solution (ml)
10	50	100	5	
15	50	100	7.5	Distilled Water
20	50	100	10	

Efficiency test against C. gloeosporioides fungi.

Dilution Susceptibility Test Method. Mixing of herbal extracts in culture medium, by weighing 19.50XX ready-made PDA into a 50 ml. beaker, using a 500 ml. Graduated Cylinder to measure 500 ml. of distilled water, pour into a 1000 ml. Duran flask, stir well, and take it to autoclave at 121 °C at 15 minutes, then put in 3 test tubes for 9 ml. each, left until the temperature drops to 45-50 °C, aspirates 1 ml. solution of each concentration (Table 1.), mix it into the prepared PDA medium by using a Vortex Mixture Machine, poured into petri dishes, repeat 3 times at each experimental concentration, 10 ml. of the herbal extract medium was obtained. As for the culture used as a comparison, 10 ml. of PDA medium was added after the medium was poured and left it for 1 day to allow the surface of the medium to dry, then transfer the tested fungi into it.

Determination of the lowest concentration of herbal extracts that can inhibit Colletotrichum gloeosporioides fungi, and take the fungi that cultured on PDA medium in a petri dish, cultivation it at 30 °C for 5 to 7 days, then use a loop with a diameter of 3 mm. to sterilize, and allow it to cool down, and puncture mycelium at the colony site, to make new fibers that are still growing then transfer pieces of agar onto a PDA medium that contains herbal extracts at concentrations of 10,15 and 20 ppm. Place the agar slices on the center of the petri dish, by turning the filamentous side of the fungus to touch the surface of the food and incubate them at 30 °C for 7 days.

By planning a completely randomized experiment (Complete Randomized Design, CRD), record the experimental results by mycelium growth, fungi that are growing on the surface of medium mixed with herbal extracts at various concentrations, by measuring the diameter of colonies that grow in the medium, compare fungal growth in the control set, stop recording the result, then used the obtained value to calculate the growth inhibition percentage from the following formula (Thanthip, B.E. 2565):

Growth Inhibition Percentage = [(A-B)/A] x100

A = Mean diameters of colonies on comparative agar plates.

B = Mean diameter of colonies germ on plates containing extract.

Testing on Nam Dok Mai mangoes by infusion method.

Take the second stage of Nam Dok Mai mangoes which have slightly yellowish green, but more on green skin color, for 1 of each, bring the lowest concentration of extract obtained based on the analysis results, soaking the whole fruits at one concentration level for 2 minutes, all concentrations must be controlled and then left at room temperature, record the results of the shoot and disease incidence by counting the number of disease incidence and daily changes can compare those from the controls, then use the obtained values to calculate the percentage inhibition of the disease, using the following formula (Chalermchon, B.E. 2565):

Growth Inhibition Percentage = [(A-B)/A] x100

A = Mean disease incidence of controls.

B = Mean incidence of herbal extracts.

Choose a Nam Dok Mai mango which the peel starts to change color from green to yellow slightly.

↓

Bring 1 Nam Dok Mai mango into the process.

↓

Dip the whole Nam Dok Mai Mango fruit into a concentration for 2 minutes.

↓

Then leave it at room temperature.

↓

Record the disease outcome by counting the number of disease spots and daily changes.

Diagram 5. Testing on Nam Dok Mai mangoes by infusion method.

Results

Result of herbal extract preparation

From the extraction of all 3 types of herbs which are betal leaves, wildbetal leafbush and kariyat with Absolute Ethanol solution at a ratio of 1: 2 (250 grams of herb powder per 1,000 ml. of Absolute Ethanol) at room temperature and immersed in absolute ethanol for 24 hours. After maturity, filter the residue with No. 4 filter paper, repeat it 2 times, and then evaporate the filtered portion with a Rotary Evaporator, resulting in a dark green viscous liquid.

Table 2. Percentage of extracts after evaporation of all 3 herbs

Herbal type	Amount of extract after evaporation (ml)	Extract percentage (%)
Betal Leaves	32	12.8
Wild BetelLeafbush	22.5	9
Kariyat	20	8

Test results of C. gloeosporioides antifungal efficacy.

In a petri dish with only fungal cultures, fungal growth was found to spread all over the agar plate. The fungus will grow from the middle of the plate to the surrounding area as shown in Figure 1.

Figure 1. C. gloeosporioides fungi were grown on PDA medium for 7 days at 30 °C. By bringing extracts from 3 types of herbs, namely; betal leaves, wildbetal leafbush, and kariyat. Extracted with Absolute Ethanol by Dilution Susceptibility Test Method, C. gloeosporioides growth was observed for 7 days.

The experiment was performed by measuring the growth of mycelium, C. gloeosporioides grown on PDA medium mixed with herbal extracts, the diameter of colonies growing horizontally was measured compared to those in the control medium (Table 1 and Figure 1).

Table 3. Comparison of the growth of C. gloeosporioides on PDA medium containing herbal extracts at various concentrations.

Herbal Type	Mean colony diameter (millimeters)[1]		
	10 ppm	15 ppm	20 ppm
Betal Leaves	0.00±0.00	0.00±0.00	0.00±0.00
Wild Betel Leafbush	3.23±0.25^{a}	0.67±0.29^{b}	0.40±0.17^{bc}
Kariyat	6.17±0.29^{a}	6.00±0.50^{a}	5.00±0.50^{b}

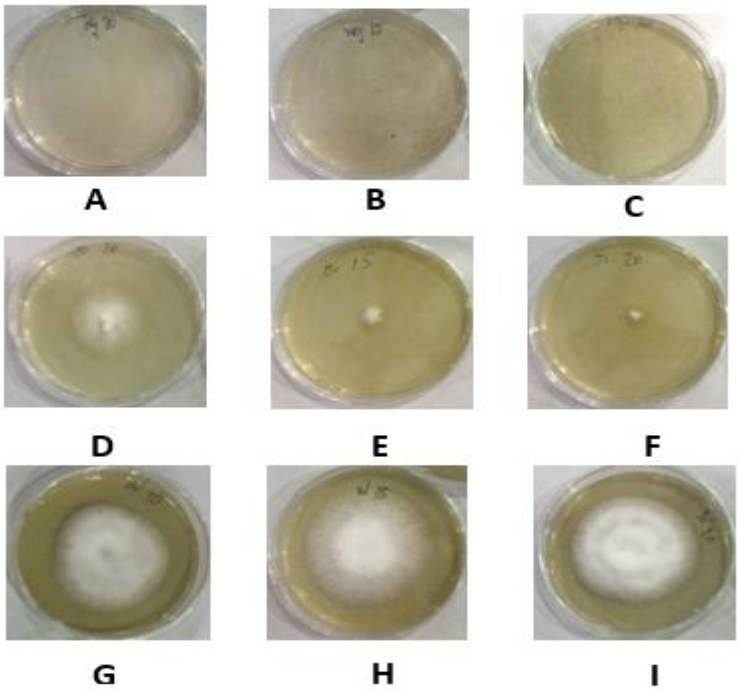
Note: 1= mean from 3 replicates of the experimental sample, different horizontal letters mean values that are statistically different ($p \leq 0.05$)

From Table 3. Shows the herb species and the lowest concentration that can inhibit the growth of C. gloeosporioides by experimenting on the PDA medium.

The efficacy of 3 herbal extracts which are betal leaves, wildbetal leafbush and kariyat, were tested at concentrations of 10, 15 and 20 ppm. in inhibiting the growth of C. gloeosporioides mycelium.

Causes of anthracnose in Nam Dok Mai mango comparison with the controller, it was found that betal leaves extracts at the concentrations of 10, 15 and 20 ppm. were not significantly different at level 0.05 ($p > 0.05$). However, the 10 ppm. extracted from wildbetal leafbush was different from the betal leaves extracted at the concentration of 15 and 20 ppm. at a statistical significance of 0.05 ($p \leq 0.05$) and kariyat extracted at concentrations of 10 and 15 ppm. were different from those of kariyat at concentrations of 20 ppm. with a statistical significance of 0.05 ($p \leq 0.05$). The betal leaves is extracted at concentrations of 10, 15 and 20 ppm. inhibited the growth of C. gloeosporioides, most of them was no different which is 100% followed by wildbetal leafbush extracted at concentrations of 10, 15 and 20 ppm. is 95, 92.5 and 60% respectively, and kariyat extracted at concentrations of 10, 15 and 20 ppm. were 37.5, 25 and 22.5% respectively. Therefore, betal leaves extract was used at a concentration of 10 ppm. to test Nam Dok Mai mango.

Figure 2. Growth of C. gloeosporioides on PDA medium mixed with herbal extracts for 7 days.



A: Mix betal leaves extract 10 ppm.

F: Mix wildbetal leafbush extract 20 ppm.

B: Mix betal leaves extract 15 ppm.

G: Mix kariyat extract 10 ppm.

C: mix betal leaves extract 20 ppm.

H: Mix kariyat extract 15 ppm.

D: Mix wildbetel leafbush extract 10 ppm.

I: Mix kariyat extract 20 ppm.

E: Mix wildbetel leafbush extract 15 ppm.

Test results on Nam Dok Mai mango fruit by infusion method (10 ppm).

Table 4. Efficacy test of Nam Dok Mai mango extract at a concentration of 10 ppm on Nam Dok Mai mango fruit for 7 days

Day	Original Nam Dok Mai Mango	Soaked in Distilled Water	Soaked the extracted
0			
1			
2			
3			

4			
5			
6			

Table 4. shows the changes of Nam Dok Mai mango fruit, the efficacy of betal leaves extract was tested at a concentration of 10 ppm. in inhibiting the growth of C. gloeosporioides on the Nam Dok Mai mango fruit.

Scientists have made a promising discovery in the effort to preserve Nam Dok Mai mango fruit. In order to inhibit the growth of C. gloeosporioides on the fruit, the authors of this study tested the efficacy of betal leaf extract at a concentration of 10 ppm. This experiment shows a significant reduction in the growth of C. gloeosporioides, indicating that betal leaf extract could be a viable solution to preventing the spoilage of Nam Dok Mai mango fruit. Mango fruit is susceptible to pathogens such as fungi, yeasts, and bacteria that can cause premature spoilage. However, with the use of betal leaf extract, this problem could be eliminated. As determined in this experiment, the extract contains compounds that could combat the fungi responsible for the spoilage of Nam Dok Mai mango fruit. This finding could lead to a decrease in waste and an increase in the longevity of the fruit, thereby benefiting both the consumers and producers of this delicious crop. In conclusion, the experiment has provided a promising result in the fight against spoilage of Nam Dok Mai mango fruit. The discovery of the efficacy of betal leaf extract at a concentration of 10 ppm in inhibiting the growth of C. gloeosporioides could lead to the development of effective preservation methods and increase the shelf life of Nam Dok Mai mango fruit.

Comparison with Nam Dok Mai mangoes that were not soaked in distilled water and immersed in distilled water and record the result as an image and found that, Betal leaves extract at a concentration of 10 ppm. was effective against C. gloeosporioides that causes anthracnose

disease. In addition, at 0.05 ($p > 0.05$) level, when comparing between soaked Nam Dok Mai mango fruit in herbal extracted and Nam Dok Mai mango fruit which is not soaked, found that 100% of those soaked fruits inhibited the growth of C. gloeosporioides and compare soaked Nam Dok Mai mango fruit in herbal extracted with Nam Dok Mai mango fruit in distilled water, the results was inhibited the growth of C. gloeosporioides by 96%.

Discussion and Conclusion

From the extraction of substances from 3 types of herbs, namely; betal leaves, wildbetal leafbush and kariyat with Absolute Ethanol for 24 hours, by repeating it 2 times, betal leaves, wildbetal leafbush and kariyat could be extracted at 32 ml. 22.5 ml. and 20 ml. representing 12.8, 9 and 8 percent extraction percentages, respectively and adjusted the concentration of herbal extracts to 10, 15 and 20 ppm. bring to test the antifungal efficacy of C. gloeosporioides with Dilution Susceptibility Test found that, from the extraction of 3 types of herbs which are betal leaves, wildbetal leafbush and kariyat and bandit with Absolute Ethanol for 24 hours, by repeated 2 times, extracts from betal leaves, wildbetal leafbush and kariyat, could be extracted at 32, 22.5 and 20 ml, respectively, representing the extract percentages of 12.8, 9 and 8 percent respectively. The concentrations of herbal extracts were adjusted to 10, 15 and 20 ppm. Then used to test the antifungal efficacy of C. gloeosporioides by Dilution Susceptibility Test, found that betal leaves extracts at concentrations of 10, 15 and 20 ppm. were able to inhibit C. gloeosporioides 100% at all 3 levels. Because betal leaves contain substances hydrocinnamic acid and ß-sitosterol, and 1-allyl-2, 6-dimethoxy-3, 4-methylenedioxy benzene that are antimicrobial. It is consistent with the research of Raporn and Ruankaew (B.E. 2565). The presence of other microorganisms in the soil or on the mango plants could introduce bias into the results. So, we ensured that the mango plants used in the study are free from any other contaminants that could impact the growth of Colletotrichum gloeosporioides. By carefully considering these potential sources of error, researchers can ensure that the results are as accurate and reliable as possible.

By comparing the betal leaves extract with E. coli and S. aureus qualitative susceptibility tests. It was found that betal leaves extract effectively inhibited the growth of microorganisms with the diameter of the inhibitory activity was 10.00 mm. MIC which is 31.25 mg/mL. but the extract from wildbetal leafbush at concentrations of 10, 15 and 20 ppm, 60, 92.5 and 95 percent of the fungi were inhibited, respectively, which was consistent with the study of Subashkumar et al. (2020). The lowest concentration of wildbetal leafbush extract was studied to inhibit contaminated microorganisms at a concentration of 0.5% found to be effective in inhibiting the growth of many microorganisms but could not

inhibit the growth of microorganisms capable of producing endospore, therefore, the effect of betal leaves extract could not fully inhibit the growth of microorganisms and kariyat extract at concentrations of 10, 15 and 20 ppm. could inhibit fungi by 22.5, 25 and 37.5 percent, respectively. Because kariyat extract contains important substances of andrographolide and dehydroandrographolide which has the ability to inhibit the growth of bacteria but is very low efficacy effective in inhibiting the growth of fungi which is consistent with Krongjit studied (B.E. 2563) which has studied the resistance characteristics of fungi, found that kariyat extract was able to inhibit certain types of fungi and was only 31.52% effective. It was found that the betal leaves extract was able to inhibit the growth of C. gloeosporioides for the most of all and at all concentrations, 10, 15 and 20 ppm. showed no significant difference in fungi inhibition efficiency at the 0.05 level ($p > 0.05$). Therefore, betal leaves extract was selected at the lowest concentration of 10 ppm. to test with Nam Dok Mai mango which can inhibit the growth of C. gloeosporioides 100% and 96%, when compared to unsoaked Nam Dok Mai mangoes and Nam Dok Mai mango soaked with distilled water, respectively and extracts from kariyat at the concentration of 20 ppm, the maximum inhibition of fungi was 95 and 37.5%, when compared to unsoaked Nam Dok Mai mangoes and Nam Dok Mai mango soaked with distilled water, respectively.

While the study results were promising, more research is necessary to understand the scope of these plants' antifungal properties fully. Future research could include investigating the effects of variations in extraction methods and plant growth conditions on the efficacy of these extracts. Additionally, researchers could explore using these extracts as preventative measures against fungal growth during cultivation. Further studies could also investigate the potential for these extracts to inhibit the growth of other fungal pathogens, expanding their potential as natural fungicides. Through continued research on the antifungal properties of plant extracts, such as those from Piper betle Linn., Piper Sarmentosum Roxb., and Andrographis Paniculata (Burm.f.) The agricultural industry may have access to adequate, eco-friendly alternatives to traditional fungicides.

The research found that all three plant extracts showed inhibition activity against Colletotrichum gloeosporioides. However, the article also highlights some limitations of the study. For example, while the extracts effectively inhibited fungal growth in vitro, their effectiveness in real-world settings must be clarified. Additionally, the article mentions that more research is needed to fully understand the mechanisms by which these plant extracts inhibit the growth of the fungus and to determine the optimal dosage and application methods for practical use in mango orchards. Overall, the article provides valuable insights into the potential use of plant extracts as natural alternatives to chemical fungicides in protecting crops from fungal pathogens. While there are still limitations

and areas for further research, the study offers promising results and new avenues for sustainable agriculture practices.

Suggestion

From this experimental study, it was found that extracts from all 3 types of herbs, namely; betal leaves, wildbetal leafbush and kariyat were interesting substances to be studied and developed, because extracts from these herbs can inhibit C. gloeosporioides fungi. And there are many other properties that can be used as food additives to extend the shelf life of various food products.

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