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Investigating the Fungicidal Property of Senna Alata and Azadirachta Indica Against Phytopthora Palmivora in Cacao Plant

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ABSTRACT

The study aimed to determine the fungicidal ability of Azadirachta Indica and Senna Alata against Phytophthora palmivora found in cacao fruit, specifically the number of inhibition zone across four plant extract concentrations. True-experimental design was used to gather relevant data. The experiments, using the Kirby-Bauer disc diffusion bio-assay, were conducted at the Research Laboratory of Davao Medical School Foundation Inc., Bajada, Davao City. Mean and non-parametric Kruskal-Wallis tests were employed to analyze the data, revealing a higher inhibition zone of Phytophthora palmivora when treated with Azadiractha indica compared to Senna alata and the difference was significant. These findings unequivocally establish the antifungal potential of Neem tree against Phytophthora palmivora in Cacao plant. To further enhance the reliability of these results, it is recommended to expand the scope of trials and replicates, performing phytochemical analysis, and exploring additional indigenous plant species with potential antifungal properties.

Keywords: *Phytophthora palmivora, Azadirachta Indica, Senna Alata, antifungal property*

Introduction

Cacao is considered a very important commodity today because it offers a good source of income for local smallholders in Davao City. That is why, it is important to generate income that helps our economy. However, some farmers are still suffering from the different kinds of fungi that can cause harm to cacao, including the *Phytophthora palmivora*. It is caused by fungi and infects many parts of the cacao plant including the pods causing black pod rot disease. However, it is considered an economic problem because it has a significant impact on cacao production. It can be managed by using chemical control, phytosanitary culture, genetics resistance, etc. but remains inevitable. Solpot and Cumagun (2020) affirmed that cacao is a good source of income for smallholder farmers, yet the cacao black pod disease can cause low yields in crops, which resulting in bankruptcy.

In Ghana, it is known that primary infections usually occur around June, but the peak of *P. Palmivora* black pod disease generally occurs between August and October. Such information on periods for attaining disease infection peaks is useful in forecasting the pattern of disease development and it is an important tool for disease management since conditions immediately preceding the infection peaks must be favorable for disease development (Briggs, 2021). This is supported by the study of Adeniyi (2019) claims that the black pod disease situation in Nigeria is similar to that of Ghana and depends on growing ecologies, patterns of rainfall, high humidity, and farmers' management practices. The disease inoculum can remain in the soil for a long time, the spores are brought back to viability at the onset of rain and other conditions are suitable. *Phytophthora palmivora* which causes pod losses of less than 30% in the year of 1985 it was only known causal agent of black pod disease of cocoa in Ghana. in 2012, the country loss over 25% of its annual output of 850, 00 MT of cocoa beans to black pod disease outbreak.

Kolpin the Philippines that could provide a potential source of income among large and small-scale farmers (Perez-Sierra et al., 2018). However, production constraints due to diseases are one of the major challenges facing the industry nowadays. The major disease of cacao in the country is cacao pod rot caused by *Phytophthora palmivora* (Solpot et al., 2020). These diseases accounted higher 30% of the crop which ultimately caused a consistent of the soil and are used in biotechnology to achieve sustainability in agriculture. (Fasusi et al., 2021). Solpot and Camagun (2022) even reported pod damage on cacao along the three-monthly monitoring periods increased in Sorsogon from 70.83% to 87.50%, decreased in Albay with 93.75% to 48.75%, and decreased in Camarines Sur from 87.92% to 85.42%. Zero pod damage in Camarines Norte for 3 months. Continuous monitoring of the cacao mirid bug infestation and cacao pod borer occurrence is recommended as the basis for timely control.

Most cacao growing areas are located in the Mindanao Island. The Davao Region contributes 78% of the national cacao production having 7,257.85 metric tons (MT) planted on 19,975 hectares of the island. (Carjaval, 2023). Production of cacao in Calinan, Davao City, the majority of the farmers were on a small scale and most of them adopted good agricultural comical practices (Lingatong, 2018). However, the level of productivity by most of the cacao

farmers was low because of the *Phytophthora palmivora* which contributed largely to the production loss of plantation crops of the cacao. *Phytopthora*-infected cacao occured in major producing provinces in South-Central Mindanao namely North Cotabato, South Cotabato, Davao Province, Bukidnon, and Sarangani. Cacao black pod, particularly is an economically serious problem in all areas in some areas of Mindanao, causing significant pod losses of up to 30% and killing up to 10% of *Theobroma cacao (*Solpot & Camagun, 2021).

Walker (2020) and Puig et al. (2021) explored the molecular exchange between the oomycete and the plant host and, the role of plant immunity during the development of such structures, to understand the infection of cocoa pods by *Phytophthora Palmivora*, and provides valuable preliminary information on fungicide resistance and temperature response that can be used to improve disease management. The findings of Geroche (2024) emphasized the potential of *Trichoderma harzianum* as a sustainable and effective solution for combating cacao black pod rot and promoting sustainable agriculture. Paguntalan et al. (2022) arbuscular mycorrhizal fungi (AMF) are recently studied for their increasing role in disease resistance in many crops, including cacao. The results showed that there was an interaction between treatment and cacao variety on disease incidence. Moreover, Carvajal (2023) evaluates the efficacy of clay particles as a coating agent of cacao pods and carrier of entomopathogens for the control of cacao mirid big (CMB). Among recent research, no research has explored and investigated the potential of *Senna Alata* and *Azadirachta Indica* as fungicides of *Phytophthora Palmivora*. With this, the researchers want to investigate the capacity of *Azadirachta Indica* and *Senna Alata* extracts against *Phytophthora* cacao fruit.

Statement of the Problem

The study aimed to determine the fungicidal ability of *Azadirachta Indica* and *Senna Alata* against Phytophthora fungi found in cacao fruit.. Specially, it sought to answer the following questions;

1. What is the number of inhibition zone of Phytophthora palmivora when treated by Senna alata in terms of concentration level;
    1.1 100% concentration extract;
    1.2 75% concentration extract;
    1.3 50% concentration extract; and
    1.4 25% concentration
2. What is the number of inhibition zone of Phytophthora palmivora when treated by Azadirachta Indica in terms of concentration level;
    2.1 100% concentration extract;
    2.2 75% concentration extract;
    2.3 50% concentration extract; and
    2.4 25% concentration
3. Is there a significant difference in the inhibition zone of Phytophthora palmivora when analyzed according to the following treatments;
    3.1 Senna Alata extract;
    3.2 Azadirachta Indica extract;
    3.3 Positive control (Mancozeb);
    3.4 Negative control (distilled water)

Research Hypothesis

Ho_1: There is no significant difference in the inhibition zone of Phytophthora palmivora when analyzed according to the different treatments.

METHODS

This section presents the study method, including the research design, plant extract preparation, fungi culture preparation, dilution method, aseptic technique, bioassay experimentation, waste disposal, and data analysis.

Research Design

The study used the experimental quantitative method of research to gather relevant data and information. According to Stefan et. al. (2015), in order to efficiently collect, handle, and analyze data for research on modern biology as well as to comprehend and anticipate the behavior of complex systems, computational and quantitative methods are becoming increasingly important. In addition, all study plans make an effort to reduce any risks to the validity of any scientific findings. In order to provide the best results, an effective research program will ideally combine mixed methods of inquiry with experimental designs. In addition, Santini et al. (2018) stated that all study plans make an effort to reduce any risks to the validity of any scientific findings. In order to provide the best results, an effective research program will ideally combine mixed methods of inquiry with experimental designs.

Specifically, this study utilized the true experimental design to describe the number of the inhibited colony of *Phytopthora Palmivora* when it was treated with Neem Tree (*Azadiractha indica*), Akapulko (*Senna Alata*) plant extracts and Mancozeb (positive control) to determine their constituents' efficacy. Before any research is conducted, Brasier (2022) insinuated that a suitable experimental design must be established. Therefore, whenever it is feasible to do so, a true experimental design should be adopted. Particularly in the area of biology research that deals with bacteria, the true experimental design is desirable since it gives a measure of the variation among samples. This research considered true experimental research because involves manipulation of variables the treatments and comparison between the experimental group (neem extract & Acapulco extract) and the control group (Mancozeb as a positive control and distilled water as a negative control) to assess the effectiveness against *Phytophthora palmivora.*

Phase I - Plant Extract Preparation (*Azadiractha indica* and *Senna Alata*)

Collection, Authentication, and Extraction of Plant Materials

Both *Senna Alata* and *Azadiractha indica* were collected on August 22, 2024. *Senna Alata* was collected at Cabantian Forestal Rd, Buhangin, Davao City, Davao del Sur. In addition, Azadiractha indica was collected at Jose P. Rizal Elementary School Brgy.22-C Suazo St, Poblacion District, Davao City, Davao del Sur. The collected plant samples were sent to Community Environment and Natural Resources Office (CENRO) for verification. The plant samples were brought back to Carlos P. Garcia Senior High School and was washed thoroughly. The researchers will follow the air-drying method, it is a process of removing moisture from fresh material to reduce its water activity, which inhibits microbial growth and minimize deteriorative biochemical reactions (Buchaillot et al., 2009) wherein *Senna Alata* is a small tree while *Azadiractha indica* is a tree, thus the drying method is important to avoid any decay of the plant prior to extraction. Therefore, a method that is applied which is sun-drying. Then, dried plant samples were cut into smaller pieces for grinding. Once ground, the researchers used the maceration method. 450g of *Senna alata* was washed with water and was dried for 3 days. The plant was exhausted with 10 times its weight of water. The pulverized leaves *of Senna Alata* (250 g) were macerated with 70% ethanol (1.5L). The neem tree leaves was wash by water to remove impurities. During the drying process, the leaves were placed in the net and left it under sunlight for three days (Chepsergon, 2020). Then, the leaves were cut into smaller pieces using blender, and during the maceration process, 70% ethanol was added resulting in extra efficient extraction. Ethanol is the best solvent in obtaining 75% of the yield of disinfectant (Hofer, 2021). After soaking the Akapulko leaves with 70% ethanol for 2 days, the researchers proceeded to extracting the plant. After that, the researchers had finally extracted the Akapulko leaves and strained for about 3 times. The extract was transferred to the amber glass to prevent contamination and stored in the refrigerator with a temperature of -20^{o}C and sealed with aluminum foil. Moreover, neem tree leaves were soaked with 70% ethanol in 3 days. It was then extracted and strained for 3 times and transferred to the amber glass, sealed with aluminum foil, and stored in the refrigerator with a temperature of -20^{o}C. Lastly, the extracts underwent Rotary Evaporation, the process of reducing the volume of a solvent by distributing it as a thin film across the interior of a vessel at elevated temperature and reduced pressure. This promoted the rapid removal of excess solvent from less volatile samples.

Phase II - Fungi Culture Preparation

Preparation of the Medium before fungi culture.

Potato Dextrose Agar (PDA) prepared from potatoes. PDA is composed of diced potato (50 g), dextrose (5 g), and agar (5 g) in a 250 ml mixture. The procedure in making the PDA were as follows: 50 grams of diced potato was placed in the Erlenmeyer flask and boiled for 30 minutes until it became soft. After cooling down the mixture, it was filtered to obtain the extract using cheesecloth, then 5 g of dextrose powder and 5 g of agar were mixed well. After doing this, it was sealed with aluminum foil. In preparing for autoclave, the water was poured into the autoclave until it reached the platform level; then, the mixture was placed into the machine, and the temperature was set at 121^{o}C for 15 minutes. Then, the mixture was placed into the petri dish for approximately 5 minutes until it cooled down and solidifies (Janiszewska, 2021).

Phase III – Dilution Method

The Neem tree (*Azadirachta indica*) and Akapulko (*Senna alata*) plant extract was divided and diluted into four different concentrations: 100%, 75%, 50%, and 25%. The distribution represented the percentage of the pure extract and the excess percentage was the distilled water. Dilution method was used to lower the concentration of the analyte being tested and it could also eliminate interferences from other substances that might present in the sample that can artificially alter the analysis.

Phase IV - Bio Assay Experimentation on the Efficacy of the Tested Plant Extract against the Tested Fungi

Bio-Assay Test.

The disk diffusion method is among the most flexible susceptibility testing methods in terms of antimicrobial agents that can be tested. The method consists of placing paper disks saturated with antimicrobial agents on a lawn of bacteria seeded on the surface of an agar medium, incubating the plate overnight, and measuring the presence or absence of a zone of inhibition around the disks (Wu, 2018). This experimentation conducted at Davao Medical School Foundation Inc. (DMSFI). Bioassay-guided fractionation maybe defined as a technique for profiling and screening of plant extracts for bioactive compounds with potential sources of new bio-based drugs. The fractionation procedure the screening and purification of natural products in the plant extracts more efficiently.

The medium used for disc diffusion was Mueller-Hinton agar (MHA). Mueller-Hinton agar is a type of growth medium that is used in microbiology to culture bacterial isolates and test their susceptibility to antibiotics. The MHA was prepared in the Davao Medical School Foundation Inc. (DMSFI), the powder ratio is 19g for 500ml, the powder was mixed in the flask using magnetic stirrer. After 30 mins, the powder was completely dissolved and was set aside to cool down. After cooling down, the agar was poured in the petri dishes and waited until it solidifies.

Phase V – Aseptic Technique

Aseptic technique was used to transfer fungi monoculture into a fresh medium without contaminating microorganisms. The common laboratory tools were the inoculation needle, inoculation loop, and Bunsen burner. The processes of aseptic technique were as follows: first, light up the Bunsen burner, and the inoculation tools was sterilized by passing through the hottest part of the flame. Once sterilized, the inoculation tools should not be sat down on any surface.

Transfer of fungi to the medium

In this section, the researchers transferred the fungi into the petri dish. First, the loop was sterilized through the flame, and the lid of the container will then be taken off, and the mouth of the container was passed through the flame. Second, the loop was dipped into the culture, and then the mouth of the container was passed again before replacing the lid to prevent contamination. Lastly, the fungi in the inoculating tools were transferred into the petri dish, and the inoculating loop was streaked into the petri dish to ensure that the fungi spread evenly. After all, the tools were sanitized again through the flame.

Waste Disposal

Disposing of the excess cacao containing fungi was covered by foil. The researchers used a Microwave to disinfect the micro-organisms with a temperature of 300° C for 30 minutes.. Also, the left over waste during the extraction leaves was also microwaved at temperature of 100C for 30s. This was done at the STEM (Chemistry Laboratory) in Carlos P. Garcia Senior High School.

Steam Autoclave was used to decontaminate certain biological waste and sterilization of media, instruments and laboratory equipment was performed at Davao Medical School Foundation. Regulated medical wastes that might contain bacteria, viruses and other biological material were inactivated by autoclaving before disposal.

Data Analysis

To analyze the data collected, the researchers used the following statistical tools.

Mean. This used to measure the inhibition zone of *P. palmivora* when treated with neem tree leaves (*Azadirachta indica*), Acapulco (*Senna alata*) and Mancozeb (positive control) in terms of different concentration levels; 25% concentration extract;50% concentration extract;75% concentration extract; and 100%.

Kruskal Wallis. This was used to determine the significant difference in the inhibition zone of *P. palmivora* when treated with *Azadirachta indica*, *Senna alata* and Mancozeb.

RESULTS

This section presents the findings based on the data gathered. The presentation is organized into four (4) sections: first, inhibited colony of *Phytophthora palmivora* when treated by Asunting (*Senna alata*) extract; second, inhibited colony of *Phytophthora palmivora* when treated by Neem tree (*Azadiractha indica*) extract; and third, significant difference in the inhibition zone of Phytophthora palmivora when analyzed according to the different treatments.

Inhibited Colony of Phytophthora palmivora when treated by Acapulco (Senna alata) Extracts

Presented in Table 1 is the inhibited colony of *Phytophthora palmivora* when treated by Asunting (*Senna alata*) extracts according to four extracts according to four different concentration levels: 25%, 50%, 75%, and 100%. As observed, this laboratory experiment was conducted in three trials. Wherein, the first trial shows that 25% obtained the highest value of the inhibition zone among the four concentration levels (10.1mm), followed by the 50%,75%,100% that has a equal value of the inhibition zone (9.1mm).

Table 1.

Inhibited Colony of Phytopthora palmivora when treated by Acapulco (Senna alata) Extracts

Concentrations	TRIALS		Mean (mm)
	Trial 1 (mm)	Trial 2 (mm)	
25%	10.1	0.0	5.05
50%	9.1	7.5	8.3

75%	9.1	8.6	8.85
100%	9.1	8.3	8.7
Total Mean	9.35	6.1	7.73

In the second trial, 75% obtained the highest mean value of the inhibition zone among the inhibition zone among the four concentration levels (8.6mm), followed by 100%(8.3mm) then the 50% (7.5mm), with the least-attained value by 25% (0.0mm). it further shows that the overall mean inhibition zone *Phytopthora palmivora* when treated by Acapulco is 7.73. It depicts that 50 to 100 percent plant extract concentrations have almost the same number of inhibition zone, compared to the 25 percent concentration.

Inhibited Colony of Phytophthora palmivora when treated by Neem tree (Azadiractha indica) Extracts

Presented in Table 2 is the inhibited colony of *Phytophthora palmivora* when treated by Neem tree (*Azadiractha indica*) extracts according to four different concentration levels: 25%, 50%, 75%, and 100%.

Table 2.

Inhibited Colony of Phytopthora palmivora when treated by Neem tree (*Azadiractha indica*) *Extracts*

Concentrations	TRIALS		Mean (mm)
	Trial 1 (mm)	Trial 2 (mm)	
25%	14.1	13.5	13.8
50%	10.3	11.7	11
75%	9.2	10.0	9.6
100%	8.9	10.0	9.45
Total Mean	10.63	11.3	10.97

It shows that 25 percent plant extract concentration obtained the highest mean of inhibition zone ($\bar{X}$=13.8), followed by 25 percent concentration, and the least is 100 percent concentration. It further reveals that the overall mean inhibition zone of *Phytopthora palmivora* when treated by Neem tree is 10.97. it implies that the four concentrations of plant extract have almost the same number of inhibition zone.

Difference in the Inhibition Zone of Phytophthora palmivora when Analyzed According to the Different Treatments

Presented in Table 3 is the difference in the inhibition zone of Phytophthora palmivora when analyzed according to the following treatments: *Senna alata*, *Azadiractha indica*, Mancozeb and distilled water.

Table 3.

Difference in the Inhibition Zone of *Phytophthora palmivora* when Analyzed According to Different Treatments

Treatments	Mean	SD	df	H	p-value	Decision on Ho
Senna alata	7.73	1.80	3	14.55	0.00225	Failed Accept
Azadiractha indica	10.97	2.02				
Mancozeb	23.10	0.00				
Distilled Water	0.0	0.00				

It shows that, Mancozeb (positive control) obtained the highest mean zone of inhibition value among the four (4) treatments ($\bar{X}$=23.10), followed by *Azadiractha indica* ($\bar{X}$= 10.97), then *Senna alata* ($\bar{X}$=7.73), and lastly the distilled water ($\bar{X}$=0.00). This means that Mancozeb is the most effective against *Phytophthora palmivora,* followed by Need tree (*Azadiractha indica*). The Kruskal-Wallis test result shows that there is a significant difference in the inhibition zone of Phytophth*ora palmivora* when analyzed according to different treatments. Thus, this research failed to accept the null hypothesis. This implies that, though the Neem tree extract obtained the second highest mean inhibition zone, it can be inferred this plant has a high potential treatment against *Phytophthora palmivora* in cacao plant.

DISCUSSION

In counting the inhibited colony of *Phytophthora palmivora* when treated with Akapulco (*Senna alata*) extracts, it indicates that the higher the concentration, the higher the value of the zone of inhibition as per observation. In accordance with that the lower concentration, the less likely to show the zone of inhibition as well. The finding is congruent with the study that stated that the extract from neem leaves had the highest antifungal activity of both, perhaps due to a higher concentration of terpenoids with low polarity (Salazar et al.,2015). And also, it can be used as fungicidal product to prevent fungi dues to its potential antifungal effect against *Phytophthora palmivora* at the highest concentration.

However, in another study conducted on the antifungal activity of Azadirachta indica (Neem) when contrasted against the chemical compound mancozeb, this positive control has the higher value of zone of inihibition (23.1%) compared to Azadirachta indica with (10.9%). Furthermore, the water(distilled) serves as a negative control that does not contain any antifungal activity as a result there is no value of zone of inihibition. For the positive control the manzate that has an active compounds mancozeb obtained the highest diameter for zone of inhibition (23.10mm). Mancozeb is classified as a contact fungicide with preventive activity. It is widely used to control fungal diseases in conifer and fir trees (Cocco, 2022). That is why, it is effective as a control against *Phytophthora palmivora*.

On the other hand, *Senna alata* is our plant of interest. Ethanolic extracts of this plant has been prepared. The total mean of zone of inihibition of Senna alata extract in treating the *Phytopthora palmivora* obtains (7.73mm) compare to the positive control mancozeb (23,10) this means that the Senna alata is more effective in human fungi than the in the pathogenic plants. Also, In the second trial the 25% of extract along side with the negative control distilled observed as ineffective in the inhibition growth of *P. palmivora.* It is seen that this plant showed 500mg/mL concentration of antifungal activity against variety of fungi such as Penicilliumsp, Microsporum sp, Aspergillus sp and Trichophyton sp (Felsociova et al., 2020).

CONCLUSIONS

Base on the findings, the following conclusions are drawn:

- The higher the concentration level of the treatment, the higher the value of the zone of inhibition as per observation. In congruence with that, the lower the concentration, the less likely to show the zone of inhibition as well. This implies that when applied for anti-fungal purposes, it is recommended for a higher amount of concentration for the treatment to be more effective against *Phytophthora palmivora.*
- Anti-fungal capacity of Neem tree leaves (Azadirachta indica) extracts the mean value of its concentration levels are much lower than the inhibitory property of the control mancozeb, but it is also thought that the neem tree leaves is effective as a natural fungicide because unlike to the chemical control the neem extract contains only the compounds Nimbidin and nimbolide as an antifungal compound, it can be maximized if the amount of extract is equal to the number of pathogenic fungi.
- Anti-fungal capacity of Akapulco (Senna alata) extract, all the value of inihibition zone of senna alata in each concentration is consistent but the total value of mean of inhibition is not enough to be a fungicide against pathogenic fungi, to sum up senna alata is more effective in the human fungi than the plant pathogenic fungi (Phytophthora palmivora).

Recommendations

Based on the conclusions, the following recommendations are offered:

1. Other plants can be explored that contains a higher compound of anti-fungal to have an accurate and precise outcome.
2. Increase the number of trials to attain a higher level of consistency and accuracy of the results.
3. Investigate further the molecular behavior of *Phytophthora palmivora*, specifically the stages of development could lead to discoveries to inhibit the growth and reproduction of the pathogens.

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