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(RESEARCH ARTICLE)

Effectiveness of Lagnob (*Ficus septica*) crude leaf extract as a potential biological control agent against the eggplant blight pathogens (*Phomopsis vexans*)

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Abstract

This study investigates the potential of Lagnob (*Ficus septica*) crude leaf extract as a biological control agent against Phomopsis vexans, the primary fungal pathogen responsible for Eggplant blight. The Lagnob is a widespread plant whose leaf extract contains bioactive compounds with antifungal properties due to alkaloid and phenolic compounds, which are antioxidants. Phomopsis vexans is a pathogenic fungus that causes Phomopsis blight on Eggplants, a plant disease. Eggplant (Solanum melongena) is one of the cultivated plants that frequently displays symptoms of Phomopsis blight. The focus of this research was to evaluate whether Ficus septica leaf extract could inhibit the growth of *P. vexans* on Eggplant. The antifungal activity of *F. septica* leaf extract against *P. vexans* were measured using the disc diffusion test. Using an experimental research design, different concentrations of Lagnob extract were applied to fungal cultures in vitro to assess their inhibitory effect on Phomopsis vexans mycelial growth. A commercial fungicide served as a Positive control, and distilled water was used as a Negative control. The mycelial length of Phomopsis vexans was measured across treatments, which shows a dose-dependent response; higher concentrations of Lagnub extract significantly reduced fungal growth. At 100% concentration, the extract achieved an inhibition rate comparable to the commercial fungicide, suggesting its potential as an effective and eco-friendly alternative to synthetic fungicides. These findings support further investigation into the practical applications of Lagnob extract in sustainable agriculture and its potential integration into integrated pest management (IPM) programs

Keywords: Phomopsis vexans; Eggplant blight; Lagnob (*Ficus septica*); Biological control agent

1. Introduction

Eggplant (Solanum melongena) is an economically important crop that is produced and consumed around many parts of the world. It has an annual production of 59.31 million metric tons globally. China (61%) and India (23%) are the top global producers of Eggplant [1]. Meanwhile, in the Philippines, about 102.98 thousand metric tons of Eggplant production were registered in April-June 2023, according to the Philippine Statistics Authority (PSA). Despite the increase in the production of this crop, significant challenges to its cultivation, such as pathogens and pests causing high yield losses, declines in the quality of the fruit, and high economic losses [2].

Phomopsis vexans is one of the major fungal diseases affecting Eggplants [3]. It is characterized based on its features, like grayish spots that produce brown zones at later stages, which develop pycnidia covering mostly rotten fruit surfaces [4], that spread through fungal spores and cause contamination. The spread of Phomopsis blight has resulted in huge economic losses in several countries, including the Philippines, with up to 73% annual loss in Eggplant production. As

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the fungal infection increases, so does the use of chemical fungicides in controlling Phomopsis blight in Eggplant; however, applying chemical fungicides causes serious environmental pollution and human health hazards [5]. Among the two ways of controlling Phomopsis blight, biological control is more recommended as an effective alternative [6]. Because of its environmentally friendly and non-toxic nature approach in managing plant pathogens. Most plant extracts and biological agents have been reported to suppress antimicrobial activity against plant fungi [7], including the Lagnob or the *Ficus septica*.

Lagnob has a bioactive compound with antifungal property due to alkaloid and phenolic compounds [8]. *Ficus septica* contains different phytochemicals and metabolites that exhibit various biological activities [9]. Previous studies have shown the biological control potential of Lagnob (*Ficus septica*) leaf extract against fungal diseases of plants. The study of S.K. Surdiga [8] showed that the crude leaf extract of *Ficus septica* contains bioactive compounds with antifungal properties that serve as a safe alternative in controlling Carica papaya anthracnose disease. Phomopsis vexans, the fungal pathogen responsible for Eggplant blight. The research will focus on determining the length of mycelial growth of Phomopsis vexans when subjected to varying concentrations of *Ficus septica* extract (25%, 50%, 75%, and 100%). This research will be conducted in a plant pathology laboratory, located at Dacodao, Calinan, Davao City. The primary objective is to evaluate the extract's effectiveness in inhibiting mycelial growth and to establish the most effective concentration for pathogen suppression. The analysis will include comparing the effectiveness of the inhibition of Lagnob (*Ficus septica*) leaf extract across concentrations and against conventional chemical fungicides specifically Grifon (fungicide) and untreated controls. The inhibitory capacity of varying concentrations of the Lagnob extract will be determined.

This study will not include other fungal pathogens of Eggplant, nor will it include other parts of the *Ficus septica*. Testing will be conducted exclusively through in vitro laboratory conditions. Pathogen suppression metrics will include changes in colony diameter and mycelial growth rate over a period of 3 days post-treatment.

2. Conceptual Framework

The conceptual framework of this study aims to assess the effectiveness of *Ficus septica* extract in inhibiting the pathogen, Phomopsis vexans, subject to Eggplant blight. In this research, the independent variable is the application of Lagnob extract, with the following concentration of 25%, 50%, 75%, and 100%, applied to assess its impact on fungal inhibition. The dependent variable is the severity of Phomopsis vexans mycelial growth observed, which serves as an indicator of the extract's inhibitory effect on the pathogen. Lower severity levels will suggest higher efficacy of the extract in managing the pathogen. By comparing the performance of *Ficus septica* extract with commercial antifungal treatments, the research aims to establish whether this natural extract offers a more effective and sustainable option for inhibiting Phomopsis vexans. The goal is to compare the antifungal efficacy of *Ficus septica* extract with the commercially available antifungal treatments to assess their relative effectiveness in managing the Phomopsis vexans.

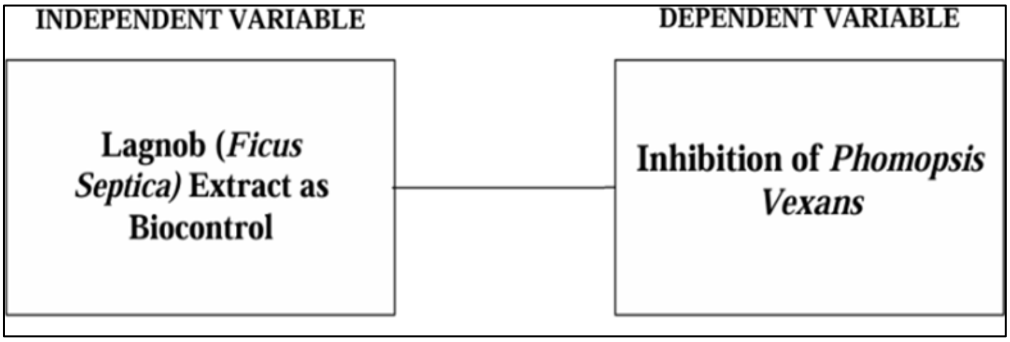

Figure 1 Conceptual Framework

3. Review of related literatures and studies

Eggplant (Solanum melongena) is a well-known crop that is being produced, and consumed globally. Eggplant belongs to the Solanaceae family that includes Potato, Tomato, Pepper, and Tobacco [10]. This crop usually grew in warmer locations in some parts of Asian countries, such as in Pakistan, Bangladesh, and the Philippines [2]. It has an annual production of 59.31 million metric tons globally [1]. The global production was contributed by two leading countries, which are China (61%) and followed by India (23%) [1]. Meanwhile, In the Philippines, according to the Philippine Statistic Authority (PSA), about 106.87 thousand metric tons of Eggplant production was registered in April- June 2022.

Eggplant provides various nutrients like calcium, magnesium, iron, polyphenolic compounds, phytonutrients, chlorogenic acid, amino acids, and vitamins that are essentially needed for enhancing the human body [11]. Aside from its use as food, in some parts of the world such as Southeast Asia, China, and in the Philippines it is being used by the people as a traditional medicine, due to its phenolic and alkaloid contents [12], it has been clinically used for treatment of several diseases, including asthma, bronchitis, diabetes, arthritis, and hypercholesterolemia [13].

According to the University of the Philippines Los Banos College of Agriculture and Food Science UPLB CAFS (2023), Eggplant production in the Philippines has been through huge losses of up to 73% per annum due to most of the catastrophic insect pest, the Eggplant fruit and shoot borer (EFSB). Furthermore, in Pakistan it was reported that alternata is causing leaf spots on Eggplant, with similar disease incidence rates. The disease manifested as circular brown spots, threatening the livelihoods of local farmers [13]. Additionally, Alternaria solani is another major pathogen responsible for early blight, with reported disease severity reaching 87.5% in affected crops [14]. These diseases not only threaten crop yields but also pose economic challenges, necessitating effective management strategies through resistance breeding and biocontrol methods [15] [14].

The prevalence of blight in Eggplant, particularly in Asia, has become a significant concern for agricultural productivity. Phomopsis blight, caused by various pathogens, is widespread in Eggplant- growing regions globally, leading to substantial yield losses [15]. In China, a recent report highlighted the emergence of Trichoderma parareesei, which caused a 35% incidence of seedling blight in Guangxi, characterized by severe leaf necrosis and defoliation [16]. A pathogen that caused Phomopsis blight occurs on Eggplant, causing a large economic loss in crop production. Phomopsis vexans produce two types of conidia such as, α and β conidia[17]. They similarly have been exposed to various carbon sources, plant components, plant leachates, and water grades. Theα-conidia are released from pycnidia and disseminated by insects, rain, and contaminated equipment, are the main cause of the disease. The conidia quickly germinate due to the presence of free moisture on the plant surface. It is both externally and internally seed-borne and remains viable for about 14 months in soil with plant debris and in the seed from infected fruits [12].

From 2017 to 2019, a total of 162 samples of Eggplant Phomopsis blight from the provinces of China were gathered to investigate the biocontrol of the disease. Eighty-seven isolates of fungus were recognized as *P. vexans*. The research was done on screening sporulation medium, analyzing spore morphology, detecting mycoviruses, and recognizing new mycoviruses that are isolated. The findings showed that Eggplant tissue medium was the most effective medium for quick sporulation, and isolates harbored mycoviruses in the form of mixed infections [18]. Phomopsis vexans cause a serious disease in brinjal known as leaf blight and fruit rot [19]. Identifying *P. vexans* in plant parts and brinjal seeds can be challenging, especially when the contamination levels are low and/or covered by rapidly multiplying saprophytic fungi or other fungi carried by seeds.

The severity of the fungal diseases has affected various factors including its economic impact, cultivation, production, and Fruit quality. It is prevailing and common, that the scientific community should integrate approaches in managing Phomopsis vexans [20]. Different managements have been applied to eliminate *P. vexans*, but it is challenging to control this disease due to the slippery skin of the infected fruit.

Synthetic pesticides have become an integral part of agriculture since the first discovery of synthetic fungicide, phenylmercury acetate, in 1913. Several management methods were used to inhibit or avoid fungal diseases, including crop protection such as RNA-based fungicides, biocontrol, or stimulation of natural plant defenses that would serve as alternative methods [21]. These alternative methods are more efficient and safer for the environment, animals, and human health. The most promising tools in ensuring plant health as well as the quality and safety of vegetal products are beneficial natural products, and microorganisms like biocontrol or biostimulant microorganisms [22] .

However, due to its devastating result of losses and the prevalence of the disease, it has been an option to use biological control. Plant growth-promoting rhizobacteria (PGPR) to replace chemicals and fertilizers through biological agents due to wide range availability, biocontrol agent production, and an environment-friendly approach [23] . This prevents the growth of phytopathogens through the activity of antagonistic suppressing the growth of the pathogen and producing antibiotics, organic volatile compounds, and hydrolytic enzymes, such as proteases and celluloses.

Lagnob or *Ficus septica* has been known for its medicinal purposes, including curing boils, anthelmintic and other human diseases like fever, appendicitis, asthma, venomous snake bites, skin diseases, eye- redness, hemorrhoids, and tuberculosis [24]. It has antioxidant compounds such as phenolics, flavonoids, and polyphenols that can suppress such as hydroperoxides, peroxides, or lipid peroxyl that can inhibit degenerative diseases [9]. Besides its other medicinal uses, it has several properties that include antifungal property. The leaf extract of *Ficus septica* contains bioactive compounds, including antifungal properties, such as alkaloid and phenolic compounds that are antioxidants [8]. *Ficus septica* contains different phytochemicals and metabolites that exhibit various biological activities [9].

Inhibitory of fungi using *Ficus septica* has significant factors when it comes to the agricultural field. In Ukraine, based on the recent study, treatment of plant extracts like Lagnob (*Ficus septica*) has served as an alternative to antibiotics. Furthermore, many sources of plant-derived agents like Botanical gardens offers a broad spectrum of biological and antimicrobial activities [25]. However, the inhibitory activity of the Lagnob *F. septica* leaf extract is not widely known [8]. The *Ficus septica* leaf extract inhibited the growth of fungi at 81.11% compared to other leaf extracts. The analysis of *F. septica* leaf extract through GV-MC shows a presence of 15 types of metabolic constituents having nine antimicrobial activities [8]. In the Philippines, the ethanolic of *F. septica* crude leaf extract has significantly inhibited the fungi activity against mammalian alpha- glucosidase at least IC50 value 8.866 (μg/mL) [26].

4. Methodology

4.1. Preparation of Lagnob extract

Lagnob leaves were gathered from Dona Andrea, Asuncion, Davao del Norte that reached about 0.75 kg. The leaves then were washed twice in running water to remove any dust or dirt, and part dried the leaves with clean tissue to remove some excess water, then air dried for at least 24 hours. After drying, the leaves were cut into small sizes, then placed on a blender to pulverize.

The crude extraction was started using the maceration extraction method by the following measurements of a 10:1 ratio, 100 ml of ethanol and 10 g of pulverized Lagnob leaves were put inside clean sterilized water and stirred with a clean stirring rod to fully submerge the dry pulverized leaves. After that, enclose the container and leave for at least 48 hours at room temperature.

After that, the extract is filtered using a filter paper and to remove the ethanol presence on the extract leaving only the Lagnob crude extract itself, Vacuum Rotary Evaporator is used in this process.

4.2. Preparation of Phomopsis vexans

The fungus (Phomopsis vexans) was cultivated at the Plant Pathology Laboratory in Dacudao, Calinan, Davao city. The blight disease isolation was conducted by acquiring fungal spores from the fruit rot of the infected Eggplant. Next was to transfer the spores on an Eppendorf tube for application preparation.

4.3. Preparation and Application of Lagnob (*Ficus septica*) Crude Extract

The Lagnob crude extract of 25%, 50%, 75%, and 100% was formulated as experimental treatments. Crude leaf extract (5 ml) and 15 ml of ethanol were mixed for a 25% concentration, 10 ml of crude leaf extract and 10 ml of ethanol for a 50% concentration, 15 ml of crude leaf extract and 5 ml of ethanol for a 75% concentration, and then 20 ml of crude leaf extract for the 100% concentration. For the positive and negative control groups, 20 ml of distilled water and Grifon fungicides for commercial spray were prepared separately.

After the extraction, the application of Lagnob (*Ficus septica*) extract to Phomopsis vexans began. The drops of fungi were added to the slides and immediately placed on coverslips and above the slides. Each slide was applied with a measurement of 100 microliters of extract using a mechanical pipette based on their corresponding concentration.

4.4. Data collection and Analysis

After the application of extract and fungi, each replicate was placed in a labeled and disinfected container box and was set aside for 3 days of observation. Statistical analysis was used to analyze the length of mycelial growth of Phomopsis vexans that germinated and failed to germinate, and the ideal concentration for the Lagnob (*Ficus septica*) was determined using a One-way Analysis of Variance (ANOVA). All the treatments were compared using a post hoc at a significant level of 5% to determine the difference in the growth of mycelial length of Phomopsis vexans when subjected to 25%, 50%, 75%, and 100% Lagnob extract concentration.

5. Results and discussion

Figure 2 and Figure 3 present the data gathered of the mean in the fungi mycelial length of Phomopsis vexans germinated among control groups and treatments.

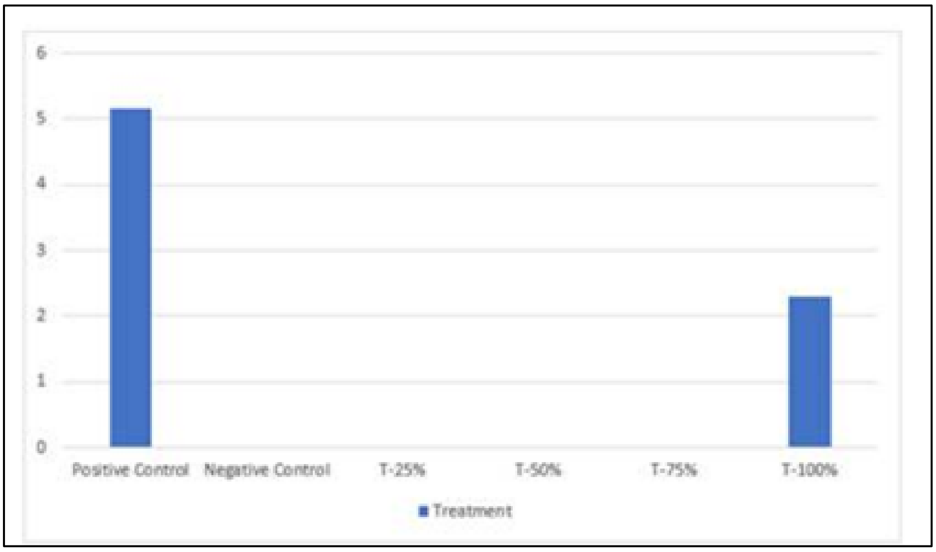

Figure 2 Mean Mycelial Growth (mm) of Phomopsis Vexans

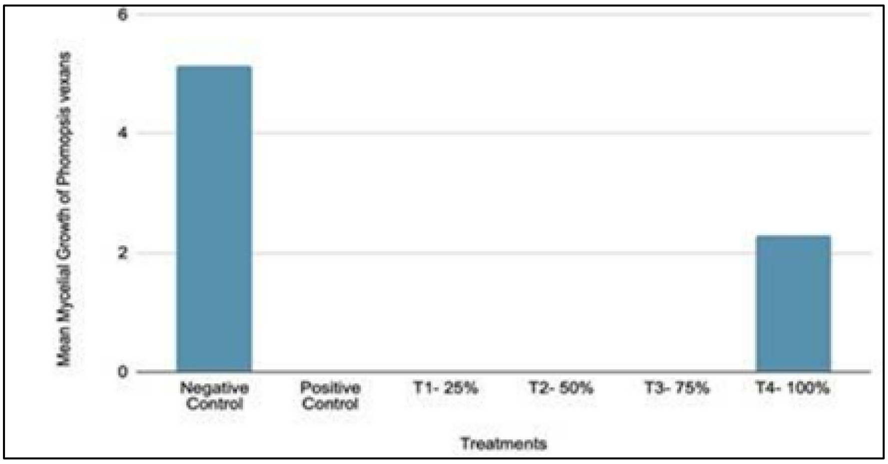

Figure 3 Mean Mycelial Growth (mm) of Phomopsis Vexans

The longer the mycelial length of the fungi is, the more effective the concentration of extract. Positive control yielded the highest measurements of mycelial length among all treatments. This indicates the efficacy of Positive control above all groups and concentrations. This shows the mean in the fungi mycelial length of Phomopsis vexans germinated among control groups and treatments. Treatments 1-3 have a zero mean, while Treatment 4 (T4) has a greater mean compared to other treatments. However, T4 has the lowest mean and less significant difference compared to the positive control group. The other treatments had no significant difference when differentiated among treatments and control groups. This indicates that T4 is more effective than the other treatments, with a mean of 2.29 than the others. To add, the positive control group is more effective with a mean of 5.15 in the above-mentioned aspect as opposed to Treatment 4 (T4) since each recorded mean is higher than the respective group.

Table 1 Growth of the fungi mycelial growth in micrometer (mm)

Concentration of Lagnob (*Ficus septica*) Extract	Replication (Mycelial Growth) (mm)			Mean	Percentage of *Phomopsis vexans* germinated
	R1	R2	R3		
Negative Control	5.75	5.5	5.25	**5.15**	**85.83%**
Positive Control	0	0	0	**0**	**0**
T1- 25%	0	0	0	**0**	**0**
T2- 50%	0	0	0	**0**	**0**
T3- 75%	0	0	0	**0**	**0**
T4- 100%	2.5	2.56	1.81	**2.29**	**38.17%**
				P-value: <0.001	

Table 2 Comparison and significance of the length of mycelial growth of Phomopsis vexans among groups and treatments

Dependent Variable	Treatment	Mean	P-value	Interpretation
Mycelial Growth	Negative Control	5.15	0.00069	*Significant*
	Positive Control	0	0	*Not Significant*
	T1-25%	0	0	*Not Significant*
	T2-50%	0	0	*Not Significant*
	T3-75%	0	0	*Not Significant*
	T4-100%	2.29	0.0109	*Less Significant*

Table 1 and 2 show the comparison of significance of the length of mycelial growth of Phomopsis vexans germinated among groups and treatments. The significance level utilized in treating is 0.005. The treatment where the extract is set to 100% has a 0.0109 probability since the value is greater than the significance level that has a difference compared to other treatments. However, the positive control is more effective since it has 0.00069 probability compared to the treatment 100%. The value is less than the significance level; the treatment with 100% concentration has the highest mean difference compared to other treatments but is lower compared to the positive control group, indicating that it is possibly better than other treatments.

6. Conclusions and recommendations

Based on the findings of the study, the crude leaf extract of Lagnob (*Ficus septica*) shows efficacy on suppressing the mycelial growth of Phomopsis vexans. At least 38.17% was suppressed by the 100% extract concentration, compared to the positive control which suppressed about 85.83%. Nonetheless, it is suggested that Lagnob could be utilized as a biocontrol agent, especially since it is an environmentally friendly and affordable approach to inhibiting Phomopsis vexans. The 100% Lagnob leaf extract is more effective in controlling the spread of the fungi than the other treatments and control groups, resulting in the ideal concentration against Phomopsis vexans, which is deemed to be within 100%.

For future research, further laboratory tests to identify the antifungal properties present in Lagnob (*Ficus septica*) leaves will provide insight into how it inhibits Phomopsis vexans. investigate the effectiveness of Lagnob (*Ficus septica*) extract in managing other plant diseases caused by different types of fungi. Furthermore, collaborating with agricultural policymakers to promote and subsidize the use of biological control like *Ficus septica* extract for smallholder farmers can support sustainable farming practices and help be more environmentally friendly in managing fungi diseases.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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