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In Vitro Evaluation of The Antifungal Activity of Breadfruit (*Artocarpus altilis*) Leaves Extract Against *Puccinia arachidis*, Causative Agent of Groundnut Rust

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ABSTRACT

Breadfruit (*Artocarpus altilis*) leaves contain several antimicrobial properties such as saponins, hydrocyanic acid, polyphenols, acetylcholine, potassium, tannin, phenol, riboflavin, and flavonoids. Phenol is a compound found in plants that causes pesticide effects. Previous studies have demonstrated that breadfruit leaf extract contains phenol compounds with antimicrobial activity and can be used as a fungicide. The purpose of this study was to determine the effect of breadfruit leaf extract on the growth inhibitory power and percentage inhibition of *Puccinia arachidis* spore germination as well as the best concentration of breadfruit leaf extract in inhibited the growth of *Puccinia arachidis, an* agent causing groundnut rust disease in peanut plants in food poisoning method. This study used a single factor CRD consisting of 5 treatment levels, namely $k_{0(-)}$ = negative control, $k_{0(+)}$ = positive control, k_1 = 10% extract concentration, k_2 = extract concentration of 20%, and k_3 = extract concentration of 30%. The results of this study indicate that the breadfruit leaf extract significantly affected *Puccinia arachidis* growth with the presence of percentage inhibition of *Puccinia arachidis* spore germination. The highest concentration of breadfruit leaf extract as a rust disease biofungicide (*Puccinia arachidis*) in peanut plants in vitro at a concentration of 30%.

Keywords:
Artocarpus altilis, biofungicide, food poisoning method, inhibition test, groundnut rust diseases.

1. Introduction

Food is an essential requirement of every human being that cannot be replaced or delayed from time to time until the future. To meet these needs, humans always strive to cultivate various crops. Humans must compete with Plant Disturbing Organisms such as weeds, pests, and diseases to fulfill food needs.

Plant disease is a disruption to plants that deviate from normal conditions, causing certain parts of the plant to not walk according to its function. Consequently, the process of plants

returning to normal conditions after being attacked by the disease takes a long time, inhibiting plant growth and development. The causative agent of plant diseases consists of fungi, bacteria, nematodes, and viruses.

Peanut plants (*Arachis hypogaea* L.) are food crops in the form of shrubs originating from South America, precisely from Brazil. One obstacle in increasing the production of peanut plants is the attack of diseases, and one of the most critical diseases in peanut plants is leaf rust caused by the fungus *Puccinia arachidis*. This rust disease is a chronic disease and, in severe attacks, will cause a decrease in yield up to 50% to 60%. In Indonesia, this disease is spread throughout peanut plantations, with the intensity of attacks varying depending on season and location. Therefore, it is necessary to control plant diseases.

One way to control peanut plant diseases is by using pesticides, namely chemical pesticides and botanical pesticides. Chemical pesticides harm humans and can contaminate the environment or leave fungicide residues on food products (Moenne-Loccoz et al., 1998). In addition to ozone layer depletion, constituent molecules of synthetic fungicides have been related to chronic human illnesses in intake or exposure scenarios due to their low biodegradability and high tendency to accumulate in the environment (Santra and Banerjee, 2020; Makhuvele et al., 2020; Bhandari et al., 2021). Several other methods have been tried to address the issues with synthetic fungicides that have been discussed, among these are botanical fungicides, which can be an effective and long-lasting substitute for synthetic fungicides (Deresa and Diriba, 2023). Botanical pesticides are pesticides whose essential basic ingredients come from plants; they are environmentally friendly and not persistent in the environment (Silva et al., 2020). Numerous studies have shown plant-derived phytochemicals to have fungicidal properties (Bhandari et al., 2021; Lengai et al., 2020). Many bioactive compounds that offer defense against pests and diseases and restrict the growth of other plant species are produced by plants through secondary metabolism (Suteu et al., 2020). The study of medicinal plants as possible natural sources of active chemicals against phytopathogens has drawn more attention recently for various reasons (Ramírez-Gomez et al., 2019). Research on the use of botanicals in plant disease control is being performed worldwide, and it has been found that crude extracts from many different plant species are effective against various phytopathogenic fungi without creating severe side effects (Okwute, 2012). In plant pathology research, managing fungi pathogens with unrefined extracts and oils is the most typical scenario (Borges et al., 2018).

Plant extracts from a variety of plants were tested for antifungal properties against crucial crop fungal pathogens (Adnew et al., 2022; Sales et al., 2016; Hernández-Ceja et al., 2021; Okla et al., 2021). Various plants express complex mixtures of secondary metabolites within each species (Arguedas and Coley, 2005). Phenols, terpenoids, flavonoids, glycosides, tannins, alkaloids, steroids, saponins, and resins are important plant phytochemicals (Tiwari et al., 2011). The phytochemical was reported to have antimicrobial activity with strong antioxidant activity (Bottari et al., 2017; Duval and Diriba, 2016; Reddy et al., 2007).

The potential plants that can be used as botanical pesticides are breadfruit plants (*Artocarpus altilis*). The existence of abundant breadfruit plants in tropical regions such as Indonesia makes this plant very potential for development (Maharani et al., 2014). Recently, studies have been carried out to find the benefits of breadfruit leaves; it turns out that the content of secondary metabolites is high enough in breadfruit leaves, such as saponin, hydrocyanic

acid, polyphenols, acetylcholine, riboflavin, phenol, and tannins (Maharani et al., 2014). Phytochemicals found in *A. altilis* leaf and steam bark extract have antimicrobial activity, as Sivagnanasundaram and Karunanayake (2015) reported. Satish et al. (2008) conducted an antimicrobial assay to test the efficacy of leaf extracts of 46 plants, including A. altilis, against 14 pathogenic bacteria, including *E. coli*. Another study showed that the silver nanoparticles synthesized using an aqueous leaf extract of *A. altilis* as a reducing agent increased the inhibitory effect of six antibiotics against *Salmonella paratyphi* and *Klebsiella pneumonia.*

In addition, it was reported that, among the 54 chemical components % of the aqueous leaf extract, 37.4% were alcohol, and 32.2% were carboxylic acids (Thirumurugan et al., 2010). Kuete et al. (2011) tested the effectiveness of crude methanolic extract of *Artocarpus altilis* Stem bark, terpenoids, and flavonoids were isolated from the bark against selected bacteria and fungi. As a result, the extract's antifungal effects may be influenced by different extracts or the same compounds in varied concentrations (Jimenez-Reyes et al., 2019).

This research is the preeliminier of the manufacture of botanical pesticide products, namely bio fungicides, and it is expected that their presence in cultivating environmentally friendly peanut plants has received a positive response from all circles.

2. Materials and Methods

This research was conducted in the Chemical and Industrial Environment Laboratory of the Agricultural Industrial Technology Study Program and Production Laboratory of the Agroecotechnology Department of the Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru, South Kalimantan.

2.1 Isolation of Pathogenic Puccinia arachidis Causing Groundnut Rust

The rust leaves of the groundnut are cut into small pieces. The leaf pieces were sterilized using 3% NaOCl for 60 seconds and 70% alcohol for 30 seconds and then rinsed twice using sterile distilled water. After the pieces have been sterilized and rinsed, the cut leaves are dried on sterile filter paper and then cut again with a size of approximately 0.5 × 0.5 cm. The leaf pieces measuring 0.5 × 0.5 cm were placed in a petri dish containing PDA media. As many as four pieces of leaf are placed in one petri dish. The fungi that grow from within the plant tissue have gone through the isolation stage and then proceed to the purification stage. The fungal mycelia that grew were then re-cultured on new PDA media and incubated for seven days.

2.2 Extraction of Breadfruit Leaves

The dried breadfruit leaves were ground using a blender, and the simplicia obtained was then macerated using 90% methanol at a ratio of 1:4 for 48 hours. The maceration results were evaporated using a rotary evaporator at 60-70°C for 5 hours. After the solvent was reduced to 80%, the results of the rotary process were then evaporated again using a water bath at a temperature of 80°C for 1 hour. The extraction result from breadfruit leaves is a thick, black paste with a small amount of oil. The extract is then stored in a refrigerator freezer at 0°C to prevent spoiling (Harborne, 1998).

2.3 Inhibitory Power Test of Breadfruit Leaves Extract Against Puccinia arachidis

This experimental study includes testing *in vitro* culture using a single randomized complete design (CRD) factor. The factors studied were variations in the concentration of breadfruit leaf extract consisting of 5 levels of treatment with five replications. So that 25 experimental units were obtained, the treatment given was $k_{0(-)}$ = giving aquades on PDA medium (negative control), $k_{0(+)}$ = Topsin-M 70 fungicide giving 30% concentration, k_1 = giving breadfruit leaf extract with a concentration of 10%, k_2 = giving breadfruit leaf extract with a concentration of 20%, k_3 = giving breadfruit leaf extract with a concentration of 30%.

The antifungal activity of botanical extracts is conducted using the poisoned food technique (Ramaiah and Garampalli, 2015; Rizali et al., 2021). Percentage inhibition of radial growth is calculated using the formula;

$$L = [(C - T)/C] \times 100\%$$

Note:
L =percent inhibition;
C =radial growth colony of the pathogen in the control dish
T =the radial growth of the pathogen in the treatment dish

However, the percentage of inhibition of *P. arachidis* germination spore using the formula,

$$K = [(\Sigma \text{ control} - \Sigma \text{ treatment}) / \Sigma \text{ control}] \times 100\%$$

Note:
K =percent inhibition of spore germination;
Σ control = total amount of spore germination on the control plate
Σ treatment = total amount of spore germination on the treatment plate

The variables observed were the inhibition power of *P. arachidis*, radial growth, and the percentage inhibition of *P.arachidis* germination spore. Saha et al. (2005) refer to the method to estimate spore germination. The data were analyzed using Bartlett's homogeneity test. Analysis of variance was carried out using the F-test at the 5% and 1% levels. Furthermore, suppose the treatment has a significant or genuine effect. In that case, it will be followed by a middle-value difference test using the Least Significant Difference Test (LSD) at the 5% confidence level.

3. Results and Discussion

The results of the variance analysis (ANOVA), which was obtained by giving various concentrations of breadfruit leaf extract to each observation variable, are presented in Table 1. The results of the recapitulation of the variance analysis showed that the multiple concentrations of breadfruit leaf extract on media growth were significantly different for all variables.

Table 1. Recapitulation of the results of the analysis of the variety of breadfruit leaf extracts given to the observation variables

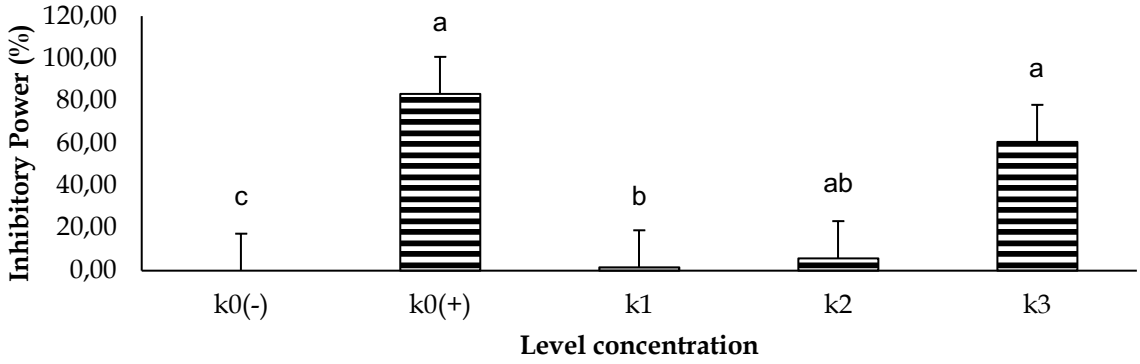
No.	Observation variable	Significance of Variable Analysis Results (ANOVA)

1	Inhibitory potency	S
2	Percentage of inhibition of spore germination	S
3	Diameter colony	S
4	The total amount of germination spora	S

Note: s = significant

3.1 Effect of Breadfruit Leaf Extract on the inhibitory power of *P. arachidis* fungus growth

The inhibition of breadfruit leaf extract on the growth of *P. arachidis* fungus *in vitro* is presented in Figure 1. The inhibition of breadfruit leaf extract to the growth of *P. arachidis in vitro* on the fifth day of observation ranged from 1.60% to 60.74%. Positive control (k_0(+)) in the treatment was used to compare inhibitory work by giving breadfruit leaf extract to *P. arachidis* growth. The treatment of k_3 (the concentration of breadfruit leaf extract 30%) showed the highest inhibitory power of 60.74%, while treatment of k_1 (the concentration of breadfruit leaf extract 10%) showed the lowest inhibition power of 1.60%. In this case, the treatment of k_3 (the concentration of breadfruit leaf extract 30%) has the best inhibitory work in inhibiting the growth of *P. arachidis* fungi or is comparable to the treatment of positive control (k_0(+)), which shows an inhibition of 83.36% on the fifth day.



*) $k_{0(-)}$ = negative control (0%), $k_{0(+)}$ = positive control (Topsin-M 70 fungicide), k_1 = 10%, k_2 = 20%, k_3 = 30%
**) The same letter above the line shows that the treatment has a different effect based on the Least Significance Difference Test (LSD) at the α level of 5%.

Figure 1. Percentage of inhibition of *P. arachidis* growth on observation of the fifth day applied with various concentrations of breadfruit leaf extract

The inhibitory potency of breadfruit extract against *P. arachidis* was observed by measuring their colony growth compared to control without breadfruit extract (Figure 2). The result showed that breadfruit leaf extract influences the diameter growth of *P. arachidis*. The fastest growth of the colony *P. arachidis* was found in the treatment k_0(-). In contrast, the other treatments have a lower radial growth colony of *P. arachidis*. The higher the concentration of breadfruit leaf extract given, the slower the *P. arachidis* diameter colony. The percentage of inhibition indicates the effect of an antifungal of *P. arachidis* mycelium. The results showed that the treatment of k_3 (30% breadfruit leaf extract concentration) produced the highest inhibition on the fifth day, 60.74%.

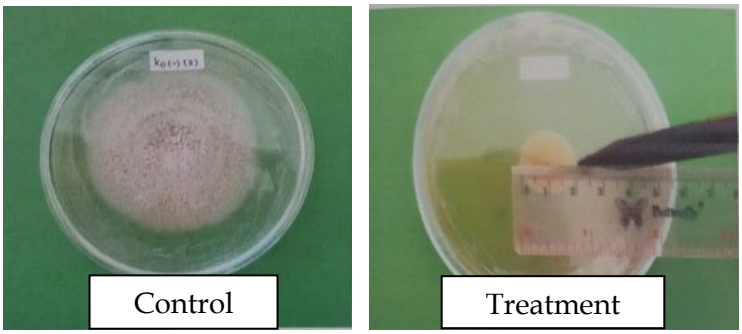
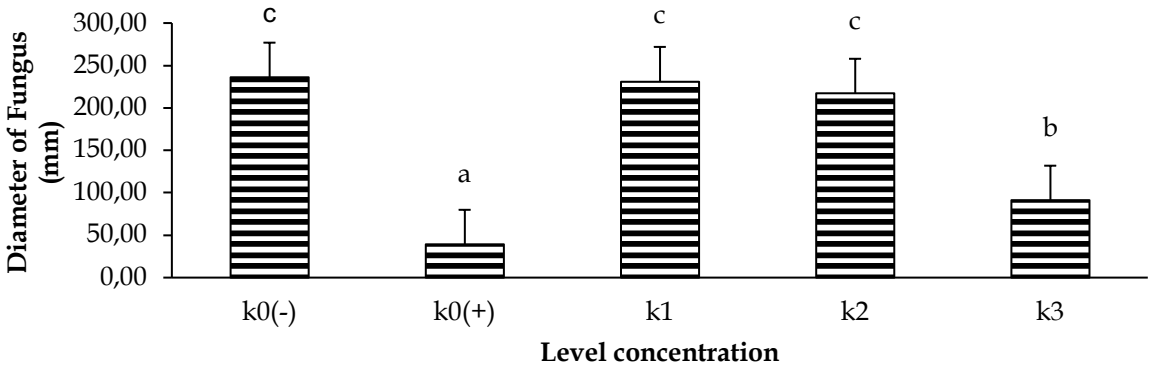


Figure 2. The inhibitory power of *P. arachidis* fungus growth on a medium of PDA



*) $k_{0(-)}$ = negative control (0%), $k_{0(+)}$ = positive control (Topsin-M 70 fungicide), k_1 = 10%, k_2 = 20%, k_3 = 30%
**) The same letter above the line shows that the treatment has a different effect based on the Least Significance Difference Test (LSD) at the α level of 5%.

Figure 3. Diameter colony of *P. arachidis* radial growth on the fifth day applied with various concentrations of breadfruit leaf extract

The diameter colony of *P. arachidis* on a medium of PDA (Potato Dextrose Agar) mixed with breadfruit leaf extract in petri dishes with various concentrations is presented in Figure 3. The results of variance analysis showed that the concentration of breadfruit leaf extract affected the diameter colony of *P. arachidis* on the day. The k_3 treatment showed the smallest diameter growth of 9.1 mm; in contrast, the treatment of k_1 showed the largest diameter growth of 23.1 mm.

Based on the results of the LSD test, the effect of breadfruit leaf extract on the growth diameter of *P. arachidis* fungus on the fifth day was found that treatment of k_1 (10% breadfruit leaf extract concentration) and k_2 treatment (breadfruit leaf extract concentration 20%) were not significantly different from the treatment $k_{0(-)}$ (negative control). Still, if the concentration of breadfruit leaf extract is increased to 30% k_3 treatment, then the two treatments have a significant effect.

As Maharani et al. (2014) reported, breadfruit leaves are one of the plants with relatively high antifungal activity. In one of the studies reported by Nisa et al. (2018), *Penicillium* sp. was most effectively inhibited by an aqueous extract of *A. altilis* with one of its components,

phenol compounds—Pholyphenol known as antifungal agents (Daglia, 2012). The phenol compounds in breadfruit leaf extract proved to have antimicrobial activity so that they can be used as fungicides. It has high antioxidant activity within breadfruit's total phenol and flavonoid content (Jalal et al., 2015).

According to Pelezar and Chan (1988), an antimicrobial can be fungistatic or fungi toxic. Fungistatic is a condition that describes the work of a material (fungicide) that inhibits the growth of fungi. This might occur because the antimicrobial concentration given is too low. A fungi toxic is a condition that acts as a material (fungicide) that controls the growth of fungi. Fungistatics can be transformed into fungi by increasing the concentration of an antimicrobial to a critical point where the fungicide can kill the fungi. On the contrary, reducing the effect of fungicides from fungi toxic levels to fungi is obtained by lowering the fungicide concentration. Similar conditions occur in the administration of breadfruit leaf extract to the growth of *P. arachidis* fungi, the higher the concentration of breadfruit leaf extract given, the slower the growth of *P. arachidis* fungus will be.

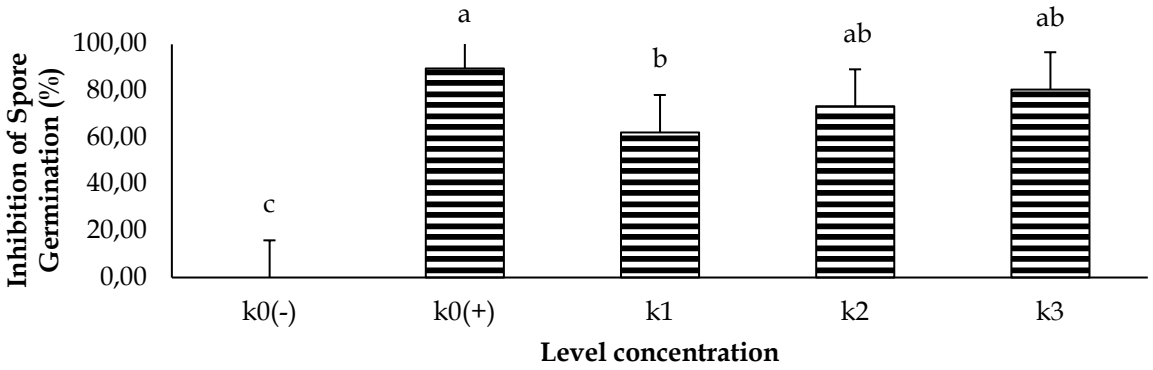
Phenol compounds can cause the lysis of microbial cells and phenol compounds from breadfruit leaf extract. According to Prindle and Wright (1971), if the microbial cells are damaged, toxins from outside the cell can enter, resulting in reduced essential metabolites needed by microbes. Once inside the cell, the phenol compound will damage the cell's working system. Phenol compounds can cause inactivation of crucial enzymes in cells. Phenol compounds as antimicrobials are active against vegetative bacteria, viruses, fungi, and vice versa, inactivity to spores and bacteria.

Nwaoha and Onwuka (2014) investigated the antimicrobial properties and Phytochemical composition of *A. altilis* leaf extracts using ethanol, n-hexane, and water. The ethanol extracts (at MIC 5 mg/ml) were more effective against bacteria, including *E. coli*, and fungi, including *Aspergillus niger*, compared to the other solvents. The n-hexane extracts inhibited all the test organisms (at MIC 8 mg/ml), while water extracts did not inhibit.

Any of the test organisms. Kuete et al. (2011) and Rahman et al. (2012) found that methanolic and ethanolic leaf extracts of *A. altilis* were effective against *Candida* sp. and *Mucor* sp.

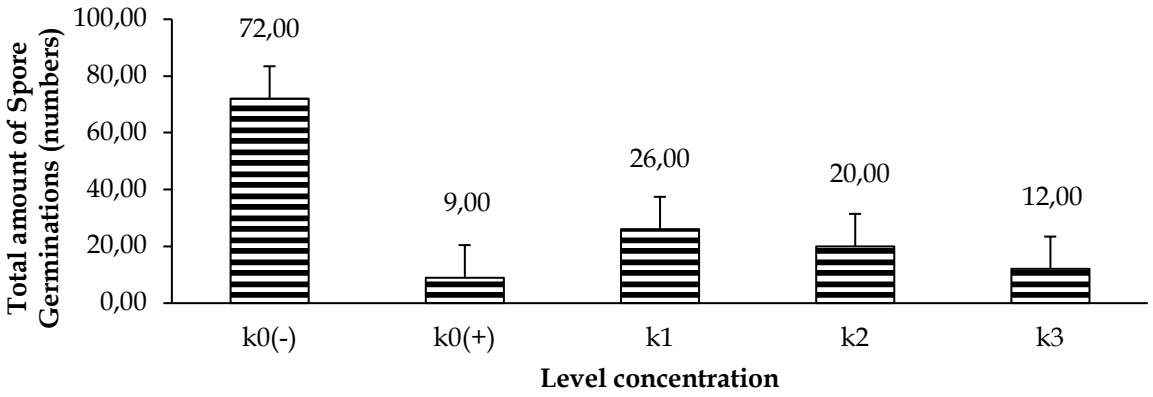
3.2 Effect of Breadfruit Leaf Extract on Percentage of *P. arachidis* Spores Germination

As a result, we can see if the application of breadfruit extract hurts the spore germination of *P. arachidis*. Based on statistical analysis, the percentage of *P. arachidis* spores' germination inhibition did not show a significant difference between treatments k_2 and k_3; nevertheless, it was significantly different in treatment k_1. The results of the percentage inhibition of *P. arachidis* germination spores on the level concentration of breadfruit leaf extract were obtained data as shown in Figure 4.



*) $k_{0(-)}$ = negative control (0%), $k_{0(+)}$ = positive control (Topsin-M 70 fungicide), k_1 = 10%, k_2 = 20%, k_3 = 30%
**) The same letter above the line shows that the treatment has a different effect based on the Least Significance Difference Test (LSD) at the α level of 5%.

Figure 4. The percentage of inhibition *P. arachidis* germination spores during one day of observation applied with various concentrations of breadfruit leaf extract



*) $k_{0(-)}$ = negative control (0%), $k_{0(+)}$ = positive control (Topsin-M 70 fungicide), k_1 = 10%, k_2 = 20%, k_3 = 30%
**) The same letter above the line shows that the treatment has a different effect based on the Least Significance Difference Test (LSD) at the α level of 5%.

Figure 5. The total amount of *P. arachidis* germination spore during one day of observation applied with various concentrations of breadfruit leaf extract

Based on the observation of the number of spores germination of *P. arachidis* on PDA media mixed with various concentrations of breadfruit leaf extract, there was a significant difference between the treatment of k_0(-) (negative control) with breadfruit leaf extract treatment in Figure 5. The application of breadfruit leaf extract shows spore germination. *P. arachidis*, carried out for one day, found that the highest germination occurred in treating k_1, with the number of spores germinating as many as 26. In contrast, the lowest germination occurred in the treatment of k_3, with the number of spores germinating as many as 12 spores. The k_3 treatment (breadfruit leaf extract concentration 30%) showed the lowest number of *P. arachidis* spores germination compared to other extract treatments. Compared to treatment k_3 with the treatment of $k_{0(-)}$ (negative control), which contained 72 spores, the

time of the percentage of germination spore inhibition of *P. arachidis* was calculated at 80.66% and the highest rate of inhibition of spore germination.

Treating breadfruit leaf extract containing phenol can reduce the permeability of cell membranes. If this membrane is damaged, it can inhibit cell growth or even cell death. Fern extract may have inhibited the development of these two significant groundnut fungi due to changes in hyphal structure and cellular compartment disarray (Sahayaraj et al., 2009). As reported by Kamble and Patil (2019), in the field, it was discovered that all of the plant extract using *Eupatorium odoratum* L., *Eucalyptus globulus* Labill., *Azadirachta indica* A. Juss., *Vitex nigundo* L. and *Datura metel* L. were more effective than the control (distilled water spray) against the groundnut rust disease.

A recent study reported by Zubarno (2021) revealed that *A. altilis* has antifungal activity against *A. niger*. The component as an antifungal in breadfruit leaf extract is phenol. The phenol compound can break the crosslink of peptidoglycan in its attempt to break through the cell wall. After the phenol compound breaks through the cell wall, nutrient leakage will occur inside the cell. Because phenol can damage the hydrophobic bonds of cell membrane constituents such as proteins and phospholipids and the dissolution of other hydrophobically bound components, these conditions decrease cell permeability. Damage to cell membranes inhibits the activity and biosynthesis of specific enzymes needed in the metabolic process (Ingram, 1981). The exact mechanism also occurs in *P. arachidis* spores, which are treated with breadfruit leaf extract so that the growth of *P. arachidis* spores is inhibited. With the increasing concentration of breadfruit leaf extract, the number of spores germinating is getting smaller, and the percentage of inhibition of *P. arachidis* sperm germination will be even higher. Phytochemicals derived from plants have the most promising natural sources of antifungal activity that have been discovered are flavonoids, tannins, coumarins, quinones, lignans, and neolignans (de Andrade Monteiro and Ribeiro Alves dos Santos, 2020).

Natural plant extracts control plant growth and participate in plant defense responses, including stopping pathogen growth (Hassan et al., 2021). The antimicrobial substance can damage or interfere with the development of microorganisms through inhibited mechanisms. Many factors influence those mechanisms, so it impacts any variation of the diameters of the inhibition zone (Sari et al., 2020). In addition to the concentration or intensity of antimicrobial substances, several factors affect the inhibition of microorganisms by antimicrobials, including the number of microorganisms, temperature, species of microorganisms, the presence of organic matter, and the degree of acidity (pH). The degree of acidity decreases with the increasing concentration of breadfruit leaf extract, the rising acidity of the media, and the antimicrobial activity will increase because the phenol compound will be more active in an acidic atmosphere. Following the study's results, it is confirmed that the higher the percentage of inhibition of spore germination, the more significant concentration of breadfruit leaf extract was given.

4. Conclusions

Breadfruit leaf extract as an alternative biofungicide in controlling rust disease in peanut plants affects the inhibitory power and the percentage inhibition of *P. arachidis* spore germination in vitro. Provision of breadfruit leaf extract with a concentration of 30% (k_3) was the best concentration in inhibiting *P. arachidis* fungus growth in vitro on PDA media, with a growth inhibition power of 60.74% and a percentage of inhibition of spore germination of 80.66%. Further research is suggested to determine the effect of directly applying breadfruit leaf extract as a rust bio-fungicide on peanuts.

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