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Application of *Piper betle* L. leaf extract to control major diseases of peanut (*Arachis hypogaea* L.)

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Abstract
Peanut plants are susceptible to major fungal diseases, which significantly affect yield. This study evaluated the effects of *Piper betle* leaf extract on leaf spot (*Cercospora* sp.), rust (*Puccinia arachidis*), and stem rot (*Sclerotium rolfsii*) occurrence on peanut. The experiment was laid out in a Randomized Complete Block Design (RCBD) with seven treatments replicated three times. The treatments were: T_1 - Negative Control: Water, T_2 - Positive Control: Ridomil Gold, T_3 - 1 ml *Piper betle* Leaf Extract/L of Water, T_4 - 2 ml *Piper betle* Leaf Extract/L of Water, T_5 - 3 ml *Piper betle* Leaf Extract /L of Water. T_6 - 4 ml *Piper betle* Leaf Extract /L of Water, and T_7 - 5 ml *Piper betle* Leaf Extract /L of Water. Results revealed that the 5 ml *Piper betle* leaf extract per liter of water significantly reduced leaf spot and rust severity, though it was still less effective than the commercial fungicide. However, this extract had no significant effect on stem rot incidence. The study highlights the promising antifungal properties of *Piper betle* leaf extract as a natural alternative for disease management in peanut production.

Keywords: Disease severity, leaf spot, peanut, rust, *Piper betle*

Introduction
Peanut (*Arachis hypogaea* L.) is a legume of high nutritional and economic value, especially in developing countries like the Philippines. It serves as a great source of plant-based protein, containing essential minerals, antioxidants, and vitamins, and is also considered one of the major field legumes grown by local farmers. In 2022, the Philippines ranked among the top 48 global producers of peanuts, with 31.52 thousand metric tons nearly half from Cagayan Valley (Philippine Statistics Authority, 2023) [27]. Peanuts are rich in protein, healthy fats, and micronutrients, helping combat malnutrition in rural communities (Garcia & Reyes, 2019) [11]. Efforts to boost production include breeding disease-resistant varieties, promoting sustainable practices, and enhancing post-harvest handling (Cruz *et al*., 2023) [8]. Despite its potential, peanut production in the country faces challenges due to constraints such as pest infestations and fungal diseases, particularly leaf spot (*Cercospora sp.*), rust (*Puccinia arachidis*), and stem rot (*Sclerotium rolfsii*), which cause significant yield losses.
Rust, caused by the fungus *Puccinia arachidis* Speg., is a major disease affecting peanut crops worldwide. First reported in Brazil in 1920, it has since spread to key peanut-growing regions in Asia, Africa, and North America (Tsukiboshi, 2018) [36]. The fungus produces and disperses urediniospores, enabling rapid spread under favorable conditions (Mehan, 2020) [24]. In addition, rust is characterized by distinctive symptoms such as yellow-orange to dark brown rust pustules on the undersides of leaves, accompanied by chlorosis and premature defoliation (Chiteka *et al.*, 2017) [6].
Leaf spot is the most common peanut disease, known for causing severe defoliation and yield losses of over 75% if left unmanaged, but less than 5% with effective control (Kucharek, 2000) [19]. Early symptoms appear as small, round to reddish spots on older leaves. As the disease progresses, these spots increase in size and number under favorable conditions. Lesions develop yellow halos with necrotic centers and dark edges. Typically emerging late in the growing season, leaf spot can reduce yield by up to 40% (Narayana & Angamuthu, 2021) [25], and when defoliation reaches 50%, losses become significant and may exceed 50% if uncontrolled (Damicone, 2017) [9].
Southern blight, also known as white mold, southern stem rot, or Sclerotium rot, is caused by the fungus *Sclerotium rolfsii*. This widespread pathogen affects a broad range of hosts and thrives in high temperatures common to major peanut-growing regions. While typical yield losses are under 25%, severe infections can cause up to 80% loss (Backman & Kokalis-

Burelle, 1997; Punja, 1985) [2, 30]. Early symptoms include yellowing and wilting of the main stem, branches, or entire plant. White mycelium often appears near the soil line, and under warm, humid conditions, the fungus rapidly spreads to neighboring plants (Punja, 1985) [30].

The traditional management of diseases usually depends on chemical fungicides. They are useful, but highly toxic to the environment, costly for farmers, and can cause pathogen resistance. As a reaction to the growing demand for sustainability in agriculture, there is a pressing need to identify eco-friendly alternatives that are efficient and economically viable for farmers.

In the Philippines, traditional medicine is one of the primary healthcare systems. Natural products of plants may give a new source of antibacterial and anti-fungal agents with possible novel mechanisms of action (Bhalodia & Shukla, 2011) [3]. Plant extracts are widely claimed to have a broad-spectrum antibacterial and are considered a main source of lead compounds (Hsouna & Hamdi, 2012) [16].

Extracted plants contain a wide range of biochemical substances having anti-fungal properties. Their utilization is less detrimental to the environment. Various plant extracts have been used instead of synthetic chemical substances to enhance the growth and yield of many crops (Rioba & Stevenson, 2017) [31]. *Piper betle* L., or betel vine, is a medicinal plant from the *Piper*aceae family, widely used in Asia. Known locally as *buyo*, *gaoed*, *gaued*, and *ikmo*, it is a twining, slightly woody vine likely native to Malaysia and cultivated in India and the Philippines (Concha, 1982) [7]. It has simple, ovate leaves with a tar-like odor and pungent taste, and produces male and red, fleshy female spikes (Philippine Pharmacopeia, 2004) [28]. Studies have shown that betel leaves, roots, and extracts have strong antimicrobial properties (Jenie *et al*., 2001) [18]. Besides health benefits, *P. betle* is also used in agriculture to promote plant growth. *Piper betle*, locally known as "buyo," has long been recognized for its antimicrobial properties. Betel leaf extract contains various active ingredients that have antimicrobial properties (Datta *et al*., 2011) [10], such as alkaloids, fatty acids, phenols, alcohol, flavonoids, terpenes, coumarin, and organic acids (Foo *et al*., 2015) [15]. Although it is widely used in traditional medicine, there have been relatively few studies on its potential for managing crop diseases.

This study was conducted to evaluate the incidence and severity of major diseases on peanut as affected by the application of different rates of *Piper betle* L. leaf extract. This research explores the use of *P. betle* leaf extract as an alternative to chemical fungicides, aiming to offer a safer and more sustainable option for managing peanut diseases.

2. Materials and Methods

The study was conducted at the Agricultural Experiment Center (AEC), Central Mindanao University, Musuan, Maramag, Bukidnon, from August to December 2024. The isolation and identification of pathogens associated with observed diseases on the test plants were done at the laboratory of the Department of Plant Pathology, College of Agriculture, CMU, Musuan, Bukidnon.

The materials used in the study were the following: peanut seeds (NSIC Pn11), leaves of *Piper betle*, fertilizer, bolo, meter sticks, plastic twine, ruler, marker, transparent folder, bamboo sticks, plain sheet, paint scissors, container, notebook, ballpen, and camera. Laboratory materials/apparatus include a weighing scale, glass slide, coverslip, scalpel, syringe, lactoglycerol, colorless nail polish, microscope, disease specimen, and blender.

The experiment was laid out in a Randomized Complete Block Design (RCBD) with seven treatments replicated three times. The plot measured 5 m x 3 m with seven plots representing the treatments and three blocks representing the replication.

These were the treatments of the study

- Treatment 1 - Negative Control (Water)
- Treatment 2 - Positive Control (Ridomil Gold)
- Treatment 3 - 1 ml *Piper betle* Leaf Extract/L of Water
- Treatment 4 - 2 ml *Piper betle* Leaf Extract/L of Water
- Treatment 5 - 3 ml *Piper betle* Leaf Extract/L of Water
- Treatment 6 - 4 ml *Piper betle* Leaf Extract/L of Water
- Treatment 7 - 5 ml *Piper betle* Leaf Extract/L of Water

To determine the amount of fertilizer to be administered, a composite soil sample from the experimental area was collected and submitted for analysis at the Soil and Plant Analysis Laboratory (SPAL), Department of Soil Science, College of Agriculture, Central Mindanao University, Musuan, Maramag, Bukidnon.

The experimental area of approximately 600 m^2 was plowed once and harrowed twice to eliminate weeds and crop residues in the field and to form a finer tilt. When the land preparation was completed, the area was divided into three blocks, which constitute the replication, and each block was further divided into seven plots, which constitute the treatments.

The planting of seeds was done manually. The seeds were sown at a rate of two seeds per hill, spaced at 10 cm between hills and 60 cm between rows. Based on the soil analysis, 185.71 g of 18-46-0 fertilizer was recommended by SPAL and applied three weeks after planting.

Fresh leaves of *P. betle* were collected from a local garden, washed with running water, surface sterilized with 10% NaOCl, and air dried. In the preparation of the extracts, 1 kg of the prepared leaves was macerated using a mortar and pestle to obtain the sap and filtered using a sterile muslin cloth. The filtrate was stored in a sterile glass container and kept for field application.

The rate of spray application was based on the desired treatment. This was applied at 14 days after planting (DAP) and at 14-day intervals thereafter. The application of treatments was terminated 14 days before harvest. The amount of fungicide applied was based on the manufacturer's recommended rate of application.

Plants were watered daily or as needed, depending on the soil type and weather conditions. The area was thoroughly weeded and kept clean to avoid nutrient competition. Hand weeding was done regularly to control the harmful weeds. Appropriate pesticides were applied at the time when insect pests were observed on the test plants. Peanut pods were harvested manually through direct hand pulling at 100 days when most of the leaves started to mature, and the interior of the pod was dark in color. The harvested pods from each treatment were placed separately in labeled plastic bags.

3. Data Gathered

3.1. Percent Disease Severity

3.1.1 Leaf Spot (*Cercospora arachidicola*)

Twenty randomly selected plants per plot were assessed for leaf spot based on the modified rating scale of Chiteka *et al.* (1988) [5]. The assessment was done following this rating scale:

Scale	Description
0	No disease
1	Very few lesions (none on the upper canopy)
2	Few lesions (very few on the upper canopy)
3	Some lesions have more on the upper canopy than for rank 2, and slight defoliation is noticeable.
4	Lesions are noticeable even on the upper canopy with noticeable defoliation.
5	Lesions are numerous and very evident on the upper canopy with significant defoliation (more than 50%)
6	Lesions are numerous on the upper canopy with much defoliation (more than 75%)
7	Upper canopy covered with lesions with high defoliation (more than 90%)
8	Very few leaves remaining, and those covered with lesions (some plants completely defoliated)
9	Plants are dead

The percent disease severity was computed using this formula:

$$\% \text{ Disease Severity} = \frac{0n_0 + 1n_1 + \ldots + 9n_9}{9N} \times 100$$

Where;

- $0n_0 + 1n_1 + 2n_2 + \ldots + 9n_9$ - Number of infected plants showing a scale of 0, 1, 2, …, and 9, respectively
- N - Total number of samples per plot
- 9 - Represents the highest scale

Rust (*Puccinia arachidis*)

Twenty randomly selected plants per plot were assessed for rust based on the modified rating scale by Subrahmanyam *et al.* (1995) as cited by Power *et al.* (2019) [29]. The assessment was done following this rating scale:

Scale	Description
0	No disease
1	Sparsely distributed lesions, primarily on lower leaves (1 to 5% severity)
2	Many lesions on the lower leaves with evident necrosis and very few lesions on the middle and upper leaves (6 to 10% severity)
3	Numerous lesions on the lower and middle leaves, with severe necrosis on the lower leaves (11 to 20% severity)
4	Severe necrosis of middle and lower leaves and fewer lesions on top leaves (21 to 30% severity)
5	Extensive damage to lower leaves, lesions densely present on middle leaves with necrosis, and lesions may be on top leaves as well (31 to 40% severity)
6	Severe damage to lower and middle leaves, and lesions are densely distributed on top leaves (41 to 60% severity)
7	100% damage to lower and middle leaves and lesions on top leaves with severe necrosis (61 to 80% severity)
8	Almost all leaves are withering, and bare stems are present (81 to 100% severity)

The percent disease severity was computed using this formula:

$$\% \text{ Disease Severity} = \frac{0n_0 + 1n_1 + \ldots + 8n_8}{8N} \times 100$$

Where:

- $0n_0 + 1n_1 + 2n_2 + \ldots + 8n_8$ - Number of infected plants showing a scale of 0, 1, 2, … and 8, respectively
- N - Total number of samples per plot
- 8 - Represents the highest scale

3.2. Percent Disease Incidence

Stem rot was assessed based on its characteristic symptoms and signs. The percent disease incidence was computed using the formula:

$$\% \text{ Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

3.3. Occurrence of Insect Pests

This was monitored and documented in the experimental area through visual inspection and sweep net sampling over the study period.

3.4. Meteorological Data

The data on rainfall was obtained from the record of the Department of Science and Technology - Philippine Atmospheric Geophysical and Astronomical Services Administration (DOST- PAGASA) of Central Mindanao University, Musuan, Maramag, Bukidnon.

3.5. Statistical Analysis

The data were analyzed using the analysis of variance (ANOVA). The difference between treatment means was determined using Tukey's HSD test at a 5% level of significance.

Results and Discussion

Percent Severity of Leaf Spot (Cercospora sp.) on Peanut (NSIC Pn11) Applied with *Piper betle* Leaf Extract

Table 1 shows the mean percent severity of leaf spot on peanuts at 30, 45, 60, and 75 days after planting (DAP). The distinctive symptoms of leaf spot on peanuts and conidiophores of the pathogen, *Cercospora* sp., are shown in Figures 1a and 1b, respectively. Analysis of variance revealed no significant difference in the percent severity of leaf spot among treatments at 30 and 45 DAP, a significant difference at 60 DAP, and a highly significant difference at 75 DAP.

At 30 DAP, the severity ranges from 7.22% in T_2 (Positive Control: Ridomil Gold) to 10.37% in T_1 (Negative Control: Water). At 45 DAP, the mean severity of leaf spot ranges from 8.89% in T_2 (Positive Control: Ridomil Gold) to 15.00% in T_4 (2 ml *Piper betle* Leaf Extract/L of Water) and T_5 (3 ml *Piper betle* Leaf Extract/L of Water).

At 60 DAP, T_2 (Positive Control: Ridomil Gold) had the lowest severity with a value of 21.11%, which was comparable to T_7 (5 ml *Piper betle* Leaf Extract/L of Water) with 30.00%, followed by T_1 (Negative Control: Water) and T_4 (2 ml *Piper betle* Leaf Extract/L of Water) with 30.37% and T_6 (4 ml *Piper betle* Leaf Extract/L of Water) with 32.59%. Meanwhile, T_3 (1 ml *Piper betle* Leaf Extract/L of Water) had the highest severity with a value of 30.93%, which is comparable with T_5 (3 ml *Piper betle* Leaf Extract/L of Water) with 30.74%.

At 75 DAP, the lowest severity was observed in T_2 (Positive Control: Ridomil Gold) with 34.07%, followed by T_7 (5 ml *Piper betle* Extract/L of Water) with 36.29%, T_5 (3 ml *Piper betle* Extract/L of Water) with 37.96% and T_6 (4 ml *Piper betle* Extract/L of Water) with 38.89%. The highest severity of leafspot was recorded in T_1 (Negative Control: Water) and T_3 (1 ml *Piper betle* Leaf Extract/L of Water) with 44.26%, but comparable to T_4 (2 ml *Piper betle* Leaf Extract/L of Water) with 44.07%.

Table 1: Mean percent severity of leaf spot (*Cercospora* sp.) on peanut (NSIC Pn11) applied with *Piper betle* leaf extract at 30, 45, 60, and 75 days after planting (DAP)

Treatment	Percent Leaf Spot Severity			
	30 DAP	**45 DAP**	**60 DAP**	**75 DAP**
T_1 - Negative Control (Water)	10.37	12.59	30.37^{a}	44.26^{a}
T_2 - Positive Control (Ridomil Gold)	7.22	8.89	21.11^{b}	34.07^{b}
T_3 - 1 ml *Piper betle* Leaf Extract/L of Water	9.63	12.41	30.93^{a}	44.26^{a}
T_4 - 2 ml *Piper betle* Leaf Extract/L of Water	8.33	15.00	30.37^{a}	44.07^{a}
T_5 - 3 ml *Piper betle* Leaf Extract/L of Water	8.15	15.00	30.74^{a}	37.96^{b}
T_6 - 4 ml *Piper betle* Leaf Extract/L of Water	8.15	10.06	32.59^{a}	38.89^{ab}
T_7 - 5 ml *Piper betle* Leaf Extract/L of Water	9.63	9.26	30.00^{ab}	36.29^{b}
F-test	ns	ns	*	**
CV (%)	23.10	35.39	10.81	5.28

Means in the column with the same letter are not significantly different at a 5% level of probability based on Tukey's HSD test.
* - significant, ** - highly significant, ns - Non-significant

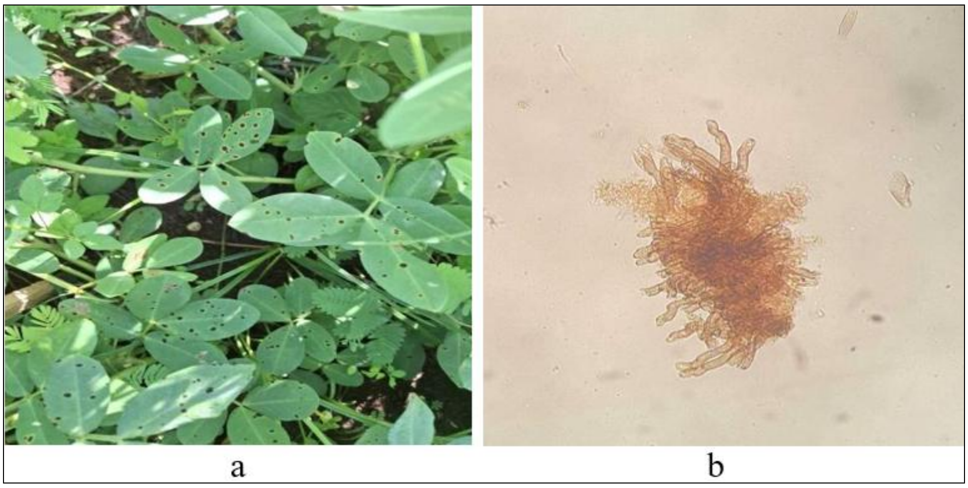

Fig 1: Symptoms of leaf spot on peanut (NSIC Pn11) at 75 days after planting (a) and the conidiophores of *Cercospora* sp. (400x) (b)

Piper betle leaf extract, particularly at higher concentrations, was found to reduce the severity of leaf spot in peanuts. While it may be less effective than Ridomil Gold, it offers a promising, eco-friendly alternative for disease management. Fazal *et al*. (2014) [14] reported that betel extract has antifungal properties, attributed to compounds such as chavicol, chavibetol, acetate, and hydroxychavicol, and showed effectiveness against *Aspergillus parasiticus*, *Trichophyton mentagrophyte*, and *Candida albicans*.

Percent Severity of Rust (Puccinia arachidis) on Peanut (NSIC Pn11) Applied with *Piper betle* Leaf Extract

Table 2 presents the mean percent severity of rust at 60 and 75 days after planting (DAP). Common symptoms include yellow-orange to dark brown rust pustules on the undersides of leaves, chlorosis, and early leaf drop. Figures 2a and 2b illustrate these symptoms and the uredospores of *Puccinia arachidis*, the causal agent.

At 60 DAP, the rust severity ranges from 14.07% in T_2 (Positive Control: Ridomil Gold) to 19.44% in T_1 (Negative Control: Water).

At 75 DAP, the lowest rust severity was recorded in T_2 (Positive Control: Ridomil Gold) with 25.74%, followed by T_7 (5 ml *Piper betle* Leaf Extract/L of Water) with 35.00%, T_6 (4 ml *Piper betle* Extract/L of Water) with 36.11% and T_4 (2 ml *Piper betle* Leaf Extract/L of Water) with 37.96%. The highest rust severity was recorded in T_1 (Negative Control: Water) with 43, 70%, followed by T_3 (1 ml *Piper betle* Leaf Extract/L of Water) with 39.07%, but comparable to T_5 (3 ml *Piper betle* Extract/L of Water) with 38.52%. According to Datta *et al*. (2011) [10], betel leaf extract contains various active ingredients that have antimicrobial properties, such as alkaloids, fatty acids, phenols, alcohol, flavonoids, terpenes, coumarin, and organic acids (Foo *et al*., 2015) [15].

Percent Incidence of Stem Rot (Sclerotium rolfsii) on Peanut (NSIC Pn11) Applied with *Piper betle* Leaf Extract: The mean percent incidence of stem rot on peanuts applied with *Piper betle* leaf extract at harvest is presented in Table 3. The symptoms of stem rot include the yellowing and wilting of the main stem, lateral branches, or the entire plant. White mycelium was observed at the base of the plant near the soil line (Figure 3).

A non-significant difference among treatments was observed on the percent wilting, with values that range from 20.00% in T_2 (Positive Control: Ridomil Gold) to 33.33% in T_5 (3 ml *Piper betle* Leaf Extract/L of Water).

Table 2: Mean percent severity of rust (*Puccinia arachidis)* on peanut (NSIC Pn11) applied with *Piper betle* leaf extract at 60 and 75 days after planting (DAP)

Treatment	Percent Rust Severity (60 DAP)	Percent Rust Severity (75 DAP)
T_1 - Negative Control (Water)	19.44	43.70^{a}
T_2 - Positive Control (Ridomil Gold)	14.07	25.74^{c}
T_3 - 1 ml *Piper betle* Leaf Extract/L of Water	17.78	39.07^{ab}
T_4 - 2 ml *Piper betle* Leaf Extract/L of Water	18.52	37.96^{ab}
T_5 - 3 ml *Piper betle* Leaf Extract/L of Water	17.22	38.52^{ab}
T_6 - 4 ml *Piper betle* Leaf Extract/L of Water	16.85	36.11^{ab}
T_7 - 5 ml *Piper betle* Leaf Extract/L of Water	15.00	35.00^{b}
F-test	ns	**
CV (%)	11.26	8.18

Means in the column with the same letter are not significantly different at a 5% level of probability based on Tukey's HSD test.
** - highly significant, ns - Non-significant

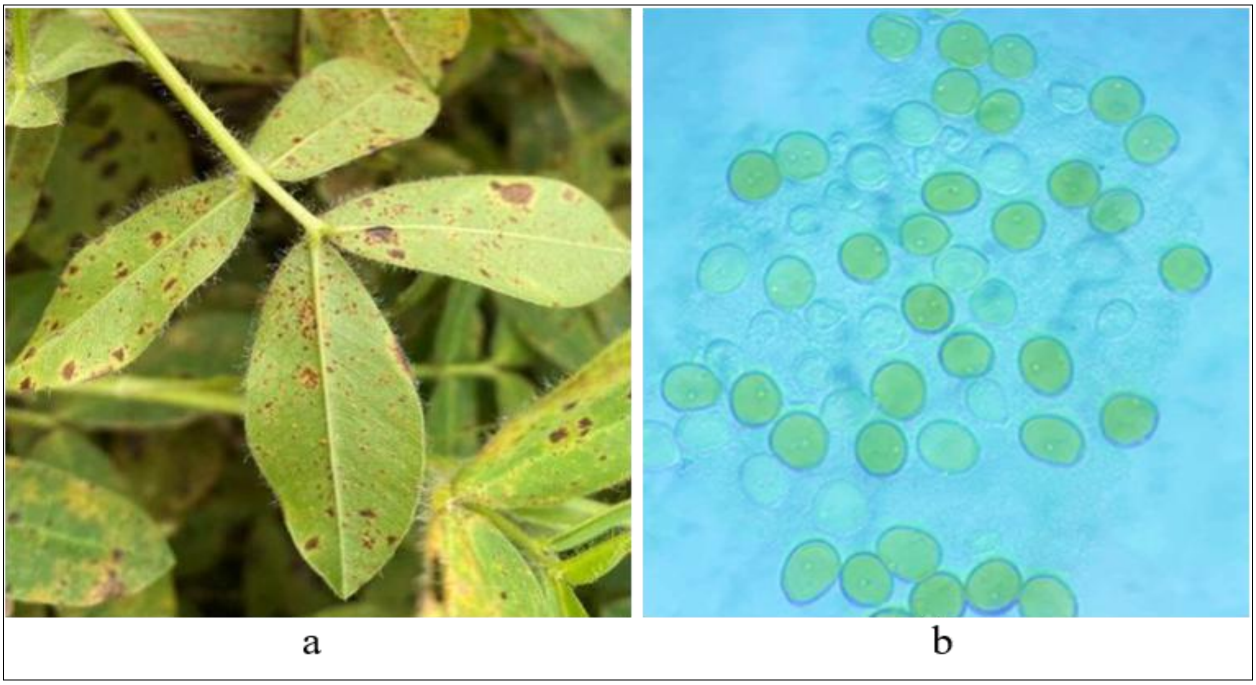

Fig 2: Symptoms of rust on peanut (NSIC Pn11) at 75 days after planting (a) and the uredospores of *Puccinia arachidis* (100x) (b)

Table 3: Mean percent incidence of stem rot (*Sclerotium rolfsii*) on peanut (NSIC Pn11) applied with *Piper betle* leaf extract

Treatment	Percent stem rot incidence
T_1 - Negative Control (Water)	28.33
T_2 - Positive Control (Ridomil Gold)	20.00
T_3 - 1 ml *Piper betle* Leaf Extract/L of Water	28.33
T_4 - 2 ml *Piper betle* Leaf Extract/L of Water	31.67
T_5 - 3 ml *Piper betle* Leaf Extract/L of Water	33.33
T_6 - 4 ml *Piper betle* Leaf Extract/L of Water	28.33
T_7 - 5 ml *Piper betle* Leaf Extract/L of Water	25.00
F-test	ns
CV (%)	18.92

ns - Non-significant

Fig 3: Symptoms of stem rot (*Sclerotium rolfsii*) on peanut (NSIC Pn11)

Occurrence of Insect Pests on Peanut (NSIC Pn11) Applied with *Piper betle* Leaf Extract

During the data collection period, the insects observed in the experimental area were semi-looper, particularly the Litter Moth Caterpillar (*Chrysodeixis sp.*) and lady beetles (*Cheilomenes sp.*), both of which were actively feeding on peanut leaves (Figure 4).

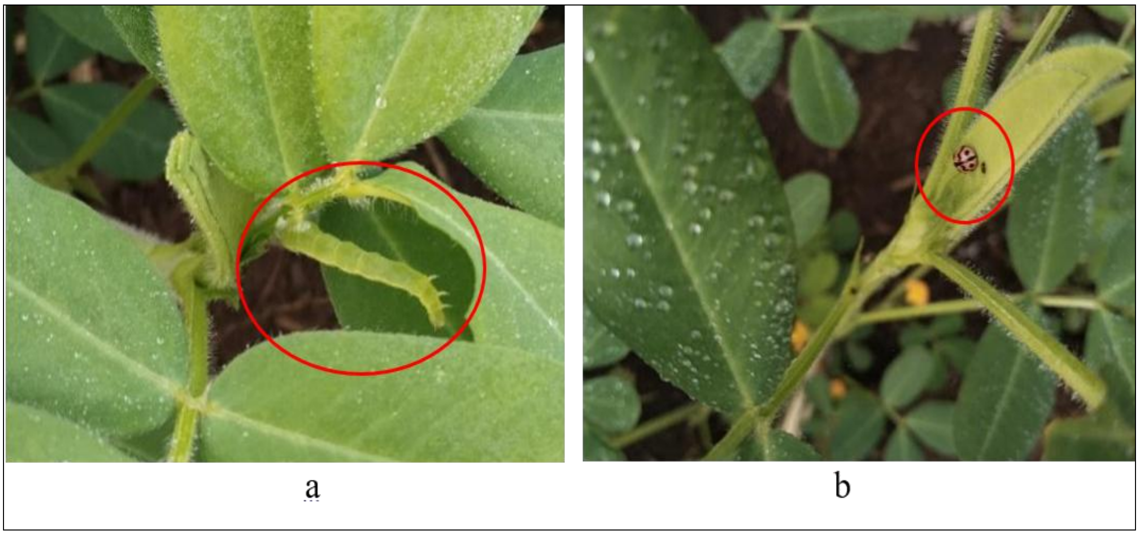

Fig 4: Occurrence of insect pests on peanut (NSIC Pn11): semi-looper caterpillar (a) and lady beetle (b)

Conclusion

Based on the results, it can be concluded that *P. betle* leaf extract has potential in managing peanut diseases, particularly leaf spot (*Cercospora* sp.) and rust (*Puccinia arachidis*), though its efficacy remains lower than that of the commercial fungicide, Ridomil Gold. Higher concentrations of *Piper betle* extract showed a notable reduction in disease severity, suggesting antifungal properties that could help control infections while offering an environmentally friendly approach.

Future researchers are encouraged to use or increase the concentration of *Piper betle* leaf extract, calibrate the treatment per plot before application to ensure uniformity, and measure the seed weight per pod in each plot for precise data collection. Additionally, it is recommended to test *Piper betle* leaf extract on other crops, particularly those with different characteristics or disease susceptibilities, to assess its potential effectiveness in various agricultural contexts.

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