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Antifungal Properties of *Phyllanthus amarus* Against *Phytophthora infestans* Disease of Tomato (*Solanum Lycopersicum*)

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Abstract: *Phytophthora infestans is a pathogenic fungus known for its destructive characteristic against tomato and potato plants. The pathogen is notorious for its mutative ability against very effective antifungal compounds. This research, therefore, focused on the possible effects of aqueous leaf extract of Phyllanthus amarus (P. amarus) in the treatment of the disease. Tomatoes were planted in nursery beds and transplanted into sacks. The experimental treatment consisted Completely Randomized Design (CRD), with six treatments and 3 replicates, which include normal control (plants not inoculated with Phytophthora infestans), untreated control (infested plants not being treated), 0.2 mg/ml extract (infested plants were treated with 0.2 mg/ml of P. amarus extract), 0.4 mg/ml extract (infested plants were treated with 0.4 mg/ml of P. amarus), 0.6 mg/ml extract (infested plants were treated with 0.6 mg/ml of P. amarus) and 0.2 mg/100 ml mancozeb (infested plants were treated with 0.2 mg/ml of mancozeb standard drug). Phytochemical analysis of P. amarus was conducted. The phytochemical analysis result showed the presence of alkaloids, tannins, saponins, flavonoids, steroids, terpenoids and anthraquinones. Application of P. amarus expressed significant effects ($P < 0.05$) on the growth parameters, with distinct remediation occurring in 0.6 mg/mL in the plant height (36.04±3.29 cm), leaf count (160.00±25.33), stem girth (8.68±0.56 cm) and fruit count (5.85±0.79). The yield parameter showed significant increase in the yield weight of 0.2 mg/ml (323.67 g), 0.4 mg/ml (339 g) and 0.6 mg/ml (375.33 g) extract when compared with the untreated control (212.8 g). The aqueous leaf extract of P. amarus expressed promising antifungal effect against Phytophthora infestans disease of tomato, especially at 0.6 g/ml application rate.*

Keywords: *Antifungal, Mancozeb, Phyllanthus amarus, Phytophthora infestans, Solanum lycopersicum, Tomato.*

Introduction: Tomato (*Solanum lycopersicum*) is one of the most widely cultivated crops globally due to its nutritional, economic, and industrial importance. Tomato serves as an abundant source of vitamins, antioxidants, lycopenes and dietary fiber, making it an essential component of human diets (Ali, Sina, Khandker, Neesa, Tanvir, Kabir, Khalil and Gan, 2020). In addition to their nutritional value, tomatoes generate income for millions of smallholder farmers and contribute significantly to the gross domestic product (GDP) of several agricultural economies, particularly in developing nations (Singh, Kumar and Patel, 2022). Despite its significance, tomato production is severely hampered by diseases, with late blight caused by *Phytophthora infestans* (*P. infestans*) being the most devastating (Arafa, Moussa, Soliman, Shirasawa, Kamel and Rakha, 2017). Late blight disease, caused by *P. infestans*, is known to be notorious for its devastating effects on tomato crops (Jehani, Mohamed, Cheemala, Nath, Chonzik and Srivastava, 2025). The pathogen responsible, *Phytophthora infestans* meaning "plant destroyer" in Greek-originates from the Andean region, which is also where tomatoes and potatoes are believed to have originated (Jehani *et al*., 2025). The organism is a water mold that spreads rapidly in cool, moist conditions, producing spores that can travel through wind or water, infecting plant tissues and causing rapid decay. This pathogen has led to significant yield losses worldwide, as it has the tendency to affect the leaf, tuber and

the stem of plants which can result in 100% yield loss (Singh and Mer, 2023).

Tomatoes are a critical crop for food security and economic stability in many countries. However, late blight disease caused by *P. infestans* poses a significant threat to tomato production, leading to substantial yield losses annually (Kesho and Tadesse, 2023). Late blight is a fast-spreading disease that thrives under cool, humid conditions. The pathogen is known for its adaptability and resistance to conventional control methods, including chemical fungicides. Overuse of synthetic chemicals not only increases production costs but also poses risks to the environment and human health (Haverkort, Boonekamp, Hutten, Jacobsen, Lotz, Kessel, Visser and van der Vossen, 2016). The reliance on chemical fungicides to manage this disease has led to several challenges, including development of fungicide-resistant strains of *P. infestans* (Ivanov, Ukladov and Golubeva, 2021), negative environmental and health impacts associated with the overuse of synthetic chemicals (Choudhary, Goswami, Singh and Chakdar, 2018) and high production costs, which are unsustainable for smallholder farmers. Consequently, there is a pressing need for sustainable and eco-friendly solutions.

Plants with natural antifungal properties have garnered attention for their potential to combat agricultural pathogens. Phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids have demonstrated antimicrobial and antiviral activities against a range of pathogens (Balkrishna, Reddy and Kumar, 2020). *Phyllanthus amarus* (*P. amarus*) is a notable plant for its bioactive compounds with antifungal properties (Balkrishna *et al*., 2020). *P. amarus* is a member of the family *Euphorbiaceae*. It is known with many common names such as "carry me seeds", "stone breaker", "gala of wind", "bhumi amla" and "jangli amli" (Deora, Nehra and Sharma, 2021). The extract of the plant has been reported to express broad range of pharmacological activities such as antioxidant effect, antiviral effect, antimicrobial effect, antidiabetic effect and hepatoprotective effect (Atampugbire, Adomako and Quaye, 2024). The plant has been extensively studied for its medicinal application, but the specific interactions with *P. infestans* of tomato remains under-explored. This research, therefore focused on the phytochemical constituents and antifungal activity of aqueous extract of *P. amarus* against *P. infestans* of tomato. Late blight disease remains a critical challenge in tomato production, threatening food security and the livelihoods of farmers. The reliance on chemical fungicides to manage this disease has led to several challenges, including: development of fungicide-resistant strains of *P. infestans* (Ivanov *et al*., 2021), negative environmental and health impacts associated with the overuse of synthetic chemicals (Choudhary *et al*., 2018) and high production costs, which are unsustainable for smallholder farmers. The search for sustainable, eco-friendly alternatives is imperative. Many plant extracts and natural products have showng promising effects as alternatives towards management of plant diseases due to their biodegradability, low toxicity and their antimicrobial activity (Alok, Raghuvanshi, Dhiman, Kumar, Sharma, Yeliya and Ramachandran, 2025). *P. amarus* is widely available, affordable, and have been reported for its antifungal activity through ethnobotanical survey. Exploring their potentials against *P. infestans* could provide a cost-effective and sustainable solution for managing late blight disease of tomato.

Materials and Methods: Material : The materials used for this research include tomato seeds, planting materials and tools such as wheel barrow, used cement bags, soil sample, shovel, organic manure, hand trowel which were gotten from CPT Department, Federal College of Forestry, Jos. The leaves of *P. amarus* was obtained from Sherri Hills, Jos East Local Government Area of Plateau State. Chemicals and reagents such as H_2SO_4, dilute NH_3, chloroform, $FeCl_3$, NH_4OH, ninhydrin etc were gotten from Sigma Chemical Company, St. Louis, Mo, USA.

Collection of Plant Samples: The fresh leaves of *P. amarus* was harvested from Sherri Hill, Jos South Local Government Area of Plateau State. The plant sample was immediately taken to the herbarium of Federal College of Forestry, Jos for identification and authentication where a voucher number (FHJ857) was obtained.

Plant Sample Preparation: The dried sample was turned into powdered form using electric blender (Model: CB8231-O) until evenly distributed finely divided powder was obtained. Exactly 500 g of the powdered sample was (percolated) in 500 mL of ethanol (1:1) for 24 hours. The sample was then filtered with muslin clothe and then with Whatman No.1 filter paper to collect the liquid filtrate which was concentrated using waterbath (Model: H H-42D PEC Medical, USA) at 35 ^{0}C to obtain the crude extract.

Method: Plant Extraction : The *P. amarus* plant sample was washed under running tap water to remove dirts. They were then drained-off water before spreading them thinly on the slab in Chemistry Laboratory for proper drying. The dried sample was pulverized to obtain finely divided powdered sample. About 500 g of the powdered sample was weighed and percolated in 1000 mL of distilled water for 24 hours after which it was filtered. The filtrate was freeze dried using lyovilizer (Model: BK-FD12S) to obtain the crude extract, which was later kept in a refrigerator until when needed.

Phytochemical Analysis: Phytochemical analysis of the plant extract was carried out using the methods of Trease & Evans (1989), Sofowora (1993) and Harborne (1973). The phytochemical screening conducted on the plant sample include flavonoids, tannins, terpenoids, coumarins, proteins, saponins, cardiac glycosides, phenols, carbohydrates, anthraquinones, phlobatannins, reducing sugar and steroids.

Collection, Identification and Inoculation of *P. infestans*: Isolation of the fungal pathogen was done following the method described by Shabiu, Dangora, Kutama, Bello, Zakari, Musa and Dahiru (2023) with some modifications. The pure culture of the pathogen was obtained from the Department of Plant Science and Technology, University of Jos. The pathogen was sub-cultured using the method of Subhani, Sahi, Hussain, Munir, Abbas and Bilal, (2014) with

Potato Dextrose Agar (39 g PDA in 1 litre of distilled water). It was then inoculated onto the plants (except the control) by weighing 0.1 g of the cultured pathogen and dissolving it in 100 ml normal saline. Exactly 5 ml was sprayed on all the plants (except for the normal control) and injected at the leaf petiole and the stems, three weeks after transplanting. The plants were allowed until manifestation of symptoms such as water-soaked leaves, blackish lesions on the stems, blackish leaves and withering of plants before spraying of the *P. amarus* extract.

Experimental Design and Treatment: The experimental design consisted of Completely Randomized Design (CRD) with six (6) treatment, replicated three times. The treatments include normal control (T_0) in which the plants were not inoculated with pathogen. Untreated control (T_1), plants were inoculated with *P. infestans* but were not treated. Treatment three (0.2 mg/ml extract) have the plants infested with *P. infestans* pathogen and treated with 0.2 mg/ml of *P. amarus* extract. Treatment four (0.4 mg/ml extract), plants were inoculated with *P. infestans* and treated with 0.4 mg/ml of *P. amarus* extract, while 0.6 mg/ml group plants were inoculated with *P. infestans* treated with 0.6 mg/ml of *P. amarus* extract. The last treatment, the plants were inoculated with the *P. infestans* and treated with 0.2 mg/ml mancozeb standard drug.

Planting Operation: A 2 m by 2 m nursery bed was made with the soil properly loosened and organic manure evenly applied and allowed to decompose. Tomato seeds were planted on the bed by broadcasting and germinated seedling were transplanted 3 weeks after germination. The pathogen was inoculated to the plants 3 weeks after transplanting and the management of the disease commenced one week after spraying (at the onset of browning of leaves with stem dark spots) by spraying the plants with various concentrations of the *P. amarus* extract every 5 days. Data collected include plant height, leaf count, stem girth, fruit count and yield weight.

Statistical Analysis: The data collected were subjected to statistical analysis, making use of Statistical Package for Social Sciences (IBM SPSS) Version 25. The data were analyzed using One Way Analysis of Variance (ANOVA) in a Complete Randomized Design (CRD). The means were separated for their level of significant difference using Duncan Multiple Range Test (DMRT) at 95% confidence interval. All graphs were plotted with the use of GraphPad Prism, Version 10 (GraphPad Software, California, United States).

Results and Discussion: Results: Phytochemical Constituents: Table 1 shows the various phytochemical constituents in the ethanol leaf extract of *P. amarus*. Phenolics occurred to be the most abundant, followed by alkaloids, cardiac glycosides, flavonoids, steroids and terpenoids. Tannins, saponins, triterpenes and anthraquinones occurred in trace quantity. Amino acids and phlobatanins were observed to be absent (Table 1).

Growth Parameters: The treatment effect of *P. amarus* on the growth parameters of *P. infestans*-infested tomatoes is presented in Table 2. The result showed that significant reduction ($p < 0.05$) occurred in the mean plant height of untreated control, relative to normal control, due to the induction of *P. infestans* disease. However, significant increase ($p > 0.05$) was observed in the plant height of the 0.4 mg/ml, 0.6 mg/ml and 0.2 mg/100 ml (mancozeb), in comparison to the untreated control. In addition, treatment effect of *P. amarus* restored the plant height of the 0.4 mg/ml (extract) and 0.2 mg/100 ml (mancozeb) to the range of the normal control group. *P. infestans* effect on the inoculated tomatoes reflected on the significant decrease ($p < 0.05$) in the leaf count of untreated control, in comparison to normal control. On the other hand, significant increase ($p > 0.05$) occurred in the leaf count of treatment 0.4 mg/ml and 0.2 mg/100 ml mancozeb group, compared to the untreated control after their treatment with ethanol leaf extract of *P. amarus*. The effect of *P. infestans* late blight disease of tomato expressed significant reduction ($p < 0.05$) in the stem girth and fruit count of the untreated control with mean values of 5.60±0.39 and 1.72±0.20 for stem girth and fruit count respectively (Table 2). However, treatment of the pathogen with methanol crude extract of *P. amarus* expressed significant increase ($p > 0.05$) in the stem girth and fruit count of 0.4, 0.6 mg/ml extract and the 0.2 mg/100 ml mancozeb standard drug. Treatments 0.4, 0.6 mg/ml and 0.2 mg/100 ml mancozeb expressed mean values of 8.15±0.45, 8.68±0.56, 8.73±0.59 and 4.91±0.58, 5.85±0.79 and 6.07±0.96 for the stem girth and fruit count respectively.

Yield Weight (g): Figure 1 displays the yield of tomatoes after pathogen infestation and treatment with *P. amarus* extract. The result showed that significant reduction ($p < 0.05$) occurred in the yield of the untreated control in comparison with the normal control. Also, the application of 0.2 mg/ml, 0.4 mg/ml and 0.6 mg/ml of *P. amarus* significantly increased the yield of the tomatoes when compared with the untreated control (Figure 1). The application of the extract at 0.6 mg/ml and that of 0.2 mg/100 ml mancozeb reverted the yield back to the normal control range (Figure 1).

Discussion: Tomato is one of the most important crops, especially in Africa as all parts of the plant serve nutritional or medicinal functions (Ali *et al*., 2020). In Sub-Saharan Africa (SSA), tomato is extensively grown as a food and cash crop, and contributes significantly to nutrition, employment, and income generation (Malherbe and Marai, 2015). According to Shabiu *et al*. (2023), fungal diseases are the major threat and limiting factor in the production of economic crops, leading to tremendous yield loss annually. To combat this threat, there is the need for disease control measure that are effective and environmentally friendly for maximum crop growth and yield. Biological control methods for the control of *Phytophthora infestans*, such as the use of biocontrol agents and plant extracts, hold promise for environmentally friendly disease suppression (Jehani *et al*., 2025). This study therefore focused on the use of *Phyllanthus amarus*, a plant known for various medicinal functions for the control of late blight disease of tomatoes. The presence of some phytochemicals in the aqueous leaf extract of *Phyllanthus amarus* might have contributed to the

various bioactivity and medicinal functions expressed by this plant. Saponins, alkaloids, terpenoids and flavonoids have been reported for their insecticidal activity (Gindaba, Negeri, Abdisa, Nemo and Kitila, 2024). This result is similar to the previous research works that stated that terpenoids (Khwaza and Aderibigbe, 2023), phenols, alkaloids (Chaudhary *et al*., 2018) and flavonoids and phenols could serve as antifungal activity against *Phytophthora infestans*. Muhaimin, Harizon and Chaerunisaa, (2025) reported the antifungal activity of three antifungal plants, namely *Cassia torosa*, *Cassia javanica* and *Cassia fistula* L. against *Fusarium oxysporum* and *Rhizhoctonia solani* through the presence of isolated flavonoids and anthraquinones isolated from the plants. These two phytochemicals were also identified to be present in the ethanol leaf extract of *P. amarus*, indicating their possible contribution to the antifungal activity of this plant. Furthermore, wild *Origanum elongatum* extracts was reported to have acted as biopesticide in controlling *P. infestans* and potentially mitigating its devastating impact Hari, Echchgadda, Darkaoui, Taarji, Sahri, Sobeh, Ezrari, Laasli, Benjelloun and Lahlali, 2024).

The reduction of the growth parameter as observed in the leaf count, plant height and stem girth of the untreated group might have been due to the effect of the infestation of late blight (*P. infestans*) disease on the tomato plant. This is not different from the published works of Ndala, Mbega and Ndakidemi, 2019); Shobhita, Singh and Musheer, 2025) who reported similar incidences in their studies. The amelioration of the stunted growth and yield parameters as observed in the various treatments, especially in the 0.4 mg/ml and 0.6 mg/ml might have been due to the medicinal properties of the plant extract. *P. amarus* is reported to contain various organic compounds, such as lignans, flavonoids, alkaloids, hydrolyzable tannins (Ellagitannins), polyphenols, triterpenes, sterols, and volatile oil (Ghosh, Banerjee and Chattopadhyay, 2022). Various phyto-constituents have been reported to be present in the leaf of *Phyllanthus amarus* (Achikanu, Ujah and Ezenwali, 2022; Omoregie, Uchenna, Enigbokan and Olude, 2020) and they might have impacted in the antifungal activity of the plant extract. Furthermore, the enhanced growth and yield parameters exhibited by the plant could also be due to the ability of the plant to serve as a bio-stimulant, thereby promoting shoot growth and stem girth expansion, ultimately leading to improved yield. This is evidenced in the reported bio-stimulant characteristics of *Phyllanthus niruri*, *Moringa oleifera*, *Aegel marmelos* and *Terminalia arjuna* extracts, through enhancement of seed germination, seedling vigor and maize plant growth (Bhowmich, Rai, Mishra, Bisht and Chauhan, 2024). Jena and Topno (2023) also reported the stimulatory activity of seed weed at the rate of 4 ml/L in the enhancement of plant height, number of leaves, number of flowers, seed yield per plant and reduced days taken for first flowering of pansy plant. In addition, Nahak and Kanta (2017) made mention of the effect of marigold flower to stimulate the shoot length, number of leaves, number of flowers and fruit count of tomatoes after its effect in reducing canker disease by 62.82% and reduction of early blight disease of tomato by 61.53%.

Conclusion & Recommendation: Conclusively, the aqueous leaf extract of *Phyllanthus amarus* expressed insightful information about its potential efficacy against late blight (*Phytophthora infestans*) disease of tomatoes. The extract of *Phyllanthus amarus* was able to successfully ameliorate the effect of stunting growth in terms of leaf count, plant height and stem girth deterioration of the tomato plants as well as under-development of tomato yield, probably owing to the various phytochemicals contained in the extract. Consequently, *Phyllanthus amarus* expressed the presence of various phytochemicals which might have contributed to the anti-fungal effect of the plant against *Phytophthora infestans* of tomatoes. *Phyllanthus amarus* ameliorated the stunting growth of tomatoes, caused by *Phytophthora infestans* infection, in a concentration dependent manner. The best application rate for the treatment of *Phytophthora infestans* disease of tomatoes is 0.4 g mL as it is the minimum effective dose. The effect of other antifungal plants, in their single entities and their possible synergistic effects against *Phytophthora infestans* should be investigated.

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Table 1: Phytochemical Constituents of *Phyllanthus amarus* Leaf Extract

S/N	Phytochemicals	Inference
1.	Alkaloids	++
2.	Tannins	+
3.	Phenolics	+++
4.	Cardiac glycosides	++
5.	Saponins	+
6.	Flavonoids	++
7.	Steroids	++
8.	Phlobatanins	-
11.	Terpenoids	++
12.	Amino acids	-
13.	Anthraquinones	+

Key: "+++" = highly present; "++" = moderately present; "+" = sparingly present; "-" = Not present

Table 2: Effect of *P. amarus* Treatment of Late Blight-Infested Tomatoes on Plant Height (cm), Leaf Count, Stem Girth (cm) and Fruit Count

Treatments	Plant Height (cm)	Leaf Count	Stem Girth (cm)	Fruit Count
Normal control	36.20 ± 3.28^{a}	158.67 ± 26.4^{a}	8.56 ± 0.51^{a}	7.09 ± 0.91^{a}
Untreated Control	19.79 ± 1.91^{c}	86.73 ± 11.39^{b}	5.60 ± 0.39^{b}	1.72 ± 0.20^{b}
0.2 mg/ml *P. amarus* extract	22.77 ± 1.13^{bc}	113.47 ± 3.28^{ab}	7.20 ± 0.53^{ab}	3.85 ± 0.10^{ab}
0.4 mg/ml *P. amarus* extract	33.92 ± 2.83^{ab}	141.20 ± 18.83^{ab}	8.15 ± 0.45^{a}	4.91 ± 0.58^{a}
0.6 g/ml *P. amarus* extract	36.04 ± 3.29^{a}	160.00 ± 25.33^{a}	8.68 ± 0.56^{a}	5.85 ± 0.79^{a}
0.2 mg/100 ml mancozeb	36.98 ± 3.74^{a}	159.33 ± 26.20^{a}	8.73 ± 0.59^{a}	6.07 ± 0.96^{a}

All means are expressed as mean (±) standard deviation of 5 replicates. Means within the same column, having different superscript alphabets are significantly different ($p \leq 0.05$) from each other

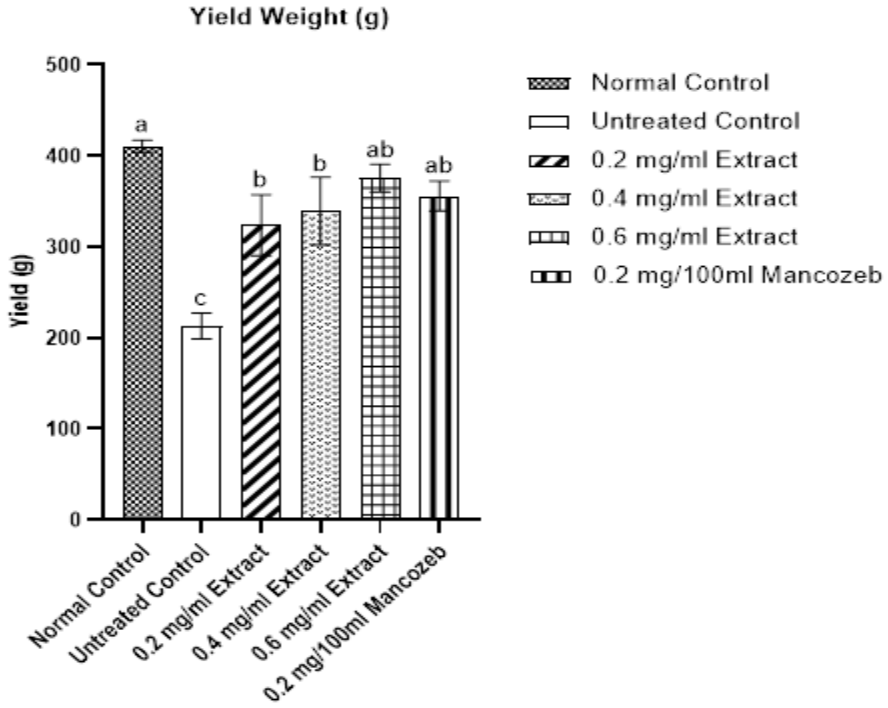

Figure 1: Yield of Tomatoes Infested with *P. infestans* and Treated with *P. amarus* Leaf Extract