

In Vitro Antifungal Activity Of Selected Plant Extracts Against Mango Stem End Rot Pathogen: *Lasiodiplodia Theobromae*

Jonathan F.T.^{1*} and Mahendranathan C.¹

Department of Botany, Faculty of Science, Eastern University of Sri Lanka

Abstract

Phytopathogens are primarily controlled through the application of synthetic products, which harm human and environmental health. The use of plant-derived extracts as eco-friendly bioagents has become increasingly popular due to the biologically active compounds present in the plants. Thus, a study was conducted to determine the antifungal activity of the ethanol and acetone leaf extracts of *Argyrea pomacea*, *Coccinia grandis*, *Erythrina subumbrans*, and *Gymnema sylvestre* against the causal pathogen of mango stem-end rot. Pathogen was isolated from diseased mangoes and identified as *Lasiodiplodia theobromae*. The pathogenicity of the isolated *L. theobromae* was confirmed on healthy samples of mangoes. The antifungal activity of leaf extracts was determined by the food poison technique. The leaf extracts were tested at different concentrations of 50, 25, and 12.5 mg/ml. Results indicated that the ethanol extract of *E. subumbrans* (29.21 ± 0.65 %) at the concentration of 50 mg/ml showed the highest percentage of mycelial growth inhibition followed by the acetone extract of *G. sylvestre* (28.97 ± 0.79 %) at the concentration of 25 mg/ml against the *L. theobromae*. The percentage of mycelial growth inhibition was significantly different among all treatments ($P < 0.05$). This might be due to the different kinds of phytochemicals in the plant leaves. Further studies are required to isolate and identify the specific bioactive compounds from the leaves, responsible for these antifungal activities.

Keywords: Antifungal activity, Eco-friendly approach, *Lasiodiplodia theobromae*, Leaf extract

Introduction

Medicinal plants have potential natural sources of active compounds against phytopathogens which can be used as botanicals for managing plant diseases sustainably and ecofriendly (Topolovec-Pintaric, 2020). Plant extracts possess antimicrobial properties due to their biologically-active compounds. The use of these natural products not only extends the shelf life but also maintains the microbiological stability and safety of the agricultural produce (Rafiullah Khan *et al.*, 2020). The bioactive compounds found in them are responsible for their antimicrobial effect

(Vijayaraj *et al.*, 2022). So, these compounds could be potential alternatives to the conventional chemical control of phytopathogens. Natural bioactive compounds used in plant protection kill pathogens or limit their development, as well as induce plant defense reactions as elicitors (Righini *et al.*, 2021). Plant extracts offer advantages in terms of low production costs, low toxicity, and good biodegradability.

Postharvest loss of mango in Sri Lanka is estimated to be 30 – 40 % (Karunanayake *et al.*, 2014). Stem-end

¹Department of Botany, Faculty of Science, Eastern University of Sri Lanka

rot and anthracnose are the two major postharvest diseases of mango, which reduce the fruit quality, shelf life and marketability (Krishnapillai and Wijeratnam, 2012). The availability of fresh mango for local and international consumers has been limited by its highly perishable nature and its susceptibility to postharvest diseases. Wang *et al.* (2021) stated that the use of chemical fungicides increased the expression of resistance genes of *Lasiodiplodia* spp. which mostly caused stem-end rot in mango. So, these postharvest diseases cause major economic losses to the producers and limit the market availability.

Farmers have gradually increased their indiscriminate use of chemical pesticides to reduce postharvest losses by lessening the negative effects that various phyto-pathogens have on plants. But pesticide poisoning poses a growing concern for public health, resulting in approximately 300,000 fatalities annually across the globe. Even in the absence of detectable levels of pesticides, the human body experiences increased DNA damage when exposed to these substances (Kapeleka *et al.*, 2021). The use of synthetic pesticides in agriculture has been under increasing pressure in recent years due to the negative effects and concerns they have on the environment and natural ecosystems. Concerns have been raised related to their impact on potential health risks to consumers and farmers. Therefore, the need for the development of safer antifungal agents gained much importance in controlling phytopathogens. The use of plant extracts impose no adverse effects on the quality of the produce and human health. So, the objective of this study is to determine the antifungal activity of selected plant leaf extracts against the phytopathogen of mango stem-end rot.

Materials and Methods

Preparation of leaf extracts

Young leaves of the selected plant species (*Argyrea pomacea*, *Coccinia grandis*, *Erythrina subumbrans*, and *Gymnema sylvestre*) were collected. To ensure effective drying, leaves were kept in the shade to minimize photo-oxidative changes. Collected plant leaves were examined and the old, insect and fungus-infected leaves were removed. Leaves were dried at room temperature (27 ± 2 °C) for about two weeks until obtaining the constant weight and leaves were brittle enough to break easily. The dried leaves were ground to a fine powder using a laboratory grinding machine and stored in airtight bottles in the dark (Mahlo *et al.*, 2013).

The powder (100 g) was soaked in 500 ml of each solvent which were ethanol and acetone. They were left shaking for 72 h at room temperature, on an orbital shaker. The extracts were filtered separately through double-layered muslin cloth followed by Whatman No.1 filter paper and the solvent was evaporated at 40 °C using a rotary evaporator under reduced pressure. They were stored in air-tight glass bottles (Moyo *et al.*, 2012). A similar procedure was followed to obtain the acetone extract as well.

Isolation and identification of pathogen

Diseased mangoes (cv. Karthakolomban) were purchased from the market, showing symptoms of dark rot that develops from the stem end. Diseased fruit tissues (4 mm²) were cut from the margins of healthy and stem end rot symptom areas under aseptic conditions. The tissues were surface sterilized by soaking in freshly prepared NaOCl (1 % w/v) for 5 minutes. After three serial washings in sterile distilled water, mango tissues (4 pieces per plate)

were placed on sterile Potato Dextrose Agar (PDA; HiMedia, India) amended with amoxicillin (200 mg/l) to prevent bacterial growth. Plates were incubated at room temperature (27 ± 2 °C) for 5 days. Fungal mycelia that developed from the margins of infected tissues were sub-cultured on water agar with autoclaved mango leaves as the substrate to induce sporulation and transferred to a new PDA plate. Cultures were incubated at room temperature (27 ± 2 °C) with a 12 h photoperiod. All cultures obtained were maintained and preserved at 4 °C (Munirah *et al.*, 2017). The morphological and cultural features of obtained fungal isolates were used for the identification of fungi and compared with previous studies (Huda-Shakirah *et al.*, 2022; Munirah *et al.*, 2017).

Pathogenicity test

Fresh and healthy mango fruits (cv. Karthakolomban) were purchased from the market and surface sterilized with freshly prepared NaOCl (1 %) for 5 minutes. The wound (1cm) was made on the mango fruits by using sterile cork borer. The mycelium plugs taken from 7 days old cultures were placed downward on the wounds and incubated in a moisturized chamber at 27 ± 2 °C for 3 days. Disease symptom development was observed and after lesion development, small pieces from the active lesion surface were taken as described above and transferred to PDA. The resultant cultures were checked for colony and spore morphology as stated above to confirm Koch's postulate (Rafiullah Khan *et al.*, 2020).

In vitro screening of antifungal activity

Leaf crude extracts were dissolved in sterile distilled water and filtered

through a filter with a pore size of 0.22 µm. The extracts and the chemical fungicide (Captan; Oasis, Sri Lanka) were prepared at concentrations of 12.5, 25, and 50 mg/ml and tested against the growth of the isolated fungus using the food poison technique (Schmitz, 1930). Each of the plant extracts (2 ml) with different concentrations was aseptically added into the freshly prepared liquid PDA (20 ml) plates (90 mm) mixed thoroughly and allowed to solidify. The mycelium plugs (7 mm) were taken from the margins of 7-day-old pure cultures of pathogen using a cork borer and placed in an inverted position at the center of each extract-amended PDA plate. The petri plates were incubated at 27 ± 2 °C for 03 days. The control plates contained the mycelial disc without extracts. When the mycelial growth in the control treatment was complete, mycelial growth was measured in all plates in three different directions and an average was taken. The experiment was done in triplicates for each concentration. The percentage of mycelial growth inhibition (GI %) was determined by using the following formula reported by Attrassi *et al.* (2007).

$$GI (\%) = [(Do - Dc) / Do] \times 100 \%$$

Where, Do = Average mycelial growth diameter in control Petri dish, Dc = Average mycelial growth diameter in Petri dish at the concentration (c) (Treatment).

Statistical analysis

Fungal growth inhibition percentage was subjected to one-way ANOVA and the mean values were compared using Tukey's multiple comparison test. Statistical analysis was done using the Minitab statistical package (V. 17.1.0., Minitab Inc.). The level of $P < 0.05$ was considered statistically significant.

Results and Discussion

Isolation and identification of the pathogen

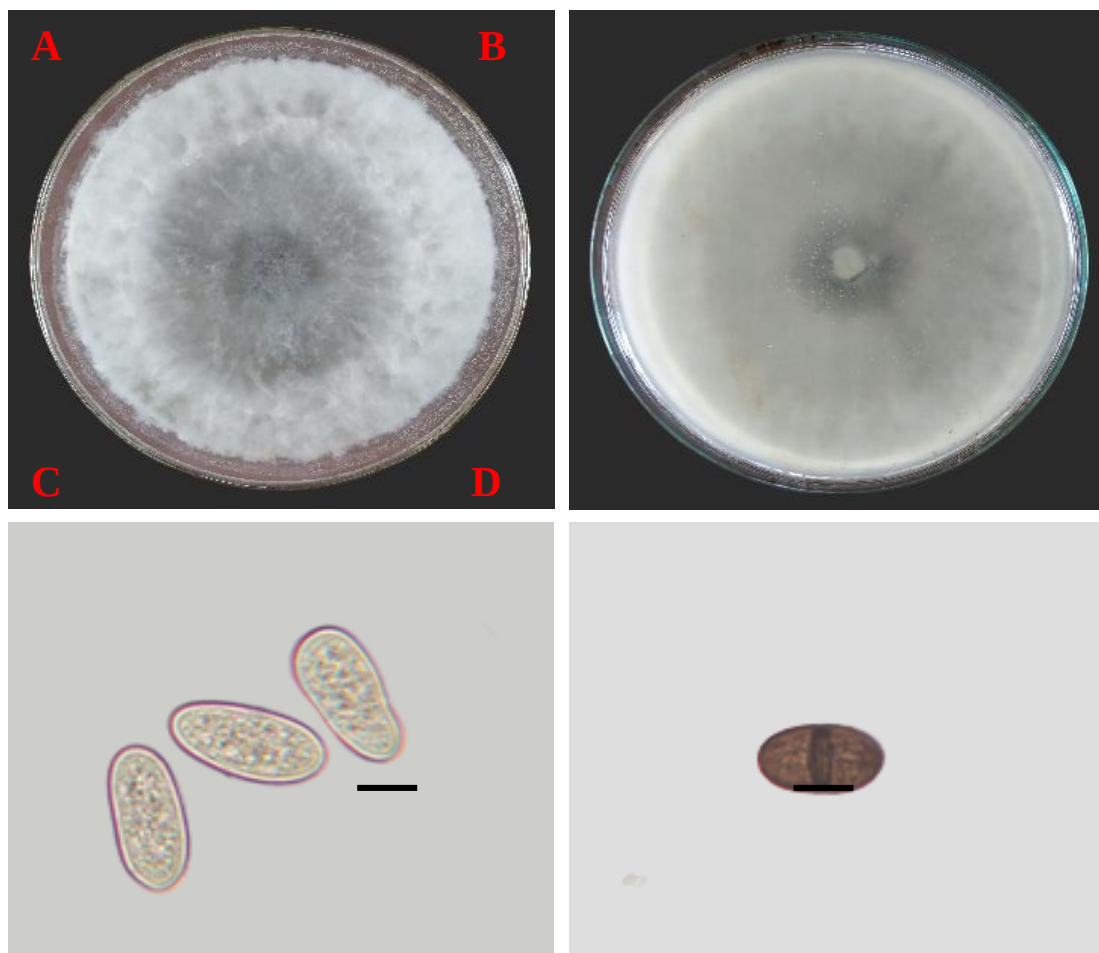


Figure 1: Morphological characteristics of *Lasiodiplodia theobromae* recovered from stem end rot of mango. (A) Upper view of the colony on PDA; (B) Lower view of the colony on PDA; (C) Immature conidia; (D) Mature conidia. Scale bars: (C) = 11 μ m; (D) = 14 μ m.

Fungal growth on the PDA media plate was white to light or dark gray aerial mycelia (Fig.1A), and later produced abundant black pigmentation, which can be viewed from the reserve side of the PDA culture (Fig.1B). According to microscopic features of the pathogenic fungus, the immature conidia (Fig.1C) were one-celled, granular and hyaline while mature conidia (Fig. 1D) were

dark brown, ellipsoid or ovoid, two-celled, with characteristic striations at maturity and contain one septum. All these phenotypic traits aided in the identification of *Lasiodiplodia theobromae* based on the study of Huda-Shakirah *et al.* (2022) and Munirah *et al.* (2017).

Antifungal activity of leaf extracts

The inhibition percentage of fungal mycelial growth by different plant leaf extracts at various concentrations (12.5, 25, and 50 mg/ml) using different solvents (ethanol and acetone) is shown in Table 1. The percentage of fungal growth inhibition significantly differs among all treatments ($P = 0.000$).

Table 1: Antifungal activity of ethanol and acetone leaf extracts, and captan fungicide on the growth of *Lasiodiplodia theobromae* *in vitro* conditions

Extraction solvent	Plant leaf extracts	Fungal growth inhibition (%) $\pm$ SE*		
		12.5 mg/ml	25 mg/ml	50 mg/ml
Ethanol extract	<i>C. grandis</i>	0.38 ⁿ $\pm$ 0.37	0.38 ⁿ $\pm$ 0.37	3.75 ^{lmn} $\pm$ 0.99
	<i>A. pomaceae</i>	4.49 ^{klm} $\pm$ 0.65	11.61 ^h $\pm$ 0.75	25.09 ^e $\pm$ 0.37
	<i>G. sylvestre</i>	4.49 ^{klm} $\pm$ 0.65	10.11 ^{hi} $\pm$ 0.65	16.77 ^f $\pm$ 0.72
	<i>E. subumbrans</i>	15.36 ^{fg} $\pm$ 0.37	23.97 ^e $\pm$ 0.75	29.21 ^d $\pm$ 0.65
Acetone extract	<i>C. grandis</i>	3.75 ^{lmn} $\pm$ 0.37	5.24 ^{klm} $\pm$ 0.37	7.49 ^{ijk} $\pm$ 0.37
	<i>A. pomaceae</i>	2.25 ^{mn} $\pm$ 0	6.37 ^{jkl} $\pm$ 0.99	12.36 ^{gh} $\pm$ 0.65
	<i>G. sylvestre</i>	8.86 ^{hij} $\pm$ 0.75	28.97 ^d $\pm$ 0.79	17.06 ^f $\pm$ 0.91
	<i>E. subumbrans</i>	7.17 ^{ijkl} $\pm$ 0.92	4.49 ^{klm} $\pm$ 0.65	4.62 ^{klm} $\pm$ 0.75
Positive control (Captan)		83.15 ^c $\pm$ 0.65	88.01 ^b $\pm$ 0.37	100 ^a $\pm$ 0

*Standard error. Means with the same letter are not significantly different at 0.05 level of probability.

The results indicated that both ethanol (Fig.2) and acetone (Fig.3) leaf extracts displayed varying degrees of antifungal activity. Notably, the ethanol extract of *E. subumbrans* demonstrated the highest inhibition of mycelial growth, with percentages ranging from 15.36 ± 0.37 to 29.21 ± 0.65 % at concentrations of 12.5 to 50 mg/ml, respectively. According to Utami (2019), ethanol extracts from the leaves of *E.*

subumbrans showed significant antifungal activity against *Candida albicans*. Maharini (2019) and Utami (2019) reported that the leaves of *E. subumbrans* contain alkaloids, flavonoids, saponin, triterpenoids, isoflavones, and lectins. So, these constituents of ethanolic extract of *E. subumbrans* might be a reason for the highest inhibition of mycelial growth of *L. theobromae* in the present study.

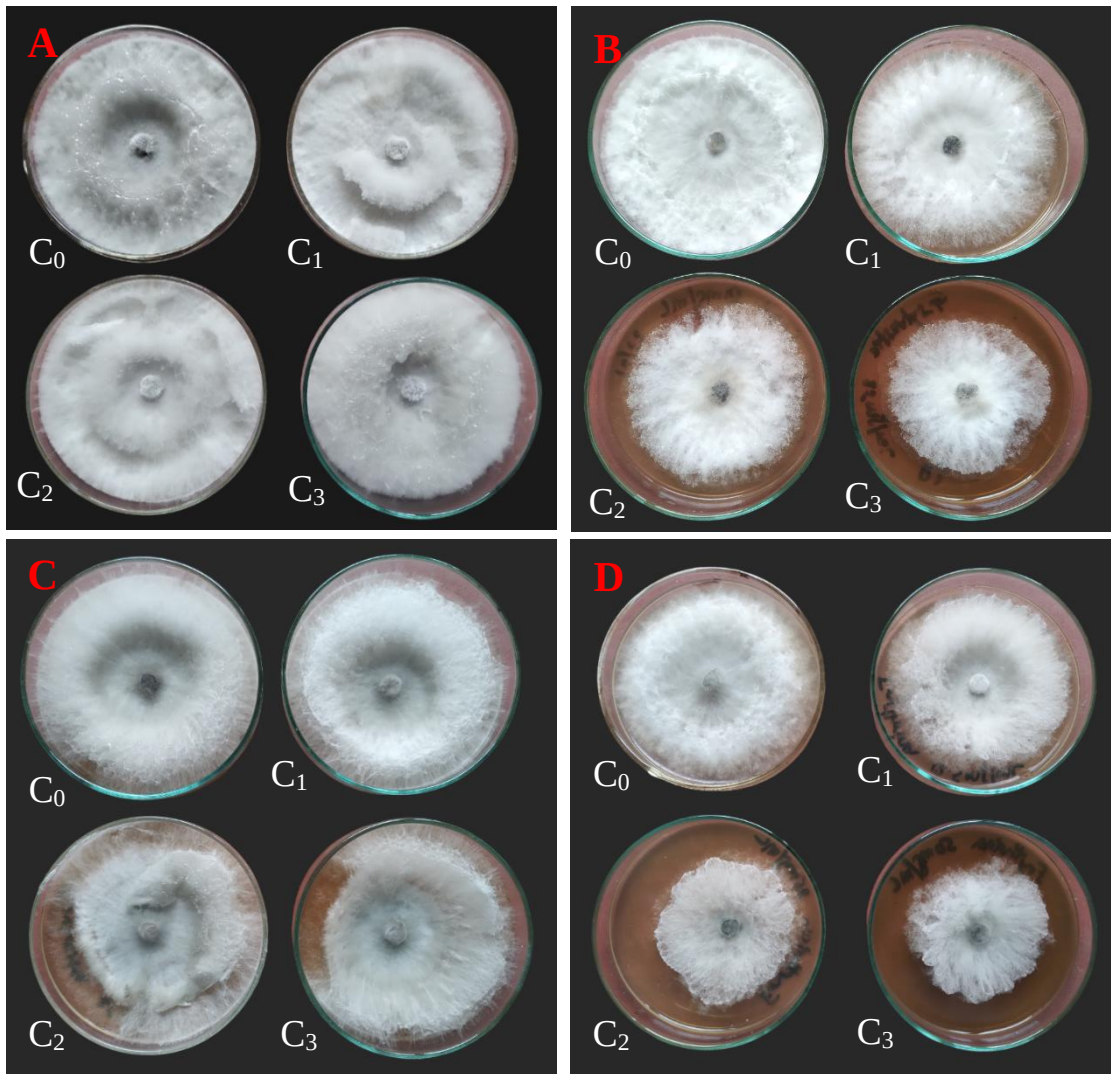


Figure 2: Effect of ethanolic leaf extracts on the mycelial growth of *Lasiodiplodia theobromae* after 3 days of incubation on PDA medium. (A) *C. grandis*; (B) *A. pomacea*; (C) *G. sylvestre*; (D) *E. subumbrans*. Co= Control; C1= 12.5 mg/ml; C2= 25 mg/ml; C3= 50 mg/ml.

Similarly, the acetone extract of *G. sylvestre* also exhibited the highest antifungal activity, particularly at a concentration of 25 mg/ml (28.97 ± 0.79 %). There was no significant difference in the percentage of fungal growth inhibition between the ethanol extract of

E. subumbrans (50 mg/ml) and the acetone extract of *G. sylvestre* (25 mg/ml). A study by David and Sudarsanam (2013) revealed that *G. sylvestre* showed antifungal activity against *Candida albicans*, *C. tropicalis*, *C. krusei* and *C. kefyr*. This result is consistent with the present study. Muddapur *et al.* (2024) stated the presence of alkaloids, glycosides, flavonoids, tannins, steroids, anthraquinones, resins, and saponins in the *G. sylvestre* extract which may possess antifungal properties in the present study.

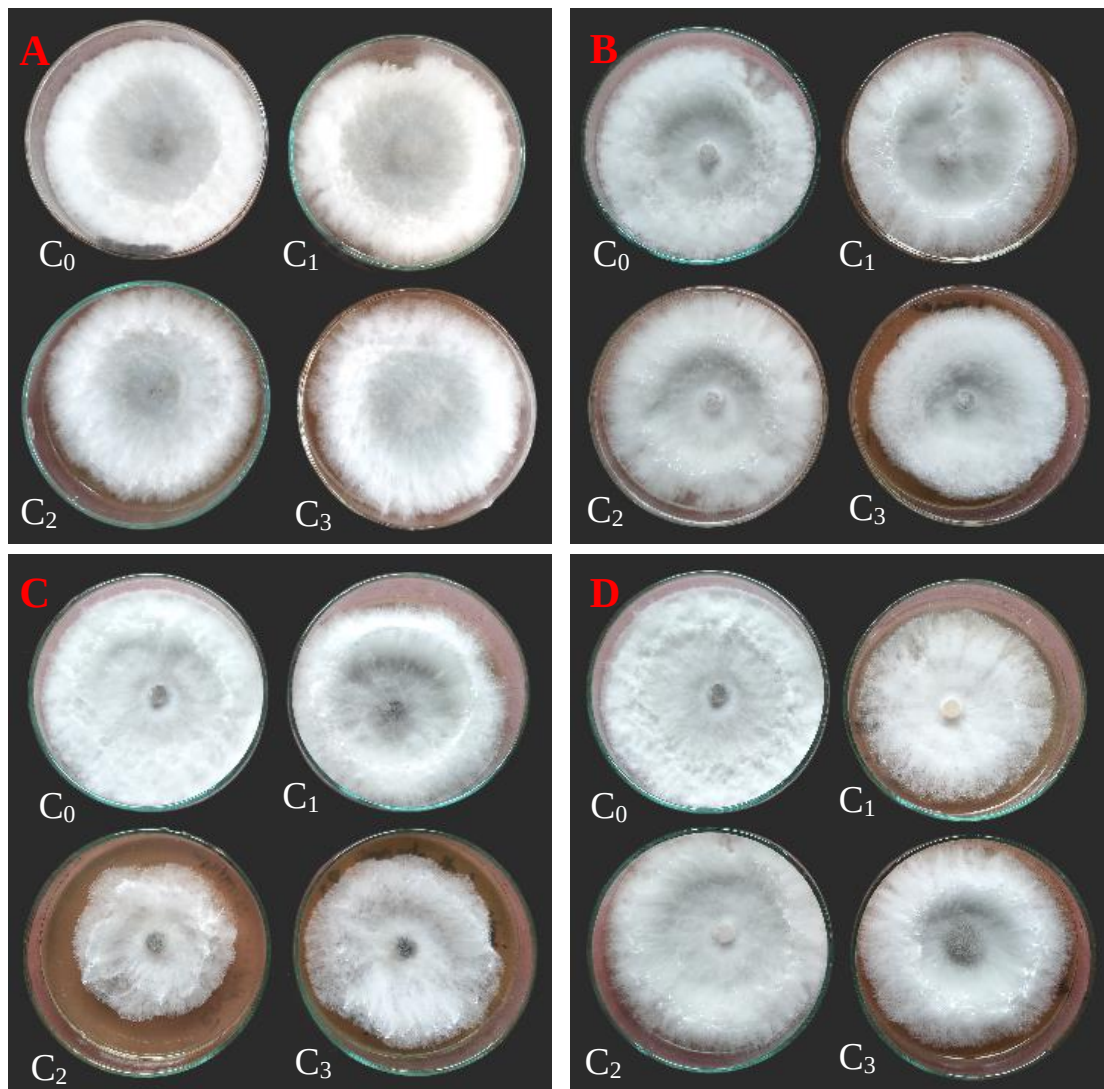


Figure 3: Effect of acetone leaf extracts on the mycelial growth of *Lasiodiplodia theobromae* after 3 days of incubation on PDA medium. (A) *C. grandis*; (B) *A. pomaceae*; (C) *G. sylvestre*; (D) *E. subumbrans*. Co= Control; C1= 12.5 mg/ml; C2= 25 mg/ml; C3= 50 mg/ml.

However, ethanol extract of *A. pomaceae* displayed significant inhibition at 50 mg/ml, indicating its moderate antifungal potential. This might be due to the presence of saponins, flavonoids, and tannins in the ethanol extract of *A. pomaceae* (Mahendrarajah *et al.*, 2020). Findings from the present study suggest that ethanol extract of *E. subumbrans* and acetone extract of *G. sylvestre* possess bioactive compounds with significant

antifungal potential against *L. theobromae*.

Conclusion

In terms of antifungal activity against *L. theobromae*, ethanol extracts exhibited notable inhibitory effects, with *E. subumbrans* demonstrating the highest percentage of mycelial growth inhibition at all concentrations tested. Acetone extract of *G. sylvestre* also exhibited a significant percentage of mycelial growth inhibition. Thus, it could be concluded that ethanol extract of *E. subumbrans* (50 mg/ml) and acetone extract of *G. sylvestre* (25 mg/ml) have greater antifungal activities against *L. theobromae* compared to other plant leaf extracts. Further investigations are required to

isolate and identify the specific bioactive compounds responsible for these antimicrobial activities. Additionally, conducting *in vivo* studies and assessing the safety profiles of these leaf extracts would be crucial steps towards their potential application as natural antifungal agents.

Acknowledgement

The authors would like to thank the academic and nonacademic personnel of the Department of Botany, Faculty of Science, Eastern University of Sri Lanka, for their kind assistance to make this study a success.

References

- Attrassi, K., Benkirane, R., Attrassi, B. and Douira, A. (2007). Effect of the association of certain fungicides with calcium chloride on the development of a fungal complex responsible for apple rot in conservation. *Phytoprotection* 88(1):17-26.
- David, B.C. and Sudarsanam, G. (2013). Antimicrobial activity of *Gymnema sylvestre* (Asclepiadaceae). *Journal of Acute Disease* 2(3):222-225.
- Huda-Shakirah, A.R., Mohamed Nor, N.M.I., Zakaria, L., Leong, Y.H. and Mohd, M.H. (2022). *Lasiodiplodia theobromae* as a causal pathogen of leaf blight, stem canker, and pod rot of *Theobroma cacao* in Malaysia. *Scientific Reports* 12(1):8966.
- Kapeleka, J.A., Sauli, E. and Ndakidemi, P.A. (2021). Pesticide exposure and genotoxic effects as measured by DNA damage and human monitoring biomarkers. *International Journal of Environmental Health Research* 31(7):805-822.
- Karunanayake, K.O.L.C., Sinniah, G.D., Adikaram, N.K.B. and Abayasekara, C.L. (2014). Cultivar differences in antifungal activity and the resistance to postharvest anthracnose and stem-end rot in mango (*Mangifera indica* L.). *Australasian Plant Pathology* 43:151-159.
- Krishnapillai, N. and Vijayakumaran, W.R.S. (2012). Aspergillus rot of ripe mangoes (*Mangifera indica* L.) var. Ambalavi, Willard and Karuthakolumban. *Journal of the National Science Foundation Sri Lanka* 41(1):69-70.
- Maharini, I. (2019). *In vitro* determination of sun protective factor (SPF) of Dadap serep (*Erythrina subumbrans* (Haks.) Merr.) leaf extract using spectrophotometric method. *Journal of Chemical Natural Resources* 1(1):64.
- Mahendrarajah, H., Krishanthiny, K. and Mahendranathan, C. (2020). Antibacterial activity and preliminary screening of phytochemicals of locally available Green Leafy Vegetables in Batticaloa district, Sri Lanka. *AGRIEAST: Journal of Agricultural Sciences* 14(2):9-16.
- Mahlo, S.M., Chauke, H.R., McGaw, L.J. and Eloff, J.N. (2013). Antioxidant and antifungal activity of selected plant species used in traditional medicine. *Journal of Medicinal Plants Research* 7(33):2444-50.
- Moyo, B., Masika, P.J. and Muchenje, V. (2012). Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. *African Journal of Biotechnology* 11(11):2797-2802.
- Muddapur, U.M., Manjunath, S., Alqahtani, Y.S., Shaikh, I.A., Khan, A.A., Mannasaheb, B.A., Yaraguppi, D. and More, S.S. (2024). Exploring bioactive phytochemicals in *Gymnema sylvestre*: Biomedical uses and

computational investigations. *Separations* 11(2):50.

Munirah, M.S., Azmi, A.R., Yong, S.Y.C. and Nur Ain Izzati, M.Z. (2017). Characterization of *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* causing fruit rot on pre-harvest mango in Malaysia. *Plant Pathology & Quarantine* 7(2): 203.

Rafiullah Khan, M., Suwanamornlert, P., Sangchote, S. and Chonhenchob, V. (2020). Antifungal activity of propyl disulphide from neem against *Lasiodiplodia theobromae* and *Neofusicoccum parvum* causing stem end rot in mango. *Journal of Applied Microbiology* 129(5):1364-1373.

Righini, H., Francioso, O., Di Foggia, M., Martel Quintana, A. and Roberti, R. (2021). Assessing the potential of the terrestrial cyanobacterium *Anabaena minutissima* for controlling *Botrytis cinerea* on tomato fruits. *Horticulturae* 7(8):210.

Schmitz, H. (1930). Poisoned food technique. *Industrial and Engineering*

Chemistry-Analytical Edition 2(4):361-363.

Topolovec-Pintaric, S. ed. (2020). *Plant Diseases: Current Threats and Management Trends*. BoD–Books on Demand, pp. 49-68.

Utami, D.T. (2019). Antimicrobial activity of dadap serep (*Erythrina subumbrans* (Hassk.) Merr.) leaves extract. *Journal of Chemical Natural Resources* 1(1):45.

Vijayaraj, R., Altaff, K. and Kumaran, N.S. (2022). Volatile bioactive compounds from marine macro-algae and their pharmacological properties. *Indian Journal of Geo-Marine Sciences (IJMS)* 51(10):832-842.

Wang, C., Xu, L., Liang, X., Wang, J., Xian, X., Zhang, Y. and Yang, Y. (2021). Molecular characterization and overexpression of the difenoconazole resistance gene CYP51 in *Lasiodiplodia theobromae* field isolates. *Scientific Reports* 11(1):24299.