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ORIGINAL ARTICLE

Harnessing the power of Sacha inchi leaf extract against fruit anthracnose disease

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Abstract

Anthracnose disease, primarily caused by *Colletotrichum* species, poses significant challenges to fruit production, resulting in serious global economic losses. While chemical fungicides are effective, their environmental and health risks underscore the need for sustainable alternatives. This study evaluated the antifungal potential of Sacha inchi (*Plukenetia volubilis*) leaf ethanol extract against *Colletotrichum gloeosporioides*, *C. scovillei*, and *C. acutatum*. Gas Chromatography-Mass Spectrometry (GCMS) analysis identified 20 chemical constituents, including bioactive compounds such as unsaturated fatty acids and saturated fatty acids known for their antimicrobial properties. In vitro assays demonstrated a dose-dependent inhibition of mycelial growth, with complete suppression of *C. gloeosporioides* at 500 mg · l^{-1}. The antifungal activity is likely attributed to the extract's ability to disrupt fungal cell membranes and interfere with metabolic pathways. These findings support the potential of Sacha inchi leaf extract as a promising, eco-friendly alternative to synthetic fungicides in the management of anthracnose disease in fruit crops. Further research into its field application, synergistic effects, and mechanisms of action is warranted to enhance its integration into sustainable crop protection strategies.

Keywords: anthracnose disease, antifungal activity, *Colletotrichum* spp., phytoanticipins, phytochemicals, Sacha inchi, sustainable plant protection

Introduction

The fruit industry is a key sector in Malaysia's economy, with the National Agrifood Policy 2021–2030 (DAN 2.0) projecting an increase in self-sufficiency levels from 78.2% in 2019 to 83.0% by 2030. Focus crops under the 12th Malaysian Plan include durian, mango, rambutan, jackfruit, mangosteen, watermelon, pineapple, and tomato, are central to achieving these targets. However, fruit production faces significant threats, particularly from anthracnose disease (Uda *et al.* 2020). Yield losses due to anthracnose can reach up to 100% in unmanaged plantations and account for 30–60% of economic losses in tropical and subtropical regions (Paudel *et al.* 2022). The disease is especially problematic during the rainy season, with incidence rates as high as 90% (Kankam *et al.* 2023).

Anthracnose, caused by the Colletotrichum species complex, adversely affects fruit quality and yield both pre- and post-harvest (Dofuor *et al.* 2023). The pathogen remains latent in unripe fruits and becomes symptomatic only at maturity (Grice *et al.* 2023). Pathological symptoms begin as black spots on the fruit surface, which later transform into large, irregular blotches (Dofuor *et al.* 2023). This latency, coupled with its ability to thrive under specific environmental conditions, makes Colletotrichum a highly adaptable pathogen. The disease, caused by the Colletotrichum

species complex, is capable of cross-infecting different host plants and shifting between life modes (De Silva *et al.* 2017). Optimal growth conditions for Colletotrichum include temperatures of 25–30°C and a pH range of 6–7 (Lima *et al.* 2015). Spores, the primary source of infection, are easily dispersed by air, water, and contact with adjacent infected plant parts or fruits.

Key members of the Colletotrichum species complex include *C. gloeosporioides, C. scovillei,* and *C. acutatum*, each causing substantial damage to specific crops. *C. gloeosporioides* is a major pathogen in mango (*Mangifera indica*), leading to yield losses of up to 100% under favorable conditions, significantly affecting both preharvest and postharvest quality (Kamle and Kumar 2016). *C. scovillei*, the primary cause of anthracnose in chili peppers (*Capsicum* spp.), results in severe fruit rot, rendering produce unmarketable and causing significant economic losses (Gao *et al.* 2024). *C. acutatum* impacts crops like strawberries, olives, and citrus fruits. In strawberries, anthracnose fruit rot causes substantial yield losses under warm and humid conditions (Garrido *et al.* 2009). Olive anthracnose caused by *C. acutatum* can result in up to 80% yield loss in severely affected orchards (Gouvinhas *et al.* 2019).

Managing anthracnose involves implementing various strategies, including cultural practices, physical control, fungicides, biological control agents, and integrated approaches. Chemical fungicides remain the primary method due to their efficiency in controlling postharvest anthracnose (Ciofini *et al.* 2022). However, overuse of fungicides has led to resistance in Colletotrichum isolates, such as resistance to procymidone, fludioxonil, and pyraclostrobin in China (Usman *et al.* 2021). Excessive fungicide use has also increased production costs and raised concerns about harmful residues affecting the environment and human health (Hua *et al.* 2018). In Malaysia, the misuse of hazardous pesticides has resulted in public health risks, with pesticide poisoning ranking as the second leading cause of poisoning after pharmaceuticals (Kamaruzaman *et al.* 2020). Chronic exposure to these chemicals has been linked to serious health issues, including Parkinson's, end-stage renal disease, and various cancers (Narayan *et al.* 2017; Martin *et al.* 2018).

To address these challenges, biopesticides have emerged as sustainable alternatives to synthetic fungicides. Among these, plant-based compounds show promising antimicrobial properties with minimal environmental impact (Abbey *et al.* 2018). Crude plant extracts, a subset of plant-based compounds, have garnered significant attention for their potential to yield novel antifungal compounds. Employing biopesticides, including plant extracts, mitigates harmful residues, and reduces negative impacts on living organisms and the environment, making them a viable and eco-friendly alternative to synthetic fungicides (Koul *et al.* 2011).

One promising candidate for natural fungicides is Sacha inchi (*Plukenetia volubilis* Linneo), a climbing shrub from the Euphorbiaceae family native to the Amazon region (Kodahl and Sørensen 2021). Renowned for its high nutritional value, Sacha inchi offers numerous health benefits, including reducing the risk of cardiovascular disease, managing chronic inflammatory conditions, dermatitis, and diabetes, as well as controlling tumor proliferation, particularly through its seeds (Cárdenas *et al.* 2021). Additionally, extracts from both the seeds and leaves exhibit high antioxidant and antiproliferative activities (Puangpronpitag *et al.* 2021; Ismail *et al.* 2022).

The leaves of Sacha inchi have pronounced antifungal properties due to phytochemicals such as alkaloids, flavonoids, and tannins (Cárdenas *et al.* 2021). Since these antifungal compounds are present in healthy plant tissues and are not induced by pathogen attack, they are classified as phytoanticipins, the pre-formed antimicrobial substances that contribute to the plant's constitutive defense mechanisms. However, their specific activity against *Colletotrichum* species remains unexplored.

This study investigated the phytochemical profile and in vitro antifungal activity of Sacha inchi leaf ethanol extract, aiming to support sustainable, plant-based disease management.

Materials and Methods

Plant materials

Mature leaves of Sacha inchi were purchased from a local farm in Kota Bharu, Kelantan. Once harvested, they were brought to the Postharvest Laboratory, Blok Pakar, Universiti Pendidikan Sultan Idris. The leaves were rinsed under running tap water to remove dirt, shade-dried for one week, and oven-dried at 40°C for 4 hours. The dried leaves were then ground for two minutes using a high-speed grinder. The powdered sample was stored in an airtight container for further extraction processes.

Preparation of Sacha inchi crude extracts

Analytical-grade ethanol (99% minimum purity) was used in the crude extraction process. A 1 : 20 ratio of powdered Sacha inchi leaf sample to ethanol was carefully measured. The mixture was macerated for 48 hours at ambient temperature. The extracts were then concentrated using a Buchi rotary evaporator until a sticky, dark green crude extract was obtained.

The crude extract (yield: 7.3%) was transferred to an airtight jar and stored at 4°C until further use.

Screening for Sacha inchi chemical constituents using gas chromatography--mass spectrometry (gcms) analysis

Crude samples (0.02 g) were dissolved in 1 ml of ethanol (HPLC grade). The solution was vortexed and filtered using a 0.02 µm syringe filter (Sartorius). The sterile crude extract was transferred to an HPLC vial and analyzed using GC-MS. GC-MS analysis was performed on an Agilent 7890A gas chromatograph (GC) coupled to an Agilent 5975C inert mass spectrometer system (MS) with a triple-axis detector. Compound separation was conducted using a DB-5MS UI column (30 m length, 0.25 mm diameter, and 0.25 µm film thickness) from Agilent Technologies. Chemical constituents were identified by comparing mass spectra with the NIST/EPA/NIH library version 2.0.

Preparation of fungal cultures

Colletotrichum gloeosporioides, C. scovillei, and *C. acutatum* cultures were obtained from the Taxonomy Microbe Laboratory, MARDI. Pure cultures were maintained on potato dextrose agar (PDA) medium through regular subculturing.

In vitro evaluation of the antifungal activity of Sacha inchi leaf extract

The effect of Sacha inchi leaf extract on the mycelial growth of tested *Colletotrichum* spp. was measured in vitro using the poison agar technique. PDA was amended with six concentrations of the extract (100, 200, 300, 400, and 500 mg · l^{-1}). Fifteen milliliters of the amended PDA were poured into 9 cm Petri dishes. A fungal plug (5 mm) from a pure culture of *Colletotrichum* spp. was placed at the center of each Petri dish. Petri dishes containing unamended PDA served as controls. The plates were incubated at room temperature, and the diameter of fungal growth was measured daily until the fungus reached the edge of the plate, which occurred within 5 days at most. The percentage inhibition of diameter growth (PIDG) was calculated after 5 days of incubation using the formula (Darlis *et al*. 2023):

$$PIDG = [(D1\text{-}D2)/D1] \times 100,$$

where: D1 is the diameter of fungal growth on the control plate (cm), and D2 is the diameter of fungal growth on the extract-containing plates (cm).

Experimental design and statistical analysis

The *in vitro* bioassay was conducted using a completely randomized design (CRD) with four replications. Data were analyzed using analysis of variance (ANOVA), and mean differences were separated using the least significant difference (LSD) test at $p \leq 0.05$.

Results and Discussion

Gas chromatography-mass spectrometry (GCMS) analysis of Sacha inchi chemical constituents

The GC-MS analysis revealed 20 chemical constituents in the Sacha inchi crude extract (Fig. 1). The identified compounds are listed in the order of their elution on the capillary column. The five most abundant compounds in the extract were 6-Octadecenoic acid (13.58%), 1,15-Pentadecanedioic acid (9.81%), oxirane (9.51%), 9-Octadecenal (9.29%), and Octadecanoic acid (8.81%).

In terms of chemical groups, unsaturated fatty acids (trans-13-Octadecenoic acid, Oleic acid, cis-Vaccenic acid, 9-Octadecenoic acid, 6-Octadecenoic acid) are the major contributors to the antifungal properties of Sacha inchi extract, followed by saturated fatty acids (Heptadecanoic acid and Octadecanoic acid), other compounds like aldehydes (e.g., 9-Octadecenal, cis-9-Hexadecenal) and dicarboxylic acids (Table 1).

Palmitic acid (n-hexadecanoic acid) can inhibit fungal growth by disrupting fungal cell membranes (Desbois and Smith 2010). Studies suggest that it may act as a supporting agent in combination with stronger antifungal compounds. Oleic acid (OA) demonstrated strong antifungal and anti-biofilm effects (Muthamil *et al*. 2020; Guimarães and Venâncio 2022). It disrupts fungal cell membranes by integrating into them, increasing permeability, and causing leakage of cell contents, which ultimately leads to cell death (Guimarães and Venâncio 2022). Muthamil *et al*. (2020) demonstrated that OA effectively inhibits biofilm formation and virulence in *Candida albicans*. Proteomic analysis revealed that OA disrupts proteins involved in glucose metabolism, ergosterol biosynthesis, and iron homeostasis, compromising cell membrane integrity and increasing permeability. Charlet *et al*. (2022) further investigated the combined effects of OA and palmitic acid (PA) against fungal pathogens, showing enhanced antifungal activity, reduced fungal viability, and biofilm formation. The study suggested that OA and PA synergistically disrupt fungal cell membranes, increasing permeability and causing cell death. Several studies have also confirmed OA's effectiveness against various

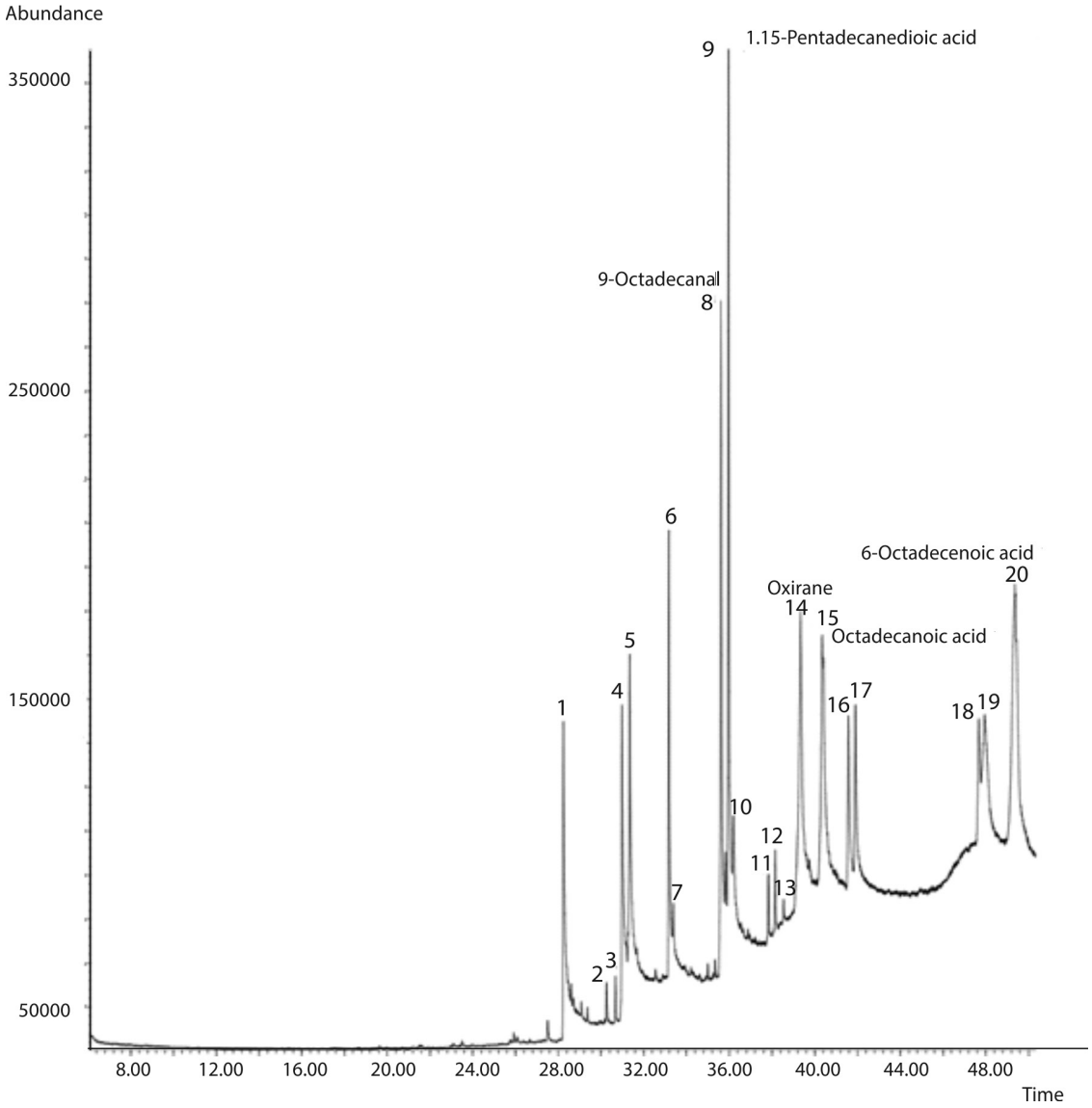

Fig. 1. Ion chromatogram of Sacha inchi crude extract was analyzed using GC-MS, with a focus on antifungal properties

fungal pathogens (Xue *et al.* 2023; Gutierrez-Perez *et al.* 2024).

Octadecanoic acid (stearic acid), detected at 7.61% and 8.81% in two peaks, is a saturated fatty acid that exhibits some degree of antifungal activity, though it is less potent than unsaturated fatty acids (Joujou *et al.* 2024). It may enhance the activity of more potent compounds, such as oleic acid, by stabilizing or disrupting fungal cell membranes. Cis-vaccenic acid, detected at 0.79%, is an unsaturated fatty acid structurally similar to oleic acid. While specific studies on its antifungal effects are limited, it has been noted for its potential antimicrobial activity (Semwal *et al.* 2018). Its ability to disrupt fungal cell membrane function suggests that it may contribute to antifungal action when combined with other fatty acids.

While the antifungal activity of key compounds such as oleic acid has been well-documented in previous studies, the present study did not experimentally evaluate the specific mechanisms of action of the Sacha inchi extract against fungal cells. Nevertheless, the presence of these antifungal compounds in healthy, untreated Sacha inchi leaves indicates that they are constitutively produced, rather than being synthesized in response to infection. Such compounds are classified as phytoanticipins, the pre-formed, low-molecular-weight antimicrobial substances that contribute to the plant's basal immunity and are distinct from phytoalexins, which are induced only after pathogen exposure (Parthasarathy *et al.* 2021). Fatty acids like oleic acid and palmitic acid, which are consistently found in healthy plant tissues and exhibit proven antimicrobial activity, have been described as phytoanticipins (Pedras and Yaya 2015). These results underscore the role of Sacha inchi's phytochemicals in natural disease resistance and highlight their potential as leads for the development of botanical fungicides.

Table 1. Chemical composition of Sacha inchi ethanol crude extract

Peak	Time [min]	Compound name	Chemical group	Area [%]
1	28.24	n-Hexadecanoic acid	fatty acid	5.9
2	30.27	trans-13-Octadecenoic acid	unsaturated fatty acid	0.39
3	30.67	Heptadecanoic acid	saturated fatty acid	0.35
4	30.99	Oleic Acid	unsaturated fatty acid	5.46
5	31.35	Octadecanoic acid	saturated fatty acid	7.61
6	33.18	Tetradecanedioic acid	dicarboxylic acid	4.39
7	33.39	Ether	ether	1.23
8	35.62	9-Octadecenal	unsaturated aldehyde	9.29
9	35.97	1,15-Pentadecanedioic acid	dicarboxylic acid	9.81
10	36.19	Cyclohexanecarboxylic acid,	carboxylic acid	2.67
11	37.83	Cinnamyl cinnamate	ester of cinnamic acid	0.51
12	38.15	Thiophene	heterocyclic compound	0.73
13	38.55	cis-Vaccenic acid	unsaturated fatty acid	0.79
14	39.34	Oxirane	epoxide	9.51
15	40.35	Octadecanoic acid	saturated fatty acid	8.81
16	41.58	Oleic Acid	unsaturated fatty acid	2.96
17	41.9	4,4,5,6-Tetramethyltetrahydro-1	cyclic hydrocarbon derivative	3.36
18	47.68	9-Octadecenoic acid	unsaturated fatty acid	5.09
19	47.96	cis-9-Hexadecenal	unsaturated aldehyde	7.57
20	49.37	6-Octadecenoic acid	unsaturated fatty acid	13.58

Effect of Sacha inchi leaf extract against mycelial growth of *Colletotrichum gloeosporioides, C. scovillei* and *C. acutatum*

The graph in Figure 2 illustrates the percentage inhibition of diameter growth (PIDG) for the three *Colletotrichum* species after 5 days of incubation. As the concentration of Sacha inchi leaf extract increased, there was a significant increase in the inhibition of mycelial growth ($p < 0.05$). Among the species tested, *C. gloeosporioides* exhibited the highest antifungal activity, with complete inhibition (100%) observed at the highest concentration of 500 mg · l^{-1}. In contrast, *C. scovillei* and *C. acutatum* also showed significant

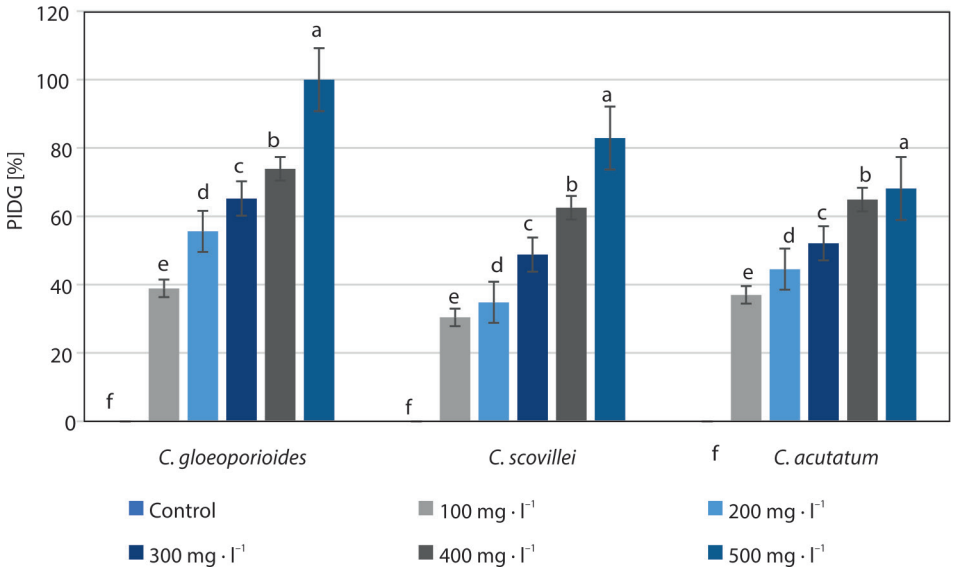

Fig. 2. Effects of Sacha inchi leaf extract on percentage inhibition of diameter growth (PIDG) of *Colletotrichum gloeosporioides, C. scovillei* and *C. acutatum* after 5 days of incubation. Means with different letters within each respective treatment are significantly different using LSD test at $p < 0.05$

inhibition, though it was less pronounced than *C. gloeosporioides*. Overall, all species displayed a dose-dependent increase in PIDG, indicating that higher concentrations of the extract were more effective in suppressing fungal growth.

The variability in antifungal activity among the species may be attributed to differences in their taxonomic classification, genetic diversity, and pathogenic characteristics. *C. gloeosporioides* belongs to a distinct species complex within the *Colletotrichum* genus, encompassing species with broad host ranges, while *C. scovillei* is part of the *C. acutatum* species complex and predominantly associated with chili anthracnose (Noor and Zakaria 2018). Structural adaptations, such as variations in pectic polymers in fungal cell walls, further influence antifungal efficacy (academic.oup.com). Resistance mechanisms also differ; for example, *C. gloeosporioides* isolates may develop resistance to benzimidazole fungicides through specific genetic mutations (Ren *et al*. 2020). Additionally, *C. gloeosporioides* is more abundant in tropical and subtropical regions, where environmental conditions favor its growth, while *C. acutatum* is distributed across a wider range of climates (Sharma and Kulshrestha 2015).

This pattern of increased inhibition with higher extract concentrations supports the finding that the ethanol extract of Sacha inchi leaves contains bioactive compounds with potent antifungal properties, which are more effective at elevated doses. The inhibition of fungal growth is likely attributed to the phytochemical components in the extract, such as various fatty acids, which have been reported in previous studies to exhibit antifungal activity by disrupting fungal cell walls or inhibiting the synthesis of essential fungal enzymes (Bhattacharyya *et al*. 2020). Fatty acids like oleic acid and palmitic acid have been reported to compromise fungal cell membranes by increasing permeability or inhibiting the synthesis of essential enzymes involved in fungal metabolism (Liu *et al.* 2008). Ethanol-soluble compounds may further enhance the penetration of bioactive molecules into fungal cells, amplifying antifungal effects.

Figure 3 visually demonstrates the growth of the three *Colletotrichum* species on poisoned potato dextrose agar (PDA) medium containing varying concentrations of Sacha inchi extract. Reduced mycelial growth in *C. gloeosporioides*, *C. scovillei*, and *C. acutatum* with increasing extract concentrations aligns with the quantitative results in Figure 2. The potential synergistic interaction between fatty acids and other bioactive compounds, such as flavonoids and alkaloids, could explain the robust antifungal effects observed. For example, oleic acid disrupts fungal membranes, while palmitic acid may act as a supporting agent, enhancing the delivery or stability of active compounds (da Silva *et al*. 2020).

The inhibitory effect of the extract likely stems from its ability to penetrate the mycelial network, slowing or completely halting fungal growth. The phytochemicals in Sacha inchi, particularly the ethanol-soluble compounds, have been reported in previous research to compromise the integrity of fungal cell membranes or disrupt fungal metabolism, ultimately reducing mycelial expansion (Rashid *et al.* 2024). This complex blend of fatty acids and bioactive compounds indicates a potential synergistic interaction, which may underlie the Sacha inchi extract's efficacy as a natural antifungal agent. Beyond fatty acids, other bioactive components, such as flavonoids or alkaloids, may contribute to antifungal activity by targeting key metabolic pathways or structural proteins in fungal cells. Similar mechanisms of membrane disruption have been observed with other natural antifungal agents, such as garlic, which disrupts fungal cell membranes through allicin (Yang *et al*. 2023). Comparing these results with neem or garlic extracts suggests that Sacha inchi extract offers competitive efficacy with added eco-friendly benefits (Mohideen *et al*. 2022).

Although this study did not include a direct comparison with synthetic fungicides, previous reports have documented the rising resistance of Colletotrichum species to commonly used chemicals such as fludioxonil and pyraclostrobin (Usman *et al.* 2021), as well as concerns over environmental and health risks associated with pesticide residues (Martin *et al*. 2018; Kamaruzaman *et al.* 2020). Plant-based alternatives like Sacha inchi offer a more sustainable profile, though their cost-effectiveness and field performance require

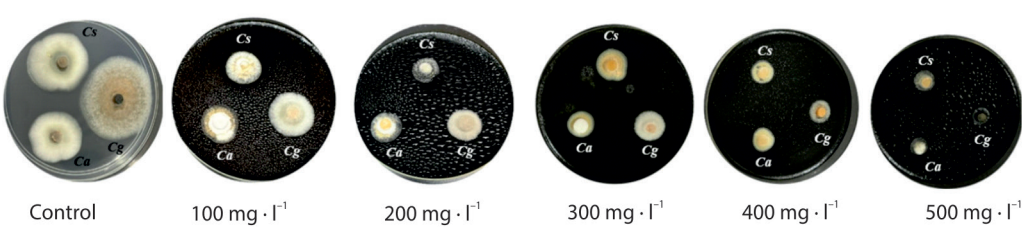

Fig. 3. Antifungal effect on growth of *Colletotrichum gloeosporioides* (Cs), *C. scovillei* (Cs) and *C. acutatum* (Ca) on poisoned PDA medium containing *Sacha inchi* ethanol extract after 5 days of incubation

further study. In this context, the present study extends the application of Sacha inchi from nutritional to plant protective uses. It complements earlier research on its antioxidant and anti-inflammatory properties and demonstrates its utility in sustainable agriculture (Abbey *et al.* 2018; Cárdenas *et al.* 2021).

Conclusions

Sacha inchi leaf ethanol extract exhibits strong antifungal activity against key Colletotrichum species causing anthracnose. Its bioactive compounds, especially oleic and 6-octadecenoic acid, disrupt fungal membranes and metabolism. The presence of phytoanticipins enhances its suitability for preemptive crop protection. These findings support the potential of Sacha inchi as a viable botanical fungicide in sustainable agriculture and highlight the need for further field testing and mechanistic studies to clarify its mode of action at the cellular and molecular levels, including assays targeting membrane disruption, apoptosis markers, and ergosterol biosynthesis interference.

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