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African Journal of Microbiology Research

Full Length Research Paper

Phytochemical screening and antifungal activity of *Cassia alata* (Linn.) crude leaf extracts

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The antimycotic effect of *Casia alata* (Linn.) commonly used for the treatment of skin diseases by local people in Nigeria was evaluated against dermatophytes (*Trichophyton tonsurans*, *Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum audouinii*, *Microsporum canis*, *Microsporum gypseum, Epidermophyton floccosum*). Ten-fold serially diluted extract concentrations were subjected to phytochemical screening and the antimycotic activity of the phytochemicals was evaluated via broth microdilution. The percentage yield of crude aqueous, n-hexane, chloroform, ethanol, and methanol leaf extracts of *Cassia alata* Linn was 5.6, 5.7, 1.6, 4.8, and 2.8%, respectively. Important phytochemicals including glycosides, anthraquinone, proteins, cardiac glycosides, steroids, alkaloids, phlabotannin, phenols, saponins, tannins, flavonoids, and carbohydrates were present in all the extracts. All the extracts inhibited the dermatophytes but at varying degrees. Similar minimum inhibitory concentration (MIC) values ranging from 1.56 to 3.125 mg/mL each for n-hexane and chloroform leaf extracts and MIC of 0.78 to 1.56 mg/mL each for ethanol and methanol leaf extracts were estimated. The minimum fungicidal concentration (MFC) of 6.25 mg/mL, 3.125 to 6.25 mg/mL, 1.56 to 6.25 mg/mL, 0.78 to 1.56 mg/mL for n-hexane, chloroform, ethanol, and methanol leaf extracts were observed and all the extracts were maximally active against *E. floccosum.* In conclusion, the potent anti-dermatophytic effect of the leaf extract of *Casia alata* Linn may be driven by their numerous phytochemicals, which justify its traditional uses for the treatment of skin diseases and could considerably illicit interest for the development of new drug leads.

Key words: Dermatophytes, *Cassia alata* Linn.*,* Crude leaf extract, Phytochemicals, Antifungal.

INTRODUCTION

Over the centuries, medicinal plants have served as remedies for infectious diseases due to the presence of bioactive components with therapeutic value (Fatmawati et al., 2020). Besides the nutrients such as vitamins, carbohydrates, lipids, proteins, and essential minerals that are provided by plants, man has long realized that the consumption of some plants offered additional benefits through improved health conditions (Lim, 2014),

hence the birth of the medicinal application of plants. Importantly, the parts of the plant used for medicinal purposes differ among plant species. For some plants, the bioactive component is in the roots while others have theirs in leaves, seeds, fruits, flowers, or stem bark (Selvi et al., 2012).

Cassia alata Linn (family, Fabaceae) is a tropical shrub, though can also grow well in the temperate. *C. alata* serves the purpose of both an ornamental and medicinal plant (Fatmawati et al., 2020). Bioactive compounds such as flavonoid, tannin, saponin, alkaloid, Phenol, steroid, alatinon, alanonal, and β-sitosterol-β-D-glucoside have been isolated from *C. alata* (Fatmawati et al., 2020) and the anti-inflammatory, anti-allergic, antioxidant, antidiabetic, anticancer, and antifungal potentials of the plants have been credited to the bioactive compounds (also called phytochemical substances) in different studies (Reezal et al., 2002; Sule et al., 2011; Akinmoladun et al., 2010; Patrick-Iwuanyanwu et al., 2011; Chatterjee et al., 2013; Fatmawati et al., 2020). The stem bark of *Cassia alata* Linn (*C. alata* L.) is used in the treatment of parasitic skin diseases, and fungal infections like ringworm and used as a common chemical ingredient in shampoos, lotions, and soaps due to the antifungal properties (Sule et al., 2011). In addition, the leaf extract of *Cassia alata* is credited for the treatment of intestinal parasitosis, constipation, inguinal hernia, venereal diseases (syphilis and gonorrhea), and diabetes (Dutta and Chatterjee, 2012). In Nigeria, the leaves of the plant in the form of an infusion are used as a purgative, and a strong decoction is sometimes given as an abortifacient or during labor to hasten delivery. Usually, the juice from the fresh leaves is taken alongside lime juice to treat worms (Dalziel, 1937).

Globally, health statistics have shown that conventional antimicrobial agents currently in use have met numerous challenges due to the increased drug resistance in human pathogens and the undesirable side effects associated with most drugs (WHO, 2020). Further, antimicrobial resistance contributes to an increase in health care costs owing to longer duration of illness, the requirement for additional tests, use of more expensive drugs as well as intensive care (WHO, 2020). Thus, there is a need to develop alternative, efficient, safe, and cost-effective natural medicines from plants (Toh et al., 2023). Over the last decades, human dependence on natural products from plants for maintaining health has increased dramatically (Samson et al., 2021). Therefore, studies to discover more medicinal plants and/ or highlight the scientific basis for the efficacy of plants employed in herbal medicine have become a priority globally. In this study, we evaluated the phytochemical properties and *in vitro* antifungal activity of crude leaf extract of *C. alata* (Linn.) on field strain dermatophytes.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh leaves in the upper part of *C. alata* plants (Figure 1) were collected from three different locations (7°15'-7°29'' N, 7°11'-7°32'' E) of the eastern part of Kogi State, Nigeria (Omatola et al., 2020). The leaf samples from the under one-year matured plants were obtained in 2019 from Anyigba, Egume and Ochaja. The leaves were carefully plucked from the stem using a sharp knife and were transported to the Department of Plant Science and Biotechnology, Prince Abubakar Audu University, where they were identified and authenticated by Mr. Momoh Theophilus.

Test organism

The dermatophyte isolates namely, *Trichophyton tonsurans*, *Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum audouinii*, *Microsporum canis*, *Microsporum gypseum, Epidermophyton floccosum* were field isolates obtained from previous (M. Sc) work in the Department of Microbiology, Prince Abubakar University Anyigba, Kogi State (Edegbo and Odama, 2015).

Preparation of plant materials

The leaves of each plant sample were separated from the stalk, spread separately on clean tarpaulins, and air-dried for about 10 days on the workbench. Using the Grindomix GM 200 grinding machine, the properly dried plant materials were pulverized individually into a fine powder (about 143 microns) and then packed in an airtight container made from plastic for further processing.

Sample processing

Aqueous extraction

The modified method of Adegoke et al. (2010) was used. Briefly, one hundred grams (100 g) of the powdered leaf of *Cassia alata* was macerated in 400 ml of sterile distilled water for 72 h. The resulting extract was filtered through Whatman no 1 filter paper and then concentrated using a water bath following the procedure described by Timothy et al. (2012). Further, the extract was placed in a desiccator to dry to a constant weight and then weighed. The extract was kept for further use following the determination of the percentage yield.

Gradient extraction of the powdered plant materials (soxhlet extraction)

Five hundred grams (500 g) of powdered leaves of *Cassia alata* were packaged into a cellulose thimble in the Soxhlet extractor compartment. Four extraction solvents (hexane, Chloroform, Ethanol, and Methanol) of different polarities were used to extract bioactive compounds from the plant material. An exhaustive

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Figure 1. Field image of *Cassia alata* Linn.
Source: Fatmawati et al., 2020.

extraction of the plant material was carried out with each solvent until the extracting solvent was clear showing no extracted materials were visible again. In between the solvent and the next, the powdered material in the cellulose thimble was emptied onto filter paper spread out, and then dried to a constant weight. It was then repacked into thimble and exhaustive extraction with the next solvent continued. After the extraction process, the hexane, chloroform, ethanol, and methanol extracts of the plant material were subjected to rotary evaporation in a water bath to concentrate the extracts. The residue or dried extracts were transferred into pre-weighed sample bottles, labeled appropriately, and placed in a desiccator, to remove any excess solvent and moisture. These extracts were weighed periodically until a constant weight was obtained for each. Then the final weights were taken for the purpose of determining the percentage yield of each solvent.

Determination of the extracts yields

Extract yield (X_3) = $X_1 - X_2$
Where; X_1 = weight of beaker + crude extract
X_2 = weight of beaker only

Percentage yield (%) = ($X_1 - X_2 \div$ weight of powder used) × 100 (Samson et al., 2021)

Preliminary phytochemical screening of plant extracts

Employing the standard qualitative procedures described by Evans and Trease (2002), Harborne (1998), and Sule et al. (2011), the powdered crude leaf extracts of *Cassia alata* Linn. were screened for phytochemical compounds and secondary metabolites such as Alkaloids, glycosides, carbohydrates, cyanogenic glycosides, Anthraquinones, Flavonoids, cardiac glycosides, Saponins, Steroids, phlobatannins proteins, Tannins, and Terpenoids. Briefly, for alkaloids detection, half-gram (0.5 g) of crude extracts of *Cassia alata* was dissolved in 10 ml of aqueous HCl in a steam bath and filtered. Two (2) drops of Mayer's reagent were added to 2 ml of the filtrates and the presence of alkaloids was indicated by a yellowish precipitate. For carbohydrate detection, 0.5 g of crude extracts of *Cassia alata* were dissolved in 10 ml of water and filtered. Two drops of Benedict's reagent were added to 2 ml of the filtrate and heated in a water bath. The presence of carbohydrates was indicated by an orange-red precipitate. For glycosides detection, half a gram (0.5 g) of crude extracts of *Cassia alata* were dissolved in 10 ml of water and hydrolyzed with aqueous HCl. Ten (10) ml of Fehling's solutions A and B were added to the resultant solution and heated for 15 min. Glycoside presence was indicated by the formation of a brick-red precipitate. For the detection of Anthraquinone, half a gram (0.5 g) of crude extracts of *Cassia alata* dissolved in 10 ml of water were hydrolyzed with aqueous HCl. Two drops of ferric chloride solution were added to 2 ml hydrolysates and the mixture was heated for 5 min in boiling water. After cooling, the solution was shaken with an equal volume of benzene.

Furthermore, the separated benzene layers were treated with half of its volume of ammonia solution and observed for the formation of rose-pink or cherry red. For Cardiac glycoside, half a gram (0.5 g) of crude extracts of *Cassia alata* dissolved in 10 ml of water was hydrolyzed with aqueous HCl. The hydrolysates (2 ml) were dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solutions and then underlaid with 1 ml of concentrated H_2SO_4 and observed for a brown ring. For cyanogenic glycosides detection, half a gram (0.5 g) of crude extracts of *Cassia*

alata dissolved in 10 ml of water were hydrolyzed with aqueous HCl. A piece of filter paper was saturated in a freshly prepared solution of guaiac resin and was dissolved in absolute ethanol and dried completely in the air. The filter paper was carefully moistened with a dilute solution of $CUSO_4$ and placed in contact with the hydrolysates and observed for the distinct strain of paper. For saponins, half a gram (0.5 g) of crude extracts of *Cassia alata* was diluted with 20 ml of distilled water separately and further shaken for 15 min in a graduated cylinder and observed for the formation of foam. For the detection of phytosteroids, 2 drops of concentrated Sulfuric acid were added to a solution containing 0.5 g extracts of *Cassia alata* and 5 ml of chloroform. After vigorous shaking, the solution was allowed to stand for some time. The presence of phytosteroids was indicated by the appearance of red or yellow colour. For flavonoids, half a gram (0.5 g) of crude extracts of *Cassia alata* dissolved in 10 ml of water was filtered and 2 drops of sodium hydroxide solution was added to 2 ml of the filtrates. The presence of flavonoids was indicated by a yellow color which becomes colourless with the addition of dilute acid. For phenols, a half gram (0.5 g) of crude extracts of *Cassia alata* dissolved in 10 ml of water were filtered and 2 drops of $FeCl_3$ solution were added to the 2 ml of the filtrates Presence of phenols was indicated by colour change, from green to black. For detection of tannins, 0.5 g of crude extracts of *Cassia alata* treated with 2 drops of vanillin hydrochloride reagent was observed for a pink to red colour formation. For proteins and amino acids, half a gram (0.5 g) of crude extracts of *Cassia alata* first dissolved in 10 ml of distilled water was filtered and 2 drops of concentrated Nitric acid were added to the filtrates (2 ml) and then observed for the presence of yellow colour. For fixed oils and fats detection, 0.1 g of the crude extract of *Cassia alata* was pressed between two filter papers separately and observed for an oily stain on the filter paper.

Evaluation of the antimycotic activity of various crude extracts of *Cassia alata* leaves

Preparation of inoculum

Stock inoculum suspension of dermatophytes isolates was prepared from a 7 to 15-day-old culture grown on SDA at ambient temperature (28±2^{0}C). Matured colonies were covered with 10 ml of sterile saline (0.85%) with a drop of polysorbate (tween 80 sigma). The surfaces were scraped using the tip of a pasture pipette. The resulting mixture of conidial and hyphal fragments was withdrawn and transferred to sterile test tubes. Heavy particles were allowed to settle for 15 min at room temperature (28±2°C). The upper suspension was mixed with a vortex mixer for 15 sec. The turbidity of the supernatants was measured using a spectrophotometer at a wavelength of 530 nm and transmission was adjusted to 65-70%. Each suspension was diluted 1:50 in SDA to obtain conidia suspension of 0.5 Mcfarland (approximately 10^6 SFU / ml) standards respectively. Plate counts were performed to verify the conidial concentration by plating 0.0 1 ml of the adjusted conidial suspension to determine the viable number of conidia and millimeters (NCCLS, 2002).

Preliminary antimycotic screening of aqueous, n-hexane, chloroform, ethanol, and methanol crude leaf extracts of *Cassia alata*

The preliminary screening for antimycotic activity of aqueous leaf extracts of *Cassia alata* was carried out using the agar incorporation method as described by Zacchino et al. (1999) and Hassan et al. (2007). 0.5 g/ ml of the aqueous extracts of *Cassia alata* was aseptically mixed with 15 ml of Sabouraud dextrose agar after cooling and solidification of the medium. The seeding was carried out by inoculating the test organisms on the plates. A control plate which contains the organism and the medium alone was also set up. The treated and control Petri dishes were incubated at ambient laboratory conditions for 72 h. The presence of growth (+) is a negative test (indicating the non-potency of the extracts) and the absence of growth (-) is a positive test (indicating potency of the extracts).

Determination of the antimycotic activity of various crude leaf extracts of *Cassia alata* Linn.

Preparation of Extracts

The stock solutions of all the extracts were prepared in water at varying concentrations. All the extracts were ten-fold serially diluted as described by National Committee for Clinical Laboratory Standards (NCCLS) (2002) followed by a further two (2) fold dilution in Sabouraud dextrose broth to yield the final concentrations required for testing.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the extracts

The NCCLS broth microdilution reference method was performed according to the NCCLS M27-P (National Committee for Clinical Laboratory Standards (1992) and the modified method of Adegoke et al. (2013). A total number of 11 test tubes (Khan Tubes) were used for the determination of MIC. 1 ml of SDB was dispensed into test tubes 2-11 each. From the stock solution of the extracts (100 mg/mL), 1 ml was dispensed into tube 1 and another ml into tube 2. From the content in tube 2, two-fold serial dilutions were carried out up to tube 9. From tube 9, 1 ml was pipetted out and discarded. The concentrations in the tubes were 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.78, and 0.39 mg/mL. A total of 0.05 ml of dermatophytes spore suspension of each organism previously diluted to give 0.5 spore/ ml was dispensed into tubes 1 to 11 except for tube 10. To tube 10 1 ml of sterile SDB was added. Tube 1 which contained 1 ml of the extract and 1 ml of the spore solution of the test organism served as the control for the extract, tube 10 which contained 1 ml of sterile SDB served as the control for the sterility of the medium, and tube 11 which contain 1 ml of the spore solution of the test organism and 1 ml of sterile SDB served as control for the viability of the test organism. All tubes were incubated at ambient temperature for 72 h or until growth was apparent in the growth control tubes. Tubes were observed visually for growth based on turbidity or cloudiness. The lowest concentration of extract which produced complete inhibition was regarded as the MIC. After the MIC determination, 0.05 ml of broth was transferred from all tubes showing no growth and from the first tube in which growth was detectable to plates of SDA. The plates were incubated at ambient laboratory conditions for 72 h. The lowest concentration of extracts for which subculture did not show any growth was regarded as the MFC.

RESULTS AND DISCUSSION

The extracts from plants have demonstrated a significant level of activity against important microbial pathogens because of their numerous phytochemicals. The risen trends in antibiotic resistance, serious side effects, costs of hospital care, and the high costs of developing synthetic drugs are driving the focus to natural medicine from plants (Fatmawati et al., 2020; Samson et al., 2021; Toh et al., 2023).

Table 1. Percentage yields of aqueous n-hexane, chloroform, ethanol and methanol leaf extracts of *Cassia alata.*

Extracts	Aqueous	N-hexane	Chloroform	Ethanol	Methanol
Yield g (%)	4.6 (5.6)	25.5 (5.7)	7.0 (1.6)	21.3 (4.8)	12.4 (2.8)

Source: Authors

Table 2. Qualitative phytochemical constituents of n-hexane, chloroform, ethanol and methanol leaf extracts of *Cassia alata.*

Phytochemicals	N-hexane	Chloroform	Ethanol	Methanol
Glycosides	+	+	+	+
Terpenoids	-	+	+	+
Steroids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Carbohydrates	+	+	+	+
Essential oils	+	-	-	-
Anthraquinone	+	+	+	+
Cardiac glycodides	+	+	+	+
Phlabotannins	+	+	+	+
Cyanogenic glycosides	-	-	-	-

+ (present); - (absent).
Source: Authors

In the current study, the percentage yield (%) of crude aqueous, n-hexane, chloroform, ethanol, and methanol leaf extracts of *Cassia alata* was 4.6 g (5.6%), 25.5 g (5.7%), 7.0 g (1.6%), 21.3 g (4.8%), and 12.4 g (2.8%), respectively (Table 1). Various phytochemical compounds such as glycosides, anthraquinone, proteins, cardiac glycosides, steroids, alkaloids, phlabotannin, phenols, saponins, tannins, flavonoids, and carbohydrates were present in all the extracts. All the metabolites found in this study have been credited to the therapeutic action of *C. alata* plants in different studies (Akinmoladun et al*.,* 2010; *Sule* et al., 2011; Patrick-Iwuanyanwu et al., 2011; Chatterjee et al., 2013). However, terpenoids present in the chloroform, ethanol, and methanol extracts were not detected in the hexane extracts, contrary to reports of Saha et al. (2020) who detected terpenoids in all the extracts. This observation is likely pointing to the high volatility of the terpenoid solvent. Likewise, essential oil was detected in the n-hexane extracts only while cyanogenic glycosides were absent in all the extracts (Table 2). This finding is not unusual as a similar study by Ndukwe et al. (2020) in another geographical location did not detect most of the bioactive compounds in *C. alata*. Previously, differences in phytochemicals detection were ascribed to variations in geographical location because of soil mineral concentration (Samson et al*.,* 2021). Similar to our study, variations in bioactive substance detection in the same species of plant have been observed (Ndukwe et al. 2020), probably a result of process differential in the plant biomolecular or secondary metabolites extraction or due to variation in bio-responsiveness in plant estrogen levels which may affect their detection.

In our preliminary screening for antidermatophytic activity of the crude aqueous, n-hexane, ethanol, and methanol leaf extracts of *Cassia alata* shown in Table 3, all the extracts demonstrated antimycotic activity against the tested organisms. Further, the result of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of aqueous, n-hexane, ethanol, and methanol leaf extracts of *Cassia alata* against the test organisms shown in Table 4 confirmed the results of the preliminary screening as it showed that none of the selected organism plated on Sabouraud dextrose agar plates incorporated with the crude extracts preparations could grow against the controls (pure SDA plates) where normal growth was observed. The inhibited growth by the crude extracts suggested the presence of some phytoconstituents with antimicrobial potential. The antifungal activity of the aqueous extract of *Cassia alata*

Table 3. Preliminary qualitative antimycotic activity of aqueous, n-hexane, chloroform, ethanol and methanol leaf extracts of *Cassia alata*.

Organisms	Extracts					
	Aqueous	n-hexane	Chloroform	Ethanol	Methanol	Control
Epidermophyton floccosum	-	-	-	-	-	+
Trichophyton mentagrophytes	-	-	-	-	-	+
Trichophyton tonsurans	-	-	-	-	-	+
Trichophyton rubrum	-	-	-	-	-	+
Microsporum canis	-	-	-	-	-	+
Microsporum audouinni	-	-	-	-	-	+
Microsporum gypseum	-		-	-	-	+

- = Inhibitory (that is, no growth); + = Not inhibitory.
Source: Authors

Table 4. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of leaf extracts of *Cassia alata*.

Organisms	Extracts (mg/ml)							
	n-hexane		Chloroform		Ethanol		Methanol	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Epidermophyton floccosum	1.56	6.25	1.56	3.13	0.78	1.56	0.39	0.78
Trichophyton mentagrophytes	3.13	6.25	3.13	6.25	1.56	3.13	1.56	1.56
Trichophyton tonsurans	3.12	6.25	3.13	6.25	1.56	3.13	1.56	1.56
Trichophyton rubrum	3.13	6.25	1.56	3.13	1.56	3.13	0.78	1.56
Microsporum canis	1.56	6.25	1.56	6.25	1.56	3.13	0.78	1.56
Microsporum audouinni	3.13	6.25	3.13	6.25	1.56	3.13	1.56	1.56
Microsporum gypseum	3.13	6.25	3.13	6.25	1.56	6.25	1.56	1.56

MIC – minimum inhibitory concentration, MFC – minimum fungicidal concentration.
Source: Authors

reported in this present study is in agreement with the findings of Hassan et al. (2007), in which aqueous extracts of *Casia alata* were active against some fungal isolates including dermatophytes (*T. rubrum* and *M. gypseum*). Inhibition of fungal cell walls due to pore formation in the cell and leakage of cytoplasmic constituents by the active components of leaf extracts of *Cassia alat*a has been suggested (Hassan et al., 2007).

Cassia alata leaves extract obtained using n-hexane demonstrated high antifungal activity with MIC values ranging from 1.56 to 3.125 mg/mL and MFC values of 6.25 mg/mL and *E. floccosum* and *M. canis* were the most susceptible. A low susceptibility level was observed for *T. mentagrophyte, T. tonsurans, T. rubrum, M. audouinii* and *M. gypseum*. This agrees with Sujatha and Asokan (2018), who reported the antifungal activity of hexane extract of *Cassia alata* leaves against strains of *M. canis, T. mentagrophytes, T. rubrum, E. floccosum,* and *M. audouinii* but with varying concentrations.

Cassia alata leaves extract obtained using chloroform showed a high antifungal activity with MIC values ranging from 1.56 to 3.125 mg/ml and MFC values ranging from 3.125 to 6.25 mg/ml. Its highest activity was observed against *E. flocossum* and *T. rubrum*. Other tested isolates had similar activities. This finding corroborates the report of Selvi et al. (2012), which indicated high antimycotic activity of *Cassia alata* leaves extracts of chloroform on fungal isolates including *E. floccosum, M.gypseum,* and *T. mentagrophyte. Cassia alata* leaf extract obtained using ethanol showed a high antifungal activity with MIC values ranging from 0.78 to 1.56 mg/ml and MFC values ranging from 1.56 to 6.25 mg/ml. The *E. floccosum* was the most susceptible to this extract, closely followed by *T. mentagrophyte, T. tonsurans, T. rubrum, M. canis, M. audouinii,* and *M. gypseum*. This antifungal activity level recorded is in direct agreement with the report of Sule et al. (2011) who marked a good antifungal effect of ethanol extract of *Casia alata* leaf on *Microsporum canis, T. mentagrophytes, Epidermophyton fluccossum* and *Trichophyton verrucosum*.

Cassia alata leaf extract obtained using methanol solvent showed the highest antifungal activity among all

other plant extracts with MIC values ranging from 0.39 to 1.56 mg/mL and MFC values ranging from 0.78 to 1.56 mg/mL of which *E. floccosum* was the most susceptible to this extract. All other isolates tested showed considerable susceptibility levels. Methanol by these findings has proven not only to be the best solvent for extraction but also the solvent that yielded the most active antimycotic agent. This could be attributed to its relatively higher polarity and ability to extract both lipophilic and hydrophobic substances (Fawehinmi et al., 2013). Hence a better extraction of the phytoconstituents of the plants is obtainable. Therefore, methanol is undoubtedly a good solvent source for the extraction of potent pharmacological agents.

CONCLUSION

This study revealed that leaf extracts of *Cassia alata* contain several phytoconstituents such as glycosides, anthraquinone, terpenoids, steroids, alkaloids, saponins, tannin, flavonoids, phenols, carbohydrates, proteins, and essential oil. From this study, it was deduced that the presence of these active compounds in the leaf extracts may be responsible for the antidermatophytic activity exhibited by the plant. Aqueous, n-hexane, chloroform, ethanol, and methanol leaf extracts of *Cassia alata* showed activity on all the tested dermatophytes. Both the minimum inhibitory concentration and minimum fungicidal concentration of the n-hexane, chloroform, ethanol, and methanol leaf extracts of *Cassia alata* revealed that the methanol extract exhibited the highest antimycotic activity. Finally, the *in vitro* antimycotic activity of *Cassia alata* (Linn.) against the field strain dermatophytes observed in our study depict the plants as promising vegetal drug candidate and viable alternative to conventional drugs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Beneficial microorganisms as affecting root development of upland rice

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Multifunctional microorganisms can significantly affect root and shoot development of upland rice seedling that could provide an increase in the crop grain yield. The objective of this study was to determine the effect of single and combined microorganism on the root and shoot development of upland rice seedlings. The experiment was arranged in a completely randomized design with treatments consisting of the upland rice seeds treated with single and combined multifunctional microorganisms (M01 (*Serratia marcescens*), M02 (*Bacillus toyonensis*), M03 (*Phanerochaete australis*), M04 (*Trichoderma koningiopsis*), M05 (*Azospirillum brasilense*), M06 (*Azospirillum* species), M07 (*Bacillus* species), M08 to M28 (combination of these microorganisms) and M29 (control – no microorganism). *S. marcescens* with *B. toyonensis* led to the greatest increase (296%) in root length relative to the control. *B. toyonensis* with *A. brasilense* greatly increased root surface area by 209% in comparison to the control. An increased root diameter by 36% was recorded for upland rice inoculated with *A. brasilense* with *Bacillus* spp. in relation to the control. *P. australis* with *Bacillus* spp. greatly increased root volume (47%) in comparison to the control. It can be concluded that multifunctional microorganisms enhanced root length, root surface area, root diameter and volume, and provided better root development.

Key words: Microbiolization, root length, germination, beneficial bacteria, beneficial fungi.

INTRODUCTION

The application of multifunctional microorganisms improves plant development through direct and indirect mechanisms, and has shown that it is possible to make crop management practices more environmentally sustainable (Cruz et al., 2023; Silva et al., 2023). These mechanisms are a result of production of specific metabolites, such as growth stimulants (phytohormones), hydrolytic enzymes, siderophores, antibiotics, and carbon and nitrogen hence acting as a growth promoter (Silva et al., 2022; Rezende et al., 2021). The multifunctional microorganisms also ensure that the plant receives solubilized nutrients which are fixed in soil minerals and not available to the plants (Sousa et al., 2021). The study of these microorganisms is now currently intense due to the elevating demand for sustainable technologies, which reduces cost of production, increases the productivity,

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profitability of agribusiness and increases efficiency of fertilizer usage (Couto de Araujo et al., 2021).

The root system is the main organ for plants to absorb nutrients and water, and the root growth condition directly affects the growth of plant shoots (White, 2009; Fernandes et al., 2021). Plant roots can adapt to the changes of soil nutrient conditions through active physiological regulation to absorb more nutrients (Sun et al., 2020). The rhizosphere is the center of the interaction among plant roots, soil, microorganisms, and environment. It is a nutrient-rich habitat and harbors a huge variety of bacteria and fungi that each can have neutral, beneficial or deleterious effects on the plant (Berendsen et al., 2012). The beneficial effect of rhizosphere include effect on plant growth by response to root exudates in the root microorganism environment and carbon sources for the microbial growth activities and the community composition through the process of rhizosphere deposition. The plant will establish beneficial connections with some microorganisms in the rhizosphere (Toju et al., 2018) thereby leading to a positive impact on plant health and productivity.

In an experiment to determine the effect of six multifunctional microorganisms on upland rice seedling performance, Fernandes et al. (2021) found that upland rice seedlings treated with multifunctional microorganisms showed increments in all parameters evaluated in the root system. Furthermore, they stated that microorganism *Azospirillum* species provided higher values in the parameters of total root length, root volume, and dry root mass in upland rice seedlings compared to the control treatment. However, it is imperative to carry out research on the sole and combined effect of these multifunctional microorganisms on the root development of upland rice. Additionally, there is need to evaluate the probable response of other root development parameters (root diameter, root surface area) to multifunctional micro-organisms, hence the need for this study. The objective of this study was to determine the effect of single and combined microorganism on the root and shoot development of upland rice seedlings.

MATERIALS AND METHODS

Site description

The experiment was conducted in the Agricultural Microbiology Laboratory at Capivara Farm, headquarters of Embrapa Rice and Beans, located in the Santo Antônio de Goiás, Goiás, Brazil in January 2022.

Experimental design and treatments

The experimental design was a completely randomized with twenty-nine (29) treatments and ten replications. The treatments consisted of the microbiolization of the seeds (a process that puts the seeds in contact with the microorganism solution to be tested) of upland rice cultivar BRS A501 CL with the multifunctional microorganisms *Azospirillum* spp. (BRM 63574), *Azospirillum brasilense* (AbV5), *Phanerochaete australis* (BRM 62389), *Serratia marcescens* (BRM 32114), *Bacillus toyonensis* (BRM 32110), *Bacillus* species (BRM 63573), *Trichoderma koningiopsis* (BRM 53736) and the control treatment (without microorganisms), then the combination of two microorganisms were also used. The multifunctional microorganisms used in this experiment were deposited in the collection of microorganisms of Agricultural importance of Embrapa Rice and Beans.

Preparing suspension of microorganism

To generate the suspensions of each microorganism, the methods used by Nascente et al. (2017) were used. Briefly, isolates of cultures of multifunctional microorganisms grown on a solid medium (nutrient agar) were used, and the suspensions were prepared in liquid medium 523 (nutrient broth) (Kado and Heskett, 1970) in a shaking incubator for 24 h at 28°C.

Seed sterilization and microbilization

The seeds were disinfected by soaking in 7.5% sodium hyplochlorite for 7 min, and drained after 3 min, afterwards the seeds were soaked in 70% alcohol for 30 min, drained, then rinsed with distilled water three times. The upland rice seeds were dried by placing them in a paper towel contained in a tray in a drying room of temperature at 29°C before microbilization. Microbilization was done with the immersion of the upland rice seeds in the rhizobacterial suspension, and for the control treatment, seeds were immersed in distilled water, for 24 h at a temperature of 25°C, under constant agitation, following the proposed methodology of Filippi et al. (2011).

Planting and germination of upland rice

The seeds were placed into the test tube after drying with the tip of a spatula. Each tube of experimental unit composed of two upland rice seeds cultivar (BRS A501 CL), placed to germinate in a 50 mL volume test tube containing 15 mL of agar-water medium (0.8 m/v). The tubes were placed in a germination room at 28°C with a 12-h photoperiod and removed 10 days after seeding.

Determination of growth parameters

The seedlings were removed from the test tubes, photographed with a camera and image processing was performed. The images obtained were analyzed using WinRHIZO 2012b software. With the software, these parameters were determined: Total Root Length (Comp R, cm), Root Diameter (Diam R, mm), and Root Volume (Vol R, cm^3). After recording the parameters, the dry biomass of the rice seedlings was determined after dehydration in a forced air chamber at 65°C until a constant mass was gotten by checking the values on a precision scale.

Statistical analysis

The data were subjected to analysis of variance and, when significance was detected, the means were compared using the Scott Knott test ($p<0.05$). The SAS statistical package was used to process these data. Additionally, a multivariate principal component analysis (PCA) was performed to describe the correlation between response variables (shoot, root and total biomass, yield components and grain yield) with isolated and mixed microorganisms.

The main components (MCs) were loaded with response variables when the correlation test produced r >0.50. The first three MCs responsible for >62% of the data variation were maintained. Biplots (two-dimensional graph) using these three MCs that correlate isolated and mixed microorganisms and response variables were built with the "FactoExtra" package on the R platform.

RESULTS AND DISCUSSION

The root length (ranged from 6.76 to 26.77 cm), volume (ranged from 0.095 to 0.234 cm³), surface area (ranged from 4.30 to 28.54 m²) and diameter (ranged 0.28 from to 0.60 mm) of upland rice seedlings were affected by microorganisms and differed from the control (no microorganisms) (Figures 1 to 4). The highest values for the root length (Leng R, cm) of the upland rice seedlings were obtained with the treatments: *B. toyonensis* (BRM 32110), *A. brasilense* (AbV5), *S.marcescens* – (BRM 32114) + *B. toyonensis* (BRM 32110), *S. marcescens* (BRM 32114) + *A. brasilense* (AbV5), *B. toyonensis* (BRM 32110) + *A. brasilense* (AbV5), *Bacillus* spp. (BRM 63574) + *B. toyonensis* (BRM 32110), *P. australis* (BRM 62389) + *S. marcescens* (BRM 32114) which were 18.58, 20.74, 26.77, 23.07, 18.12, 16.95, and 20.82 cm, respectively and significantly much higher than that of the control treatment (6.76 cm) (Figure 1). This could be attributed to increased physiological processes of the treated plants relative to untreated ones. According to Nascente et al. (2017), when comparing treated to untreated plants, the treated rice plants had a more effective photosynthesis, stomata permeability, and transpiration process for exchanging gases which could contribute to the growth of rice plants. Furthermore, the combination of *S. marcescens* and *B. toyonensis* led to the greatest increase (296%) in root length relative to the control. This could be attributed to the fact that *S. marcescens* and *B. toyonensis* were able to fix nitrogen, solubilize phosphate and also produce indoleacetic acid (IAA) which aided in the elongation of the root relative the control. It was reported that *S. marcescens* (BRM 32114) was observed to be the most effective microbe in promoting increases in rice shoot biomass (Nascente et al., 2017).

The inoculation of upland rice seedling was observed with M02, M04, M05, M06, M09, M11, M16, M19, M22, M23, M24, M25, and M27 which led to a significantly greater increase in root volume (Vol R), with 0.182, 0.177, 0.203, 0.168, 0.192, 0.170, 0.226, 0.217, 0.227, 0.234, 0.212, 0.190, and 0.204 cm³, respectively, higher than those of the control treatment (Figure 2). Additionally, the mixture of *P. australis* and *Bacillus* spp. greatly increased root volume (47%) in comparison to the control. Fernandes et al. (2020) reported that the use of multifunctional microorganisms significantly affects the total biomass production of the plants, as well as the accumulation of nutrients in the shoots and roots, and in the yield components, resulting in significant increases in the grain yield of the crop. All sole microorganisms except *S. marcescens*, *P. australiani* and *Bacillus* spp. had a significantly higher effect on root volume as compared to the control. However, all combination of *P. australiani* with other microorganism except *B. toyonensis* and *T. konigiopsis* significantly increased the root volume of upland rice in comparison to other combinations.

The inoculation of upland with sole and combined microorganism significantly affected the root area (Figure 3). Inoculation of upland rice seedling with M02 (20.85 cm²), M05 (24.27 cm²), M06 (21.31 cm²), M08 (26.45 cm²), M09 (22.97 cm²), M11 (28.54 cm²), M16 (25.58 cm²), M22 (18.32 cm²), M23 (18.93 cm²), M24 (20.53 cm²), M25 (18.00 cm²) and M27 (24.74 cm²) led to a significantly greater total root surface area relative to the control (9.21 cm²) and other treatments. The mixture of *B. toyonensis* and *A. brasilense* greatly increased root surface area by 209% in comparison with the control. Banayo et al. (2012) reported that *A. brasilense* positively influenced the increase in the production of biomass and grain yield of rice crop by producing growth hormones and stimulating greater root development and greater absorption of nutrients, with direct effects on the development of the plant. Among single microorganism treatments, *B. toyonensis, A. brasilense,* and *Azospirillum* spp. were superior in terms of effect on root area.

The inoculation of multifunctional microorganism significantly affected the root diameter (Figure 4). This is supported by the findings of Wijayanto et al. (2021) who reported that the use of PGPR was able to increase root length, dry weight of roots and stems, root development, and N content in roots of rice plants. Inoculation of upland rice seedlings with M4 (0.057 mm), M11 (0.048 mm), M16 (0.060 mm), M19 (0.055 mm), M22 (0.053 mm), and M23 (0.056 mm) significantly increased the root diameter relative to the control (0.044 mm) and other treatments. Furthermore, the highest percentage increase in root diameter of upland rice was observed with the inoculation with M16: *A. brasilense* and *Bacillus* spp. The combination of *A. brasilense* and *Bacillus* spp. led to the highest increase in root diameter (36%) of upland rice inoculated in comparison to the control. All treatments except M4, M11, M16, M19, and M23 were similar to the control. Among sole microorganism, only the inoculation of upland rice seedling with *T. koningiopsis* led to significant increases in root diameter. Species of *Trichoderma* have been reported to promote increases of up to 300% in plant growth (Brotman et al., 2010).

With principal component analysis, it can be seen that the variability of treatments with isolated and combined microorganisms with respect to total root length, root diameter, total root surface area, root volume, and root dry mass of rice seedlings treated with multifunctional microorganisms were best described by two principal components (PCs), accounting for 98.1% of the variation in the data, that is, PC1 (62.1%) added to PC2 (36%) (Figure 5). The factor map (biplot) shows groups of

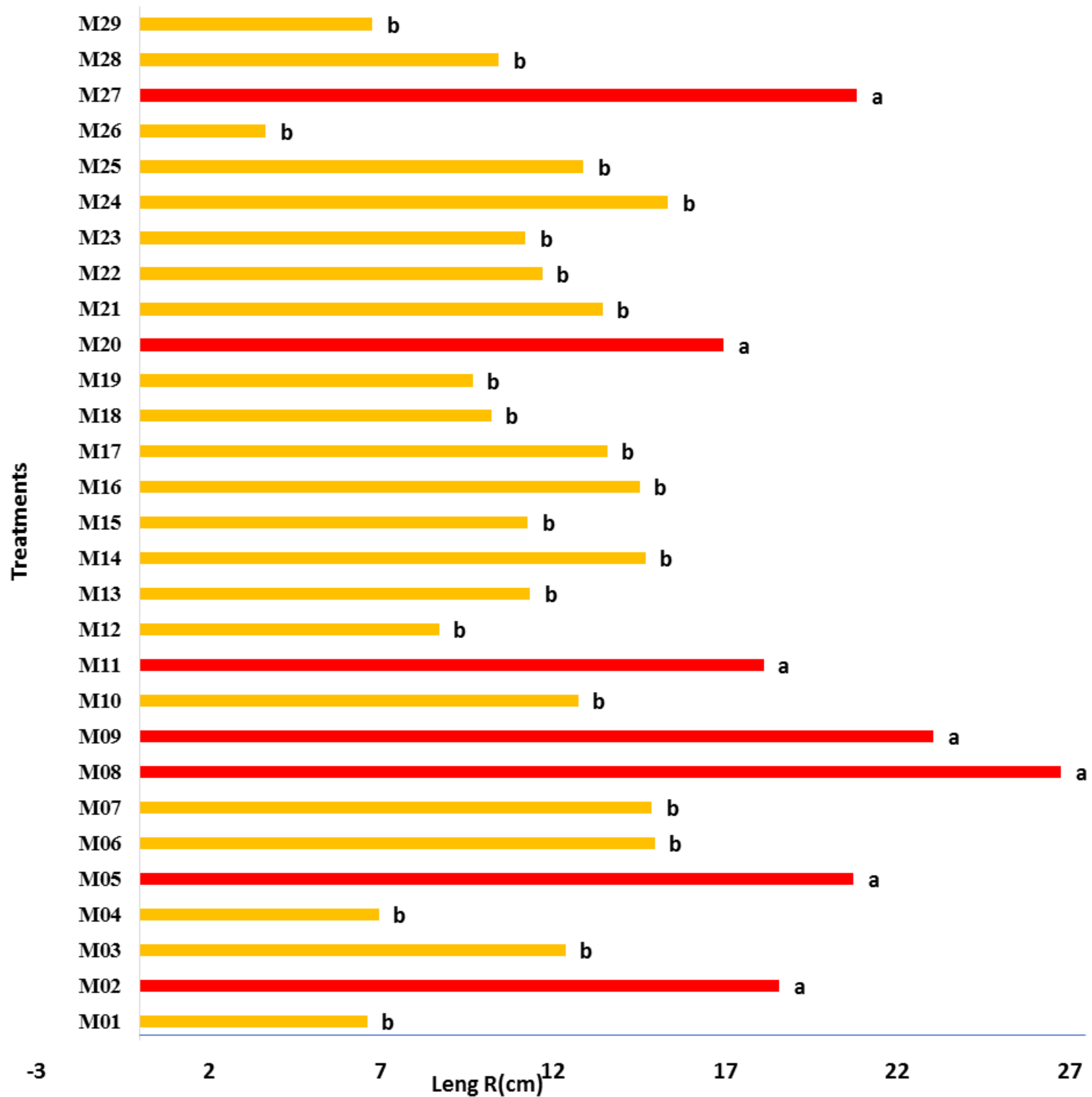

Figure 1. Effect of growth promoting microorganism on total root length of upland rice seedlings cultivar A 501 CL at ten days after sowing. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at p< 0.05. **Treatments: M01- BRM 32114 (*Serratia marcescens*), M02-BRM 32110 (*Bacillus toyonensis*), M03- BRM 62389 (*Phanerochaete australiani)*, M04 -BRM 53736 *(Trichoderma koningiopsis)*, M05-AbV5 (*Azospirillum brasilense)*, M06 -BRM 63574 *(Azospirillum sp.*), M07-BRM 63573 *(Bacillus* spp.), M08-BRM 32114 + BRM 32110 , M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control.
Source: Authors

variables (arrows) denoting positive and negative correlations with each principal component (PC), with the length of the arrow indicating the magnitude of each response for each PC (Figure 5B). For example, PC1 was negatively correlated to all variables analyzed. On the other hand, PC2 was positively correlated with root length and root surface area, and negatively correlated with root diameter and root volume.

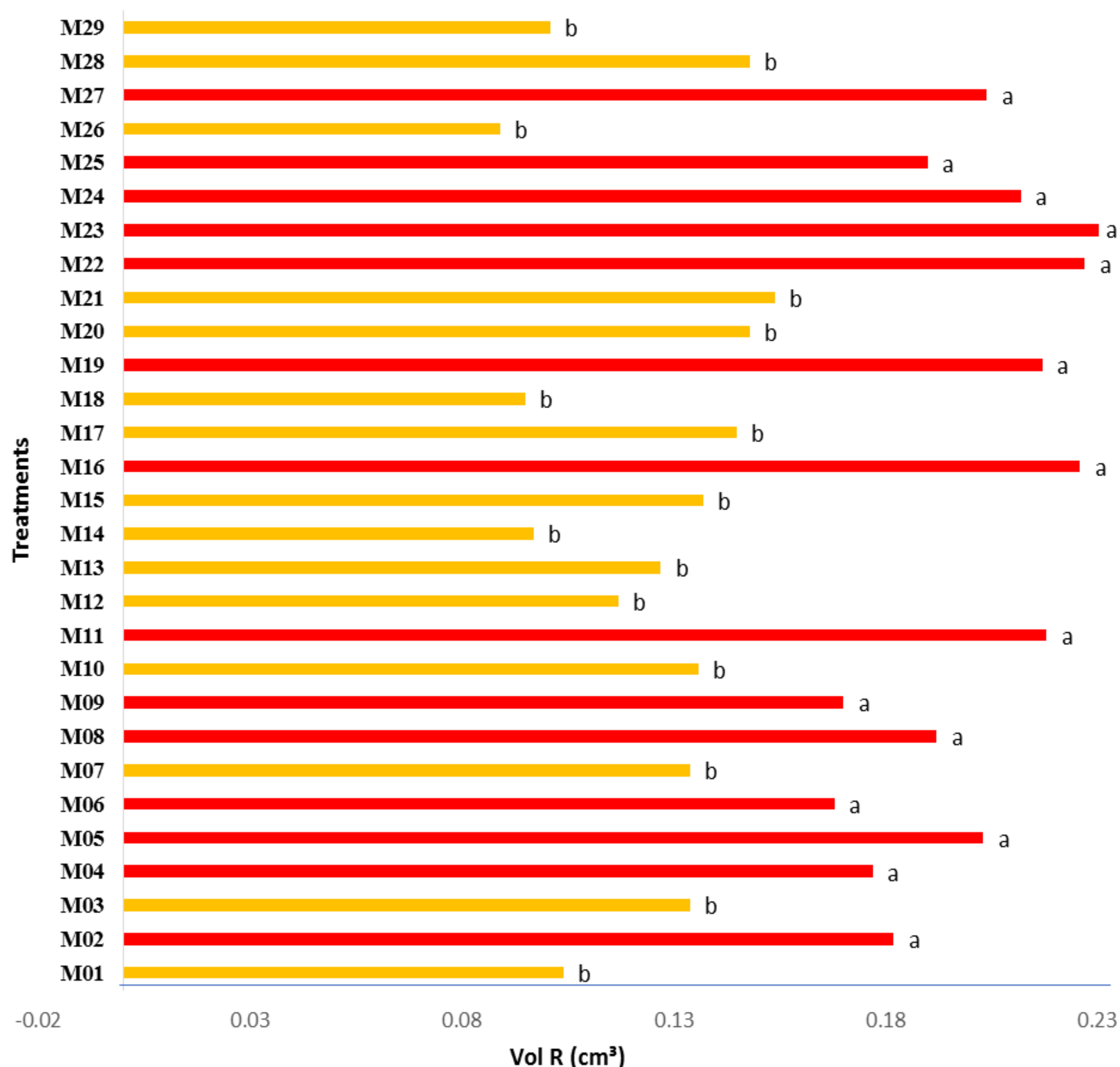

Figure 2. Effect of growth promoting microorganism on root volume of upland rice seedlings cultivar A 501 CL at ten days after sowing. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$. **Treatments: M01- BRM 32114 (*Serratia marcescens*), M02-BRM 32110 (*Bacillus toyonensis*), M03-BRM 62389 (*Phanerochaete australiani)*, M04 -BRM 53736 *(Trichoderma koningiopsis)*, M05-AbV5 (*Azospirillum brasilense)*, M06 -BRM 63574 *(Azospirillum* spp.), M07-BRM 63573 *(Bacillus* spp.), M08-BRM 32114 + BRM 32110, M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control.
Source: Authors

Based on the representational quality of the treatments with microorganisms alone and in combination for the analyzed variables, the treatments M08 and M09, highlighted in purple (Figure 5A), obtained the highest positive correlation for total root length (Figure 5B). The variable total surface area correlated positively to treatments M02, M05, M06 and M27, highlighted in red. The treatments M11, M24 and M25, in blue, correlated positively with the root volume variable. Regarding the remaining root diameter variable, a positive correlation

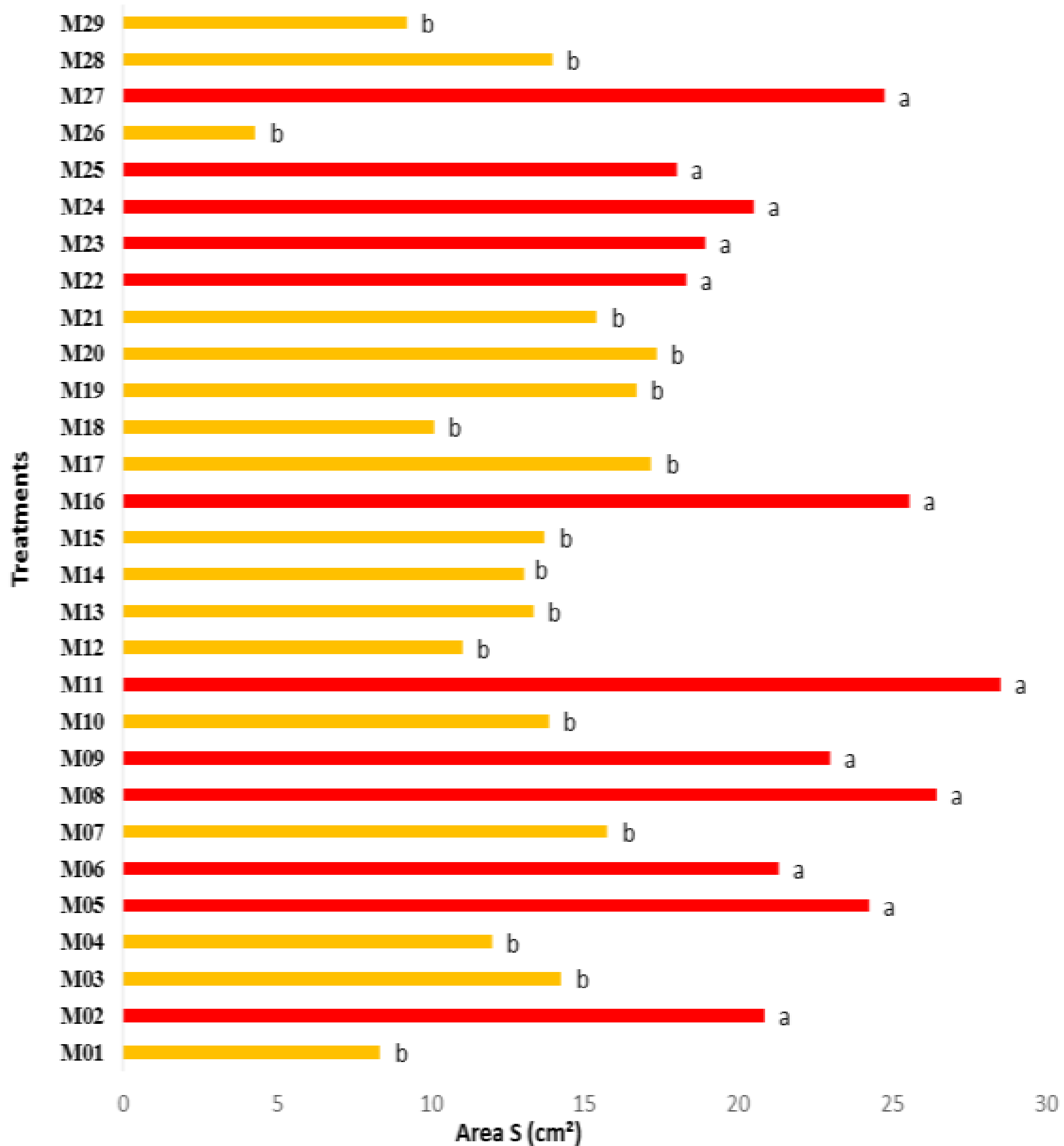

Figure 3. Effect of growth promoting microorganism on surface area of upland rice seedlings cultivar A 501 CL at ten days after sowing. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$. **Treatments: M01- BRM 32114 (*Serratia marcescens*), M02-BRM 32110 (*Bacillus toyonensis*), M03- BRM 62389 (*Phanerochaete australiani)*, M04 -BRM 53736 *(Trichoderma koningiopsis)*, M05-AbV5 (*Azospirillum brasilense)*, M06 -BRM 63574 *(Azospirillum* spp.), M07-BRM 63573 *(Bacillus* spp.), M08-BRM 32114 + BRM 32110 , M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control.
Source: Authors

was noted with treatments M16, M19, M22 and M23, highlighted in green color. The remaining treatments did not correlate positively with any of the variables analyzed.

The results of the present study show that microbiolization of rice seed with multifunctional microorganisms is a promising approach to improve rice root development.

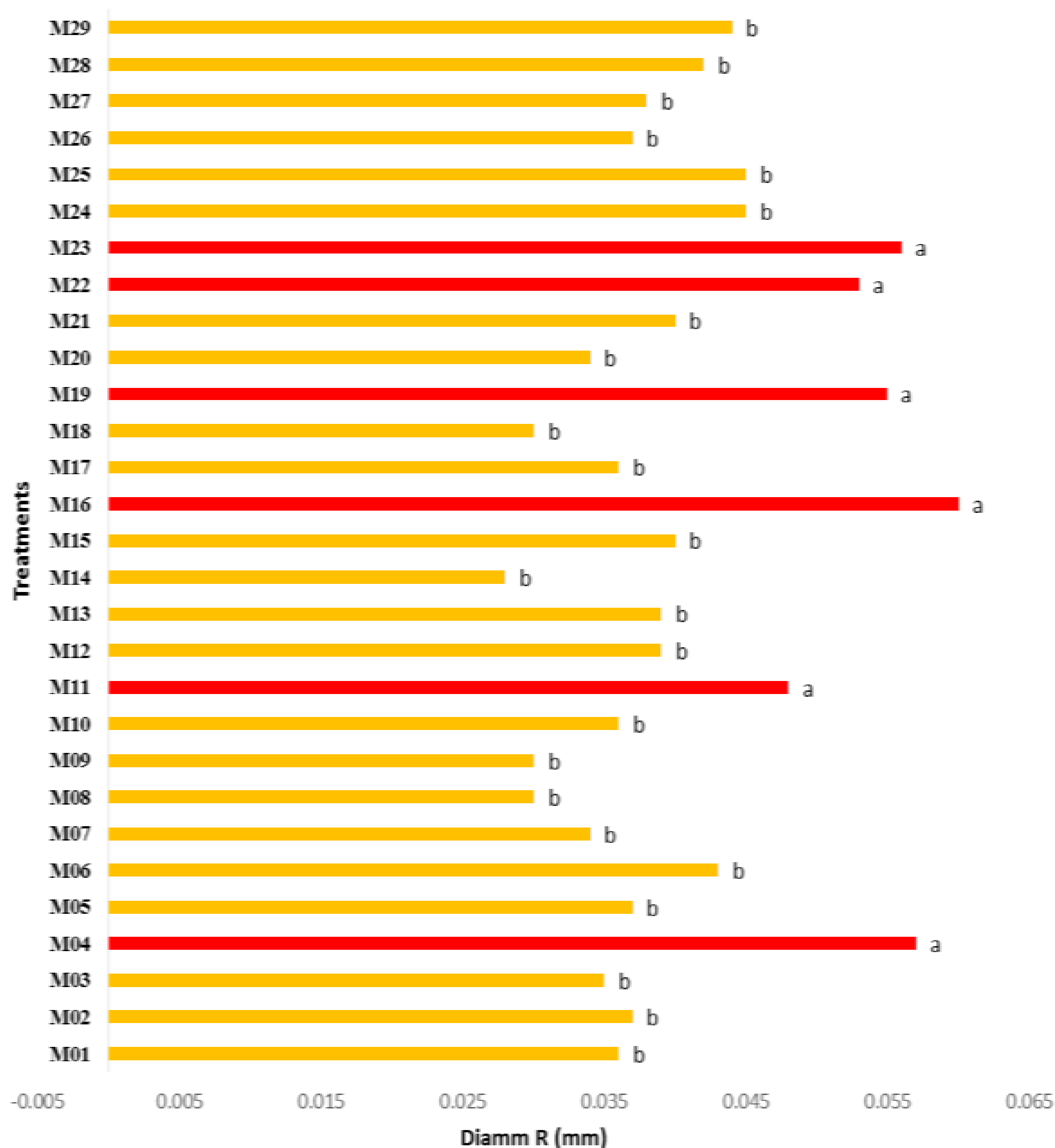

Figure 4. Effect of growth promoting microorganism on root diameter of upland rice seedlings cultivar A 501 CL at ten days after sowing. * Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p< 0.05$. ** Treatments: M01- BRM 32114 (*Serratia marcescens*), M02-BRM 32110 (*Bacillus toyonensis*), M03- BRM 62389 (*Phanerochaete australiani)*, M04 -BRM 53736 *(Trichoderma koningiopsis)*, M05-AbV5 (*Azospirillum brasilense)* , M06 -BRM 63574 *(Azospirillum* spp.), M07-BRM 63573 *(Bacillus* spp.), M08-BRM 32114 + BRM 32110, M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control.
Source: Authors

We could see that these microorganisms enhanced root length, root surface area, root diameter and volume of upland rice. As a result of greater root development, it is possible that absorption and accumulation of nutrients

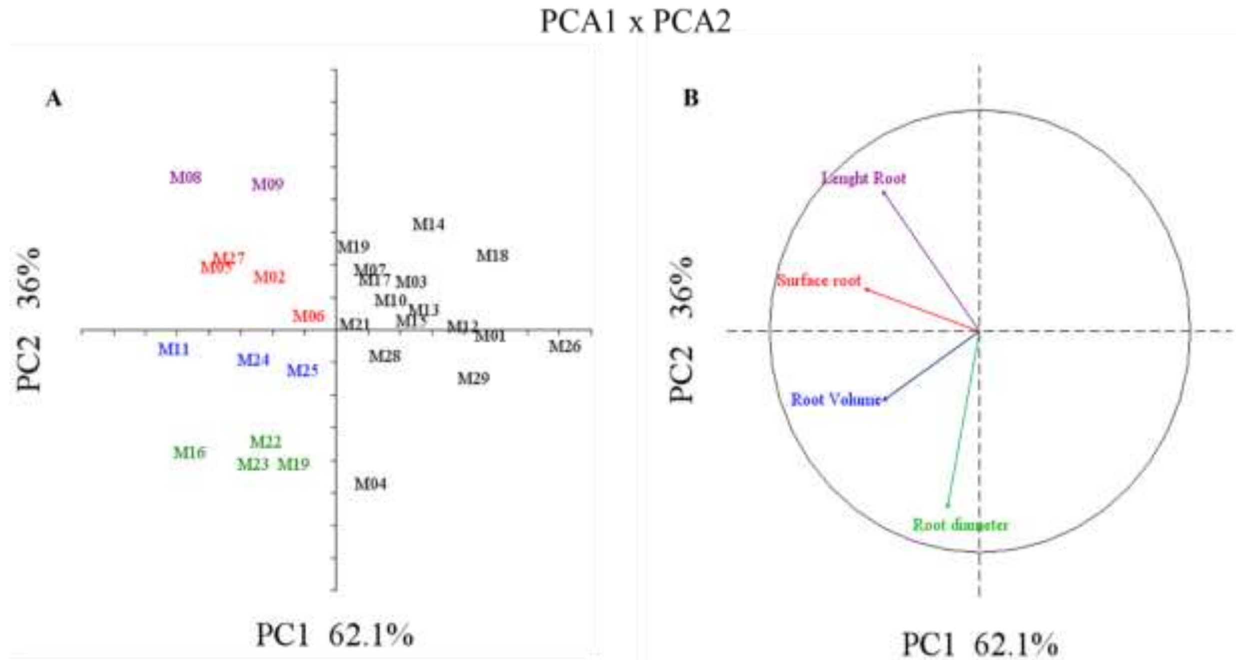

Figure 5. Principal component analysis (PCA) PCA1 X PCA2, explaining the correlations between the evaluated variables and seven treatments with isolates of rhizobacteria and the control (without the microorganism). (A) Biplot graph for treatments: M01- BRM 32114 (*Serratia marcescens*), M02-BRM 32110 (*Bacillus toyonensis*), M03- BRM 62389 (*Phanerochaete australiani*), M04 -BRM 53736 (*Trichoderma koningiopsis*) , M05-AbV5 (*Azospirillum brasilense*) , M06 - BRM 63574 (*Azospirillum* sp.), M07-BRM 63573 (*Bacillus* sp.), M08-BRM 32114 + BRM 32110 , M09-BRM 32114 + AbV5, M10-BRM 32114 + BRM 53736, M11-BRM 32110 + AbV5, M12-BRM 32110 + BRM 53736, M13-AbV5 + BRM 53736, M14-BRM 63574 + BRM 63573, M15-BRM 63574 + AbV5, M16-BRM 63573 + AbV5, M17-BRM 63574 + BRM 32114, M18-BRM 63573 + BRM 32114, M19-BRM 63574 + BRM 32110, M20-BRM 63573 + BRM 32110, M21-BRM 63574 + BRM 53736, M22-BRM 63573 + BRM 53736, M23-BRM 62389 + BRM 63573, M24-BRM 62389 + BRM 63574, M25-BRM 62389 + AbV5, M26-BRM 62389 + BRM 53736, M27-BRM 62389 + BRM 32114, M28-BRM 62389 + BRM 32110, M29-control. (B) Graph with the variables correlation circle.
Source: Authors

occur because rice plants are able to explore a greater volume of the soil. This will therefore provide a decrease for application of synthetic fertilizer for rice development.

Conclusion

Inoculation of upland rice with sole and combined microorganism on upland rice increased the yield components and grain yield of the crop. The combination of *S. marcescens* and *B. toyonensis* led to the greatest root length while combination of *B. toyonensis* and *A. brasilense* greatly increased root surface area. The highest increase in root diameter was recorded for upland rice inoculated with mixture of *A. brasilense* and *Bacillus* spp. The mixture of *P. australis* and *Bacillus* spp. led to the highest root volume.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Morphological, pathogenic and molecular characterization of fungal species associated with mango fruits in Mexico

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The characterisation of the causal agents of postharvest diseases, as well as their pathogenicity in different mango cultivars of economic importance in Mexico was evaluated. In total, 37 fungi were isolated from diseased mango (*Mangifera indica* L.) of the cvs Ataulfo, Hayden, Manila, Peach, Haden and creole. For the morphological characterization, the isolates were seeded in potato dextrose agar media and described by 4 variables. For the molecular characterization, DNA extraction was performed using the commercial Kit. For amplification, primers ITS5 and ITS4 were used and phylogenetic tree was built using RaxML. Six species were identified, namely *Aspergillus niger*, *Colletotrichum asianum*, *Lasiodiplodia theobromae*, *Neofusicoccum oculatum*, *Pestalotiopsis mangiferea* and *Talaromyces variabilis*. The most abundant group was the genus *Aspergillus*, with an appearance frequency of 0.35. Phylogenetic analysis showed that *C. asianum* and *P. mangiferae* belong to the families Glomerellaceae and Pestalotiopsidaceae, respectively, whereas *A. niger* and *T. variabilis* belong to the family Trichocomaceae. The fungi *L. theobromae* and *N. occulatum* belong to the family Botryosphaeriaceae. The pathogenicity of all isolates was demonstrated, except for *T. variabilis*. In contrast, *L. theobromae* and *N. oculatum*, were the most pathogenic isolates in all evaluated cultivars. Susceptibility to each pathogen differed among the cultivars, and Creole was most susceptible to the fungi evaluated.

Key words: Cultivars, disease postharvest, *Mangifera indica* L., phylogenetics analysis.

INTRODUCTION

Mango is a popular and economically important tropical fruit throughout the world, mostly because of its excellent eating quality and nutritional composition (Kim et al., 2009). Global production reached 50.65 million tons in 2017 (Shahbandeh, 2018), and India is the principal mango producer with 35% of the world's production (13.6 million tons), followed by China, Thailand, Indonesia, Mexico and others (FAOSTAT, 2009). The Ataulfo,

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Haden, Tommy Atkins, Kent and Keitt varieties are the most in demand (Luna-Esquivel et al., 2006). Of these, the Ataulfo mango is one of the perennial cultivars with the largest cultivation area in Mexico (114,403 ha planted, 77,993 ha harvested), with an annual production of 510,700 t, and has been listed by Servicio de Información Agroalimentaria y Pesquera (SIAP, 2019) as one of the most important cultivars due to its growing demand on the foreign market. A significant amount, however, is wasted, estimated at 2 to 33% because of fruit drop cracking, immaturity and postharvest decay, mainly due to anthracnose and stem end rot (Nuevo and Apaga, 2010). Anthracnose is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Dodd et al., 1997; Arauz, 2000; Kamle and Kumar, 2016) whereas stem-end rot is caused by *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. (Prusky et al., 1997; Kobiler et al., 2001). Infection of fruit occurs during production and postharvest operations (Johnson and Hofman, 2009); it compromises storage life of the fruit leading to a decline in market value. In this study, due to a lack of information on this fungus in the host, this investigation was carried out, in order to detect the presence of the pathogens with certainty. Because of this, the aim of our investigation was to characterize pathogenically and molecularly different isolations obtained from diseased mango (*Mangifera indica* L.) and determine the causal agent.

MATERIALS AND METHODS

Sampling, isolation and morphological identification

The investigated mango fruits were of the commercial cultivars Ataulfo and Manila from the state of Nayarit, cv. Hayden, Peach and Haden from the state of Guerrero and Creole from the state of Morelos, México. The fruits were of top quality and uniform in size. Six fruits per variety, without physical damage or diseases caused by pathogens, were selected and taken to the Postharvest Technology Laboratory for Agricultural Products of CEPROBI-IPN for processing.

Isolation of phytopathogenic fungi from mango fruits

The mango fruits were washed with soap and water; this will reduced the number of contaminants. Six fruits placed into single humid chamber (95% humidity) and incubated at room temperature (28 ± 2°C), every 2 days were reviewed for a period of 10 days. Once fungal development was observed, mycelium samples were taken with the help of a dissection needle under aseptic conditions, seeded in Petri dishes with potato-dextrose agar (PDA) and incubated at room temperature. Once the colonies had developed, they were purified by reseeding in new PDA dishes and incubated at a temperature of 28 ± 2°C.

Establishment of morphological groups

Each morphology found was grouped according to its cultural characteristics, taking into account the colour of the colony and its appearance, zoning, colour of the underside and edge. After the grouping of each morphology, the frequency of appearance was calculated using the following equation:

$$fa1 = N1/Nt \quad (1)$$

where N1 is the number of times a morphology appears, and Nt is the total number of morphologies found (Pérez-Bocourt et al., 2010).

Cultural and morphological characterisation

The cultural characterisation of six fungi was determined from the growth rate of the isolates in the culture media Sabouraud-Dextrose (SDA), nutrient agar (AN), cellulose agar (AC), Czapek agar (Cz), cornmeal agar (AHM), flour agar oatmeal (AHA), water agar (AA) and PDA as control. Mycelium discs with a diameter of 5 mm and a growth period of 15 days in PDA were seeded in the centre of the 50-mm Petri dishes containing each culture medium in triplicate. The dishes were then incubated at room temperature (28 ± 2°C) until the colony reached its maximum mycelial growth, and the colony diameter was measured daily. The data were graphed, and the growth rate was determined by an equation of the straight line of each growth curve for each fungus in the different culture media. A Scott-Knot multivariate analysis was carried out using Infostat 2020.

Micromorphological characterisation was carried out by preparing microcultures in duplicate with two revisions at eight and fifteen days. These consisted of 8-mm discs of PDA, AA and SDA, placed on the ends of slides and inside sterile Petri dishes. They were inoculated with 10 uL of spore solution at a concentration of 1×10^7 mL^{-1} of each fungus, and each disk was covered with a cover slip. Two slides were prepared for each culture medium.

For the analysis of the microcultures, the coverslip was placed on a new slide with a drop of lactophenol blue at 1% v/v and sealed with enamel. The microcultures were observed under an optical microscope (Nikon Olympus CI, Japan) with a 10 and 40x objective. Taxonomic structures such as conidiogenic cells, conidiophore type, conidium type and mycelium type were determined and compared with specialised bibliography.

Molecular identification

Molecular characterisation was performed from an isolate of each of the species obtained from mango fruits. From each selected isolate, 10-day-old mycelium was recovered with the help of a sterile spatula and placed in a 2-mL microtube. Subsequently, DNA extraction was performed using the commercial DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. The DNA integrity was verified by 1.5% w/v agarose gel electrophoresis, and the DNA was quantified by spectrophotometry using a Nanodrop® ND-1 V 3.2.1 (Thermo Fisher Scientific, Waltham, MA, USA); the concentration was adjusted to 50 ng/µL. For amplification, the primers ITS5 and ITS4 (White et al., 1990) were used, which delimit the Internal Transcribed Spacer 1 and 2, as well as the 5.8 gene of the nuclear ribosomal RNA.

Amplification was performed using a Mastercycler Pro thermocycler (Eppendorf, Hamburg, Germany) with a total reaction volume of 25 µL, with two replicates. The amplification conditions consisted of an initial denaturation step at 94°C for 4 min, followed by 36 denaturation cycles at 94°C for 60 s, annealing at 55°C for 60 s and extension at 72°C for 60 s; final extension was performed at 72°C for 10 min. The amplified products were separated by ethidium bromide-stained 1% agarose gel electrophoresis and visualised using a Chemi Genius 2 Bio Imaging System photodocumenter (Syngene International Ltd, Karnataka, India). The PCR products were purified using the QIAquick PCR

Table 1. Morphological group and frequency of appearance of fungi in different cultivars mango.

Morphological group	Genus	Frequency of appearance	cvs Mango
GM1	*Aspergillus*	0.35	Ataulfo, Hayden, Melocotón and Haden
GM2	*Colletotrichum*	0.12	Haden and creole
GM3	*Lasiodiplodia*	0.20	Ataulfo, haden, Hayden and Melocotón
GM4	*Neofusicoccum*	0.10	Ataulfo, Hayden and Haden
GM5	*Pestalotiopsis*	0.02	Hayden
GM6	*Talaromyces*	0.02	Hayden, Peach and Ataulfo

purification Kit, following the manufacturer's instructions. Purified products were sent for sequencing in both directions to Macrogen (Macrogen Inc., Seoul, Korea), using the same primers as those used for amplification.

Sequence quality was verified by reviewing the electropherograms. The "forward" and "reverse" sequences were aligned using the BioEdit v. 7.2.5 software (Hall, 1999) to obtain the consensus sequences of approximately 560 to 600 bp. The sequences of the isolates were compared with those deposited in the National Center for Biotechnology Information (NCBI), using the BLAST tool. Sequences were deposited in the NCBI Nucleotide Database

Phylogenetic tree

For phylogenetic studies, the molecular marker Internal Transcribed Spacer (ITS) was used, which represents a highly conserved region within mitochondrial DNA, highly repetitive with a slow evolution, where mutations are scarce. Despite this, this marker has a low resolution in terms of discrimination among species, most likely because it also has an area with greater variation (Suárez-Contreras et al., 2022). The orthologue search was performed using a Biopython script with NCBIWWW, with a p-value parameter of 1e-20. The BLAST results were processed with Python and Perl scripts to facilitate multiple alignments. The Mafft v.7.475 program (Yamada et al., 2016), was employed to obtain multiple alignments with the --reorder--auto parameters. Each alignment was edited with Trimal v. 1.4.rev 22 (Capella-Gutiérrez, 2009) with the parameters -gt 0.3 -st 0.001. We used the script Fasta_to_phylip.py to convert to Phylip format https://github.com/josephhughes/Sequence-manipulation), and the phylogenetic tree was built using RaxML v. 8.2.12 (Stamatakis 2014), with the following parameters: -f a -T 15 -m GTRGAMMA -N 100 -x 12345 -p 54321. Finally, the tree was plotted with iTOL (https:/ /itol.embl.de/login.cgi).

Pathogenicity tests

Six isolates were chosen as representatives for the species identified. Inoculation was conducted on healthy mango fruits of the cvs Ataulfo, Creole, Manila and Haden. The fruits were washed with soap and water, disinfected with 1% v/v sodium hypochlorite for 3 min and rinsed three times with water. For inoculation, we used 5-mm-diameter mycelium discs from 8 to 15 days old, grown in PDA medium. Five fruits per variety were inoculated with two discs from the fungi, one per side in the equatorial zone of each fruit. For control, we used un-inoculated fruits, which were kept in a humid chamber with a relative humidity of 100% at room temperature (28 ± 2°C) for 10 days.

The percentage of disease incidence (% I) was determined for each fungus, using the following equation:

$$\%Incidence = Nd/Nt \times 100 \quad (2)$$

where Nd is the number of disks that caused symptoms, Nt is the total number of disks inoculated in mango fruits (Pérez Bocourt et al., 2010).

The area of damage was determined by measuring the width and height of each symptom with the help of a Vernier; for calculation, the following equation was used:

$$Area = Equatorial\ diameter \times Longitudinal\ diameter\ mm^2 \quad (3)$$

RESULTS

Establishment of morphological groups

The frequencies of appearance of the six fungi are shown in Table 1. Morphological Group 1 (GM1) showed black to pale yellow colonies and barely visible white mycelium with a granular to floppy texture, especially in the central zone, similar to *Aspergillus* colonies. In Group 2 (GM2), the colonies had a cottony appearance, dark grey mycelium in the centre with orange spores forming rings, smooth edges and concentric growth; they were included within the genus *Colletotrichum*. Morphological Group 3 (GM3) presented typical characteristics of the genus *Lasiodiplodia*, with colonies from white to black, with a fluffy-cotton appearance, irregular concentric growth and an irregular border. Morphological Group 4 (GM4) showed colonies similar to those of the genus *Neofusicoccum*, with olive-brown woolly mycelium, irregular growth, brown underside and irregular growth. Morphological Group 5 (GM5) showed white mycelium growing in rings and with a woolly appearance. In the centre, we observed black spores and irregular growth, coinciding with the characteristics of the genus *Pestalotiopsis*. Morphological Group 6 (GM6) presented regular concentric growth, smooth edges and mycelium with a powdery appearance due to the quantity of olive-green to lemon-green spores. Some colonies were reddish on the underside, characteristic of the genus *Talaromyces.* The frequency of appearance differed between the genera isolated. *Aspergillus* presented the highest frequency of appearance (0.35) and *Pestalotiopsis* and *Talaromyces* the lowest one (0.02).

Table 2. Growth rate of the six fungi species in the different culture media evaluated.

Species	Growth rate (mm/day)							
	PDA†	SDA	NA	CzA	AA	CELA	CMA	OMA
Aspergillus niger	12.17	11.80	9.11	11.14	8.28	7.58	9.84	6.87
Colletotrichum asianum	6.28	9.06	6.75	9.75	6.10	6.47	8.81	7.91
Lasiodiplodia theobromae	18.50	21.43	19.42	20.55	15.88	20.84	19.44	24.03
Neofusicoccum oculatum	2.53	19.70	8.56	15.74	10.39	14.78	14.94	16.98
Pestalotiopsis mangifereae	8.45	9.08	9.47	10.60	10.05	8.38	11.22	9.44
Talaromyces variabilis	2.93	3.25	2.53	2.73	2.73	2.46	2.81	3.23

†PDA: potato dextrose agar, SDA: Sabouraud dextrose agar, NA: nutrient agar, CzA: Czapek agar, AA: water agar, CELA: cellulose agar, CMA: cornmeal agar, OMA: oatmeal agar.

The Ataulfo and Hayden varieties were infected by the six fungi isolated.

Cultural and morphological characterisation

Growth rates (mm/day) differed among the six species in the eight selected culture media. The species *L. theobromae* (Pat.) Griffon & Maubl (15.88 to 24.03), *Neofusicoccum oculatum* Syd & P. Sid (2.53 to 19.70), *Aspergillus niger* v. Tieghem (6.87 to 12.17) and *Pestalotiopsis mangifereae* P. Henn (8.45 to 11.22) presented the highest growth rates, whereas *Colletotrichum asianum* Prihastuti (6.10 to 9.75) and *Talaromyces variabilis* C.R. Benj (2.46 to 3.23) showed the lowest values (Table 2). A high cultural variability was observed among the pathogens that cause various diseases in mango. These results not only demonstrate genetic diversity among populations, they are also preferentially distributed in varieties mango and have great potential to colonize and cause disease symptoms in some varieties more than others. For example *L. theobromae* and *N. oculatum* showed 100% incidence in all varieties evaluated. However, *C. asianum* presents 100% incidence in two varieties.

A total of 37 isolates of fungi were recovered from lesions of mango fruits, and six species were morphologically identified as *A. niger* (14 isolates), *C. asianum* (5 isolates), *L.theobromae* (8 isolates), *N. oculatum* (5 isolates), *P. mangiferea* (1 isolates) and *T. variabilis* (4 isolates). Microscopic taxonomic structures were described; *A. niger* showed conidiophores hyaline and smooth, forming spherical conidial heads, morphology biseriate with globose hyaline phialides, and bottle-shaped metulae attached to the spore chain radially. The conidia are globose, with a surface wrinkled black (4 µm). *C. asianum* presents hyaline phialides and conidia without septa, hyaline in the form of a stick (fusiform) of 8 to 12 µm. The brown appressoria, semicircular 10-15 µm; *L. theobromae* with subovoid conidia with round apices, brown in color and a septum. They have longitudinal striae (myelin deposits) 15 to 20 µm. *N. oculatum* does not present reproduction structures only mycelial development, reason why it is not included in Figure 1. *P. mangiferea* presents conidia formed by five cells, two hyaline at the ends and three versicolor central. Fusoid, ellipsoid or straight, 12 to 15 µm, basal cell conical, hyaline; with basal and apical tubular hyaline appendages. *T. variabilis* showed right conidiophores, hyaline biverticillates, with ampulla-shaped phialides and straight metulas. Globose conidia of green color and smooth walls, 3.5-4 µm, in chains (Figure 1).

Molecular identification

Coverage and identity values for all strains exceeded 99%, based on analyses performed on all sequences using the Targeted Loci Nucleotide BLAST tool (Table 3). For phylogenetic studies, the molecular marker ITS (Internal Transcribed Spacer) was used, which represents a highly conserved region within mitochondrial DNA, highly repetitive with a slow evolution, where mutations are scarce. Despite this, this marker has a low resolution in terms of discrimination among species, most likely because it also has an area with greater variation (Suárez-Contreras et al., 2022).

The results obtained from the phylogenetic analysis for each of the six isolates is as shown in Figure 2. In each of them, the name and accession number assigned by NCBI are indicated in red. The clades or branches closest to it indicate sequences similar to the fungi identified in this work, obtaining a more precise approach to the name of the species. The isolates are grouped in correspondence to the genetic distance between them. Three clades (branches) were formed, and in each of them, two bifurcations were formed, indicating two different species. In the case of *C. asianum* and *P. mangiferae*, they are genetically closer compared to *A. niger* and *T. variabilis,* and the genetic distance between *L. theobromae* and *N. occulatum* is even smaller with respect to the other isolates. This is because the fungi identified belong to the same classes, that is, *C. asianum* and *P. mangiferae* belong to the class Sordariomycetes

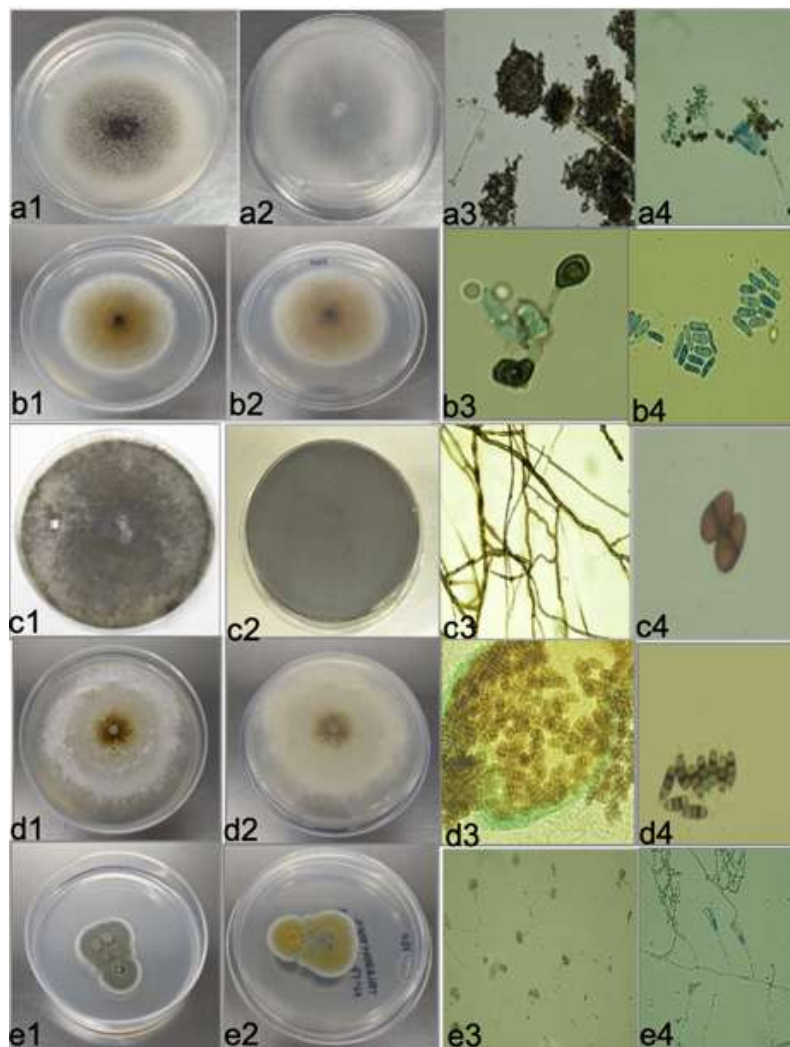

Figure 1. Macroscopic and microscopic characteristics of fungi species from mango fruit. *Aspergillus niger*: Colony upper surface (a1); Colony lower surface (a2); Conidiophore (a3); Phialide (a4). *Colletotrichum asianum*: Colony upper surface (b1); Colony lower surface (b2); Conidio (b3); Apresorio (b4). *Lasiodiplodia theobromae:* Colony upper surface (c1); Colony lower surface (c2); Mycelio (c3); Conidio (c4); *Pestalotiopsis mangifereae:* Colony upper surface (d1); Colony lower surface (d2); Picnidio (d3); Conidio (d4); *Talaromyces variabilis:* Colony upper surface (e1); Colony lower surface (e2); Conidiophore (e3); Phialide and Conidiophore (e4).

Table 3. Molecular identification using ITS markers of fungi isolated from mango fruits.

Género	No. Accession±	Morphological identification	Molecular identification	Coberage (%)	Identity (%)
Neofusicoccum	ON003476.1	*Neofusicoccum* spp.	*Neofusicoccum oculatum*	100	100
Aspergillus	ON003479.1	*Aspergillus niger*	*Aspergillus niger*	100	99.83
Colletotrichum	OM892874.1	*Colletotrichum* spp.	*Colletotrichum asianum*	100	99.82
Lasiodiplodia	ON003477.1	*Lasiodiplodia* spp.	*Lasiodiplodia theobromae*	100	100
Penicillium	ON003478.1	*Penicillium* spp.	*Talaromyces variabilis*	99.83	100
Pestalotiopsis	ON003480.1	*Pestalotiopsis* spp.	*Pestalotiposis mangiferae*	100	99.82

±Accession number in the NCBI database.

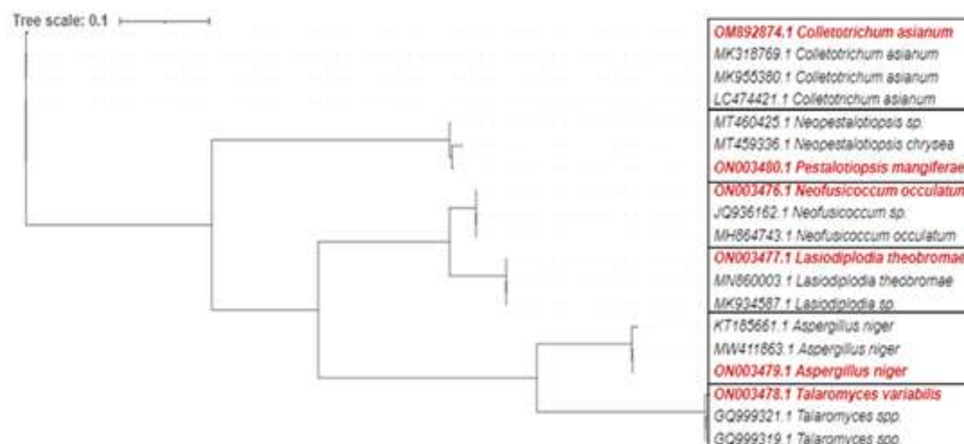

Figure 2. Phylogenetic tree of the six fungi isolated from fruits of different mango cultivars. The isolates identified in this research are marked in red with the NCBI accession code and the species name. Black are the accessions with which these sequences were aligned. Analysis was performed with Maximum likelihood.

Table 4. Incidence of fungi inoculated in mango fruits of the cv Ataulfo, Manila, Haden and criolle after 10 days.

Species	Incidence (%)†			
	Ataulfo	Criolle	Manila	Haden
Aspergillus niger	60	0	60	0
Colletotrichum asianum	0	100	0	100
Lasiodiplodia theobromae	80	100	100	100
Neofusicoccum oculatum	100	100	100	100
Pestalotiopsis mangifereae	0	0	0	0
Talaromyces variabilis	0	0	0	0

†O (%) Calculated from five fruits per variety, two wounds per fruit, total: 10 wounds.

but to the families Glomerellaceae and Pestalotiopsidaceae, respectively. The same occurs with *A. niger* and *T. variabilis*, which belong to the class Eurotiomycetes and to the same family, Trichocomaceae. The fungi *L. theobromae* and *N. occulatum* belong to the family Botryosphaeriaceae. All three classes belong to the division Ascomycetes and are therefore located in the central branch of the tree.

Pathogenicity tests

Based on the results of the pathogenicity test, isolates of *L. theobromae* and *N. oculatum* were responsible for the development of symptoms associated with peduncle scar rot disease and dieback in cvs Ataulfo, Criollo, Manila and Haden. In our study, *A. niger* caused infection on fruits of the cvs Ataulfo and Manila, and *C. asianum* was pathogenic for the cvs Haden and Criolle, with high percentages of incidence. The fungi *P. mangifereae* and *T. variabilis* did not cause symptoms in the three cvs and in Criolle (Table 4).

Figure 3 shows that *N. oculatum* induced damage on the fruit surface, with areas of 53.60 mm^2 in Ataulfo, 40.58 mm^2 in Manila, 37.51 mm^2 in Haden, and 7.74 mm^2 in Criolle. The tissue of these fruits turned dark brown at the point of inoculation, becoming lighter as the disease progressed, and abundant white mycelium and amber gutules developed on the skin. To the touch, the fruit felt firm, the colour of the pulp turned light brown, and a strong odour of ripe or rotten fruit was noticed. The fungus *L. theobromae* produced damage areas of 28.72 mm^2 for Ataulfo, 38.0 mm^2 for Manila, 95.11 mm^2 for Haden and 11.23 mm^2 for the Criolle on the surface of the fruits, with necrotic lesions on the entire surface with small yellow patches and concentric growth. Abundant white mycelium was observed at 10 days, along with the presence of small transparent gutules. The fungus *C. asianum* only caused infection on the cv Haden, with a damage area of 2.83 mm^2, and on Criolle, with an area of

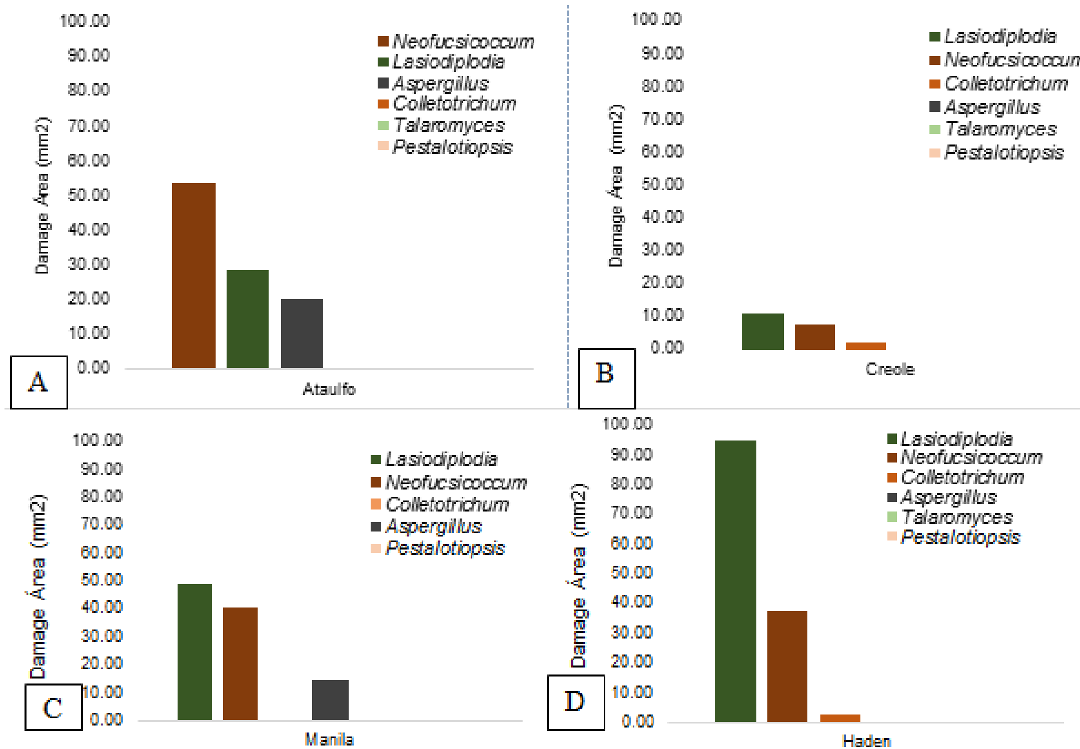

Figure 3. Damage area caused by fungi phytopathogenic in mango fruits. A) cv Ataulfo; B) Creolle; C) cv Manila; D) cv Haden.

2.09 mm^2. Necrotic and sunken lesions were observed at the inoculation site, with concentric growth. Signs such as grey or white mycelium growing in the wounds were observed. The species *A. niger* caused damage in an area of 19.75 mm^2 of the fruit surface of Ataulfo and of 14.75 mm^2 in Manila. The development of a light brown concentric sunken lesion and a darker halo was observed as the disease progressed. At the point of inoculation, white mycelium and abundant black spores were observed. Overall, we observed a high cultural, morphological and pathogenic variability among the isolates.

DISCUSSION

Several species of fungi associated with dieback in mango plantations in Gran Canaria have been reported, such as *Fusarium* spp., *Pestalotiopsis* spp., *Alternaria alternata*, *C. gloesporioides*, *Cladosporoium* spp., *Dothiorella* spp., *Penicillium* spp. and *Phomopsis* spp., with different incidence values depending on the sample type (leaf, stem or fruit), mango variety and collection season (Rodríguez et al., 2008). Santos-Mezones (2019) identified postharvest fungi from 486 fruits collected in a fruit packaging facility in Peru; the authors found two most frequent types of symptoms and identified the genera associated with these: *Alternaria* (50%), *Curvularia* (7%), *Colletotrichum* (6%), *Stemphylium* (6%), *Bipolaris* (4%), and *Pestalotia* (4%) from fruit spots and *Aspergillus* (54%), *Lasiodiplodia* (38%) and *Penicillium* (8%) from peduncular rot. These results are closely related to the findings of the present study, where most of the isolates from the brown spots on the peduncle were identified as *Lasiodiplodia, Alternaria, Aspergillus* and *Pestalotiopsis* and isolated directly from the progression of the disease *Colletotrichum* and *Neofusicoccum.* Another of the diseases of great importance in mango cultivation is "anthracnose", which has been reported in Asia and Latin America (Valenzuela et al., 2022). Anthracnose causes between 30 and 60% of losses in the field, and in some cases, up to 100% of the production is lost in humid conditions (Felipe et al., 2022). *C. asianum* may be the most important species in mango, described in Australia, Brasil, Florida (USA), Ghana, Mexico, the Philippines, South Africa and Thailand (Weir et al., 2012). In Mexico, anthracnose for varieties such as Ataulfo does not represent a serious postharvest problem compared to varieties from Florida, such as petacones (Tommy Atkins, Kent, Keiit, Haden). The latter explains the high percentage of occurrence for *Colletotrichum* in the cv Haden (19 isolates) and the absence for the cv Ataulfo. Krishnapillai and Wijeratnam (2014) were the first to report the presence of *C. asianum* in mango varieties in Sri Lanka; the pathogenicity tests carried out in varieties of petacones showed that the cvs Tommy Atkins and

Keiit were resistant to this fungus, whereas the Haden and Kent varieties were susceptible. Felipe et al. (2022), using molecular methods, reported a gene related to mango resistance to anthracnose. This was detected through the β-1,3-GLU2 gene, which synthesises the β-1,3-glucanase enzyme related to abiotic and biotic response processes in plants. The authors conclude that this gene is one of the first mango responses against *C. gloeosporioides* attack. Species of the genus *Pestalotiopsis* have been reported on mango trees in Italy (Ismail et al., 2013). *P. mangiferae* is a pathogen, that requires wounds to achieve infection and is often found as a saprophyte or associated with other diseases of the stem during postharvest storage. In addition, *P. glandicola* was reported as the causative agent of a postharvest disease of mango in Bangalore. In the present study, both *C. asianum* and *P. mangiferae* were genetically close in the phylogenetic analysis since both fungi belong to the same class, albeit to different families. However, there is not sufficient evidence for a resistance of mango cultivars to *P. mangiferae*.

Based on the results of the pathogenicity test, isolates of *L. theobromae* and *N. occulatum* produced symptoms on three cultivars and criolle. The species *L. theobromae* caused more damage to Ataulfo, Haden and Criolle on the surface of the fruits, whereas *N. occulatum* induced damage on the cvs Ataulfo, Manila and Haden. These fungi belong to the family Botryosphaeraceae, whose species are cosmopolitan, causing different diseases in different crops, such as dieback, trunk cancer and fruit rot being responsible for economic losses in fruit production; they have been reported in most mango producing areas in Asia, Africa and America. The *N. occulatum* isolate found in this research aligns with sequences reported in the NCBI database for *Neofusicoccum* isolates from mango in different areas in Mexico. Sandoval-Sánchez et al. (2013) isolated *N. parvum* from the mango cv. Ataulfo. In Egypt, *Lasiodiplodia* is considered to be the main causal agent of fruit deterioration, stem deterioration, panicle brown rot and stem dieback (Abdalla et al., 2003). Coutinho et al. (2017) identified the genus *Lasiodiplodia* through symptoms and pathogenicity in its different hosts. Taxonomic characteristics such as conidia, colony morphology, changes in coloration due to the effect of temperature, as well as changes in growth rate and molecular markers such as ITS, genes such as β-tubulin and elongation factor-α allowed the identification of more species that affect mango cultivation, emphasising that for the family Botryosphaeriacea, the use of several markers is necessary to separate the species of this group.

A. niger has been reported in fruits imported from Puerto Rico and Venezuela to England (Snowdon, 1991) placing "black rot" as an important disease in mango cultivation. Together with fungi of the genus *Penicillium* (*Talaromyces teleomorph*), they are saprophytic species and of particular importance when the fruits have a previous infection and a high degree of deterioration. The cultivars susceptible to artificial inoculation of *A. niger* were Ataulfo and Manila. As discussed by Yilmaz et al. (2014), the genus *Penicillium* is a monophyletic group with an asexual subgenus called *Biverticillium* and a sexual one called *Talaromyces*. However, Samson et al. (2011) recombined the subgenus *Biverticillium* within the genus *Talaromyces*, which allowed this group to have species that reproduce sexually and asexually, which is why the genus *Talaromyces* is reported in this research, isolated and identified in the first instance as the genus *Penicillium*. Together with *A. niger*, they are within the family Trichocomceae, with a lower genetic distance in the phylogenetic tree with respect to the remaining fungi identified.

In this work it is shown that diversity of fungal species is not only subject to the fungi that cause the symptoms, but also associated with a greater number of fungal species that are in a cryptic form and are only expressed when the physiology of the fruits changes. Also the chemical composition of each fruit is different, the cvs ataulfo presents a high variety of antifungal compounds in peel and pulp (Istúriz et al., 2022) and was more resistant to the fungi evaluated. Disease management in mango is challenging since several species or subspecies of the same genus converge with differences in terms of morphology and pathogenicity, so it is recommended to carry out the taxonomic identification of the isolates in addition to implementing the use of resistant varieties. Accordingly, such information is relevant because it can assist in the implementation of disease control measures more effectively.

Conclusions

In this study, we determined the pathogenicity and incidence of six postharvest phytopathogens on the most important commercial mango cultivars in Mexico. The greatest affectation corresponds to Haden, Manila, Creole and cv. "Ataulfo" being the most resistant, most likely because it is an endemic cultivar. However, all cultivars were susceptible to fungi of the family Botryosphareaceae and differed in their susceptibility to *A. niger* (Ataulfo and Manila) and to *C. asianum* (Criollo and Haden). We assume that the resistance or susceptibility of cultivars depends on their origin and genetics.

CONFLICT OF INTERESTS

The authors have not declared any of conflict of interests.

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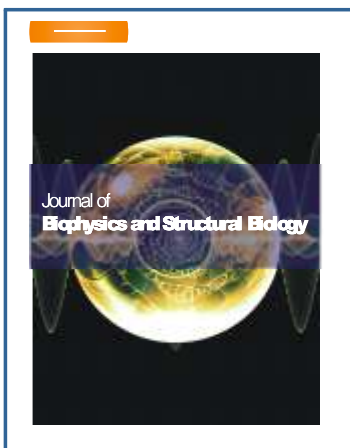

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