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Biofungicidal Potential of *Melia azedarach* Extracts against *Alternaria* spp. in Potato: Integrating Phytochemical Profiling with *In Vitro* and *In Vivo* Antifungal Efficacy

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Abstract : The development of sustainable alternatives to synthetic fungicides for controlling potato early blight, caused by *Alternaria* spp., is crucial for food security and environmental health. This study investigates the biofungicidal potential of *Melia azedarach* L. extracts. The aim was to compare the efficacy and phytochemical basis of methanolic extracts from *M. azedarach* seeds and leaves against *Alternaria* spp. The antifungal activity was evaluated *in vitro* using the poisoned food technique against three *Alternaria* isolates. The most potent extract was further tested on detached potato leaves (preventive and curative modes) and on whole potato plants under greenhouse conditions. Phytochemical profiling was conducted through TLC, quantification of total phenolics and flavonoids, and UPLC-ESI-MS/MS analysis. The seed extract demonstrated superior and consistent antifungal activity, achieving mycelial growth inhibition (MGI) of 77.8-78.6% *in vitro*, significantly higher than the leaf extract. *In vivo* trials confirmed significant disease suppression at the concentration 20%. Preventive application on detached leaves was more effective than curative. UPLC-ESI-MS/MS analysis revealed several major bioactive constituents in the seeds, including

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oleanolic acid, sinapic acid, oleuropein, and kojic acid. These compounds are known to contribute to membrane destabilization, enzyme inhibition, and the induction of oxidative stress in target pathogens. The seed extract of *Melia azedarach* demonstrates strong potential as a biofungicide against potato early blight. Its effectiveness appears to arise from a diverse array of bioactive molecules operating through multiple modes of action. Collectively, these results highlight its suitability for incorporation into sustainable potato disease management strategies.

Key words: *Melia azedarach*; early blight; biocontrol; botanical fungicide; integrated pest management (IPM).

The potato (*Solanum tuberosum* L.) stands as one of the most crucial staple crops globally, fundamental to food security and economic stability in numerous regions. In Algeria, potato represents the most developed vegetable crop in terms of both cultivated area and production volume. Among the most dynamic regions in this sector, the wilaya of Mostaganem stands out with a significant area dedicated to market gardening, reaching nearly 18,623 ha and an estimated production of 7,167,00 tons (DSA, 2022). However, potato cultivation faces persistent threats from fungal diseases, particularly early blight caused by *Alternaria* spp., which causes significant yield and quality losses worldwide. This disease is widespread in cultivation areas and can, in certain situations, represent a major constraint to production. In the Netherlands, it has been classified by some specialists as the second most concerning disease after late blight (Hamitouche, 2021). Conventional management of early blight has heavily relied on synthetic fungicides. While effective, their prolonged use has raised serious concerns about environmental pollution, effects on non-target organisms, and the emergence of resistant fungal strains (Deresa *et al*., 2023). In this context, bioactive plant compounds, particularly secondary metabolites, have attracted growing interest due to their proven antifungal properties (Martins *et al*., 2008; Negi *et al*., 2003). The use of plant extracts and natural products is highly recommended as they are safe for health and do not cause environmental pollution.

Among botanical species with documented antifungal and insecticidal bioactivity, Persian lilac (*Melia azedarach* L.) has shown promising pesticidal and antifungal properties. Previous studies have reported that *M. azedarach* extracts exhibit strong bioactivity against a range of phytopathogens and insect pests. For instance, its leaf and seed extracts have demonstrated significant inhibitory effects against *Fusarium oxysporum*, *Alternaria alternata* and *Botrytis cinerea* (Meziane *et al*., 2014; Akacha *et al*., 2016). In addition, *M. azedarach* seed oil and methanolic extracts are rich in triterpenoids, limonoids, and flavonoids that act as potent antifungal and insecticidal agents (Carpinella *et al*., 2003). These findings highlight the considerable potential of this species as a natural source of bioactive compounds for crop protection and pest management.

This study specifically focuses on a comparative analysis of leaf and seed extracts of *M. azedarach*. This comparative approach is critical, as the type, concentration, and bioactivity of secondary metabolites can vary significantly between different plant organs. Seeds often accumulate high concentrations of defensive compounds as a strategy to protect the embryo, while leaves may contain a distinct profile of compounds involved in direct defense against foliar pathogens. By systematically comparing extracts from these two key tissues, this research aims to identify the most potent source of antifungal compounds for targeting the foliar pathogen *Alternaria* spp. Further, in this study the antifungal activity of these botanical extracts against *Alternaria* spp was rigrously evaluated. Through a comprehensive approach. The specific objectives were: (i) to prepare and characterize methanolic extracts using phytochemical screening and UPLC-ESI-MS/MS analysis; (ii) to assess and compare their *in vitro* antifungal activity against different *Alternaria* isolates; (iii) to evaluate the efficacy of the most potent extract *ex vivo* on detached potato leaves under both preventive and curative treatment regimens; and (iv) to validate its bioactivity *in vivo* on whole potato plants under greenhouse conditions. By elucidating the chemical constituents and demonstrating their efficacy across multiple experimental levels, this research aims to provide a scientific foundation for developing effective and sustainable biofungicides for

potato early blight management in Algerian agriculture.

Materials and Methods

Plant materials and preparation

Potato cultivation **:** The plant material used for the *in vivo* assays consisted of potato (*Solanum tuberosum* L.). Eight varieties (Spunta, Bartina, Rudolph, Evolution, Rasmida, Manitou, Arizona, Liseta) were cultivated in a greenhouse at the École Supérieure d'Agronomie de Mostaganem. Planting was conducted in February within polyethylene bags filled with a substrate composed of one-third potting soil, two-thirds field soil, and cow manure. The bottom of each bag was lined with a thin layer of gravel to improve drainage. The plants were irrigated regularly and fertilized according to standard practices, with no application of fungicides throughout the growth period.

Botanical extract sources and processing

Two botanical sources were prepared for solvent extraction. (i) *Melia azedarach*'s Leaves and seeds were collected in February 2025. The samples were thoroughly washed with tap water to remove dust and impurities, air-dried for 2 hours, and subsequently oven-dried at 80°C for 48 hours to ensure complete dehydration. The dried material was ground and sieved to obtain a fine powder. The resulting powder were stored in airtight glass containers, protected from light and heat, until extraction.

Crude methanolic extracts were obtained using a standard maceration protocol. For plant material (*M. azedarach* leaves, *M. azedarach* seeds), 100 g of the dried powder was weighed and introduced into a flask containing 1000 mL of 80% methanol (v/v). The flasks were hermetically sealed and subjected to constant agitation for 24 hours at room temperature. After maceration, the solutions were filtered through Whatman No. 1 filter paper. The solvent was then removed from the filtrate using a rotary evaporator at 40°C to obtain a crude dry extract.

Phytochemical screening of the methanolic extracts from *Melia azedarach* seeds and leaves was conducted to quantify their total polyphenol and flavonoid content. All analyses were performed in triplicate using spectrophotometric methods (Lakhdari *et al*., 2024). The total polyphenol content (TPC) was determined using the Folin-Ciocalteu colorimetric method (Singleton *et al*. 1999). Briefly, 0.2 mL of each extract was mixed with 1 mL of diluted Folin-Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%). After 30 minutes of incubation in the dark, the absorbance was measured at 760 nm. A standard curve was prepared using gallic acid (0-1000 mg L^{-1}), and the results were expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of dry extract.

The total flavonoid content (TFC) was quantified using the aluminum chloride method (Zhishen *et al*., 1999). A 2 mL aliquot of extract was mixed with 2 mL aluminum chloride (2%). After 40 minutes, added to the mixture. The absorbance was measured immediately at 430 nm. A standard curve was prepared with quercetin (0-1000 mg L^{-1}), and the results were expressed as milligrams of Quercetin Equivalents (QE) per gram of dry extract.

Thin-layer chromatography (TLC) was employed as a preliminary technique to profile the secondary metabolites present in the methanolic extracts of *Melia azedarach* seeds and leaves (Lakhdari *et al*., 2023). The methanolic extracts were analyzed on silica gel G plates (20 × 10 cm). The solvent system used for development was n-Butanol/Water/Acetic Acid (5:3:1, v/v/v). After development, the air-dried TLC plates were first visualized under ultraviolet (UV) light at wavelengths of 254 nm and 365 nm. They were then derivatized by spraying with specific chromogenic reagents: vanillin-sulfuric acid reagent for terpenoids and steroids, aluminum chloride ($AlCl_3$) for flavonoids, and Dragendorff's reagent for alkaloids (Nakamura *et al*., 1999).

The methanolic extracts of *Melia azedarach* seeds and leaves were analyzed using a Shimadzu 8040 UPLC-ESI-MS/MS system. Separation was performed on a Restek Ultra C18 column (150 × 4.6 mm, 3 µm) with a gradient of 0.1% formic acid in water (A) and methanol (B) at a flow rate of 0.2 mL min^{-1}.

The mass spectrometer operated in electrospray ionization (ESI) mode with the following parameters: DL temperature, 250°C; heat block, 400°C; nebulizing gas flow, 3.0 L/min; and drying gas flow, 10.0 L/min. Data

were acquired in full scan mode (m/z 100-1000). Compounds were tentatively identified by comparing their mass spectra and fragmentation patterns with literature and database records.

In vitro antifungal activity assay

The antifungal activity of the methanolic extracts against three isolates of *Alternaria* spp. (I03, I21, I08) which were obtained from the fungal culture bank of the École Supérieure d'Agronomie de Mostaganem, was evaluated using the Poisoned Food Technique (El Mansouri and Moutaj, 2013).

Two experiments were caried out. In first the activity of the methanolic extracts (from *M. azedarach* leaves, *M. azedarach* seeds) was evaluated against three isolates of *Alternaria* spp. (I03, I21, I08). Each extract was reconstituted in dimethyl sulfoxide (DMSO) and incorporated into molten Potato Dextrose Agar (PDA) to achieve a final concentration 20% and 10%. A 6 mm mycelial disc of each pathogen, taken from the edge of a 7-day-old culture, was placed in the center of each Petri plate. The plates were then incubated at 25°C, and the radial growth was monitored until the mycelium in the control plates reached the edges.

Mycelial growth was monitored by measuring the colony diameter in centimeters. The inhibition percentage of mycelial growth was calculated using the following formula (De Albuquerque *et al*., 2006):

$$I\% = (C - T)/C \times 100$$

where: I% is the inhibition percentage, C is the diametrical growth of the fungus in the control plate and T is the diametrical growth of the fungus in the treated plate.

In the second the efficacy of the methanolic extract from *M. azedarach* seeds (at a concentration of 20%) was evaluated on detached potato leaves from two varieties, Spunta (white) and Rudolph (red), against two *Alternaria* isolates (I03, I31). The experimental design was a complete factorial, incorporating two treatment modalities (preventive and curative), with three replicates per isolate-variety-treatment combination. A negative control was included for each combination. In the preventive treatment, the methanolic extract solution was sprayed onto the leaves 24 hours prior to inoculation. In the curative treatment, the leaves were inoculated first and treated with the extract 24 hours post-inoculation.

For the preventive treatment, inoculation was performed by depositing 0.1 mL of a spore suspension (containing 0.01% Tween 80) on the main vein of each leaf, following a controlled wound made with a sterilized needle. A negative control, consisting of inoculated leaves treated only with the solvent solution (0.5% DMSO), was included for each isolate-variety combination. Each treatment was replicated three times. The plates containing the leaves were maintained at ambient temperature and daylight, with daily monitoring for 8 days. The contaminated leaf area (CLA) was estimated using the equation from NeSmith (1991):

$$CLA\ (mm^2) = 0.542 \times (width \times length) + 0.477.$$

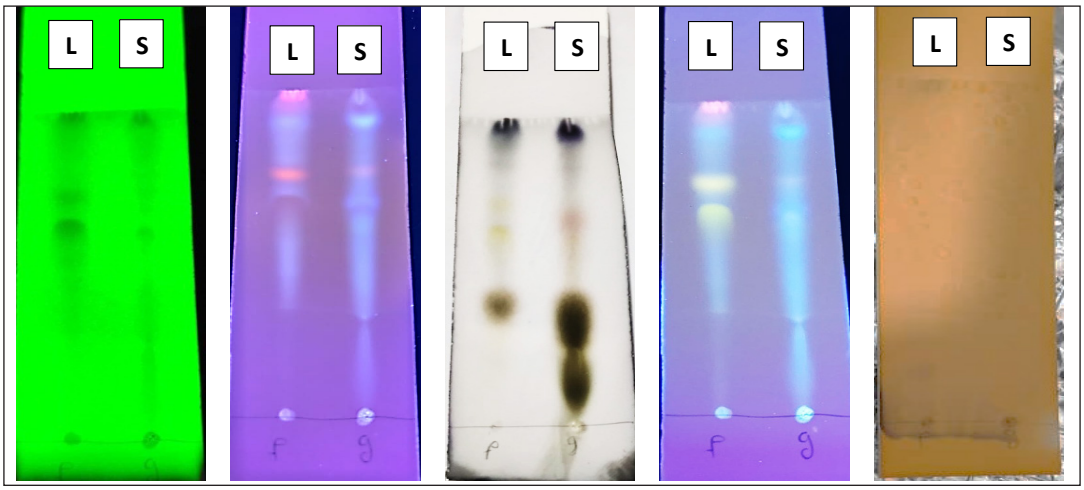
The lesion expansion rate was calculated according to the method described by Sofi *et al*. (2013). For curative treatment, whole potato plants of white (Spunta, Rasmida) and red (Evolution, Manitou) varieties grown in a greenhouse. The plants were maintained at a temperature of 22 ± 2°C, with a relative humidity of 70 ± 5% and a 16/8-hour light/dark photoperiod. The main vein of the leaves was wounded with a sterile needle and inoculated with 0.1 mL of a spore suspension containing 0.01% Tween 80. After 24 hours, plants were treated by spraying with the methanolic extract of *M. azedarach* seeds (1.0 mg/mL). Three replicates were maintained for each variety, alongside inoculated control plants treated with the solvent solution only. The progression of symptoms was monitored daily for 9 days post-inoculation.

All experiments were conducted using a completely randomized design with three independent replicates ($n \geq 3$) per treatment. When the ANOVA indicated significant differences ($p < 0.05$), post-hoc comparisons of means were performed using Tukey's Honest Significant Difference (HSD) test at a 95% confidence level. All statistical analyses were conducted using statistical software, e.g., R version 4.3.1, SPSS 28.0, or JMP Pro 16.

Results and Discussion

Thin layer chromatography (TLC)

Thin-layer chromatography (TLC) analysis of the methanolic seed extract (S) of *M.*



a *b* *c* *d* *e*

Fig.1.Thin layer chromatography (TLC) of seeds (S) and leaves (L) methanolic extracts of M. azedarach. (a): Under UV (254); (b): Under UV (365); (c): Vanillin/Sulfuric acid reagent; (d): Aluminum AlCl3; (e): Dragendroff reagent.

azedarach revealed a diverse phytochemical profile through multiple detection methods (Fig. 1). Under UV light at 254 nm, four distinct spots were observed with Rf values of 0.22, 0.55, 0.59, and 0.68. At 365 nm, six fluorescents spots were detected with Rf values of 0.22 (blue), 0.55 (yellow), 0.59 (blue), 0.68 (orange), 0.86 (blue), and 0.90 (dark blue), indicating the presence of various conjugated compound. The chromatographic profile was further characterized using specific chemical reagents. Vanillin-sulfuric acid staining revealed six distinct spots with Rf values of 0.16 (green), 0.32 (green), 0.59 (pink), 0.68 (blue), 0.86 (blue), and 0.90 (dark blue), suggesting the presence of terpenoids and other secondary metabolites. Aluminum chloride staining showed four fluorescent spots at Rf 0.22 (blue), 0.48 (yellow), 0.68 (yellow), and 0.81 (blue), indicating the presence of flavonoid compound. The Dragendorff's reagent test showed no detectable spots, suggesting the absence of alkaloids in the seed extract. The varied Rf values and distinct color reactions with different staining agents demonstrate the complex chemical composition of *M. azedarach* seed extract, particularly rich in flavonoid and terpenoid compounds, which may contribute to its observed antifungal activity.

Thin-layer chromatography analysis of the methanolic leaf extract (L) of *M. azedarach* revealed a distinct phytochemical profile differing from that observed in the seed extract (Figure 1) Under UV light at 254 nm, five spots were detected with Rf values of 0.22, 0.55, 0.59, 0.65, and 0.72. At 365 nm, seven fluorescent spots were observed with Rf values of 0.22 (blue), 0.55 (yellow), 0.59 (blue), 0.65 (orange), 0.86 (blue), 0.90 (dark blue), and 0.94 (pink), indicating a diverse array of conjugated compound (Fig.1).

Chemical staining revealed significant phytochemical diversity. The Vanillin-sulfuric acid reagent detected six spots with Rf values of 0.40 (green), 0.55 (blue), 0.65 (yellow), 0.68 (yellow), 0.86 (blue), and 0.90 (dark blue), suggesting the presence of terpenoids and phenolic compounds. Aluminum chloride staining showed three distinct spots at Rf 0.59 (yellow), 0.68 (yellow), and 0.90 (blue), indicating flavonoid content. Notably, Dragendorff's reagent revealed a single red spot at Rf 0.16, confirming the presence of alkaloids in the leaf extract - a significant difference from the seed extract where no alkaloids were detected.

The varied Rf values and distinct color reactions demonstrate that the leaf extract contains a complex mixture of phytochemicals including alkaloids, flavonoids, and terpenoids, which may contribute synergistically to its antifungal properties observed in previous assays.

Phytochemical screening: Quantification of polyphenols and flavonoids

Spectrophotometric quantification revealed significant differences in phytochemical content between seed and leaf methanolic extracts of *M. azedarach* (Table 1). The total

Table 1. Phytochemical analysis of seeds and leaves methanolic extract of M. azedarach

	Seeds extract	Leaves extract	Curve equation	R^2
Total phenolics (mg GAE/g DE)	49.56±0.092	66.20±0.81	ABS = 0.0108x - 0.1843	0.999
Total flavonoids (mg QE/g DE)	5.95±0.25	7.539±0.18	ABS =0.0218x + 0.0799	0.996

GAE: Gallic acide equivalent. QE: Quercetin equivalent. DE: Dry extract. ± : SD. ABS : Absorbance

phenolic content (TPC), expressed as gallic acid equivalents (GAE), was substantially higher in the leaf extract (66.20±0.81 mg GAE g dry extract^{-1}) compared to the seed extract (49.56±0.092 mg g dry extract^{-1}). Similarly, the total flavonoid content (TFC), calculated as quercetin equivalents (QE), demonstrated markedly higher levels in leaves (7.539±0.18 mg QE g dry extract^{-1}) than in seeds (5.95±0.25 mg QE g dry extract^{-1}).

UPLC-ESI-MS/MS

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis of the methanolic leaf extract of *M. azedarach* (Fig. 2) revealed a complex phytochemical profile with 18 identified compounds spanning multiple biochemical classes (Table 2). The analysis was performed using a UPLC-ESI-MS/MS system with electrospray ionization in both positive and negative modes, employing a C18 column with a water-methanol gradient mobile phase. The vitamin and carotenoid fractions were particularly prominent, with riboflavin emerging as the most abundant compound (peak area: 20,358,233; retention time (RT): 6.287 min), followed by beta-carotene (peak area: 1,001,947; RT: 7.891 min). Phenolic compounds constituted a major component of the extract, with sinapic acid (1,949,229; RT: 4.908 min), salicylic acid (1,769,085; RT: 4.677 min), and ferulic acid (665,215; RT: 2.968 min) representing the most significant constituents. Additional phenolic acids included caffeic acid (224,881; RT: 3.929 min), p-coumaric acid (62,913; RT: 4.269 min), and vanillic acid (680; RT: 4.013 min).

The secoiridoid and terpenoid profiles were characterized by oleuropein (742,804; RT: 5.597 min) and two detections of oleanolic acid at different retention times (376,145 at RT: 5.558 min and 43,492 at RT: 5.379 min), suggesting potential isomeric variation. The flavonoid content included rutin (22,739; RT: 4.238 min) and trace amounts of quercetin (1,571; RT: 4.297 min). Other identified compounds included vanillin (374,263; RT: 4.138 min), thymol (34,137; RT: 4.850 min), 2-methoxybenzoic acid (26,906; RT: 3.174 min), and curcumin (17,791; RT: 5.082 min).

UPLC-ESI-MS/MS analysis of the methanolic seed extract of *M. azedarach* identified 17 bioactive compounds (Fig. 3), revealing a distinct phytochemical profile compared to the leaf extract. The seed extract demonstrated particularly high concentrations of specific compounds that may contribute to its enhanced antifungal efficacy observed in previous bioassays (Table 3). The most abundant compounds in the seed extract were riboflavin (peak area: 20358233; RT: 6.287min) and beta-carotene (1001947; RT: 7.891min), both present in substantial quantities. Sinapic acid was also a major constituent (1949229; RT: 4.908 min), along with significant levels of oleuropein (742804; RT: 5.597min) and oleanolic acid (376145; RT: 5.558min). The phenolic acid profile included ferulic acid (665215; RT: 2.968min), salicylic acid (1769085; RT: 4. 4.677 min), and vanillin (381,715; RT: 4.136 min). Notably, the seed extract contained detectable levels of kojic acid (374263; RT: 4.138min), which was absent in the leaf extract. The flavonoid content was represented by rutin (22739; RT: 4.238min), while other flavonoids such as quercetin were not detected.

Comparative analysis revealed that the seed extract contained a higher β-carotene than leaves (2,687,218 vs. 1,001,947). However, the leaf extract showed significantly higher concentrations of both oleanolic acid isoforms (376,145 vs. 16,635) and oleuropein (742,804 vs. 587,624).

The seed extract uniquely contained kojic acid (area 2,074), which was absent in leaves. Regarding phenolic acids, the leaf extract exhibited a richer profile, containing caffeic acid (224,881), p-coumaric acid (62,913), and vanillic acid (680). In contrast, vanillic acid

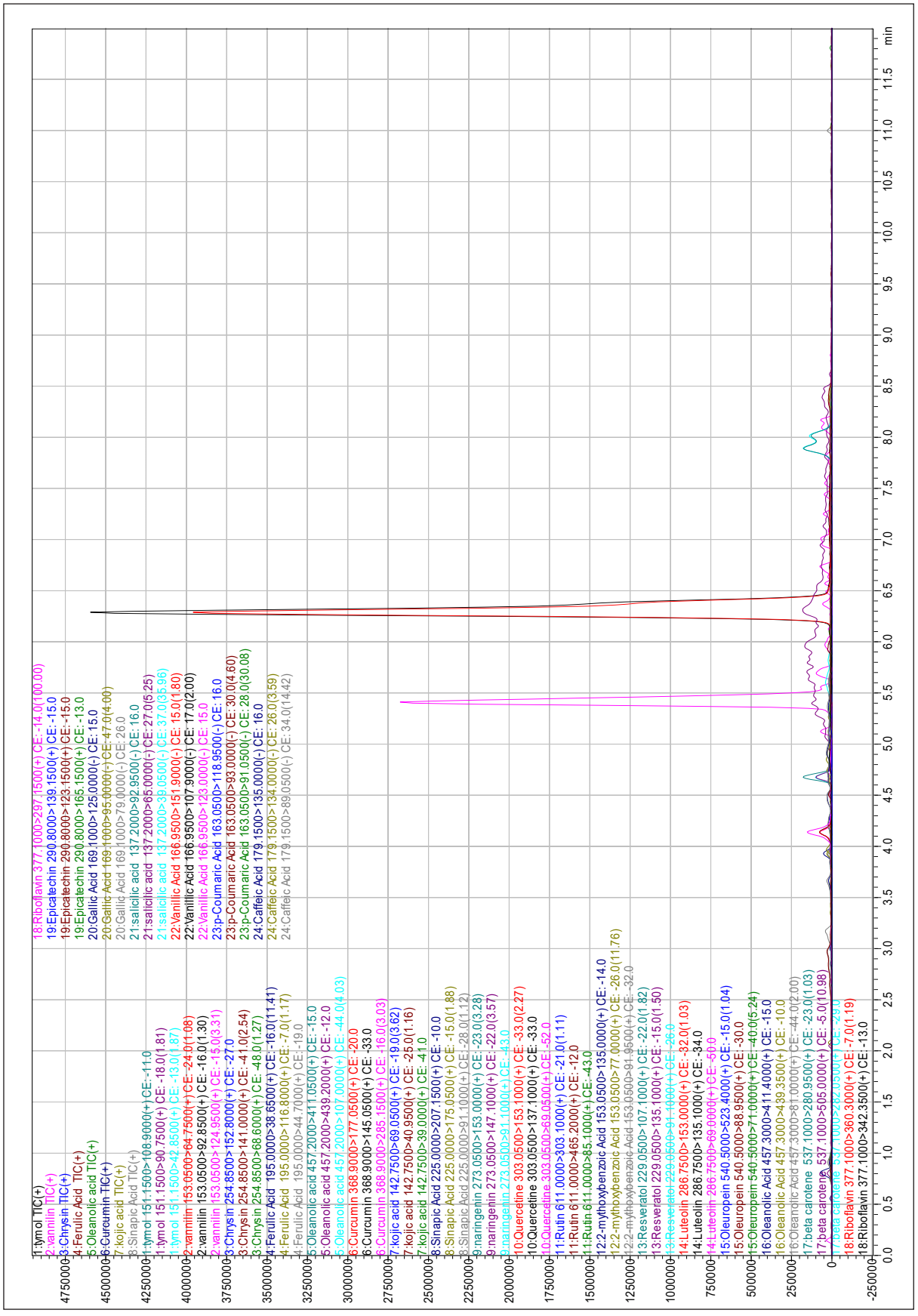


Fig. 2. UPLC-ESI-MS/MS chromatogram of the methanolic extract from Melia azedarach leaves.

Table 2. Phytochemical composition of M. azedarach leaves extract identified by UPLC-ESI-MS/MS analysis

Name	ESI charge	CE (Volt)	m/z	Ret. Time	Area	Height
Thymol	+	-11	151.1500>108.9000	4.850	34137	5365
Vannilin	+	-24	153.0500>92.8500	4.138	374263	68966
Ferulic Acid	+	-7	195.0000>116.8000	2.968	665215	25778
Oleanolic acid	+	-15	457.2000>439.2000	5.379	43492	3852
Curcumin	+	-20	368.9000>177.0500	5.082	17791	4423
Sinapic Acid	+	-10	225.0000>207.1500	4.908	1949229	28825
Quercetine	+	-33	303.0500>153.1000	4.297	1571	622
Rutin	+	-21	611.0000>303.1000	4.238	22739	6399
2-Mythoxybenzoic Acid	+	-14	153.0500>135.0000	3.174	26906	5554
Oleuropein	+	-15	540.5000>523.4000	5.597	742804	17132
Oleanolic Acid	+	-15	457.3000>439.3500	5.558	376145	6000
β Carotene	+	-23	537.1000>280.9500	7.891	1001947	165205
Riboflavin	+	-7	377.1000>360.3000	6.287	20358233	3323172
Salicylic acid	-	16	137.2000>92.9500	4.677	1769085	181791
Vanillic Acid	-	15	166.9500>151.9000	4.013	680	183
p-Coumaric Acid	-	16	163.0500>118.9500	4.269	62913	16043
Caffeic Acid	-	16	179.1500>135.0000	3.929	224881	50938

Table 3. Phytochemical composition of M. azedarach seeds extract identified by UPLC-ESI-MS/MS analysis

Name	ESI charge	CE (Volt)	m/z	Ret. Time	Area	Height
Thymol	+	-11	151.1500>108.9000	4.836	23502	4631
Vannilin	+	-24	153.0500>92.8500	4.136	381715	64970
Ferulic Acid	+	-7	195.0000>116.8000	2.963	567465	38230
Oleanolic acid	+	-15	457.2000>439.2000	5.332	573133	10623
Curcumin	+	-20	368.9000>177.0500	5.077	17255	3705
Kojic acid	+	-19	142.7500>69.0500	5.268	2074	566
Sinapic Acid	+	-10	225.0000>207.1500	4.483	1627514	26613
Rutin	+	-21	611.0000>303.1000	4.213	29391	6934
2-Mythoxybenzoic Acid	+	-14	153.0500>135.0000	3.181	9779	2960
Oleuropein	+	-15	540.5000>523.4000	5.592	587624	35509
Oleanolic Acid	+	-15	457.3000>439.3500	5.541	16635	2785
β Carotene	+	-23	537.1000>280.9500	7.896	2687218	473567
Riboflavin	+	-7	377.1000>360.3000	6.286	14239992	2404132
Salicylic acid	-	16	137.2000>92.9500	4.658	437462	9670
p-Coumaric Acid	-	16	163.0500>118.9500	4.251	6041	1226
Caffeic Acid	-	16	179.1500>135.0000	3.877	8580	1442

were absent in seeds, while p-coumaric acid was present at a much lower concentration (6,041) compared to leaves.

Anti fungal activity of M. azedarach

In vitro antifungal efficacy of botanical extracts : The antifungal potential of methanolic extracts derived from *Melia azedarach* (seeds and leaves) was assessed against three genetically distinct isolates of *Alternaria* spp. (I03, I21, I08) via the poisoned food technique (Fig. 4). Mycelial growth inhibition (MGI%) served as the primary metric for efficacy evaluation. Analysis of variance (ANOVA) revealed that the observed differences in inhibition were statistically significant ($p < 0.05$) for the main effects of plant part (seed vs. leaf), extract concentration, and Alternaria isolate.

M. azedarach seed extracts demonstrated superior antifungal performance, achieving mean inhibition rates of 78.6%±1.25 and 77.8%±0.03

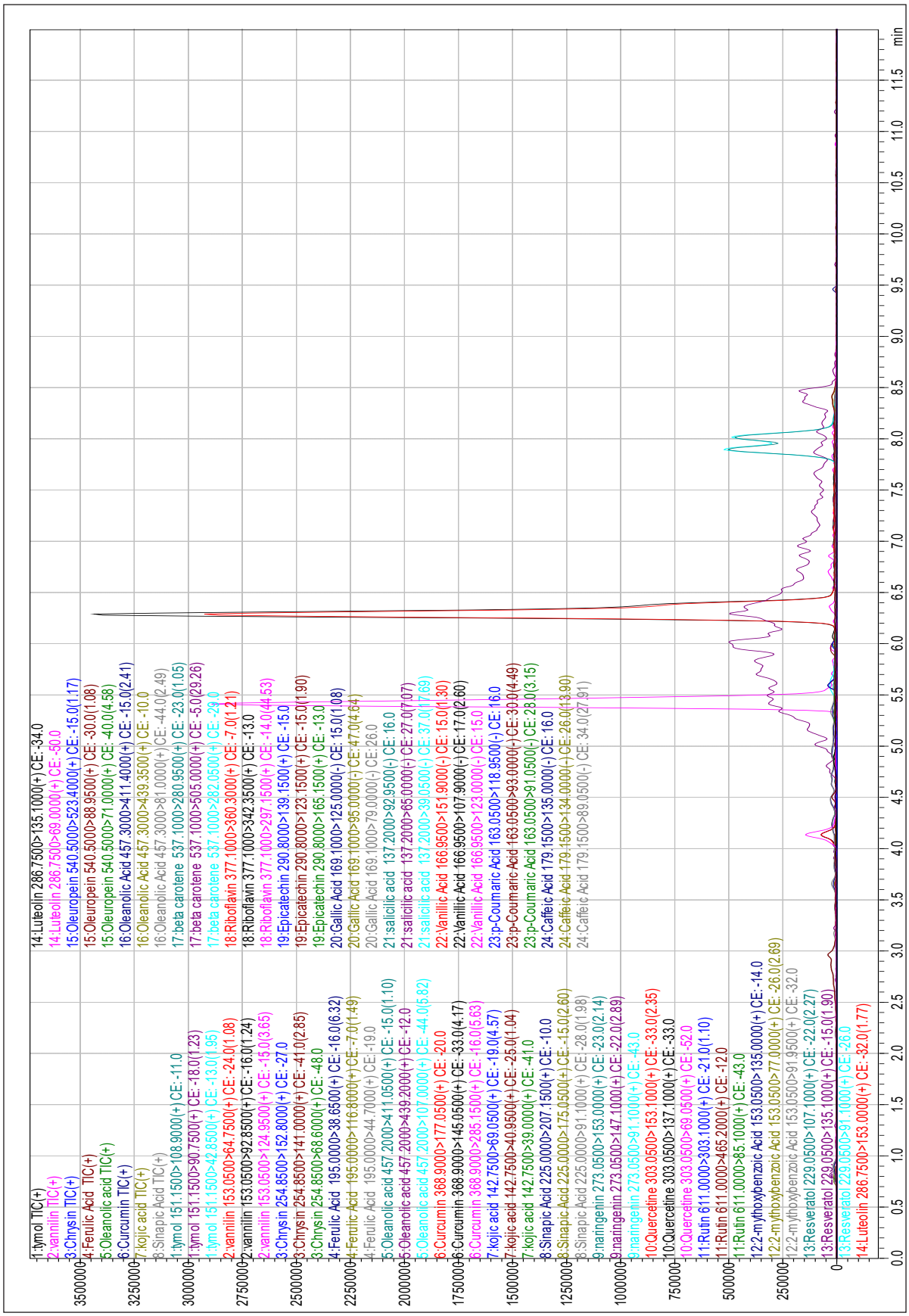


Fig. 3. UPLC-ESI-MS/MS chromatogram of the methanolic extract from Melia azedarach seeds.

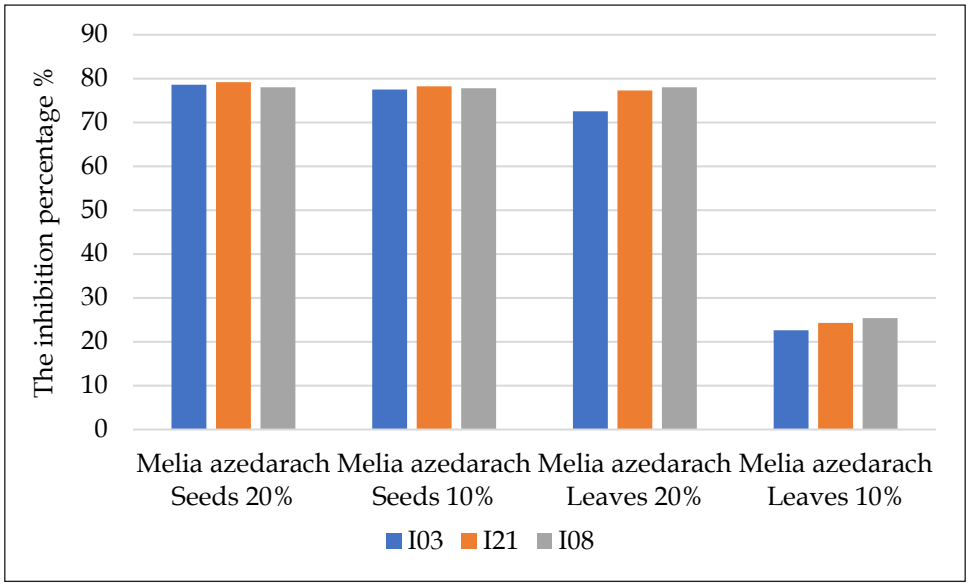


Fig. 4. In vitro antifungal activity of methanolic extracts of Melia azedarach against three isolates of Alternaria spp.

at concentrations of 20% and 10%, respectively. This robust activity, maintained even at 10% (Fig 4), signifies a high concentration of potent antifungal metabolites within the seeds. Conversely, leaf extracts from *M. azedarach* exhibited a concentration-dependent efficacy. At 20% concentration, inhibition rates varied between 72.5%±0.26 and 78.0%±1.31, which sharply declined to a mean range of 22.6-25.4%±2.69 at 10% concentration, indicating a requisite for higher dosage to achieve significant fungal suppression. Please write mean value as you have done in case of sed above

A isolate-specific response was noted. Isolates I21 and I08 were consistently more susceptible, whereas I03 demonstrated reduced sensitivity across all extract types, hinting at potential intrinsic tolerance mechanisms.

Bioactivity on detached potato leaves: preventive vs. curative action: The efficacy of *M. azedarach* seed extract (20%) was further evaluated

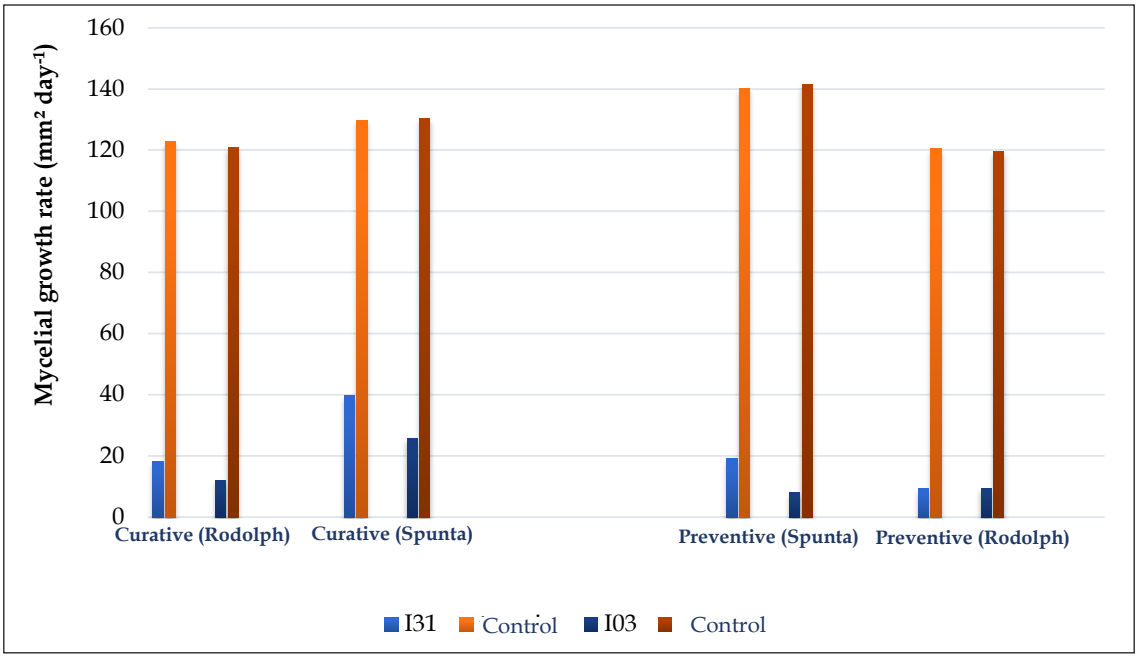


Fig. 5. Effect of M. azedarach seed extract on mycelial growth rate of Alternaria isolates I31 and I03 on detached leaves of potato varieties Spunta and Rodolph under curative and preventive treatment regimens.

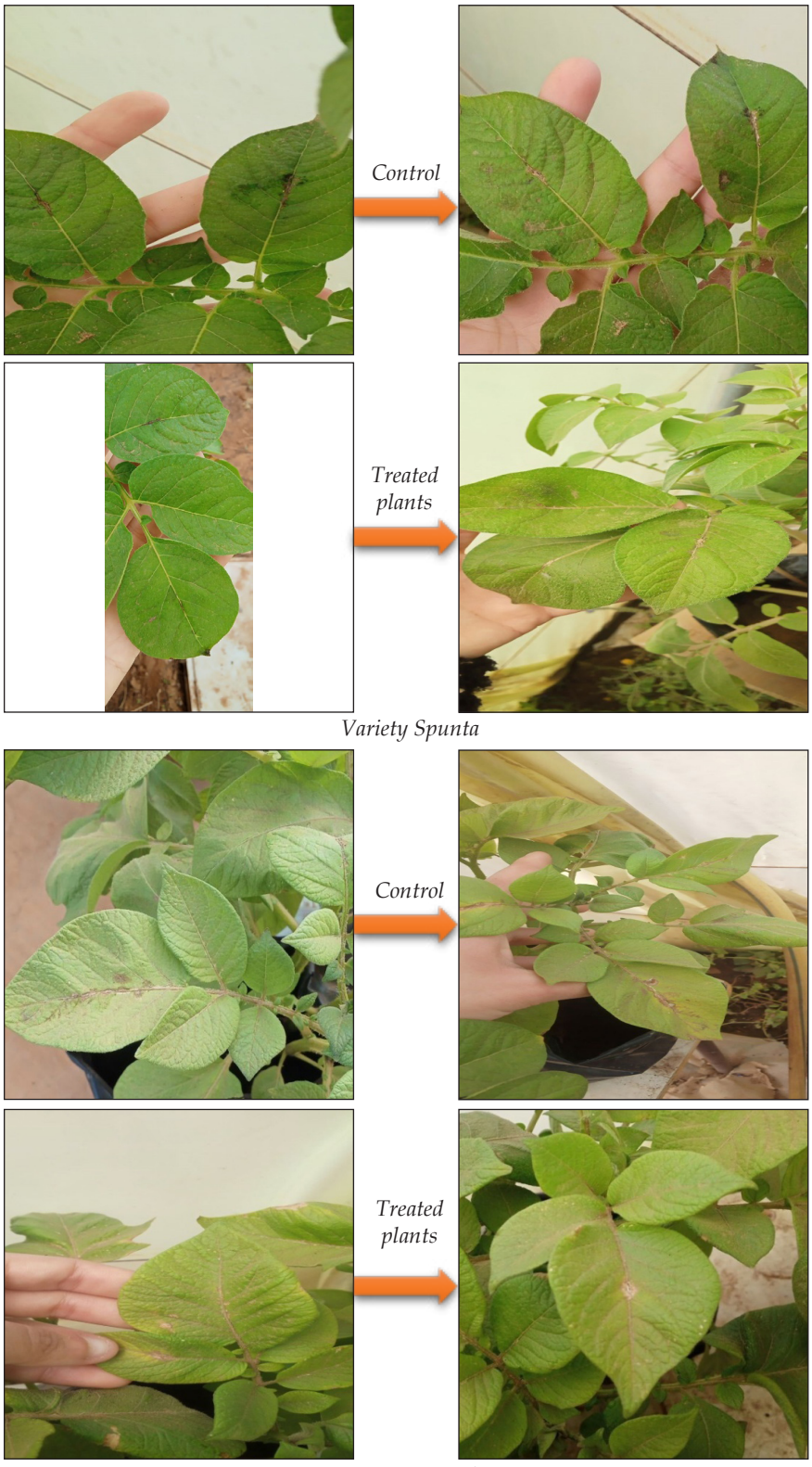


Fig. 6. Greenhouse trial using M. azedarach seed extract (20%) on potato plants (Spunta, Rasmida).

on detached leaves of two potato varieties, Spunta and Rudolph, against *Alternaria* isolates I03 and I31 using both preventive and curative treatment approaches (Fig. 5). In preventive treatments, symptoms appeared 4 to 5 days post-inoculation, whereas in curative

treatments, symptoms emerged earlier (2 to 3 days). Mycelial growth velocity (MGV), expressed as daily lesion expansion ($mm^2\ day^{-1}$), was used to quantify treatment effects. A two-way analysis of variance (ANOVA) confirmed that the differences in MGV were statistically significant ($p < 0.05$) for the main factors of treatment type (preventive vs. curative) and potato variety, as well as for their interaction. Untreated controls exhibited the highest growth rates, often exceeding 140 $mm^2\ day^{-1}$. Preventive application of the extract strongly suppressed fungal growth across all variety-isolate combinations. In Rudolph, growth rates dropped approximately 10±1.02 mm^2/ day, while in Spunta, rates remained slightly higher (10 to 20±0.96 $mm^2\ day^{-1}$). Curative treatment also reduced growth rates but was less effective than preventive application. In Rudolph, curative treatment resulted in growth rates of 10 to 20±1.82 $mm^2\ day^{-1}$, whereas in Spunta, rates reached up to 40±0.63 $mm^2\ day^{-1}$. Isolate I03 was more sensitive to the extract than I31, showing lower growth rates under both treatment regimens. In preventive treatment, I03 reached <10±2.03 $mm^2\ day^{-1}$on Spunta, compared to nearly 20±1.36 $mm^2\ day^{-1}$ for I31.

These results indicate that preventive application of *M. azedarach* seed extract is more effective than curative treatment in suppressing *Alternaria* growth. The Rudolph variety consistently showed greater responsiveness to treatment compared to Spunta, suggesting either enhanced extract efficacy or inherent resistance to fungal infection.

In vivo validation under greenhouse conditions

A curative greenhouse trial was conducted using *M. azedarach* seed extract (20%) on whole potato plants of white (Spunta, Rasmida) and red (Evolution, Manitou) varieties. Treated plants showed delayed symptom development and reduced disease severity compared to inoculated controls (Fig. 6). The slower disease progression in whole plants compared to detached leaves may be attributed to active plant defense mechanisms, including constitutive and inducible responses.

This study provides a comprehensive, multi-level evaluation of the antifungal potential of *Melia azedarach* extracts against *Alternaria* spp., integrating detailed phytochemical profiling with *in vitro*, detached leaf, and whole-plant bioassays. Our findings robustly demonstrate that the methanolic seed extract of *M. azedarach* possesses superior and more consistent antifungal properties compared to the leaf extract. This efficacy is directly correlated with its distinct and chemically complex profile, characterized by a potent combination of bioactive compounds that appear to act through multiple, complementary mechanisms of action.

The *in vitro* poison food assay unequivocally established the seed extract's exceptional potency, achieving remarkable mycelial growth inhibition (MGI) of over 77% even at a halved concentration (10%). This sustained efficacy suggests a high concentration of potent antifungal metabolites and points strongly to synergistic interactions between constituents, a feature that significantly reduces the likelihood of rapid resistance development in pathogen populations (Williamson *et al*., 2001; Bhandari *et al*., 2021). The efficacy of *M. azedarach* seed extract appears competitive with, if not superior to, other botanicals reported against *Alternaria solani*. For instance, while neem (*Azadirachta indica*) kernel extracts have shown inhibitions of 60-80% *in vitro*, and garlic (*Allium sativum*) and eucalyptus (*Eucalyptus globulus*) extracts also demonstrate significant activity, the consistent high-performance of *M. azedarach* seed extract at lower concentrations highlights its particularly strong potential for development into a biofungicide (Afifi *et al*., 2009; Tomazoni *et al*., 2017). Performance of *M. azedarach* seed extract, its multi-faceted mode of action, and its efficacy across experimental levels position it as a highly promising candidate for integrating into sustainable potato early blight management strategies. Its use could reduce dependency on synthetic fungicides, thereby mitigating environmental and resistance issues.

The phytochemical analyses provide a clear mechanistic rationale for this bioactivity disparity. Quantitative assays revealed an approximately 8-fold higher concentration of total phenolics and flavonoids in the seed extract. These compound classes are well-documented for their multi-target antifungal mechanisms, including membrane disruption, enzyme inhibition, and interference with critical metabolic pathways (Yi *et al*., 2025). UPLC-ESI-

MS/MS analysis revealed that the seed extract's superior efficacy may stems from a synergistic combination of bioactive compounds. These include membrane-disrupting oleanolic acid, enzyme-inhibiting phenolic acids, and other metabolites like oleuropein and kojic acid that induce oxidative stress and disrupt fungal metabolism. This multi-target mechanism could potentially enhances its antifungal potency (Ashfaq *et al*., 2025).

Conversely, the leaf extract's profile, while complex, was dominated by riboflavin (Vitamin B2), a compound with less direct antifungal activity. Although it contained a broader range of phenolic acids like caffeic acid, they were present at concentrations likely insufficient to elicit a strong, consistent antifungal response (Aragão *et al*., 2024). The superior efficacy of the seed extract may be attributed to synergistic interactions among its unique phytochemical constituents. The TLC analysis further confirmed these differences, with the leaf extract testing positive for alkaloids absent in seeds, which may not contribute significantly to anti-*Alternaria* activity in this context.

The detached leaf bioassay provided critical insights into the practical application dynamics, clearly demonstrating the superior efficacy of preventive treatment over curative intervention. The significantly delayed symptom manifestation and the profound reduction in mycelial growth velocity (MGV) in preventive treatments potentially underscore a fundamental principle in plant pathology: it is more effective to prevent infection than to cure it (Lucas *et al*., 2015). The superior performance of the preventive application could be attributed to the establishment of a protective chemical barrier on the phylloplane, directly inhibiting spore germination and appressorium formation (Renda *et al*., 2022). Furthermore, the identified bioactive compounds, particularly the phenolics and terpenoids, could potentially act as elicitors suggesting a possible mechanism where plant defense pathways are primed for a stronger response upon pathogen challenge, a phenomenon well-documented for similar plant extracts (Thakur and Sohal, 2013; Leiva-Mora *et al*., 2024).

In contrast, the curative application, while still effective, faced the challenge of halting an established infection. Once *Alternaria* has penetrated the epidermis, it could be more difficult for antifungal compounds to reach the pathogen in lethal concentrations, a common limitation for many natural fungicides (Perla, 2023; Deresa *et al*., 2023). The varietal differences observed, with Rudolph showing greater responsiveness, highlight the critical role of host genotype and suggest a potential synergy between the extract and the inherent resistance mechanisms of certain cultivars (Gan *et al*., 2025).

Finally, the successful validation under greenhouse conditions confirmed the practical relevance of our findings. The significant disease suppression in whole plants treated with the seed extract demonstrates that its bioactive constituents remain stable and functional within a more complex system. The slower disease progression in whole plants compared to detached leaves could be explained by the potential activation of systemic acquired resistance (SAR) and other inducible defense mechanisms in the intact hos (Jones and Dangl, 2006), underscoring the importance of whole-plant validation.

Conclusion

Our integrated approach demonstrates that *M. azedarach* seed extract is a highly promising biocontrol candidate. Its efficacy is underpinned by a rich phytochemical profile that facilitates a multi-target, synergistic attack on the pathogen, complemented by its potential to prime plant defenses. The clear superiority of preventive application provides a direct strategy for IPM integration. However, this study lacks field validation under natural conditions and phytotoxicity assessment across different cultivars-critical gaps that must be addressed before commercial adoption.

Future research should prioritize: (1) comprehensive phytotoxicity screening on diverse potato varieties; (2) formulation development to enhance rainfastness and shelf stability; (3) standardization of extraction protocols for consistent bioactive compound profiles; and (4) multi-season field trials under realistic agronomic conditions. Successfully addressing these steps will facilitate integration of this botanical fungicide into modern IPM strategies, contributing to more sustainable potato production in Algeria and beyond.

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