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Antifungal Activity of Various Plant Extracts against Aspergillus and Penicillium Species Isolated from Leather-Borne Fungus

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

This investigation aims to examine the antimicrobial properties of a few types of medicinal plants on fungi transmitted by leather. In the soxhlet method, the antifungal agents were successfully extracted from the leaves of *Azadirachta indica*, *Lantana camara*, *Wedelia chinensis, Moringa oleifera* and *Coccinia grandis* using methanolic solvent. The fungus isolates from leather bags,

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shoes and wallets were cultured in Potato Dextrose Agar (PDA) plates. The two leather-borne fungi *Aspergillus sp*. and *Penicillium sp*. were the targets of the antifungal assay. Here plant extracts were applied in concentrations of 5.0%, 10.0% and 15.0%. *Azadirachta indica, Moringa oleifera* and *Lantana camara* extract were the most successful treatments for inhibiting the controlled growth of the fungi under this investigation. Furthermore, it is stated that the pathogenic fungi's capacity to proliferate increases with the concentration of plant extracts in the culture. This two-plant showed promising results in treating two cases of fungus, suggesting that it may be used to treat fungi carried by leather.

Keywords: Azadirachta indica; anti-fungal; leather fungi; plant extract.

1. INTRODUCTION

Finished leather is often damaged by different types of molds such as *Aspergillus niger, aspergillus flavus, Trichoderma viride* and *Penicillium sp* by elaborating lipase proteases and degrading natural leather [1]. Fungi such as *A. niger, A sydoni, A. versicolor, An Amsterdam and A. flavus* have been isolated from finished leather [2]. Some common species of microorganisms found responsible for the damage of leather by previous studies were *Mucor, Aspergillus, Penicillium and Rhizopus* [3-4]. In this investigation, we extracted natural antifungal agents from *Azadirachta Indica, Moringa oleifera, Lantana camara, Wedelia chinensis*, and *Coccinia grandis* leaves for the first-time application on leather-borne fungi which dramatically killed the control fungus [4]. *Azadirachta indica, the tree belonging to the Meliaceae family, is also referred to as neem.* The neem tree has been attracted worldwide for its wide range of medicinal The Neem tree contains hundreds of compounds such as beta-sitosterol, bita-salenin, azadirachtin, nimbin, etc [5-6] *Moringa* is a tree and belongs to the family Moringaceae. The leaves, flowers, bark and pods are eaten. The plant is medicinally important and traditionally used in Asian medicine. It represents numerous classes of bioactive natural products, including glycosides, tannins, terpenoids, flavonoids and steroids [6]. One species of flowering plant in the Verbenaceae family is the *Lantana*. It is a great source of flavonoids, terpenoids, glycosides, steroids and other classes of bioactive natural products [7-8]. L. camara is the source of several significant phyto molecules that have been isolated, including phytol, oleanolic acid, lantanoside, linaroside and carminic acid [8]. *Wedelia chinensis, also referred to as "Pilabhamgara" or "Bhringraj," is a perennial herb in the Asteraceae family* [9-10]. The plant is scientifically reported to possess antioxidant, antiseptic and antimicrobial properties [10-12]. *Coccinia* is a climber-type plant and belongs to the family Cucurbitaceae. It's a great source of flavonoids, terpenoids, glycosides and tannins, among other classes of bioactive natural compounds [11-13]. Numerous antifungal properties of selected plant extracts such as Azadirachta *indica, Lantana camara, Moringa oleifera, Wedelia chinensis* and *Coccinia grandis* have outstanding antifungal properties [14-19] as well as other numerous properties have been studied [20-29]. Therefore, the main purpose of this study is to examine the antifungal properties of various plant extracts, *Azadirachta indica, Lantana camara, Moringa oleifera, Wedelia chinensis* and *Coccinia grandis* on leather-borne fungi.

2. METHODOLOGY

2.1 Collection of Different Leaves

Fresh mature leaves of *Azadirachta, Lantana, Moringa wedelia* and *Coccinia* were collected from trees abundantly available in Dhaka city. The gathered leaves were repeatedly cleaned with running tap water to get rid of any dust and dirt particles and then fresh leaf rains with distilled water were applied to prevent contamination.

2.2 Microwave Drying Process

The collected leaves drayed at 30.0-50.0 °C in a microwave dryer for residual moisture. A microwave dryer uses mechanical energy; similar to that found in freezers to transport heat from a cooler area at ambient temperature to a hotter area in the drying chamber.

2.3 Pulverization

The reduction of something to small particles or powder by crushing is called pulverization. The dried leaves were ground using an electric grinder and different sizes of fractions were

collected. The plant materials were in a grinder and then switched on. The collected powder was fined and granular that's stored in airtight containers for further investigation.

2.4 Soxhlet Method of Extraction

Samples of fresh plants are used to prepare extracts such as leaves, stems and bark. The ages chosen were young, mature and old leaf. 500.00 gm dried powders of *Azadirachta indica, Moringa oleifera, Lantana camara, Wedelia chinensis* and *Coccinea grandis* were packed for extraction with methanol using a soxhlet apparatus. At the boiling point of 64.0 ^{0}C for 4.0 -10.0 hours or until the extracted solvent becomes clear. A flask with a circular bottom was filled with the solvent at 250.0 ml of methanol. The thimble which is housed inside the Soxhlet extractor is filled with crushed plant material. By using the isomantle to heat it, the solvent will start to evaporate and go through the device and into the condenser. The condensate pours into the reservoir holding the thimble. The cycle restarts when the solvent level reaches the siphon and drips back into the flask. A rotary evaporator should be used to evaporate the methanol and leave the glass bottom flask with a tiny yield of extracted plant material of about 2.0 to 3.0 ml.

2.5 Isolation, Identification, Purification and Culture

To study their taxonomy to increase the population of infectious propagules for inoculation fungi must be cultured. For optimal growth and sporulation, all microorganisms require specific environmental conditions such as aeration, light, moisture etc. There are three categories for the range of conditions that allow for vegetative growth and sporulation: minimum, maximum and optimum. One of the most significant phases of a fungal life cycle is spore germination. The growth and sporulation of fungi are significantly influenced by the nutrients present in the growth medium, particularly carbon and nitrogen.

2.6 Infected Sample Collection

Visually infected leather was collected from the Leather Research Institute (LRI), Bangladesh Councill of Scientific and Industrial Research (BCSIR), Savar, Bangladesh. Symptoms such as white spots, black and brown spots. Symptoms that appeared in infected leather were studied. Spot color shapes were examined in detail by naked eyes as well as by hand lenses and its characteristic features were recorded. Collected samples were separately packed in sterile polythene bags which were kept in air-tight conditions.

2.7 Preparation of PDA Medium

Fungi have natural deficiencies for vitamins that are satisfied at small concentrations [13]. We use the most common and natural media based on potato dextrose agar medium. Commercial PDA medium is a good medium for fungal growth. It's a relatively rich medium for growing a wide range of fungi [30]. Add 39.00 gm. of commercial PDA powder to 1.0 L of distilled water, boil while mixing to dissolve and autoclave for 15.0 minutes at 121.0 °C.

2.8 Isolation and Culture of Leather-borne Fungi

There was 10.0 ml of PDA medium on each plate. The PDA solution was autoclaved for 30.0 minutes at 121.0 °C at 15.0 lbs of pressure. With sterile 10.0 % tartaric acid, the medium was made more acidic. To prevent bacterial growth in low pH environments about 1.0 millilitres of tartaric acid was added to the previously sterilized PDA medium. Each sterile Petri plate was filled with about 10.0 ml of the prepared media which was then allowed to cool and solidify aseptically. Spores were collected by inoculating needles from separate colonies that were growing on the leather surface and the samples were examined under a binocular microscope before being directly inoculated into agar plates in an aseptic manner. To encourage fungal growth, the plates were incubated at 28.0 ± 1.0 °C for seven days.

2.9 Purification and Identification of Fungi

When the fungus was feasible, the fungi growing out of the inocula were identified in situ and transferred to PDA slants. The isolated fungus was purified following the single spore culture method [31]. To cling spores on the agar block, a small block of solid agar medium was first taken and gently touched to the surface of the culture using the sterile inoculating needle tip. After that, a PDA plate was placed on the agar block slide. (5.0 – 10.0 ml of medium). The medium will become contaminated with spores. The petri dish

was examined under a microscope and spores were separated and collected using a needle containing a tiny piece of sterile paper. The PDA slant was used to hold the filter paper. The spores are going to germinate on the medium after absorbing the filter paper. For every spore, use a fresh piece of filter paper for upcoming research, stock cultures were kept on PDA slants and refrigerated at 5.0 -100.0 °C. Four-week interval subculturing was used to maintain the cultures. Fungi are identified after they have grown in culture using visual traits like colony morphology and colour. When assessing the microscopic morphology of yeasts and identifying whether or not molds have fruiting structures and septate or hyphae, light microscopy is a valuable tool. The fungal isolates' identities were ascertained through morphological analyses. Mycelia, spore-bearing structures and other fungal structures were preserved in lactophenol for microscopic analysis. A tiny bit of cotton blue was added to the material whenever staining was required. Over the material, a spotless cover glass was placed and any extra fluid was wiped away by soaking blotting paper. Colony characters, sporulation time of test, fungus, hyphal features, spore colour and shape were recorded. The purified fungi were identified according to their morphological character in Tables 1 and 2.

In this investigation, *Aspergillus sp.* on the PDA medium showed hyaline mycelium and conidiophores clavate swelling. The results are reported by Fontoura Fig. 1. Macroscopic observation of identified fungi *Aspergillus sp.* A (Front View), B (Bottom View) and C (Macroscopic observation views) [32].

Table 1. Morphological characteristics of the identified fungi

Strains	Mycelium	Conidiophores	Identified Fungus
01	Hyaline mycelium with interconnected hyphae was observed in the culture.	Conidiophores are simple or upright with phialides radiating from the surface or at the apex and ending in a globose or clavate swelling. Features of globose catenates and one-celled conidia were noted.	*Aspergillus sp.*

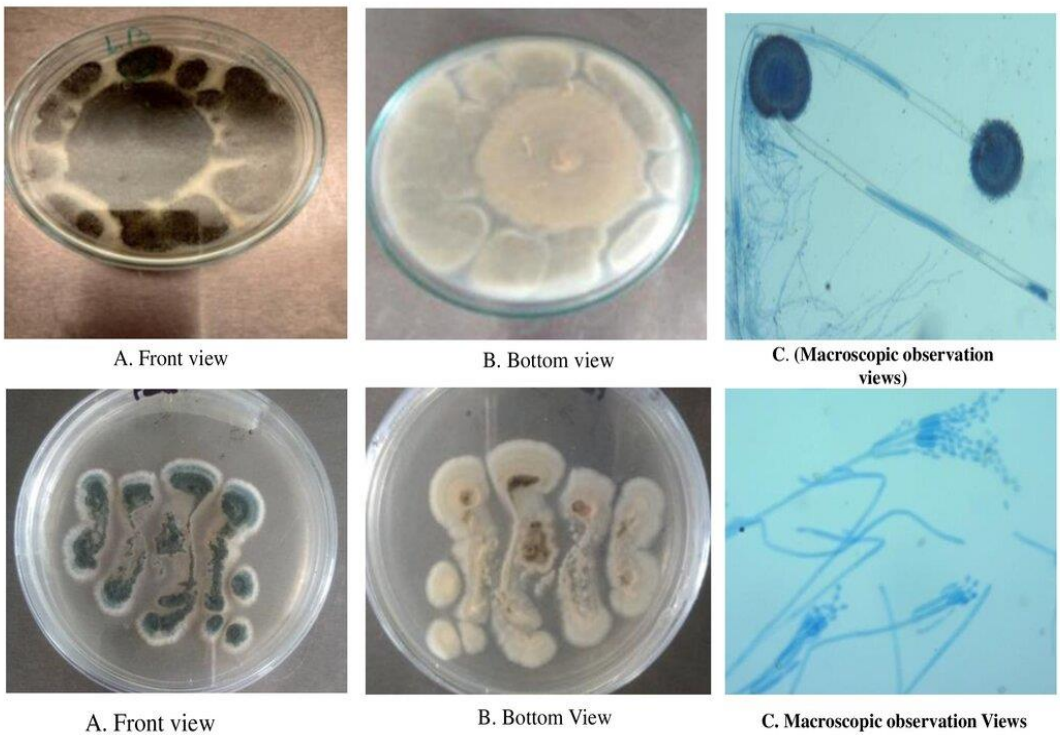

Fig. 1. Macroscopic observation of identified fungi *Aspergillus sp*. and *Penicillium sp.*

Table 2. Morphological characteristics of the identified fungi

Strains	Mycelium	Conidiophores	Identified fungus
02	The mycelium was observed as hyaline, branched and septated.	Conidiophores were single-celled, ovoid, green, granular spores that eventually transformed into phialides. They originated from the mycelium singly or less frequently in synnemata and they branched close to the apex to form a brash-like structure.	*Penicillium sp.*

In this study, *Penicillium sp*. The PDA medium showed hyaline branched mycelium and conidiophores arising from the mycelium [33] recorded such characteristics of *Penicillium sp.* In Fig. 1. Macroscopic observation of identified fungi *Penicillium sp.* A (Front View), B (Bottom View) and C (Macroscopic observation views).

2.10 Anti-fungal and Antimicrobial Test

The isolated fungi were covered to identify the active (rapidly growing) leather deteriogens and examine how well-suited they were to target various kinds of leather. The moist chamber was prepared by placing small autoclave cotton bars on petri plates. The leather was inoculated with actively growing spores of fungus that were previously grown on PDA medium and incubated for 7.0 days. After the incubation period, the test materials were visually inspected to assess the amount of fungal growth. The pathogenic potential of the test fungus was investigated. Fungi are identified after growing for seven days using visual traits like colony morphology and colour. When assessing the microscopic morphology of yeasts and identifying whether or not molds have fruiting structures and septate or non-septate hyphae, light microscopy is a valuable tool to ascertain the identity of the fungal isolates, morphological analyses were conducted. Mycelia, spore-bearing structures and spores were among the fungi that were preserved in lactophenol for microscopic analysis. The existence of *Aspergillus sp. and Penicillium sp.* is further confirmed by microscopic observation.

3. RESULTS AND DISCUSSION

3.1 Testing the Extract for Antifungal Effect

The antifungal activity of *Azadirachta indica, Moringa oleifera, Lantana camara, Wedelia chinensis* and *Coccinia grandis* leaf extract is used on different fungi. The methanolic extracts of these plants possess strong antifungal effectivity because of their strong chemical properties. Two toxigenic fungi isolated from leather and different concentrations of extracts were used. A PDA plate was swabbed with each fungal culture and a standard antifungal plate plated with plant extract served as a positive control. A negative control group of petri plates was created using no plant extracts. For nine days, all petri plates were incubated at 370.0 °C. The cultures were checked three times a week while they were being incubated. Measurements of the fungus's mycelial growth zone in petri plates were used to obtain readings illustrated in Tables 3 to 9.

Table 3. Mean diameter of colonies (cm) on PDA medium with different concentrations of *Azadirachta indica* extract

Sr. No.	Name of fungi	Control	Doses 5.0 %	10.0 %	15.0 %
01.	*Aspergillus sp.*	8.5 cm	1.0 cm	0.7 cm	0.2 cm
02.	*Penicillum sp.*	9.0 cm	1.9 cm	1.0 cm	0.5 cm

Table 4. Mean diameter of colonies (cm) on PDA medium with different concentrations of *Moringa oliefera* extract

Sr. No.	Name of fungi	Doses Control	5.0 %	10.0 %	15.0 %
01	*Aspergillus sp.*	9.0 cm	1.2 cm	0.9 cm	0.3 cm
02	*Penicillum sp.*	9.0 cm	2.1 cm	1.5 cm	0.9 cm

Table 5. The mean diameter of colonies (cm) on PDA medium with different concentrations of *Lantana camara* extract

Sr. No	Name of fungi	Doses Control	5.0 %	10.0 %	15.0 %
01	*Aspergillus Sp.*	9.0 cm	2.1 cm	1.4 cm	0.8 cm
02	*Penicillum Sp.*	9.0 cm	3.5 cm	2.2 cm	1.5 cm

Table 6. The mean diameter of colonies (cm) on PDA medium with different concentrations of *Wedelia sinensis* extract

Sr. No	Name of fungi	Doses Control	5.0 %	10.0 %	15.0 %
01.	*Aspergillus sp.*	8.5 cm	2.5 cm	1.6 cm	1.0 cm
02.	*Penicillum sp.*	9.0 cm	4.0 cm	3.2 cm	2.1 cm

Table 7. The mean diameter of colonies (cm) on PDA medium with different concentrations of *Coccinia grandis* extract

Sr. No	Name of fungi	Doses Control	5.0 %	10.0 %	15.0 %
01	*Aspergillus sp.*	9.0 cm	3.1 cm	2.2 cm	1.5 cm
02	*Penicillum sp.*	9.0 cm	4.5 cm	3.3 cm	2.5 cm

Table 8. Percentage of plant extracts at various concentrations inhibiting fungal growth against *Aspergillus sp.*

Plant	5.0 %	10.0 %	Growth inhibition percentage at various concentrations 15.0 %
Azadirachta	88.20	91.70	97.60
Moringa	86.6	90.0	96.6
Lantana	76.7	84.4	91.1
Wedelia	70.5	81.1	88.2
Coccinia	65.5	75.5	83.3

Table 9. Percentage of plant extracts at various concentrations inhibiting fungal growth against *Penicillium sp.*

Plant	5.0 %	10.0 %	Percent of growth inhibition at different concentrations 15.0 %
Azadirachta	78.8	88.8	94.4
Moringa	76.6	83.3	90.0
Lantana	61.1	75.5	83.3
Wedelia	55.5	64.4	76.6
Coccinia	50.0	63.3	72.2

To test the plant extract's antimicrobial qualities, use the agar medium technique assay. The antifungal activity of *Azadirachta indica, Moringa oleifera, Lantana camara, Wedelia chinensis* and *Coccinia grandis* leaf extracts used on *Aspergillus sp. & Penicillium sp.* Table 3-7 displays plant extracts' impact on *Aspergillus* and *Penicillium species'* radial growth The table's data demonstrated that at varying concentrations all plants extract different fungus growth inhibitors. Antifungal activities of *Azadirachta indica, Lantana camara, Moringa oleifera, Wedelia chinensis* and *Coccinia grandis* were determined against two leather-borne pathogenic fungi *Aspergillus sp. And Penicillium sp*. Plant extracts 5.0, 10.0 and 15.0 % of the concentration tested by the agar plate method significantly caused a reduction in the growth of the above-mentioned fungi. Results showed that *Azadirachta indica, Moringa oleifera, Lantana camara, Wedelia chinensis* and *Coccinia grandis* significantly inhibited the growth of all tested fungi. 15.0 % concentration of *Azadirachta indica, Moringa oleifera and*

Lantana camara extracts demonstrated remarkable antifungal activity against the two fungi [34]. Table 8 presented that out of five plant extracts *Azadirachta indica, Moringa oleifera and Lantana camara* showed (97.60 %), (96.60 %) and (91.60 %) mycelial growth inhibition of the pathogen *Aspergillus sp.* at 15.0 % concentration which is followed by *Wedelia* (88.80 %), *Coccinia* (83.30 %).

Table 9, demonstrated that at a 15.0 % concentration, three of the five plant extracts Azadirachta, Moringa and Lantana showed (94.40 %, 90.0 %) and 83.30 % mycelial growth inhibition of the pathogen *Penicillium sp.* Wedelia (76.60 %) and Coccinia (72.20 %) followed. The order of effectiveness presented in Table 8 against *Aspergillus sp*. at 5.0 % concentration was *Azadirachta* (88.20 %) > *Moringa* (86.60 %) > *Lantana* (76.60 %) > *Wedelia* (70.50 %) > *Coccinia* (65.50 %). The order of effectiveness shown in Table 8 against *Aspergillus sp.* at 10.0 % concentration was *Azadirachta* (91.70 %) > *Moringa* (90.0 %) > *Lantana* (84.40 %) > *Wedelia* (81.10 %) > *Coccinia* (75.50 %). The order of effectiveness shown in Table 8 against *Aspergillus sp*. at 15.0 % concentration was *Azadirachta* (97.60 %) > *Moringa* (96.60 %) > *Lantana* (91.10 %) > *Wedelia* (88.20 %) > *Coccinia* (83.30 %). The order of effectiveness in Table 9 against *Penicillium* sp. at 5.0 % concentration was *Azadirachta* (78.80 %) > *Moringa* (76.60 %) > *Lantana* (61.10 %) > *Wedelia* (55.50 %) > *Coccinia* (50.0 %). The order of effectiveness in Table 9 against *Penicillium sp.* at 10.0 % concentration was *Azadirachta* (88.80 %) > *Moringa* (83.30 %) > *Lantana* (75.50 %) > *Wedelia* (64.40 %) > *Coccinia* (63.30 %). The order of effectiveness against *Penicillium* sp. at 15.0 % concentration was *Azadirachta* (94.40 %) > *Moringa* (90.0 %) > *Lantana* (83.30 %) > *Wedelia* (76.60 %) > *Coccinia* (72.20 %) in Table 9. Therefore, it was demonstrated that the concentration of plant extracts in culture correlated with the inhibition of *Aspergillus sp.* and *Penicillium sp.* mycelial growth.

It is clear from the data in Table 8-9 that *Azadirachta indica, Moringa oleifera and Lantana camara* extracts were the most efficient in preventing of mycelial growth of *Aspergillus sp.* and *Penicillium sp.* It is also mentioned that other plant extracts showed antifungal activity against two pathogenic leather-borne fungi [35,36].

The methanolic plant extracts were significantly higher against leather-borne fungi. At concentrations of 5.0 –15.0 %, the tested microorganism demonstrated broad antifungal activity in extracts from *Azadirachta indica, Moringa oleifera, Lantana camara, Wedelia chinensis and Coccinia grandis.* The methanolic extracts of this plant assert strong antifungal properties because of their strong chemical properties [-22]. The fresh methanol extract of *Azadirachta indica* leaves significantly reduced the mycelial growth of test fungi in a range of 88.20-97.60 % at (5.0 -15.0 %) concentration over control in Fig. 3 to 5 [37]. Methanolic extract of *Azadirachta indica* leaves also significantly reduced the mycelial growth of *Penicillium sp* in a range of 78.8- 94.4% at (5.0 -15.0 %) concentration over control Fig. 5 to 8. who reported that 0.6gm/ 5ml of Neem extract significantly reduced the growth of fungi [15],showing how extracts from *Hibiscus rosa sin ensis, Cassia alata, Ocimum gratissimum, Azadir achta indica* and *Allium sativum* had fungitoxic eff ect.

They demonstrated how the extracts could stop t he growth of mycelial cells. The findings shown in Fig. 5 showed that all test fungi's mycelial growth was significantly inhibited by the methanol solvent extract of Moringa leaves and that the application of *M. oleifera* and *V. amygdalina* extracts at low concentrations was effective in inhibiting the growth of fungal organisms. The highest 15.0 % concentration of 96.60 % significantly suppressed the growth of fungi [38]. Data presented in Fig. 3 revealed that the methanol extract of *Lantana* effective and concentration effect was significant over control. A 91.10 % reduction in radial growth was observed in the fungus. Mycelial growth was efficiently suppressed by lantana leaf extract. These results are consistent with multiple reports that reported similar observations with the maximum activity being recorded at 80.74 % at a concentration of 15.0 % [39]. In this study, the antifungal properties of Wedelia extract have been assessed. The methanol extract of Wedelia was formulated to inhibit fungal growth significantly. The maximum reduction of fungi was 88.20 % in Fig. 3 [40]. Methanol extract from fresh leaves of *Coccinia* is effective at different concentrations against different fungi. The maximum reduction of two fungi was 83.30 % in Fig. 3 [17].

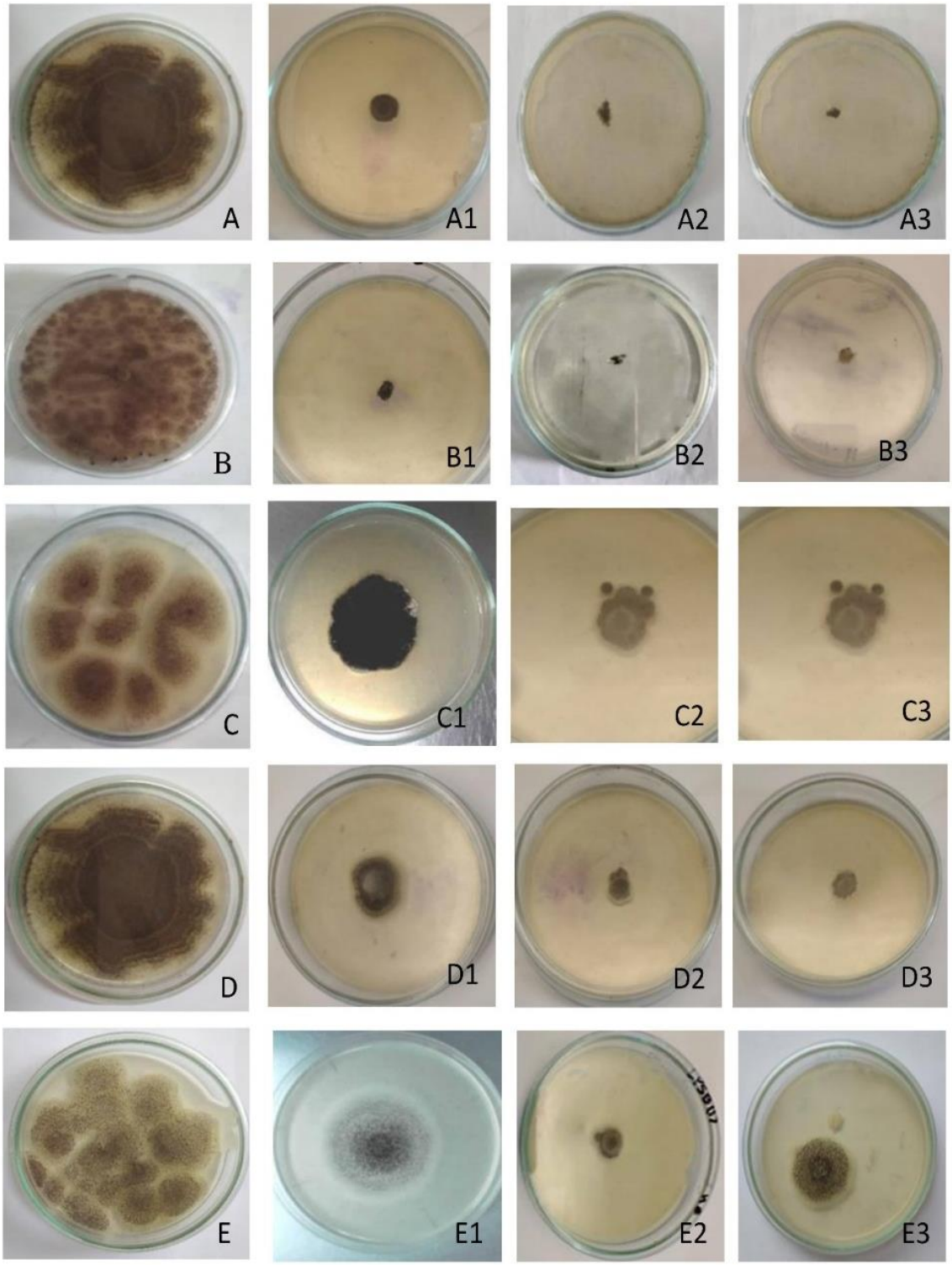

Fig. 2. *Aspergillus sp*. plate A,B,C,D,E (Control), A1,A2,A3(5.0 %,10.0 %,15.0 % concentration) of *Azadirachta indica* extracts. B1,B2,B3 (5.0 %, 10.0 %, 15.0 % concentration) of *Moringa oliefera* extracts. C1,C2,C3 (5.0 %, 10.0 %, 15.0 % concentration) of *Lantana camara* extracts. D1,D2,D3 (5.0 %, 10.0 %, 15.0 % concentration) of *Wedelia cheninsis* extracts. E1,E2,E3 (5.0 %, 10.0 %, 15.0 % concentration) of *Coccinia grandis* extracts

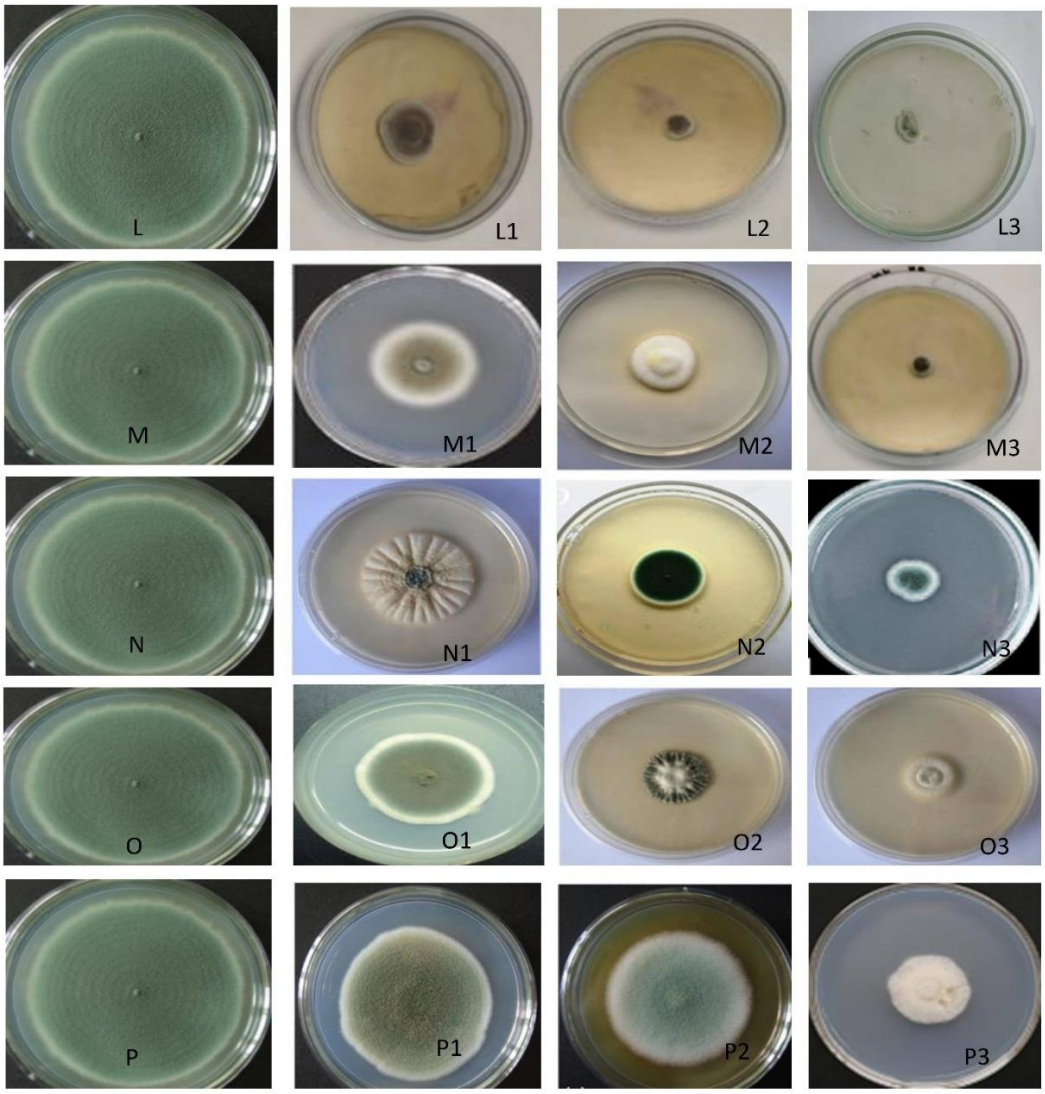

Fig. 3. *Penicillum sp.* plate L,M,N,O,P (Control), L1,L2,L3 (5.0 %, 10.0 %, 15.0 % concentration) of *Azadirachta indica* extracts. M1,M2,M3 (5.0 %, 10.0 %, 15.0 % concentration) of *Moringa oliefera* extracts. N1,N2,N3 (5.0 %, 10.0 %, 15.0 % concentration) of *Lantana camara* extracts. O1,O2,O3 (5.0 %, 10.0 %, 15.0 % concentration) of *Wedelia cheninsis* extracts. P1,P2,P3 (5.0 %, 10.0 %, 15.0 % concentration) of *Coccinia grandis* extracts

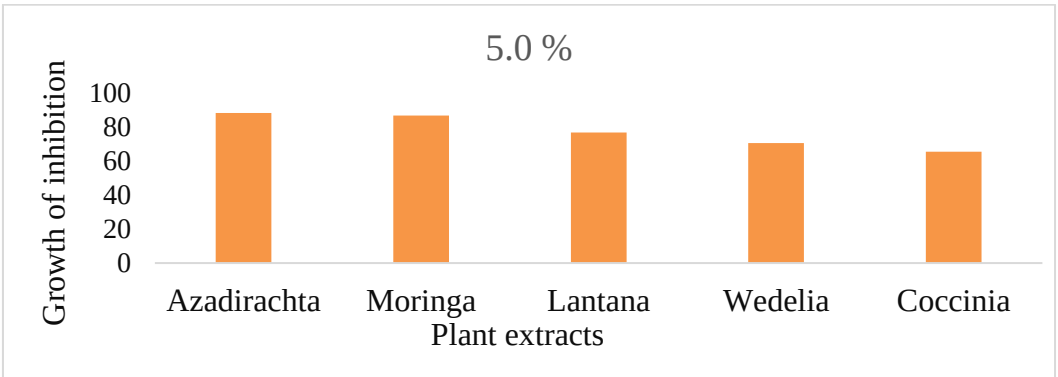

Fig. 4. Percentage of growth inhibition of *Aspergillus sp.* by plant extracts at 5.0 % concentration

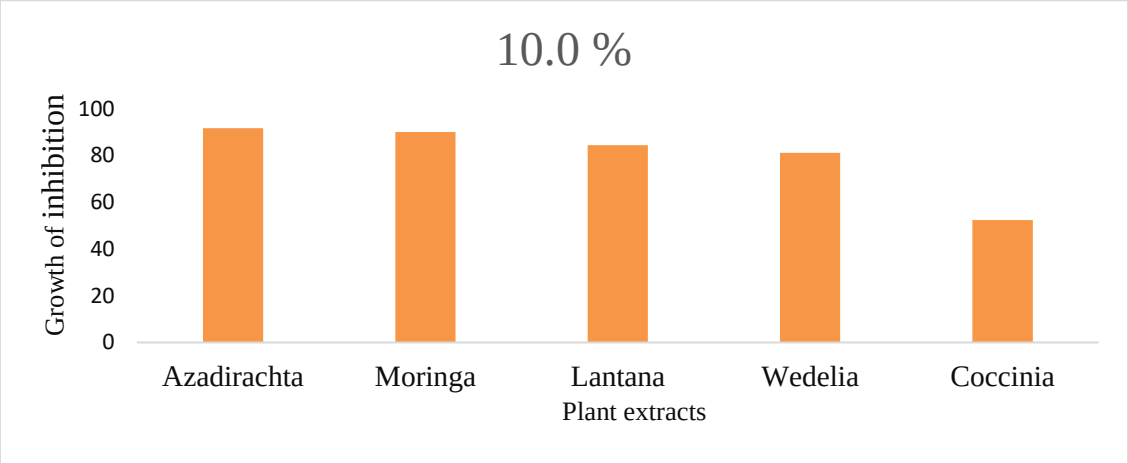

Fig. 5. Percentage of growth inhibition of *Aspergillus sp.* by plant extracts at 10.0 % concentration

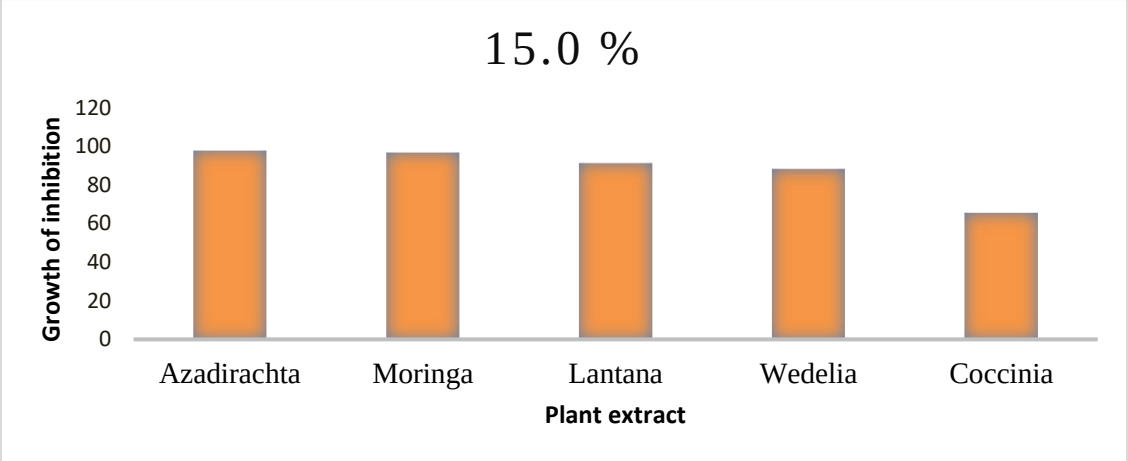

Fig. 6. Percentage of growth inhibition of *Aspergillus sp.* by plant extracts at 15.0 % concentration

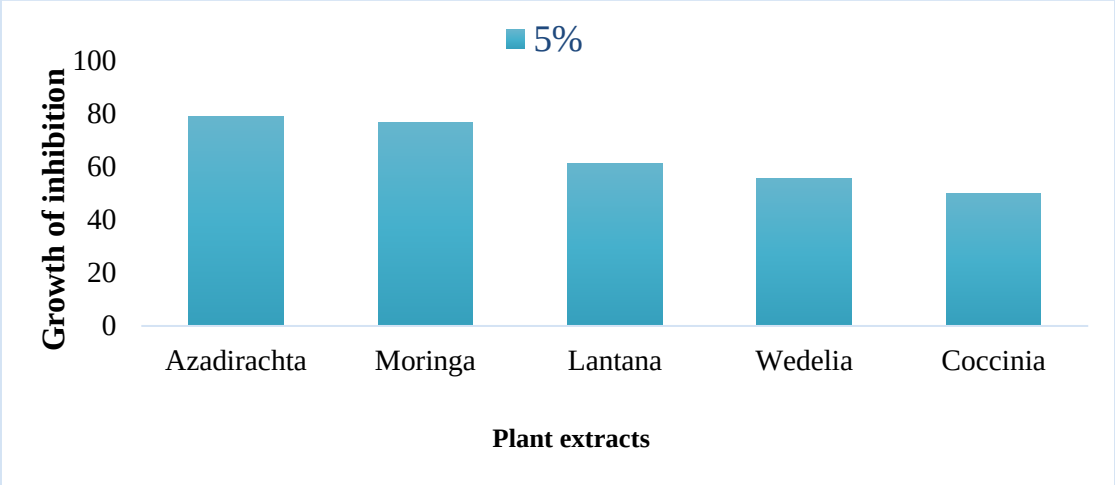

Fig. 7. Percentage of growth inhibition of *Penicillium sp*. by plant extracts at 5.0 % concentration

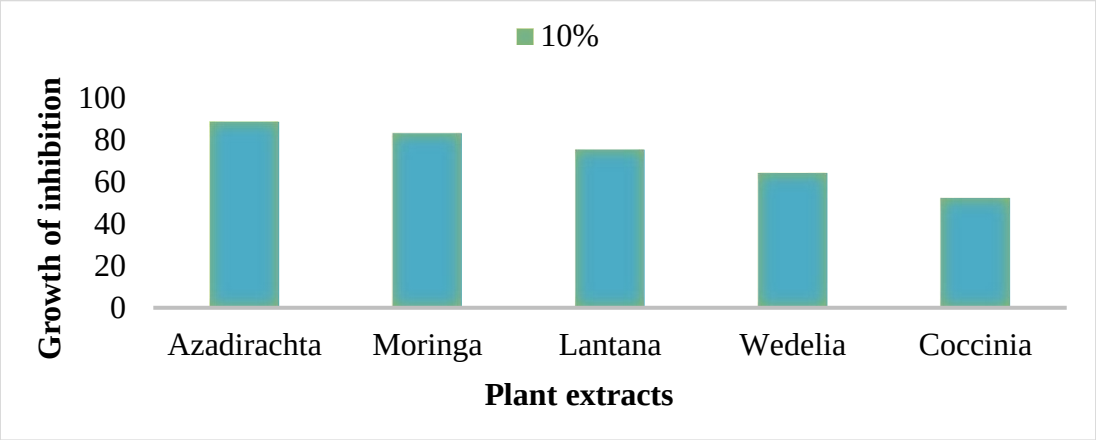

Fig. 8. Percentage of growth inhibition of *Penicillium sp.* by plant extracts at 10.0 % concentration

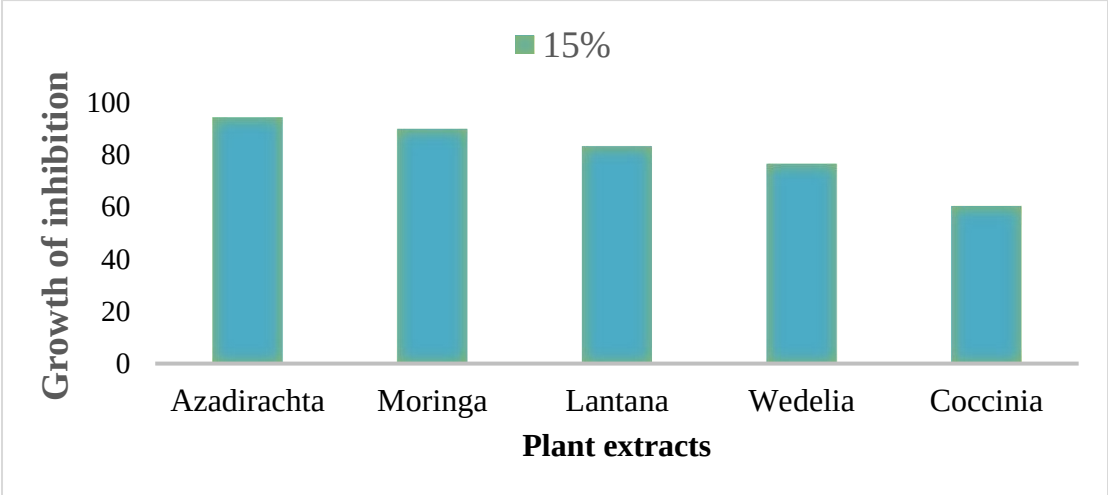

Fig. 9. Percentage of growth inhibition of *Penicillium sp.* by plant extracts at 15.0 % concentration

4. CONCLUSION

The finding of this study is that the isolated pathogens identified as *Aspergillus sp.* and *Penicillium* sp. A positive correlation was found between the biological activity of fungi and the anti-fungal activity of plant extracts at various concentrations. Solvent extracted from plant leaves and evaluated their anti-fungal activity at 5.0, 10.0 and 15.0% concentrations respectively. It was found that *Azadirachta indica*, *Moringa oleifera* and *Lantana camara* extract most significantly suppressed the fungal growth of the tested fungi. As the concentration of plant extracts in culture media increases, so does the inhibition of fungal growth. Among them, the leaf extract of *Azadirachta indica* exhibits superior fungicidal activity compared to other plant extracts. According to our findings, the two tested fungi's growth is significantly inhibited by the methanol extracts of these plants. It can also be used commercially using cost-effective According to our findings, the two tested fungi's growth is significantly inhibited by the methanol extracts of these plants. According to our findings, the two tested fungi's growth is significantly inhibited by the methanol extracts of these plants in an eco-friendly way.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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