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RESEARCH ARTICLE

IN VITRO ANTIFUNGAL ACTIVITY OF ETHANOLIC EXTRACTS OF SYZYGIUMAROMATICUM (L.) MERR. AND L.M. PERRY AND ACACIA NILOTICA (L.) WILLD. EX DELILE AGAINST PATHOGENIC FUNGI OF PEANUT (ARACHIS HYPOGAEA L.)

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Abstract

Groundnut (Arachishypogaea L.) is the main rainfed crop in Senegal, both in terms of cultivated area and the diversity of its uses. However, its production is negatively affected by diseasescaused by a wide range of phytopathogenic fungi. Most of pathogens are commonly controlled with chemical fungicides, which may have harmful effects on the environment and human health. Research for more sustainable and eco-friendly alternatives has therefore been broadened to include natural products, particularly plant extracts, among others. This study was carried outto assess the in vitro antifungal efficacy of ethanolic extracts from the floral buds of Syzygiumaromaticum and pods of Acacia nilotica against several phytopathogenic fungi responsible for diseases of groundnut.The extracts were incorporated at different concentrations into Potato Dextrose Agar (PDA) medium intended to grow the fungi. The test results revealed that S. aromaticum extract completely (100%) inhibited the mycelial growth of Aspergillussp., Rhizoctoniasp., and Rhizopussp. readily at 1000 ppm. At 5000 ppmthe growth of all tested fungi was suppressed. A. nilotica extract also showed effectiveness, however its maximum inhibition rate was79.87% at 10000 ppm against Fusarium sp. The major chemical constituents identified in these plants extracts included polyphenols, alkaloids, flavonoids, tannins, saponins, sterols, and polyterpenes. These findings suggest that such extracts could, in the future, be integrated into biological control strategies against groundnut fungal diseases.

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Introduction:-

In Senegal, the groundnut sector was, around the 1960s, the driving force behind the country's economic development. It accounted for up to 60% of the agricultural GDP and 80% of export revenues (Gueye*et al*., 2015). Today, although it no longer plays such a central role in the national economy, groundnut remains the country's main rainfed and cash crop, both in terms of cultivated area, with 1,225,172 hectares, and production, with 1,501,498 tons (ANSD, 2025). It is grown in all regions of Senegal, with a strong dominance in Kaffrine (228,368 ha), Fatick (185,396 ha), Kaolack (168,663 ha), Kolda (126,188 ha), Louga (126,108 ha), and Tambacounda (116,049 ha), according to the 2022/2023 Annual Agricultural Survey (EAA) report (DAPSA, 2023). Groundnut production aimed at family consumption, but it is also sold in the market places countrywide. The product is sold in the form of pods or kernels or after processing into several forms, including artisanal and industrial oil extraction, peanut paste and peanut flour for local consumption, and exportation to international markets (mainly oil, kernels and peanut cake as feed) (Nobaet al., 2014). Other by-products, such as peanut haulms, are widely used as feed for cattle (Mboup, 2004).

Despite its importance, the groundnut sector faces numerous constraints that significantly reduce yields. Among these are diseases caused by bacteria, viruses, and fungi. Fungi alone account for more than 50% of known plant diseases (Blancard, 2015). They severely affect this crop, causing various foliar diseases such as early and late leaf spots, rust, anthracnose, grey leaf spots, blight, and circular rust-colored lesions along leaf margins (Subrahmanyamet al., 1992). The two types of leaf spots and rust alone are estimated to cause yield losses of up to 70% (ECHOcommunity, 2022). Beyond foliar diseases, groundnut is also subjected to other fungal diseases that cause seedling damping-off, root and stem rot, pod rots, as well as seedling mortality (EzzahiriandSekkat, 2018; Djibaetal., 2025). The pathogens responsible for these afflictions include Aspergillusniger (black collar rot, seedling damping-off), Rhizoctoniasolani (dry root and pod rot), Macrophominaphaseolina (charcoal rot), Rhizopusstolonifer, and Fusarium spp. (seedling damping-off), according to Subrahmanyametal. (1992), Martin (1999), and IFDC (2020). In many countries, some of these diseases, such as collar rot, can cause up to 28–50% plant mortality (Kumar andPolavakkalipalayam, 2016).

Given the extent of the losses caused by these diseases, several control methods are employed to reduce their impact on groundnut production. Chemical control remains the most accessible and widespread approach (Konateetal., 2022). However, it poses major drawbacks, including environmental pollution, risks to human health, and the emergence of resistant fungal strains. Consequently, it has become urgent to identify more sustainable and eco-friendly management strategies. Plant extracts, by virtue of their natural origin, represent a promising alternative to chemical pesticides. Many of them are derived from medicinal plants and are therefore rich in bioactive compounds (antibacterial, antifungal, etc.) that can be exploited forthe control of crop pathogens.The objective of this study was therefore to assess the in vitro antifungal activity of ethanolic extracts from floral buds of Syzygiumaromaticumand pods of Acacia nilotica against selected phytopathogenic fungi responsible for groundnut diseases.

Materials and Methods:-

Plant material:

Clove buds and pods of Acacia nilotica were used as plant material in this study. The clove buds were purchased at the Tilene market in Dakar (Av. Blaise Diagne, Dakar, Senegal, Medina, Dakar (region) 26879 • 7.9 km), while the pods of A. nilotica were collected in the botanical garden of the Faculty of Science and Technologyofthe Cheikh Anta Diop University of Dakar.

Fungal strains:

The fungal material consisted of five phytopathogenic fungi isolated from groundnut plants showing symptoms of dry rot. These included Macrophominasp., Aspergillusniger, Rhizopus sp., Rhizoctonia sp., and Fusarium sp. They were isolated in the Laboratory of Phytochemistry and Plant Protection (LPPV) of the Department of Plant Biology (BV/FST/UCAD) and maintained in the dark in an incubator at 28 °C.

Method of plant extract preparation:

The method used to extract the active compounds from the plants was cold maceration as described by Jonathan andMahendranathan (2024). The ethanolic extract of clove buds was prepared as follows: first, 1 kg of clove buds was placed ina distillation apparatus to extract the essential oil. This extraction was carried out by steam distillation and lasted for 3 hours. After oil extraction, the marc (clove bud residues) was shade-dried for one week and then

ground into a fine powder using an electric grinder. Thereafter 100 g of this powder were mixed with 96% ethanol in an Erlenmeyer flask and left to macerate under continuous stirring for 48 h using a magnetic stirrer. After maceration, the powder–ethanol mixture was filtered twice trough filter paper. The filtrate was collected in a glass flask and concentrated using a rotary evaporator at 50 °C. The extract was then collectedin a watch glass and dried under a laminar flow hood.For the A. nilotica extract, the pods were first dried and then shelled to remove the seeds before being ground with an electric grinder. After grinding, the same procedure as for the S. aromaticum extract was followed.

Determination of extraction yield:
The extraction yield was determined using the formula developed byFallehetal. (2008):
R (%) = (Me/Ms) x 100, where
R: yield,
Me (g): mass of the dry extract obtained after solvent evaporation,
Ms (g): mass of the dry plant material.

Preparation of the concentration rangeof extract:
In order to cover a wide range of doses and better observe the dose–response effect, concentrations of 500, 1000, 5000, and 10000 ppm were selected to test the in vitro antifungal efficacy of the plant extracts. The required amount of extract for each concentration was weighed using a precision balance. The weighed extract was then dissolved in 1 ml of 96% ethanol before being mixed with Potato Dextrose Agar (PDA) medium to a final volume of 100 ml. After homogenization, the mixture was poured into Petri dishes (9 cm in diameter) at a rate of 20 ml per dish, and the plates were stored under a laminar flow hood until use.

In vitro antifungal efficacy test of the extracts on fungal mycelial growth:
After preparing the different concentration ranges, a mycelial disc of 0.5 cm in diameter was taken from a 7-day-old pre-culture of the fungus to be tested, using a cork borer, and inoculated into the test plates. In addition, a negative control (PDA + ethanol + fungus) and a positive control (PDA + mancozeb at 10000 ppm + fungus) were included. For each treatment, three replicates were performed. Mycelial growth was assessed every two days by measuring two perpendicular diameters passing through the center of the mycelial disc. The inhibition rate of mycelial growth was calculated using the following formula described by Serghatetal. (2004): IM = [(M0 – M1) / M0] *100, where
IM (%) = percentage of mycelial inhibition,
M0 (cm) = mean diameter of mycelial growth in control plates, and
M1 (cm) = mean diameter of mycelial growth in treated plates.

Phytochemical screening of plant extracts:
A phytochemical screening was carried out to determine the families of potentially bioactive compounds (polyphenols, flavonoids, alkaloids, tannins, coumarins, saponins, sterols, and polyterpenes) present in the ethanolic extracts of S. aromaticum and A. nilotica.

Polyphenols:
To detect polyphenols, 2 mL of extract were placed in two test tubes (control and test), then a few drops of a 2% alcoholic solution of ferric chloride ($FeCl_3$) were added to the test tube. The appearance of a bluish-black or dark green coloration indicates a positive reaction, confirming the presence of polyphenols.

Flavonoids:
To detect flavonoids, the SHIBATA reaction was used(Fofieetal., 2016). This involves mixing 2 mL of the solution to be tested with 2 mL of hydrochloric acid (HCl) and adding a few magnesium turnings. The appearance of a coloration ranging from orange to purplish-red indicates the presence of flavonoids.

Alkaloids:
This test is based on the ability of alkaloids to combine with heavy metals. A volume of 2 mL of extract is mixed with 1 mL of half-diluted HCl and 10 drops of Dragendorff's reagent (or Mayer's reagent). The appearance of a yellow precipitate or orange spots indicates the presence of alkaloids.

Tannins:

- ✓ Gallic tannins (hydrolyzable tannins) were detected by adding 2 mL of Stiasny reagent to 2 mL of the extract. After 15 minutes of heating in a water bath at 90 °C, the mixture was saturated with sodium acetate, followed by the addition of a few drops of 2% $FeCl_3$. The appearance of an intense blue-black coloration indicates the presence of gallic tannins.
- ✓ Catechin tannins (non-hydrolyzable tannins) were detected by adding 1 mL of concentrated HCl to 2 mL of the solution. The mixture was then boiled for 15 minutes. The appearance of a brown-green precipitate indicates the presence of catechin tannins.

Coumarins:

To 2 mL of the solution, 2 mL of hot water were added. The solution was then divided into two test tubes. The presence of coumarins was tested by adding 0.5 mL of a 25% ammonium hydroxide (NH_4OH) solution to one of the tubes, followed by observation of fluorescence under a UV lamp at 365 nm. An intense blue fluorescence in the tube where ammonia was added indicates the presence of coumarins.

Saponosides:

For the detection of saponosides, 1 g of plant material was added to 100 mL of distilled water and gently boiled for 30 minutes. After cooling, the solution was filtered. Then, 1 mL of the extract was poured into different test tubes and completed to 10 mL with distilled water if necessary. Each tube was manually shaken for 30 seconds. After 15 minutes of rest, the formation of foam with a height greater than 1 cm, persisting over time, indicates the presence of saponosides.

Sterols and Polyterpenes:

For this experiment, 1 mL of each extract was dissolved in 1 mL of acetic anhydride. Then, 0.5 mL of concentrated sulfuric acid (H_2SO_4) was added. The formation of a reddish-brown ring at the interface of the two liquids and a violet coloration of the supernatant layer reveal the presence of sterols and polyterpenes.

Statistical Analysis:

The data were compiled in an Excel spreadsheet and then analyzed using RStudio Quarto (version 4.4.2). A one-way analysis of variance (ANOVA) was performed using the aov function in R, followed by multiple comparison of means with Tukey's Honest Significant Difference (HSD) test using the HSD.test function at the 5% significance level.

Results:-

Extraction Yields of Plant Extracts:

The extraction yield varied depending on the plant species used. It was higher for A. nilotica (38.50%) as compared toS. aromaticum (20.90%) (Table 1).

Table 1. Percentage yield of extracts

Plants	Organs	Solvent	Yield
Acacia nilotica	Pods	96% Ethanol	38.50%
Syzygiumaromaticum	Flower buds	96% Ethanol	20.90%

In vitro antifungal activity of the different extracts on the mycelial growth of fungi:

Figures1 and 2 illustrate the effect of the tested ethanolic extracts on the mycelial growth of the fungi. They show a progressive inhibition strength of the extractswith increasingconcentrations. This was more pronounced with the S. aromaticum extract that induced a total inhibition of the mycelial growth for the majority of the tested fungi starting at 1000 ppm.ForMacrophomina sp. and Fusariumsp., a total inhibition was observed at a concentrationas high as 5000 ppm. For the A. nilotica extract in contrast, despite progressive increase of inhibition performance with rising concentration, a total inhibition was observed only for Rhizoctonia sp. starting at 5000ppm.

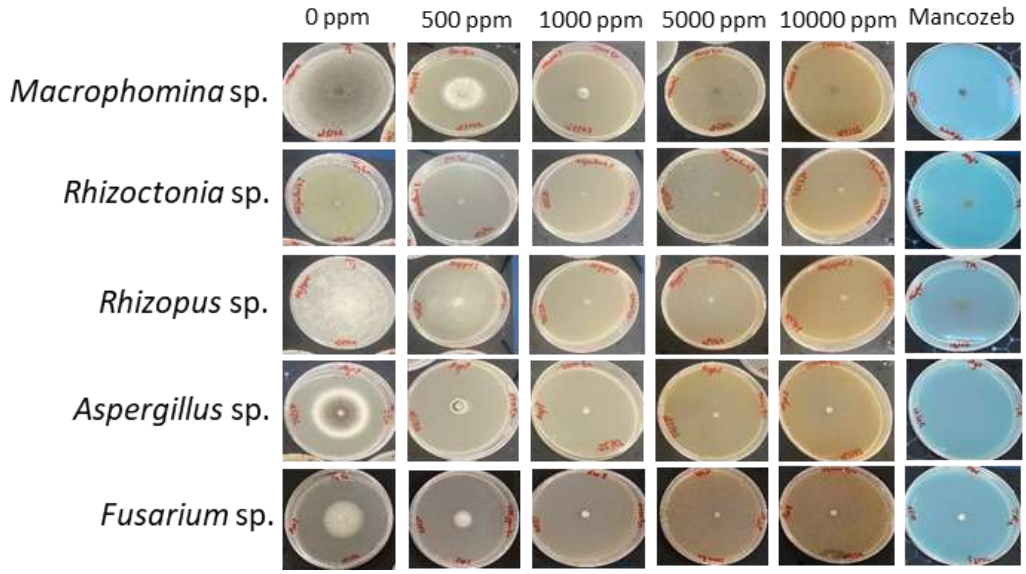

Figure 1. Effect of the S. aromaticum extract on the mycelial growth of the fungi

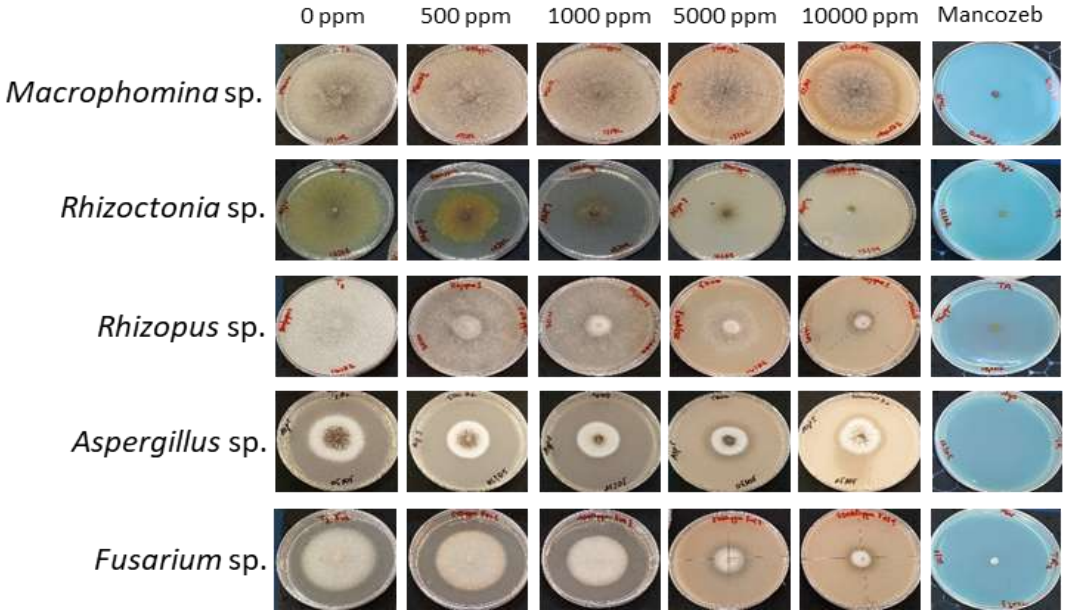

Figure 2. Effect of the A. nilotica extract on the mycelial growth of the fungi.

Important differences in the antifungal activity of the extracts are displayed in Table 2. The A. nilotica extract showed limited efficacy against Macrophomina sp., with a maximum inhibition rate of 4.31% at 10000 ppm, which was statistically comparable to the negative control ($p < 0.05$). In contrast, against the other fungi, the inhibition rates were significantly higher than those of the absolute control ($p < 0.05$), reaching 79.87% for Fusarium, 40.40% for Aspergillus, 52.35% for Rhizopus, and 100% for Rhizoctonia. However, these inhibition levels remain significantly lower ($p < 0.05$) than those achieved with the S. aromaticum extract and the positive control, both of which resulted in complete inhibition of the mycelial growth of all tested fungi. Indeed, the S. aromaticum extract completely inhibited the mycelial growth of Rhizoctonia sp. starting at 500 ppm, Aspergillus sp. and Rhizopus sp. at 1000 ppm, and Macrophomina sp. and Fusarium sp. at 5000 ppm.

Table 2. Inhibition rate of the extracts on the mycelial growth of fungi.

Extracts	Concentrations (ppm)	Inhibition rate (%)				
		Macrophomina	Fusarium	Aspergillus	Rhizopus	Rhizoctonia
Acacia nilotica	500	0±0b	28.35±2.46g	15.08±0.46d	0±0d	26.08±1.48c
	1000	0±0b	37.01±2.34f	21.15±10.08d	0±0d	60±0b
	5000	0±0b	68.61±0.99d	40.40±10.80c	24.90±6.29c	100±0a
	10000	4.31±7.47b	79.87±0.65c	35.13±1.58c	52.35±5.61b	100±0a
Syzygiumaromaticum	500	9.41±8.24b	46.75±2.34e	83.51±3.22b	0±0d	100±0a
	1000	40±4.24c	87.66±0.65b	100±0a	100±0a	100±0a
	5000	100±0a	100±0a	100±0a	100±0a	100±0a
	10000	100±0a	100±0a	100±0a	100±0a	100±0a
Mancozeb	10000	100±0a	100±0a	100±0a	100±0a	100±0a
Negative control	0	0±0b	0±0h	0±0e	0±0d	0±0d

For the same columns, means followed by the same letter are not significantly different according to the Tukey test ($p < 0.05$)

LC50 and LC90 values of plant extracts on the mycelial growth of fungal isolates:

The LC50 and CL90 values were higher with the A. nilotica extract for all tested fungi. Rhizoctonia sp. exhibited the lowest LC 50 (116.27 ppm) and LC90 (137.18 ppm) values with the Syzygiumaromaticum extract(Table 3). With the A. nilotica extract, the values for the same fungus were 716.61 ppm and 1534.18 ppm, respectively. The mycelial growth of Macrophomina sp. and Aspergillus sp., was less sensitive to the extract of A. nilotica sincethe inhibition did not reach 50% at the highest concentration tested.

Table 3. LC50 and LC90 values of plant extracts against fungal isolates

Isolates	Syzygiumaromaticum		Acacia nilotica	
	LC50 (ppm)	LC90 (ppm)	LC50 (ppm)	LC90 (ppm)
Fusariumsp.	534.24	962.94	1885.85	20521.54
Macrophominasp.	332.71	887.70	NaN	NaN
Rhizopussp.	790.06	822.81	1089.87	NaN
Aspergillussp.	129.42	677.21	NaN	NaN
Rhizoctoniasp.	116.27	137.18	716.61	1534.18

Comparative analysis of theantifungal activity of theplant extracts:

Table 4 containing the pairwise analysis of the inhibition performance of the plant extracts shows, significant differences between the treatments (A. nilotica extract, S. aromaticum extract, and Mancozeb) and the negative control. The A. nilotica extract exhibited markedly lower efficacy compared to both S. aromaticum extract and Mancozeb, while no significant difference was detected between the latter two treatments.

Table 4.Comparison of extracts based on their antifungal activity

Comparison	Mean Difference	Adjusted p-value	Significance
Syzygium vs Acacia	48.70	5.052E-14	Yes
Negative control vs Acacia	-34.66	0.0003	Yes
Mancozeb vs Acacia	65.34	4.488E-12	Yes
Negative control vs Syzygium	-83.37	4.807E-14	Yes
Mancozeb vs Syzygium	16.63	0.192	No
Mancozeb vs Negative control	100	5.063E-14	Yes

Phytochemical screening:
Phytochemical analysis revealed the presence of several classes of secondary metabolites in both extracts: polyphenols, flavonoids, gallic and catechin tannins, sterols, and polyterpenes (Table 5). However, certain compounds such as alkaloids were detected only in the S. aromaticum extract, while saponosides were detected exclusively in the A. nilotica extract. The absence of coumarins was noted in both extracts.

Table 5. Chemical compounds present in the extracts

Chemical compounds	Extraction solvent	Syzygiumaromaticum	Acacia nilotica
Polyphenols	96% Ethanol	+	+
Flavonoids	96% Ethanol	+	+
Alkaloids	96% Ethanol	+	-
Gallic tannins	96% Ethanol	+	+
Coumarins	96% Ethanol	-	-
Sterols and Polyterpenes	96% Ethanol	+	+
Catechic tannins	96% Ethanol	+	+
Saponosides	96% Ethanol	-	+

Discussion:-

Plant extracts are considered an alternative to the use of chemical products to control fungal diseases of plants. The results of this study show that the ethanolic extracts of Acacia nilotica and Syzygiumaromaticum, in addition to a good extraction yield, exhibited a remarkable antifungal effect against the targeted phytopathogenic fungi, namely Fusarium sp., Rhizoctonia sp., Macrophomina sp., Aspergillus sp., and Rhizopus sp. The S. aromaticum extract was the most effective, achieving 100% inhibition of mycelial growth at 500 ppm for the most sensitive fungus (Rhizoctonia sp.) and at 5000 ppm for the less responsive fungi (Macrophomina sp. and Fusarium sp.). The analysis of the LC50 and LC90 values also confirms that the S. aromaticum extract is the most effective.

Furthermore, it demonstrated a strong capacity to compete with Mancozeb, the reference fungicide used as a positive control in this study.Uaraksakul and Chanprapai (2022) also showed an antifungal effect of an extract from this same plant against Aspergillusflavus, with a mycelial growth inhibition rate of 27.41% at 1000 ppm. Benmakhloufetal. (2022), for their part, achieved total inhibition of the mycelial growth of this same fungus using a hexane extract of clove at 100 mg/ml. The ethanolic extract of A. nilotica pods, despiteless effectiveness compared toS. aromaticum, showednevertheless a satisfactory antifungal effect with complete inhibition at 5000 ppm against Rhizoctonia sp. and 79.87% at 10000 ppm against Fusarium sp. The inhibitory power of A. nilotica, despite showing moderate performance in our study, was reported as being strong by Abbassyetal. (2018), who obtained an inhibition of 37.48% against Aspergillusflavus and 60% against Sclerotiniasclerotiorum at 1000 ppm.

The antifungal efficacy of these plant extracts is attributed to their chemical composition. In fact, the presence of secondary metabolites such as polyphenols, flavonoids, alkaloids, tannins, saponins, sterols, and polyterpenes, with a powerful antifungal activity in these plantsis welldocumented. The majority of these compounds were reported both in the S. aromaticum and A. nilotica extracts (Uaraksakul and Chanprapai, 2022; Sabah etal. 2017). Some of

thecompounds, namely alkaloids, flavonoids, and tannins, have already demonstrated their ability to neutralize pathogenic fungi, as shown by Zhang etal. (2024), Al Aboody and Mickymaray (2020), and Huang etal. (2024). It is speculated that these secondary metabolites can cause damages in the structure and the functioning of fungal myceliumincluding a destruction of the mitochondrial ultrastructure of the fungal mycelium, a disruption in energy metabolism, a disruption of the plasma membrane, and an inhibition of the synthesis of RNA and proteins among other effects. According to Arifetal. (2009), saponins act on fungal cells by disrupting their integrity. The work of Choudharyetal. (2020) confirmed the antifungal activity of phytosterols by showing that β-sitosterol can inhibit the growth potential of Alternariaarborescens at 500 ppm. Furthermore, the ethanolic extract of clove primarily contains eugenol (54.71%) and, in smaller proportions, eugenyl acetate (17.40%), caryophyllene (11.22%), humulene (5.20%), caryophyllene oxide (4.77%), copaene (3.80%), and β-cadinene (2.89%) (Mostafaetal., 2023). According to Wang etal. (2010), eugenol causes membrane binding and an alteration of permeability, leading to the destabilization and rupture of the plasma membrane.

Conclusion:-

The ethanolic extracts of the tested plants, mainlySyzygiumaromaticum and ina lesser extend Acacia nilotica, demonstrated a highly significant antifungal potential against all tested fungi (Fusarium sp., Macrophomina sp., Rhizoctonia sp., Rhizopus sp., and Aspergillusniger) in this study. The presence of secondary metabolites with strong antifungal potential was noted in these plants. Utilizing their extracts could significantly contribute to reducing the impact of synthetic products on the environment and human health.

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