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In vitro and *In-vivo* antimicrobial activities of endophytic fungi from *Carissa edulis*, *Microglossa pyrifolia* and *Steganotaenia araliacea* against *Cercosporae zeae maydis* and *Fusarium verticillioides*

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Abstract

Understanding antimicrobial capabilities of endophytic fungi against pathogens is crucial for the development of sustainable and eco-friendly strategies for disease management in agriculture. This study explored *in vitro* and *in vivo* antimicrobial activities of fungal endophytic extracts from medicinal plants; *Carissa edulis, Microglossa pyrifolia and Steganotaenia araliacea* against maize fungal pathogens; *Cercosporae zeae maydis* and *Fusarium verticillioides*. These pathogens were identified using field identification manual (CIMMYT 2004). Diseased maize plant parts were aseptically collected in polythene bags and used for isolation of pathogens. Endophytic fungi extracts were obtained in ethyl acetate, evaporated and used for antimicrobial tests in food poison method. Plates for *in vitro* and pots for *in vivo* experiments were arranged in a completely randomized design. *In vitro* assays demonstrated that extracts from *Microglossa pyrifolia* exhibited the highest antimicrobial activities. Extracts from different plant parts, notably leaves and roots of *Microglossa pyrifolia* and *Carissa edulis*, respectively, showed significant ($P<0.05$) inhibition against pathogens. While *C. zeae maydis* was more susceptible to fungal extracts than *F. verticilloides, in vivo* experiments confirmed efficacy of fungal extracts in significantly reducing disease incidence in maize plants than in controls. The study emphasizes the agricultural potential of endophytic fungal extracts in offering sustainable strategies for fungal disease management in maize cultivation. This study had limitations on the impact of extract concentrations and environmental factors on antimicrobial activities and hence future studies should focus on these factors to optimize the efficacy of fungal extracts in agricultural applications.

Keywords: *In Vitro*; *In Vivo*; Antimicrobial; Disease Incidence

1. Introduction

Exploration of natural sources for novel antimicrobial agents has gained significant momentum in recent years due to the escalating global threat posed by antimicrobial resistance. Endophytic fungi, residing asymptomatically within plant tissues, have gained increasing recognition for their role in producing bioactive compounds that exhibit diverse biological activities, including antimicrobial properties (Emitaro *et al.,* 2021; Gunatilaka, 2020; Harun *et al.,* 2021). These fungi form mutualistic associations with their host plants, where they reside predominantly in intercellular spaces or within plant cells without causing apparent disease symptoms (Harun *et al.,* 2021). Their potential to produce secondary metabolites capable of inhibiting various phytopathogens has been unveiled through recent researches (Gu *et al.,* 2022; Anjum *et al.,* 2019), thereby offering promising alternatives to conventional chemical fungicides. Medicinal plants such as *Carissa edulis*, *Microglossa pyrifolia* and *Steganotaenia araliacea* are known for their traditional uses and ecological significance (Opande, 2022; Keter *et al*., 2012; Ochwangi *et al.,* 2014; Akimanya *et al.,* 2015). The exploration

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of endophytic fungi as potential sources of bioactive compounds from these plants represents a sustainable approach to disease management in agriculture. These fungi have evolved in close association with their host plants, producing a diverse array of secondary metabolites that serve various ecological functions, including defense against pathogens (Ochwangi *et al.,* 2014). The search for novel antimicrobial agents from natural sources has thus turned towards endophytic fungi, driven by their ability to synthesize bioactive compounds with specific activity against phytopathogens (Gu *et al.,* 2022; Hyde *et al.,* 2019).

Among the notable phytopathogens affecting maize (*Zea mays*), *Cercosporae zeae maydis* and *Fusarium verticillioides* stand out due to their devastating impact on agricultural productivity worldwide (Fisher *et al.,* 2012; Harwood *et al.,* 2011). These pathogens may lead to significant yield losses if left uncontrolled. On the other hand, *F. verticillioides* causes ear rot, reducing both yield and grain quality while also posing health risks due to its production of mycotoxins such as fumonisins (Yadeta and Thomma, 2013). Current management strategies rely heavily on synthetic fungicides, which not only raise environmental and health concerns but also contribute to the development of resistant pathogen strains (Qing *et al.,* 2015).

Understanding the antimicrobial capabilities of endophytic fungi against these pathogens is crucial for the development of sustainable and eco-friendly strategies for disease management in agriculture. Therefore, exploring *in vitro* and *in vivo* activities of these fungal isolates provides insights into their potential as biocontrol agents or as sources for the discovery of novel antimicrobial compounds (Santhanam *et al.,* 2014). Furthermore, significant gaps remain in our understanding of the full spectrum of antimicrobial activities exhibited by endophytic fungi against *C. zeae maydis* and *F. verticillioides*. Although, numerous studies have focused on characterizing individual fungal strains isolated from specific plant hosts (Hyde *et al.,* 2019; Tolo *et al.,* 2006) and their antagonistic activities, broader investigations into the ecological and evolutionary factors shaping these interactions are limited. Furthermore, the translation of *in vitro* findings to in planta efficacy and practical applications in agricultural settings requires comprehensive assessments of fungal diversity, metabolite production, and bioactivity under varying environmental conditions. In this study, we investigated the antimicrobial potential of endophytic fungi extracts isolated from *C. edulis, M. pyrifolia* and *S. araliacea* against two devastating phytopathogens, *Cercosporae zeae maydis* and *Fusarium verticillioides*, which cause significant yield losses in maize production worldwide.

2. Materials and methods

2.1. Plant material collections and processing

Leaves, stems and roots of *Carissa edulis, Microglossa pyrifolia* and *Steganotaenia araliacea* were collected from parts of Kakamega, western Kenya, 0° 17' 3.19'' N and 34° 45' 8.24'' E in Khaki bags. They were identified and authenticated by a taxonomist at Maseno Botanical Garden and transported in a cooler box to Jaramogi Oginga Odinga University of Science and Technology (JOOUST) microbiology laboratory. Maize leaves with typical symptoms of *Cercosporae zeae maydis and Fusarium verticillioides*, were identified using field identification manual (CIMMYT 2004; Viljoen *et al.,* 2017) and collected from maize fields around Jaramogi Oginga Odinga University of Science and Technology. Field experiments were carried out in green house of the same Institution.

2.2. Endophytic fungi and pathogen isolation

Fungal endophytes were isolated on Potato extract agar amended by streptomycin to inhibit bacterial growth according to Mahadevamurthy *et al.* (2016). Surface sterilized leaves, stems and roots were cut into small pieces of 5mm and plated separately on the surface of PDA. Plates were sealed and incubated at 28^{o}C in completely randomized design for seven days to recover the endophytic fungi.

Fungal pathogens were isolated according to Viljoen *et al.* (2017) protocol. Diseased maize plant parts were surface sterilized, and placed on moist bloter paper in petri dish and incubated at 28^{o}C for five days to allow sprouting of the pathogens. The emerging fungal hyphae were examined in a compound microscope LEICA DM 500 and aseptically transferred to PDA using sterilized dissecting needle as pure cultures.

2.3. Extraction of Endophytic Fungal Compounds and test concentration preparation

Endophytic fungi were isolated from healthy plant tissues, surface-sterilized using 70% ethanol and 2% sodium hypochlorite, followed by rinsing in sterile distilled water. The sterilized tissues were then plated on potato dextrose agar (PDA) and incubated at 25°C for 14 days. Pure cultures of endophytic fungi were obtained by sub-culturing. For the extraction of fungal metabolites, the pure cultures were inoculated into 250 mL flasks containing 100 mL of potato dextrose broth (PDB) and incubated at 25°C with shaking at 150 rpm for 21 days. After incubation, the fungal biomass

was separated from the broth by filtration using Whatman No. 1 filter paper. The filtrate was extracted thrice with an equal volume of ethyl acetate, and the organic phase was collected and evaporated to dryness under reduced pressure using a rotary evaporator at 40°C. The dried crude extract was weighed and reconstituted in dimethyl sulfoxide (DMSO) to prepare stock solutions. Concentration of 1mg of the crude extract was dissolved in 1ml of DMSO to yield a concentration of 1mg/ml which were then prepared for subsequent biological assays.

2.4. *In vitro* antimicrobial activity of extracts against Cercosporae zeae maydis and Fusarium verticillioides.

The growth inhibition activity of fungal extracts of endophytes from *Carissa edulis, Microglossa pyrifolia and Steganotaenia araliacea* against *Cercosporae zeae maydis* and *Fusarium verticillioides* was determined using poisoned food technique (Durgeshlal *et al.,* 2019). A volume of 4 ml of each extract was dispensed in petriplates and adding 16 ml of PDA then mixing and allowing them to set. A 5 mm mycelia plug from 7 days old mycelia was inoculated at the center of the plate then incubated for 7 days. PDA plated without extract was inoculated by the respective pathogens to serve as control. The treatments were done in triplicates. Petri plates were then arranged in a completely randomized design and incubated for 48 hours at 30°C. Radial growth of mycelia for fungi inhibition was measured with a transparent ruler in milimiters. Growth inhibition percentage was determined using the formula of Durgeshlal *et al.* (2019).

Growth inhibition % = DC-DT/DC ˣ100%Where;
DC - colony diameters of the control
DT - colony diameters of the treated.

2.5. Preparation of endophytic fungal spores suspension for *in vivo* antimicrobial activity

Antagonists DSTS2, DENS4, DSTS4 and DSTL2 were based on their *in vitro* abilities to inhibit growth of *Cercosporae zeae maydis* and *Fusarium verticillioides* pathogens in the dual culture experiment. The endophytic fungi; DSTS2, DENS4, DSTS4 and DSTL2 and fungal pathogens were grown on PDA plates for 10 days and conidia suspensions were prepared for both the endophytes and the pathogens by adding 20ml of sterile water to the plates containing the fungi and gently scrapping the conidia from the agar surface using a spreader. They were filtered with sterile gauze into a container and the concentration was determined with Neubauer haemacytometer. Three 500 ml beakers each containing 300 ml of a $1*10^6$ cfu/ml conidia suspension of the selected endophytic fungi and pathogens were prepared for each isolate and pathogen and used once for each type of inoculation.

2.6. *In vivo* antimicrobial activity of the endophytic fungi extracts against *Cercosporae zeae maydis* and *Fusarium verticillioides*

2.6.1. Greenhouse experiment (In Vivo screening)

30 Plastic pots of 30 × 30 cm (20L) with holes drilled at the bottom to facilitate drainage were surface sterilized with sodium hypochlorite and filled with steam sterilized top soil from where maize has not been grown for the past five years. Two hundred maize seeds of rachar variety were surface sterilized with half-strength standard treatment solution (15% commercial bleach + 0.01% Triton X-100) for 15 minutes. Five maize seeds were planted in a sterile pot in the greenhouse, the seedlings were uprooted at 1 week after germination and roots pruned ready for inoculations, which was carried out immediately. The pruned root dip inoculations were performed using the method of Nuangmek *et al.,* (2008) by simultaneously dipping the roots of maize seedlings into a 500 ml beaker containing fungal extracts for 5 min, followed by another 5 min dipping in the fungal pathogen suspension of the same concentration (4g spores/litre of sterile water) from freshly collected cultures having more than 70% germination (Seedlings inoculated with pathogens alone served as positive control. Each treatment was replicated three times, irrigated after every two days up to the first month where they were exposed to water stress to enhance development of fungi pathogens in the vascular system and produce symptoms. The treatments were as shown in table 1:

Table 1 Treatment and fungal isolates for the two pathogens

Treatment	Fungal Isolate / (C. maydis)	Fungal Isolate / (F. verticilloides.)
1	DSTS2+ C. maydis	DSTS2 + F. verticilloides
2	DENS4+ C. maydis	DENS4 + F. verticilloides
3	DSTS4+ C. maydis	DSTS4 + F. verticilloides
4	DSTL2+ C. maydis	DSTL2 + F. verticilloides
5	Distilled water + C. maydis	Distilled water + F. verticilloides

Invitro data was based on the observation of disease symptoms in all treatments from week 8 to 14 of inoculation, scored and calculated as disease incidence (DI) as per method developed by Dua and Sidhu (2012)

DI = Total no. of diseased leaves÷ Total no. of observed leaves× 100

Where DI is disease incidence. which was scored based on Table 2.

Table 2 Scoring scale method

Percentage of Disease Incidence	Host response
0-10%	Resistant
11-30%	Moderately resistant
31-60%	Moderately susceptible
61-100%	Susceptible

(Scale modified from Hill Crop Research Programme, Kabre, NARC)

2.7. Data analysis

The percentage growth inhibition was calculated based on the ratio between the average inhibition and the average growth of the control. Triplicate data from the *in vitro* and *in vivo* antimicrobial activity of the endophytes extracts of each medicinal plant were subjected to one way analysis of variance (ANOVA) and means separated by least significant difference at P≤0.05.

3. Results

3.1. In vitro antimicrobial activities of endophytic fungal extracts against *Cercosporae zeae maydis* and *Fusarium verticillioides*

Growth inhibition of the fungal isolates extracts from the three plant parts of the three plant species that inhibited the growth of *Cercosporae zeae maydis* and *Fusarium verticillioides* were significantly ($p<0.05$) different except for the isolates from *Carissa edulis* (Table 3 and 4). All fungal extracts inhibited *Cercosporae zeae maydis* pathogen above 50% except for DSTS5 extract whose inhibition was 49%. Growth inhibition of all fungal extracts against *Fusarium verticillioides* pathogen was below 50%.

Fungal extracts from endophytes residing in the stems of *Microglossa pyrifolia* and *Steganotaenia araliacea* possessed the highest *in vitro* antimicrobial activities against *Cercosporae zeae maydis* pathogen compared to other plant parts. Isolate DENS4 from the stem of *Microglossa pyrifolia* had the highest inhibition percentage of 84% against *Cercosporae zeae maydis* while isolate DSTS4 from stem of *Steganotaenia aralicea* produced the highest inhibition percentage of 77.33% against *Cercosporae zeae maydis*. The lowest inhibition percentage against *Cercosporae zeae maydis* was from isolate DSTS5 (49%) from *Steganotaenia aralicea* stem and isolate DENS1 (64%) from *Microglossa pyrifolia* stem..

Fungal extracts from endophytes residing in the leaves of *Microglossa pyrifolia* and *Steganotaenia araliacea* possessed the highest *in vitro* antimicrobial activities against *Fusarium verticilloides* pathogen compared to other plant parts (Table 3 and 4). Isolate DENL2 from the leaves of *Microglossa pyrifolia* had the highest inhibition percentage of 34.67% against *Fusarium verticilloides* while isolate DSTL1 from leaves of *Steganotaenia aralicea* produced the highest inhibition percentage of 22.67% against *Fusarium verticilloides.* The lowest inhibition percentage against *Fusarium verticilloides* was from isolate DSTS5 (1.33%) from *Steganotaenia aralicea* stem and isolate DENS4 (1%) from *Microglossa pyrifolia* stem.

Fungal extracts from endophytes residing in the roots of *Carissa edulis* possessed higher *in vitro* antimicrobial activities against *Cercosporae zeae maydis* compared to *Fusarium verticilloides*. Extracts from both leaves and root fungi exhibited higher disease symptoms inhibition against *Cercosporae zeae maydis* but low inhibition against *F. verticilloides*. Isolate DSRR2 from the roots of *Carissa edulis* had higher inhibition percentage of 26.33% against *Fusarium verticilloides* while the lower inhibition percentage against *Fusarium verticilloides* was from isolate DSRL3 (23%) from same plant.

Table 3 *In vitro* antimicrobial activities of fungal extracts from Microglossa pyrifolia against Cercosporae zeae maydis and Fusarium verticillioides.

Pathogen	**Fungal isolates from *Microglossa pyrifolia***							
	Leaf	Root			Stem			P value
	DENL2	DENR1	DENR2	DENR3	DENS1	DENS3	DENS4	
C. zeae maydis	68.67^{bc}	70.67^{bc}	71^{bc}	65^{c}	64^{c}	75^{ab}	84^{a}	0.005
F. verticilloides	34.67^{a}	2.67^{b}	22.67^{b}	3^{c}	19^{b}	14.67^{b}	1^{c}	<0.0001

Means followed with same letter in the columns are not significantly different

Table 4 *In vitro* antimicrobial activities of fungal extracts from Steganotaenia aralicea and Carissa edulis against Cercosporae zeae maydis and Fusarium verticilloides

Pathogen	**Fungal isolates from *Steganotaenia aralicea***							**Fungal isolates from *Carissa edulis***		
	Leaf		Stem				P value	Leaf	Root	P value
	DSTL1	DSTL2	DSTS2	DSTS3	DSTS4	DSTS5		DSRL3	DSRR2	
C. zeae maydis	58^{bc}	60.33^{bc}	72^{ab}	67.67^{ab}	77.33^{a}	49^{c}	0.02	76.33^{a}	76.33^{a}	1.00
F. verticilloides	22.67^{a}	9.67^{bc}	3^{bc}	14.33^{ab}	12.67^{abc}	1.33^{c}	0.02	23^{a}	26.33^{a}	0.67

Inhibition % followed by same super script letter is not significantly different

3.2. *In vivo* antimicrobial activities of endophytic extracts from Carissa edulis, Microglossa pyrifolia and Steganotaenia araliacea against pathogenic fungi of maize; Cercosporae zeae maydis and Fusarium verticillioides.

There was no significant ($p>0.05$) difference in the disease symptoms inhibition against *Cercosporae zeae maydis* by the extracts at weeks 6, 8 and 10 whereas at weeks 12 and 14, extracts from endophytic fungi significantly inhibited the expression of disease symptoms (Table 5). In week 6 and 8, there was progressive increase in disease incidence. Treatments with isolate DSTL2 had the highest disease incidences of 35% at week 6 while at week 8, isolate DSTS2 treatments recorded the highest disease incidence of 66%. The lowest disease incidence in week 6 and 8 were recorded in treatments for isolates DSTS2 (27%) and DENS4 (55.33%) respectively. From week 10 all the way to week 14, disease incidences recorded significantly reduced as time went by. The highest disease incidences recorded at week 10, 12 and 14 were at treatment DENS4 (30%), DSTS4 (16%) and DSTS4 (33.3%). The lowest disease incidences at week 10, 12 and 14 were from treatments DSTS4 (17%), DSTS2 (12.67%) and DSTS2 (15%).

There was no significant ($p>0.05$) difference in the disease symptom expression inhibition by endophytic fungal extracts against *Fusarium verticilloides* at week 6,8 and 12 while at week 10 and 14, inhibition was significantly ($p<0.05$) different (Table 6). As the weeks progressed, percent Disease Incidence increased in control plots since they were not treated with the endophytic extracts. This was similar case with the isolates in which disease incidence progressively increased from week 10 up to week 14, except for DSTS2 in which disease incidence reduced from week 12 to 14. Among the isolates; DSTS4 from stem of *Steganotaenia aralicea* recorded the highest percent Disease Incidence at 60.33% in week 6 while DSTS4 from stem of *Steganotaenia aralicea* recorded the highest percent Disease Incidence at 55.67% in week 8. The lowest disease incidence in week 6 and 8 were recorded in treatments for isolate DENS4 isolated from *Microglossa pyrifolia* inhibited growth of *Fusarium verticilloides* at (47.67% and 48.67%) respectively. The highest disease incidences recorded at week 10, 12 and 14 were at treatment DSTS2 (33.33%), DSTS2 (39.67%) and DSTL2 (44.33%). The lowest disease incidences at week 10, 12 and 14 were from treatments DSTL2 (9.67%), DENS4 (23%) and DENS4 (36%). Isolate DSTS2 and DSTL2 were isolated from the leaves of *Steganotaenia aralicea.*

Table 5 Mean Percentage Disease Incidence (DI) against *Cercosporae zeae maydis*

Weeks	Isolates against *Cercosporae zeae maydis*					
	DSTS2	DENS4	DSTS4	DSTL2	CONTROL	P value
6	27^{b}	29.67^{ab}	32^{ab}	35^{ab}	51^{a}	0.21
8	66^{a}	55.33^{a}	58.33^{a}	63.33^{a}	63.67^{a}	0.85
10	25.67^{ab}	30^{ab}	17^{b}	24.33^{ab}	53.33^{a}	0.15
12	12.67^{b}	16^{b}	16^{b}	14.33^{b}	24.33^{bc}	0.01
14	15^{c}	33.33^{b}	33.33^{b}	24.33^{b}	75^{a}	<.0.001

Means followed with same letter in the same row are not significantly different

Table 6 Mean Percentage Disease Incidence (DI) against *Fusarium verticillioides*

Weeks	Isolates against *Fusarium verticillioides*					
	DSTS2	DENS4	DSTS4	DSTL2	CONTROL	P value
6	48^{a}	47.67^{a}	60.33^{a}	52.33^{a}	44^{a}	0.32
8	52.67^{a}	48.67^{a}	55.67^{a}	51^{a}	52.67^{a}	0.90
10	33.33^{b}	19.67^{b}	20.67^{b}	9.67^{b}	63.33^{a}	0.01
12	39.67^{ab}	23^{b}	23.33^{b}	37.67^{ab}	70^{a}	0.11
14	36^{b}	36^{b}	38.33^{b}	44.33^{b}	80.67^{a}	0.01

Means followed with same letter in the row are not significantly different

4. Discussion

Medicinal plants derived-endophytic fingi are indisputable important and have continuously attracted considerable research attention (Emitaro *et al.,* 2024; Chauhan *et al.,* 2023; Balkrishna *et al.,* 2022). In vitro antimicrobial activity revealed the potential of these endophytic fungal extracts isolated from *Microglossa pyrifolia, Carissa edulis* and *Steganotaenia araliacea* to inhibit the growth of *Cercosporae zeae maydis* and *Fusarium oxysporum* pathogens. All the fungal extracts were active against *Cercosporae zeae maydis* at higher percentages compared to *Fusarium verticilloides.* Growth inhibition could be attributed to the secondary metabolites in the fungal extracts that may have interfered with normal metabolic activities relevant for growth of the pathogens such as cell wall and protein synthesis. The results concur with the reports by Corrado and Rodrigues (2004) that crude extracts of cultures of 13 fungal strains from *Phomopsis* sp. and other extracts from *Aspidosperma tomentosum* and *Spondias mombin* inhibited the growth of *Aspergillus niger* and *Fusarium oxysporum*.

Fungal extracts from endophytes residing in the stems of *Microglossa pyrifolia* and *Steganotaenia araliacea* possessed the highest *in vitro* antimicrobial activities against *Cercosporae zeae maydis* pathogen. The high in vitro antimicrobial activities of fungal extracts from endophytes residing in the stems of *M. pyrifolia* and *S. araliacea* against the pathogen *Cercosporae zeae maydis* can be attributed to a combination of unique chemical profiles of the host plants, symbiotic relationships, co-evolution, synergistic effects, fungal diversity, metabolic versatility, and ecological niche dynamics. These factors collectively contribute to the production of potent antimicrobial compounds by the endophytic fungi (Chaudhary *et al.,* 2022; Balkrishna *et al.,* 2022; French *et al.,* 202; Singh e*t al.,* 2020). Previous studies have noted that endophytic fungi residing in the stems of these plants are subjected to competitive pressures from other microorganisms (Singh e*t al.,* 2020; Chaudhary *et al.,* 2022). This competition can drive the production of potent antimicrobial compounds as a survival strategy. Furthermore, ecological niche within the stems of *Microglossa pyrifolia* and *Steganotaenia araliacea* may provide unique conditions that favor the growth of fungi with strong antimicrobial capabilities. These results are in agreement with report from Khalil Al-Mughrabi (2003) that extracts from stems had stronger antimicrobial activities than those from other plant organs of *Euphorbia macroclada* against selected pathogens.

Extracts from DENS4 had the highest antimicrobial activities against *Cercosporae zeae maydis* probably because the extracts could have contained tannins, terpenoids, alkaloids and flavonoids which are the active ingredients in the inhibition of cell wall or protein synthesis. These results are in agreement with the report by Salhi *et al.* (2017) that extracts from *Asphodelus tenuifolius* plants have alkaloids, quinines, flavonoids and many other secondary metabolites with antimicrobial properties against *Fusarium mycelia* and *F. graminearum*.

Generally, the inhibition against *Fusarium verticilloides* was low compared to *Cercosporae zeae maydis* probably because endophytic fungi and their host plants often co-evolve, leading to the development of specialized metabolic pathways in the fungi that are geared towards producing compounds effective against local pathogens, such as *Cercosporae zeae maydis*. This evolutionary process can result in endophytes that are particularly adapted at producing antimicrobial agents that target specific pathogens threatening the host plant and hence the observed differences in their inhibitory potential (Alam *et al.,* 2021).

Fungal extracts of endophytes from leaves of *Microglossa pyrifolia* and *Steganotaenia araliacea* possessed the highest *in vitro* antimicrobial activities against *Fusarium verticilloides* while extracts from endophytes from roots of *Carissa edulis* possessed the highest *in vitro* antimicrobial activities against *Fusarium verticilloides.* It is well known that leaves are the primary sites of photosynthesis and often contain high concentrations of secondary metabolites such as phenolics, flavonoids, and terpenoids (Li *et al.,* 2020). These compounds can enhance the antimicrobial activity of endophytic fungi residing in the leaves. Additionally, the interaction between the host plant's defensive compounds and the endophytic fungi may have led to the production of unique bioactive metabolites that were particularly effective against *Fusarium verticilloides* pathogen. Leaves are often exposed to various environmental stressors such as UV radiation, herbivory, and microbial attacks (Ogbe *et al.,* 2020). Although this study did not monitor such environmental stresses, exposure of the plants could have induced the production of stress-related secondary metabolites in both the host plant and the endophytic fungi. The need for increased defense mechanisms in *M. pyrifolia* and *S. araliacea* may have led to endophytes in leaves to produce potent antimicrobial compounds.

Roots interact directly with the soil environment, which is rich in microbial diversity, including both beneficial and pathogenic microorganisms (Chauhan *et al.,* 2023). This interaction could have conferred the production of specific antimicrobial compounds by root-associated endophytes in *C. edulis* to combat soil-borne pathogens. The unique chemical environment of the root zone, including root exudates, could have also influenced the metabolic pathways of endophytic fungi, leading to the production of effective antimicrobial agents against *Fusarium verticilloides*. It is worth noting that roots often form symbiotic relationships with various microorganisms, including endophytic fungi (Taniguchi *et al.,* 2023). These relationships can enhance the ability of endophytes to produce bioactive compounds as a part of the plant's overall defense strategy. Generally, the endophytes in roots may have evolved to produce specific antimicrobial compounds that target *Fusarium verticilloides* pathogen.

The root environment provides a rich source of nutrients and organic compounds (Li *et al.,* 2023), which can support the growth and metabolic activity of endophytic fungi. This may have enhanced the production of secondary metabolites with antimicrobial properties. Availability of specific nutrients and root exudates in the root zone possibly fostered biosynthesis of unique antimicrobial compounds by endophytes in *C. edulis* as described in other studies (Li *et al.,* 2023; Taniguchi *et al.,* 2023).

In vivo experiments demonstrated that maize plants treated with fungal extracts exhibited lower disease incidence compared to untreated controls for both pathogens. The fungal extracts could have triggered the plant's own defense mechanisms, leading to induced systemic resistance state. This state enhanced the plant's ability to fend off pathogen attacks, reducing disease incidence (Daigham *et al.,* 2023). Furthermore, treatment with fungal extracts may have upregulated the expression of defense-related genes in maize, leading to increased production of pathogenesis-related proteins, phytoalexins, and other antimicrobial compounds (Naz *et al.,* 2021).

This reduction in disease incidence could be attributed to the antimicrobial properties of secondary metabolites within the fungal extracts, which reduced the germination of pathogen spores as well as interfering with pathogen metabolic functions thus arresting their growth. The secondary metabolites present in the fungal extracts possessed direct antimicrobial properties that inhibited the growth and spread of the two pathogens (Balkrishna et al., 2022; Salhi *et al.,* 2017). This finding substantiates the potential of these extracts in agricultural applications for disease management. According to Salhi *et al.* (2017) extracts from endophytic fungi of *Artemisia herba alba* have secondary metabolites that possess antifungal properties against pathogenic fungi of wheat. Similarly, Izah *et al*. (2018) also reported that fungal extracts have quinines, alkaloids, flavonoids and several other secondary metabolites with antimicrobial properties. Extracts from isolates DSTS2, DENS4, and DSTL2 showed particularly promising results in reducing disease incidence, highlighting their potential as biocontrol agents against *C. zeae maydis* and *F. verticilloides* infections. This could be

attributed to the several secondary metabolites produced by the extracts hence conferring protection to the maize plant against the pathogen infection. It should be clarified that e even if the *in vitro* inhibition might be lower against one pathogen, the complex mixture of compounds in the extracts might still be effective in reducing pathogen viability and infection rates *in vivo* (Anjum *et al.,* 2019). It is also known that secondary metabolites possess antimicrobial properties as revealed by the abilities to lower percent Disease Incidence in the maize pots for the greenhouse experiment. Such results were reported by Izah *et al.* (2018) and Rosa *et al.* (2012) that endophytic extracts of *Smallanthus sonchifolius* fungi exhibited antifungal activities against pathogens under greenhouse conditions.

In *Fusarium verticilloides* treated pots, all the maize pots were moderately susceptible to infections at the end of study period. *Fusarium verticilloides* is a highly virulent pathogen capable of adapting to various environmental conditions and overcoming plant defenses over time (Xu *et al.,* 2023). Its persistent nature could have led to moderate infections despite initial treatments. It also produces survival structures like chlamydospores, which can remain viable in soil and plant debris, leading to recurrent infections even after treatments.

Higher disease incidence recorded under *Fusarium verticilloides* could be attributed to production of the inhibitory metabolite which could have counteracted the antimicrobial potential of the endophytic fungal extracts against *Fusarium verticilloides*. These results concur with the findings according to Akram *et al.* (2023) that fungal extracts confer moderate resistance against pathogenic diseases hence the plants are moderately susceptible to pathogenic infections. These results did not agree with the reports by Seepe *et al.* (2020) that these fungal extracts have ability to control *Fusarium* pathogen infections against maize plants *in vivo* and that extracts showed strong antifungal activities against *Fusarium* pathogens.

5. Conclusion

This study has revealed the potential of endophytic fungi from medicinal plants in producing antimicrobial compounds effective against *Cercosporae zeae maydis* and *Fusarium verticilloides*. The variability in efficacy observed among different plant species and parts underscores the influence of biotic and abiotic factors on fungal colonization and secondary metabolite production. Despite the reduced *in vitro* inhibition against *Fusarium verticilloides*, in vivo experiments demonstrated that fungal extracts significantly reduced disease incidence in maize, suggesting their utility in sustainable agricultural disease management. The variability in efficacy among plant species and parts suggests a complex interplay of biotic and abiotic factors influencing fungal colonization and secondary metabolite production. These findings contribute valuable insights into sustainable strategies for fungal disease management in maize cultivation, paving the way for further research into harnessing natural plant-microbe interactions for agricultural benefit. Findings from this study underscore the importance of exploring the diversity and bioactivity of endophytic fungi residing in different plant species as potential reservoirs of novel antimicrobial agents.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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