Plant Extracts as Alternative Seed Treatments against *Fusarium oxysporum* Associated with Cowpea (*Vigna unguiculata* (L.) Walp.)

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Abstract

Fusarium wilt, caused by the pathogen *Fusarium oxysporum*, is one of the most destructive biotic stresses affecting cowpea production in sub-Saharan Africa. As synthetic fungicides have multiple limitations, including environmental concerns and inconsistent accessibility, there is a growing need to investigate biological alternatives. Plant-derived extracts from medicinal species represent a promising option, yet their efficacy against *F. oxysporum* remains insufficiently understood.

This study evaluated the antifungal activity of four plant extracts: *Ceratonia siliqua*, *Curtisia dentata*, *Lantana camara* and *Schkuhria pinnata*. Extracts were first assessed at varying concentration in *in vitro* experiments. Best performing extracts were subsequently applied as seed treatments to inoculated seeds and evaluated using an ISTA paper-roll germination test. Afterwards, the two most promising treatments were tested in a six-week pot trial.

In vitro, all extracts except *C. dentata* inhibited mycelial growth of *F. oxysporum*, with *L. camara* showing the strongest suppression on 7.5 mg mL^{-1}. However, high concentrations of *L. camara* (10.0 mg mL^{-1}) and *S. pinnata* (7.5 mg mL^{-1}) caused phytotoxicity and completely inhibited seed germination. Lower concentrations of *L. camara* (1.25 and 2.5 mg mL^{-1}) enhanced germination but did not prevent infection, which remained high across all treatments. In the pot trial, no significant treatment effects on disease severity or plant growth were observed among the water control, fungicide control (Celest® XL) and the tested *L. camara* treatments (1.25 and 2.5 mg mL^{-1}). This lack of differences was likely due to wet growing conditions that promoted infection and reduced treatment efficacy.

Overall, although *L. camara* demonstrated clear antifungal activity *in vitro*, its suitability as a cowpea seed treatment against *F. oxysporum* was limited by phytotoxicity and environmental growing conditions. Further optimalisation of extract concentration, application techniques and seed inoculation methods is necessary before plant extracts can be developed into practical alternatives for managing Fusarium wilt in cowpea.

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Abbreviations

Abbreviation	Description
ANOVA	One-way analysis of variance
DAS	Days after sowing
f. sp.	Forma specialis
GC-MS	Gas chromatography-mass spectrometry
IPM	Integrated Pest Management
ISTA	International Seed Testing Association
LSD	Least significant differences
MIC	Minimum inhibitory concentration
PDA	Potato Dextrose Agar
SD	Standard Deviation

1. Introduction

In sub-Saharan Africa, cowpea (*Vigna unguiculata* (L.) Walp.) is a major legume crop that provides both food and income for millions of smallholder farmers (Msuku et al., 2000). The crop is well adapted to the dry savannah and semi-arid regions, where other crops often fail due to drought and poor soil fertility (Williams, 1975). Cowpea seeds are rich in protein, vitamins and minerals, making them a valuable food and feed source. In addition to its nutritional value, cowpea improves soil fertility through symbiotic nitrogen fixation, contributing to soil restoration and sustainable mixed cropping systems (Elawad & Hall, 1987; Carsky et al., 2002).

Despite these advantages, cowpea production in Sub-Saharan Africa often remains limited due to low and unstable yields. This is mainly due to abiotic stresses and a wide range of fungal and bacterial pathogens (Mekonnen et al., 2022). Among the most serious diseases affecting cowpea is Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *tracheiphilum* which is both highly destructive and persistent (Omoigoi et al., 2018; Viljoen et al., 2025). The pathogen is both seed-borne and soil-borne, allowing it to persist in fields for several seasons and infect plants at different growth stages (Biemond et al., 2013). It attacks roots and invades the vascular tissues, leading to wilting, chlorosis and necrosis of leaves. In severe cases, infection results in leaf fall, plant death and significant yield losses (Viljoen et al., 2025).

In susceptible cultivars, symptoms can appear within 20-25 days after sowing (known as “early wilt”), while resistant varieties show delayed or no visible symptoms (Omoigui et al., 2018). Environmental conditions such as high soil moisture and warm temperatures can further enhance disease development (Houssou et al., 2009). The persistence and variability of *F. oxysporum* have led to the use of management practices based on resistant varieties and integrated disease control strategies (Panth et al., 2020).

Breeding for resistance is considered the most effective and sustainable method as it minimises the need for chemical control. However, *F. oxysporum* populations have been shown to overcome single-gene resistance, leading to short-term durability of the resistance (Omoigui et al., 2018). Furthermore, resistant cultivars are not always accessible or affordable for smallholder farmers due to weak seed delivery systems and high input costs. Consequently, genetic resistance alone remains an unstable solution for many regions in Africa (Mkomo, 2020).

Next to genetic resistance, there are seed-based management strategies that target the pathogen before seedling emergence (Gwary et al., 2007). Seed treatment is particularly effective since it can protect the plant during early growth by

preventing the fungus from colonising internal tissues (Khanzada et al., 2002). Conventional management strategies often rely on synthetic fungicides and chemical seed treatments. However, the use of synthetic fungicides can have serious environmental risks. Chemical residues can contaminate soil and water systems and may be toxic to humans (Sampaio et al., 2020). In addition, repeated fungicide application can lead to the development of resistant pathogen strains (Zubrod et al., 2019). The high costs and limited availability of fungicides also make them less practical for smallholder farmers (Peters et al., 2008).

Due to these limitations of synthetic fungicides, increasing attention is being directed towards natural alternatives such as plant extracts. Many medicinal plants contain secondary metabolites that exhibit strong antifungal properties, including phenolics, alkaloids, flavonoids and terpenoids (Osbourne, 1996). These bioactive compounds can suppress fungal growth or induce host plant resistance through antioxidant and defence signalling mechanisms (Thines et al., 2004). Plant extracts are generally biodegradable and have lower environmental and health risks compared to conventional fungicides (Ahmed et al., 2023).

Several studies have reported the inhibitory effects of plant extracts on *Fusarium* spp. and other plant pathogens (Rongai et al., 2015). For example, Seepe et al. (2020) demonstrated that several South African plant extracts, including *Melia azedarach*, *Combretum molle*, *Combretum erythrophyllum* and *Quercus acutissima*, were effective. These extracts were able to suppress *F. verticilliodes*, *F. solani* and *F. proliferatum* infections in maize (*Zea mays* (L.)). The *in vivo* greenhouse experiment showed that seed treatment with these extracts did not have harmful effects on germination or plant growth. Similarly, Sharma and Kumar (2008) reported strong antifungal activity of extracts from three common weed species against *F. oxysporum* under *in vitro* conditions.

These studies demonstrate that plant extracts have strong potential as natural antifungal agents and may represent safer alternatives to synthetic chemicals for managing *Fusarium* infections. However, most research has been limited to *in vitro* experiments or crops other than cowpea. Although the findings are promising, there remains limited research focusing specifically on *F. oxysporum* f. sp. *tracheiphilum* associated with cowpea. It is important to evaluate the practical application of plant extracts as seed treatments in *in vivo* experiments, including effects on germination, seedling development and disease incidence.

In this study, the antifungal activity of four plant extracts, namely *Lantana camara* (L.), *Schkuhria pinnata* (Lam.) Kuntze ex Thell, *Ceratonia siliqua* (L.) and *Curtisia dentata* (L.f.) against *F. oxysporum* was evaluated. The study aimed to (1) assess the inhibitory effects of each plant extract on the mycelial growth of *F. oxysporum in vitro*; (2) identify the most effective extract and evaluate its potential as a seed treatment for cowpea; and (3) evaluate the effect on disease incidence and growth parameters of cowpea through *in vitro* germination and open-air pot trials.

2. Literature review

2.1 Growth characteristics of cowpea

Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume crop that belongs to the Fabaceae family native from Africa (Steele, 1976) (Figure 1). It is an annual herbaceous plant with growth habits ranging from prostrate to semi-erect or climbing, depending on the cultivar. Plant height typically varies between 0.3 and 2.0 meters. Flowers vary in colour from white to purple, and the plant produces elongated pods containing multiple seeds, which transition from green to brown upon maturation (Boukar et al., 2018).

Figure 1: Mature cowpea plant (14 weeks) (own photo).

Cowpea grows well in warm climates and is well adapted to a wide range of soil types, including sandy or slightly acidic soils (Hall et al., 2003). Its deep root system and efficient water-use make it highly tolerant to drought. This allows cowpea to produce stable yields even under low-input farming conditions (Timko & Singh, 2008).

The crop has a relatively short growth cycle, ranging from 60 to 120 days depending on variety and environmental conditions. Flowering generally begins 30-50 days after sowing, and pod development and maturation occur shortly thereafter. This rapid and flexible growth cycle allows cowpea to fit into diverse cropping systems and to perform reliably in regions with irregular rainfall (Timko & Singh, 2008).

2.2 Importance of cowpea in sub-Saharan Africa

Cowpea is cultivated on about 14 million hectares worldwide, with over 80% of production occurring in Sub-Saharan Africa, mainly in Nigeria, Niger and Burkina Faso (Food and Agriculture Organisation STAT, n.d.). In South Africa, although production remains relatively small, it is still an important crop for smallholder farmers in provinces such as Limpopo, Mpumalanga and KwaZulu-Natal (Asiwe, 2009).

Cowpea plays an important role in the diets of many African countries. In eastern and southern Africa, young cowpea leaves are consumed as spinach, whereas in West African countries such as Senegal, Ghana and Nigeria, both the green immature pods and fresh seeds are eaten as vegetables. Most commonly, however, the dried grains are consumed as beans in most parts of Africa (Boukar et al., 2016; Kim et al., 2025).

Cowpea is also used for medicinal purposes. The leaves can be applied to burns and used as a snuff to relieve headaches (Hutchings et al., 1996). Infusions made from cowpea seeds are traditionally used to treat menstrual pain, epilepsy, and chest discomfort (Van Wyk & Gericke, 2000).

Cowpea is a rich source of high-quality protein (approximately 25% crude protein) and provides essential nutrients such as iron, zinc, folate and vitamins A and C (Muñoz-Amatriaín et al., 2016; Owade et al., 2020). Cowpea is considered a key crop for climate change adaptation and smallholder resilience because of its short growing cycle, low input requirements and stable performance under variable rainfall (Timko & Singh, 2008).

Through its ability to symbiotically fix nitrogen, cowpea improves soil fertility and is often used in crop rotation systems (Ravelombola et al., 2017). It can fix up to 240 kg nitrogen (N) per hectare per season, reducing the need for synthetic N fertilisers and improving the yields of subsequent cereal crops (Belane & Dakora, 2009). Its high tolerance to drought and heat makes it one of the most resilient legumes cultivated globally (Singh, 2014). It can adapt well to harsh environmental conditions and is a well-suited crop for arid and semi-arid regions such as those in sub-Saharan Africa (Omomowo & Babalola, 2021). It enhances the nutritional as well as the ecological sustainability of farming systems. Therefore, cowpea plays an important role in traditional and mixed smallholder farming system, supporting both household food security and rural income.

2.3 Limitations in cowpea production

Cowpea production in sub-Saharan Africa is expected to rise from approximately 9.8 million tons in 2020 to almost 12.3 million tons by 2030 (Boukar et al., 2016). Despite its major contribution to food and nutritional security across the region, cowpea production is limited by several abiotic and biotic factors (Omomowo & Babalola, 2021). These stresses can lead to yield losses exceeding 50% under field conditions (Boukar et al., 2016; Kim et al., 2025).

2.3.1 Abiotic stresses

Cowpea productivity is highly influenced by environmental factors such as drought, flooding, heat, soil salinity and nutrient deficiencies (Singh, 2014; Djanaguiraman et al., 2017). Among these, drought is the most critical constraint due to its frequent occurrence in arid and semi-arid regions. Water stress during germination reduces seedling establishment and overall biomass production (Muñoz-Amatriaín et al., 2016). High temperature stress can impair pollen viability and reduce pod formation, leading to yield reductions of up to 40% (Barros et al., 2021). Soil salinity can further limit cowpea production by restricting water uptake and disrupting osmotic balance, which results in stunted growth and poor yields (Omomowo & Babalola, 2021).

2.3.2 Biotic stresses

Cowpea is highly susceptible to various insect pests, nematodes, parasitic weeds and pathogens (Singh, 2014). Among the insect pests, aphids, thrips and pod borers are the most destructive, as they damage flowers and pods, cause lower seed quality and serve as vectors for viral diseases (Boukar et al., 2016). Root-knot nematodes cause gall formation on roots and thereby reduce nodulation and nutrient uptake efficiency (Omomowo & Babalola, 2021). These nematodes are particularly prevalent in sandy soils, where they can cause significant but often unnoticed yield losses (Harouna, 2020).

Cowpea is also affected by several destructive fungal, bacterial and viral diseases (Mekonnen et al., 2022). Common fungal pathogens such as *Fusarium* spp., *Aspergillus* spp. and *Rhizoctonia solani* cause root rot, damping-off and seed decay (Viljoen et al., 2025). Some fungi like *A. flavus*, *A. ochraceus* and *A. niger* also contribute to post-harvest losses and mycotoxin contamination in stored grains (Ashiq et al., 2014). Mycotoxins are secondary metabolites that negatively affect livestock production and cause serious risks to human health (Fleurat-Lessard, 2017).

Among the cowpea pathogens, *F. oxysporum*, the causal agent of Fusarium wilt, is one of the most destructive seed-borne pathogens to cowpea worldwide (Omoigui et al., 2018). *Fusarium oxysporum* has been isolated from cowpea seeds in several sub-Saharan African countries, including Botswana and Nigeria (Khare et al., 2016). Viljoen et al. (2025) found a high incidence of *F. oxysporum* on cowpea seeds collected from major production areas in South Africa.

2.4 Fusarium Wilt and management strategies

2.4.1 The genus *Fusarium*

The genus *Fusarium* includes some of the most important plant-pathogenic fungi affecting global agriculture (Dean et al., 2012). Members of this genus cause wilt, rot and blight diseases on economically important crops such as wheat (*Triticum aestivum* (L.)), tomato (*Solanum lycopersicum* (L.)), cotton (*Gossypium hirsutum* (L.)), banana (*Musa* × *paradisiaca* (L.)) and various legumes (Ploetz, 2015; Summerell, 2019). Many *Fusarium* species also produce mycotoxins including trichothecenes fumonisins and zearalenone (Desjardins, 2006; Cortinovis et al., 2013).

Fusarium oxysporum is one of the most significant plant pathogens worldwide (Dean et al., 2012). It exists as a species complex with over 120 formae specialis (f. sp.), each adapted to specific hosts (Gordon, 2017). The fungus causes vascular wilt and stem rot in many crops such as tomato (f. sp. *lycopersici*), banana (f. sp. *cubense*), chickpea (f. sp. *ciceris*) and cowpea (f. sp. *tracheiphilum*) (Armstrong & Armstrong, 1981; Biemond et al., 2013). It can survive for years in the soil or infected plant remains as chlamydospores, enabling persistence across cropping cycles.

2.4.2 *Fusarium oxysporum* in cowpea

Infection of *F. oxysporum* can occur at nearly all growth stages through the seeds, the roots or the hypocotyl (Armstrong & Armstrong, 1981; Omoigui et al., 2018). The infection process follows a similar pattern in many susceptible hosts, including cowpea. The pathogen invades the xylem vessels, blocking water and causing wilting, yellowing and stunted growth (Figure 2).

The pathogen penetrates the roots, colonises the xylem, produces conidia that spread systemically, and ultimately causes wilting, yellowing, defoliation, and plant death (Jackson et al., 2024). After plant collapse, conidia and mycelia are returned to the soil, where chlamydospores enable long-term survival. Severe infections can lead to leaf fall and total plant death before pod formation, resulting in significant yield losses (Beckman, 1987).

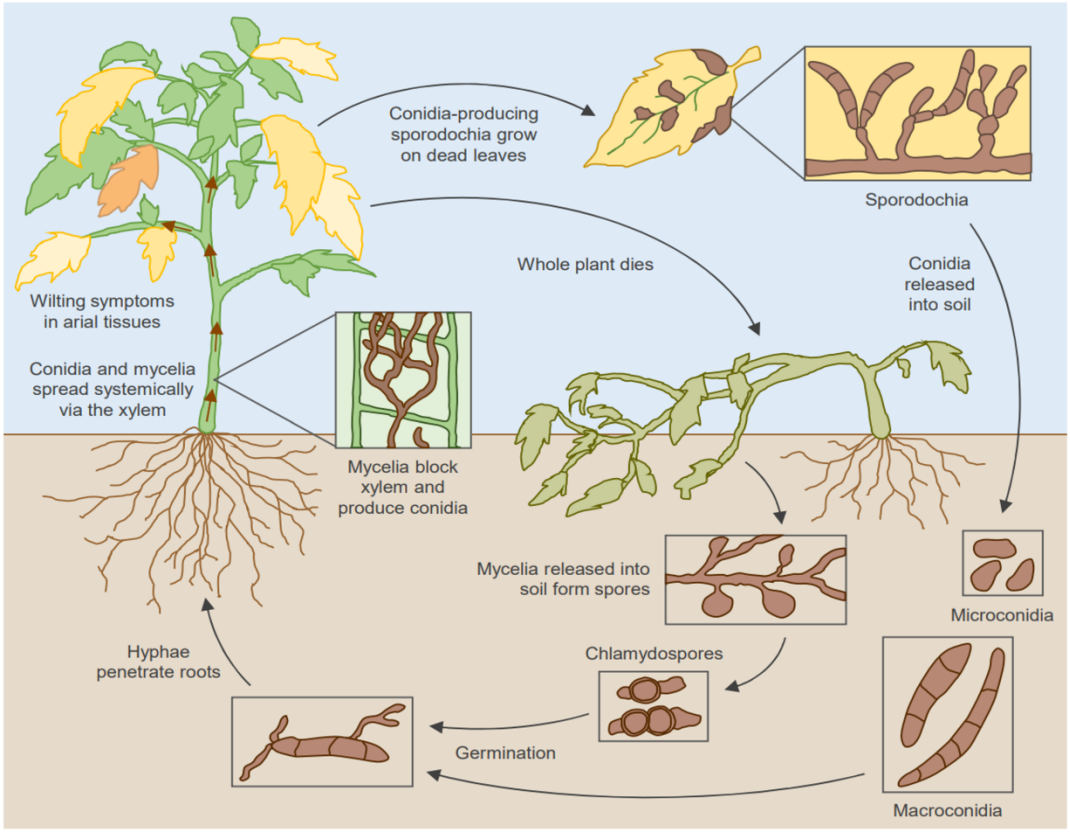

Figure 2: Schematic representation of the disease cycle of F. oxysporum infecting tomato (Jackson et al., 2024).

2.4.3 Management strategies for *Fusarium* Wilt

Managing Fusarium wilt is difficult as *F. oxysporum* is both seed- and soil-borne and genetically diverse. Therefore, it is capable of surviving for long periods of time. Effective management requires an Integrated Pest Management (IPM) approach that combines genetic, cultural, chemical and biological strategies (Jiménez-Díaz et al., 2015; Panth et al., 2020).

Breeding for resistance remains the most effective and environmentally friendly method for controlling Fusarium wilt. Several cowpea genotypes resistant have been identified through breeding programs in Africa (Boukar et al., 2016; Ibrahim, 2023). However, resistant seeds are often expensive and inaccessible, with most rural smallholder farmers still relying on the use of recycled seeds from landraces (Gyasi et al., 2022). Moreover, breeding programs are often time-consuming and new *F. oxysporum* races can overcome single-gene resistance (Kim et al., 2025).

Chemical fungicides, applied as seed treatments or foliar sprays, can inhibit mycelial growth of *F. oxysporum*. For example, fungicides such as prochloraz and bromuconazole have been shown to be effective foliar treatments against *F.*

oxysporum on tomato plants (Amini & Sidovich, 2010). Most chemical fungicides can suppress initial infection but were found to be ineffective once the pathogen colonised plant tissues. Aditionally, excessive use of synthetic fungicides causes environmental and health risks, including soil and water contamination and toxic residue accumulation in food products (Sampaio et al., 2020).

In addition to resistance breeding and chemical control, cultural farming practices such as improving soil drainage, maintaining high organic matter content and practicing crop rotation or intercropping can help suppress disease outbreaks (Panth et al., 2020; Yan & Nelson, 2022). However, these methods alone are insufficient.

Given the limitations of conventional management strategies, there is growing interest in biological control options for managing *Fusarium* species (Gyasi et al., 2022). Several biocontrol strategies have shown potential against Fusarium wilt in crops such as cowpea. For example, beneficial microbes in the rhizosphere (e.g., *Bacillus subtilis* and *Pseudomonas* spp.) inhibit fungal growth and spore germination (Zhu et al., 2025). These microbes can be applied as seed coating before planting or inoculated directly into the soil.

Another promising approach is the use of plant extracts rich in bioactive compounds to inhibit *Fusarium* growth. Plant extracts have demonstrated antifungal properties against a range of seed- and soil-borne pathogens and can therefore function as eco-friendly alternatives to synthetic pesticides within agriculture (Osbourne, 1996; Jamiolkowska, 2020).

2.5 Plant extracts as alternative biocontrol

Plant extracts represent a sustainable alternative to synthetic fungicides for controlling Fusarium wilt in cowpea. Plant extracts are a type of botanical that are more biodegradable than chemical fungicides. The rapid breakdown means they are considered to be more eco-friendly (Rice, 1983; Guleria & Tiku, 2009). Plant extracts are often active against a limited number of specific target pests and therefore have a reduced risk to kill beneficial pests (Kim et al., 2003).

Historically used for medicinal purposes, many plant extracts have proven antibacterial and antifungal properties (Cowan, 1999; Martins et al., 2015).

2.5.1 Mode of action of secondary metabolites

The antimicrobial effects from plant extracts derive from the plant secondary metabolites, such as phenolics, alkaloids, terpenoids, saponins, tannins, flavonoids or essential oils. Secondary metabolites are bioactive compounds and play an important role in plant defence mechanisms (Osbourne, 1996).

Pathogenic fungi are especially vulnerable during the early infection stages between spore germination and host penetration (Thines et al., 2004). Many plant-derived metabolites act as antifungal agents by preventing fungal entry into host tissues. Different metabolites act through different mechanisms, with some disrupting fungal cell membranes, others inhibiting spore germination or triggering plant defense responses (Sales et al., 2016).

The terpenoids thymol and carvacrol, derived from thyme (*Thymus vulgaris* (L.)) and oregano (*Origanum vulgare* (L.)), can degrade fungal cell walls (Cowan, 1999). Dalleau et al. (2008) reported that these compounds inhibited biofilm development of *Candida* spp. Similarly, Salari et al. (2016) found that the phenolic eugenol derived from cloves (*Syzygium aromaticum* (L.) Merril & L.M. Perry) damaged fungal membranes, causing cytoplasmic leakage.

Flavonoids have also been reported to actively inhibit spore germination of *F. oxysporum*, *Botrytis cinerea* and *Alternaria alternata* (Ma et al., 2022). Microscopic analysis revealed wrinkled and lysed conidia, indicating direct structural damage. The composition and concentration of metabolites will determine how effective a particular extract is against specific fungal species (Sales et al., 2016).

2.5.2 Phytotoxicity

Plant extracts may have antifungal properties, while some can also exhibit phytotoxic effects on crops. Plants can display so called ‘allelopathy’ through secondary metabolites such as phenolic substances, fatty acids, terpenes and alkaloids (Yoneyama & Natsume, 2013). When these secondary metabolites act allelopathically, they are referred to as allelochemicals. These compounds can be secreted by a plant and can affect the development, germination, survival or reproduction of neighbouring plants. For this reason, secondary metabolites not only have antifungal potential but can also be applied as botanical herbicides (Puig et al., 2021).

For example, Lam-Gutiérrez et al. (2025) investigated the antifungal activity of *Plantago major* leaves against several phytofungi as *F. oxysporum* and

Corynespora cassiicola. The bioextract inhibited both fungi, while inhibition of seed germination was observed at higher doses.

The degree of phytotoxicity depends on the plant species used for the extract, the target crop, the extraction method and the concentration applied (Yoneyama & Natsume, 2013). It is therefore important to carefully test plant extracts to determine whether they may have phytotoxic effects on the host plant. Extract concentration should be optimised to maximise antifungal activity while minimising the potential phytotoxic effects (El-Kenany & El-Darier, 2013).

2.6 Plant extracts against *Fusarium* species

Several studies have demonstrated the antifungal activity of plant extracts against *Fusarium* species. Seepe et al. (2020) investigated the antifungal properties of several indigenous South African plants and found that combined leaf extracts from *Quercus acutissima* and *Combretum erythrophyllum* inhibited the growth of *F. verticillioides*, *F. solani*, and *F. proliferatum*, identified from maize seeds. These results were obtained through *in vitro* assays, where the extracts were incorporated into growth media to test their inhibitory effects on mycelial growth. An *in vivo* greenhouse experiment showed that a seed treatment with these extracts did not have harmful effects on the germination or plant growth of the maize seedlings.

Similarly, Sharma and Kumar (2008) examined extracts from three common weed species (*Lantana camara*, *Tridax procumbens*, and *Capparis decidua*) for their antifungal potential against *F. oxysporum* under *in vitro* conditions. The extracts significantly inhibited spore germination, indicating strong antifungal activity. Further phytochemical analysis indicated that flavonoids and alkaloids in the tested plants were responsible for the inhibitory effects on *F. oxysporum*.

Gyasi et al. (2022) evaluated the use of botanical extracts for managing seed-borne fungi of cowpea, including *F. verticillioides*. Their experiments used aqueous extracts of grains of paradise (*Aframomum melegueta*), which showed up to 98% inhibition of fungal growth under laboratory conditions.

These studies suggest that plant extracts have strong potential as a natural seed treatment against *Fusarium* infections in cowpea. Despite promising outcomes, there remains limited research focusing on the *in vivo* effects of seed treatments with plant extracts. Most studies focus on *in vitro* testing of the antifungal activity of selected plant extracts, while germination and pot trials are important to test real life effects on seeds and crop performance. Given the high genetic and pathogenic variability among *Fusarium* species and formae speciales, the effectiveness of plant extracts can vary depending on the specific pathogen strain and host plant.

Therefore, the present study focused on evaluating selected plant extracts for their antifungal activity against *F. oxysporum* infecting cowpea.

2.7 Selected plant extracts for this study

The plant species used in this study were selected based on their reported antimicrobial properties in the literature and their availability in the University of Pretoria's research collections. Each of the selected species has demonstrated potential bioactivity that may be useful for managing *Fusarium* infections. The following subsections summarise their background, known bioactive compounds and reported antifungal properties.

2.7.1 Curtisia dentata

Curtisia dentata (family Cornaceae), commonly known as the Assegai tree, is native to Southern Africa and valued both as a medicinal and ornamental species (Lemmens & Louppe, 2008) (Figure 3). The bark and leaves are traditionally used by various cultures as "blood purifiers" and for treating sexually transmitted infections, cancer, diabetes and stomach ailments (Shai et al., 2008; Manning & Goldblatt, 2012).

Figure 3: Curtisia dentata (Trees-SA, n.d.).

Extracts from *C. dentata* have shown antifungal activity against human pathogenic fungi such as *Aspergillus fumigatus, Candida albicans* and *Candida spicata* (Shai et al., 2008). The compounds ursolic acid, lupeol and betulinic acid were identified as the most active constituents. However, no studies to date have tested *C. dentata* against plant pathogenic fungi, leaving its potential role in phytopathogen control largely unexplored. This research, therefore, aims to investigate its possible antifungal effects on *F. oxysporum* infecting cowpea.

2.7.2 Ceratonia siliqua

Figure 4: Ceratonia siliqua (Sabambu, 2025).

Ceratonia siliqua, commonly known as the carob tree, is a leguminous species in the Fabaceae family (Figure 4). Native to the Middle East, *C. siliqua* has spread throughout the Mediterranean, Western America, and parts of South Africa, where it grows well in limestone-rich and subtropical areas (Dahmani et al., 2023).

Traditionally, *C. siliqua* has been used to treat diarrhea, diabetes, and other ailments (Ali-Shtayeh et al., 2013). The plant contains high levels of phenolics, flavonoids, and tannins, which exhibit antioxidant and antimicrobial properties (Zahra et al., 2021). Najem and Alhamdani (2025) reported strong antifungal activity of *C. siliqua* extracts against several *Candida* species. Secondary metabolites such as methyl esters and siloxanes may contribute to its antimicrobial function. Although *C. siliqua* has not yet been evaluated against *Fusarium* species, its broad-spectrum antifungal activity suggests potential for further testing in crop protection.

2.7.3 Lantana camara

Figure 5: Lantana camara (Bromilow, C. 2023).

Lantana camara (family Verbenaceae) is a flowering shrub commonly known as wild sage, Spanish flag or tea plant (Figure 5). Originally introduced as an ornamental species, it has become one of the ten most invasive weeds globally (Lonare et al., 2012). In South Africa, *L. camara* is widespread, especially along riverbanks, where it reduces native plant diversity (Agricultural Research Council – Plant Protection Research Institute, n.d.).

Despite its invasive nature, *L. camara* is widely used in traditional medicine for treating fever, swelling, cancer and malaria (Kalyani et al., 2011). Research has shown that *L. camara* extracts exhibit strong antimicrobial and antibacterial properties, suggesting potential applications in agriculture. For example, Seepe et al. (2020) isolated bioactive compounds from *L. camara* and tested their antifungal effects against several *Fusarium* species. The compounds lantadene A and boswellic acid exhibited strong inhibitory activity, with a minimum inhibitory concentration (MIC) below 0.65 mg/mL, against *F. subglutinans*, *F. semitectum*, *F. proliferatum*, *F. solani* and *F. graminearum*. Although *F. oxysporum* was not

directly tested, the results indicate that *L. camara* extract could serve as an effective biological seed treatment to prevent Fusarium wilt in cowpea.

2.7.4 Schkuria pinnata

Figure 6: Schkuria pinnata (EarthOne, n.d.)

Schkuhria pinnata (family Asteraceae) is an annual herb commonly known as dwarf Mexican marigold or klein kakiebos (Taylor, 2006) (Figure 6). It is an introduced species in South Africa, often found along roadsides and in cultivated areas. Traditionally, extracts of *S. pinnata* have been used in African medicine to treat stomach pain, diarrhea, and malaria (Watt & Breyer-Brandwijk, 1962).

Several studies have reported antibacterial properties of *S. pinnata* extracts. Mupfure et al. (2014) demonstrated its effectiveness against *Staphylococcus aureus, Streptococcus agalactiae* and *Escherichia coli*. Although its antifungal activity against plant pathogens has not been widely studied, *S. pinnata* has shown inhibitory effects on *Candida* species, suggesting the presence of bioactive compounds with potential antifungal activity (Masevhe, 2013). These properties need further investigation into their role as a biocontrol agent against phytopathogens such as *F. oxysporum*.

2.8 Literature summary & research gaps

In summary, the literature highlights cowpea as a vital crop for food security and resilience in Sub-Saharan Africa, yet its production is limited by both abiotic and biotic stresses. Among the biotic stresses, *F. oxysporum* f. sp. *tracheiphilum*, the causal agent of Fusarium wilt, is one of the most destructive diseases affecting cowpea.

Although IPM strategies by combining host resistance, cultural practices and chemical fungicides are commonly used, each has its limitations. This has led to increasing interest in plant-based alternatives. Plant extracts offer a promising, environmentally friendly alternative due to their rich content of secondary metabolites that have shown strong antifungal potential.

However, research on *F. oxysporum* infecting cowpea remains limited and many South African medicinal plants with potential antifungal activity have not been investigated within agricultural practices. Only a few studies link *in vitro* antifungal findings to greenhouse or field applications.

Therefore, the aim of this study was to address these gaps by evaluating the antifungal activity of selected plant extracts against *F. oxysporum* through three sequential experiments. The extracts were evaluated *in vitro* at three different concentrations. The most effective extracts and concentrations from the *in vitro* screening were tested as seed treatments in a seed germination trial. The best-performing extract and concentration were eventually applied in a six-week pot trial, contributing to the search for sustainable biocontrol options for cowpea production.

2.9 Hypotheses

It was hypothesised that most of the selected plant extracts would exhibit antifungal activity against *F. oxysporum* under *in vitro* conditions. The degree of inhibition was expected to differ among the extracts and concentrations used, as extracts rich in bioactive secondary metabolites would show stronger antifungal effects.

It was further hypothesised that extracts demonstrating strong *in vitro* antifungal activity may not directly translate to improved cowpea seed germination or early seedling development under pathogen pressure. Potential phytotoxicity of secondary metabolites or other plant-extract interactions may influence the outcomes.

Finally, application of the most effective plant extract and concentration identified in the seed germination test might reduce Fusarium wilt severity and improve seedling growth in the open-air pot trial. Given the limited research in this area, the extent of these effects was expected to be small or uncertain.

3. Materials and Methods

3.1 Cowpea variety

The cowpea seeds used in this study were the brown variety, PAN 311, obtained from the Department of Agriculture in Mbombela, Mpumalanga, South Africa.

3.2 Crude plant extracts

The antifungal activity of four plant leaf extracts was evaluated: *C. dentata* (methanol extract), *C. siliqua* (ethanol extract), *L. camara* (acetone extract) and *S. pinnata* (chloroform extract). The first three 100% crude plant extracts were provided from the extract library of Professor Namrita Lall, Department of Plant and Soil Sciences, University of Pretoria, South Africa, where they had been previously used for research purposes and stored at –20°C.

The *L. camara* extract was freshly prepared for this study. Dried aerial plant material (500 g) was finely chopped and soaked in 2 L of acetone (1:4 w/v). Acetone was selected as the extraction solvent due to its intermediate polarity, allowing efficient extraction of both polar and nonpolar plant compounds, and its ease of removal after extraction (Kumar et al., 2023). The mixture was placed on a laboratory shaker for 72 hours and then vacuum filtered through Whatman No. 1 filter paper to remove plant residues. The solvent was removed using a rotary evaporator and further dried in a fume hood to obtain a 100% crude plant extract. The dried extract was stored at 4°C until use.

Stock solutions were prepared by dissolving the crude plant extracts in acetone to a final concentration of 150 mg mL^{-1} (150:1 w/v), as all extracts fully dissolved at this ratio. These stock solutions were used for all experiments and were further diluted with sterile distilled water as required.

3.3 Fungal strain and pathogenicity trial

The fungus *F. oxysporum* was previously isolated from cowpea seeds obtained from an informal market in Pretoria, South Africa. Identification of the isolate was confirmed by Sanger sequencing and phylogenetic analysis. The isolate was sub-cultured on full-strength Potato Dextrose Agar (PDA), incubated at 25°C for seven days and stored at 4°C until further use.

A fungal spore suspension was prepared by gently removing mycelia from seven-days-old *F. oxysporum* cultures using a sterile forceps and transferring them into sterile distilled water containing 0.1% Tween 80, followed by thorough mixing.

Spore concentration was determined using a hemacytometer and adjusted to 2×10^5 spores mL^{-1}.

To confirm that the isolated strain was pathogenic to cowpea, a preliminary pathogenicity test was conducted. Cowpea seeds were surface sterilised in 1% sodium hypochlorite (NaOCl) for 5 minutes and rinsed three times with sterile distilled water. The seeds were then artificially inoculated with *F. oxysporum* by soaking them in a spore suspension (2×10^5 spores mL^{-1}) and incubating in closed flasks at 25°C in the dark. After 4 hours, the spore suspension was poured out, and the flasks were kept closed to maintain a humid environment and incubated for an additional 16 hours at 25°C.

Seeds were sown in potting soil and grown under open-air conditions with shade netting and were watered every two days. Typical signs of Fusarium wilt such as reduced seed germination, leaf chlorosis and vascular browning were observed within four weeks after sowing. This confirmed that the isolate was virulent on cowpea and suitable to use for the experiments.

3.4 *In vitro* antifungal assays

3.4.1 Disk diffusion assay

A disk diffusion assay was conducted to evaluate whether this method was suitable for testing the antifungal activity of the plant extracts against *F. oxysporum*. Full strength PDA was poured into 90-mm Petri dishes (15 mL per plate) and allowed to solidify. Each plate was inoculated by spreading 20 µL of *F. oxysporum* spore suspension across the agar surface with an L-shaped cell spreader. Two spore concentrations were tested: 6×10^4 spores mL^{-1} (1,200 spores per plate) and 5×10^5 spores mL^{-1} (10,000 spores per plate). Spore suspensions were prepared as described under section 3.3.

Stock solution of *L. camara* (150 mg mL^{-1}) was diluted with sterile distilled water to obtain concentrations of 10 mg mL^{-1} and 25 mg mL^{-1}. Sterile 5-mm filter paper disks were impregnated with 15 µL of extract at the respective concentrations; 0.15 mg per disk for 10 mg mL^{-1}, 0.375 mg per disk for 25 mg mL^{-1}. After drying for 2 minutes in a laminar flow cabinet, four disks of the same extract concentration were placed onto each inoculated plate. For each combination of spore concentration and extract concentration, two replicate plates were prepared, resulting in a total of eight plates. Additionally, four plates were included as a fungicide control using Celest® XL (9 µg fludioxonil mL^{-1}, 3.6 µg mefenoxam mL^{-1}), with 20 µL applied per disk, resulting in 0.18 µg fludioxonil and 0.072 µg mefenoxam per disk.

All plates were incubated at 25°C for five days and observed daily for signs of fungal inhibition.

3.4.2 Poisoned food assay

The poisoned food technique described by Kritzinger et al. (2005) was followed with slight modifications. PDA media were prepared according to the manufacturer's instructions. Stock solutions of the leaf extracts (150 mg mL^{-1}) were diluted with sterile distilled water and added to prepared PDA media at approximately 50°C to obtain concentrations of 2.5, 5.0 and 7.5 mg mL^{-1}.

The fungicide Celest® XL (25 g L^{-1} fludioxonil, 10 g L^{-1} mefenoxam) served as positive control (9 µg fludioxonil mL^{-1}, 3.6 µg mefenoxam mL^{-1}), while PDA with acetone (0.05 mL mL^{-1} (5% v/v)) acted as solvent control and unamended PDA as negative control. A solvent control was added to exclude that acetone influenced *F. oxysporum* growth.

Fifty-millimetre Petri dishes were prepared with 4.5 mL of amended PDA per plate (four replicates per treatment). A seven-millimetre diameter mycelia plug of seven-days-old *F. oxysporum* cultures was placed in the centre of each petri dish, sealed with parafilm and incubated for five days at 25°C in the dark (Figure 7).

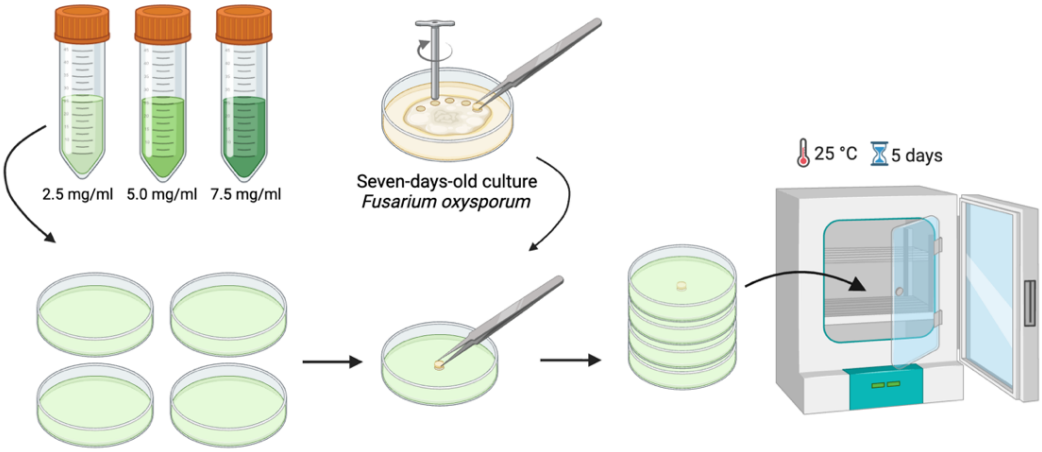

Figure 7. Schematic representation of the poisoned food technique. Created with BioRender.

The mycelial growth was measured on the Petri dishes at all four radial axes on day 5 of incubation. The percentage of inhibition of mycelial growth was calculated with the formula: % inhibition = (dc – dt)/dc x 100

Whereby dc = average radial growth of the unamended PDA
dt = average radial growth of the amended PDA (extract, fungicide, solvent)

The total area of the mycelial growth on each Petri dish was determined by photo analysis with the software ImageJ. The average area of each treatment was compared to the area of the control plates.

3.5 Detached leaf assay

A detached leaf assay was tested to evaluate whether this method could be used to assess the antifungal activity of the plant extracts as foliar spray. Fresh leaves were collected from four-week-old cowpea plants using scissors disinfected with 70% ethanol. The leaves were rinsed with sterile distilled water and dried in a laminar flow cabinet, then placed in two plastic boxes (250 × 250 mm) prepared with five layers of moistened paper towel.

Each leaf was inoculated with *F. oxysporum* by creating two small wounds on either side of the midrib using a sterile pipette tip. A 20 µL droplet of spore suspension (5 x 10^5 spores mL^{-1}) was placed onto each wound (Figure 8). Each box contained eight inoculated leaves. The boxes were sealed with plastic tape to maintain high humidity and incubated at room temperature under natural daylight conditions (21°C ± 5°C; 12/12h day/night).

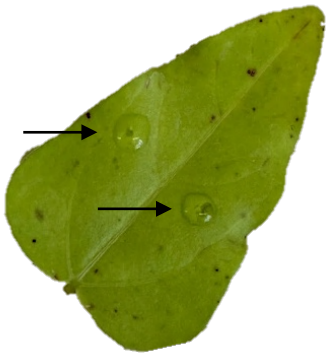

Figure 8: Representative picture showing a cowpea leaf inoculated with F. oxysporum on both sides of the midrib.

The inoculated leaves were observed daily for infection development and lesion formation around the wounds.

3.6 Seed germination test

Seed germination was evaluated following a modified method based on the International Seed Testing Association (ISTA) rules using rolled germination paper (ISTA, 2025). This method also involved monitoring of both seedling growth and infection levels caused by *F. oxysporum*. Plant extracts for seed treatment were selected based on the poisoned food assay: *L. camara* on 1.25, 2.5, 5.0, 7.5 and 10 mg mL^{-1}; *S. pinnata* on 7.5 mg mL^{-1}; and *C. siliqua* on 7.5 mg mL^{-1}. Sterile distilled water and the fungicide Celest® XL (9 µg fludioxonil mL^{-1}, 3.6 µg mefenoxam mL^{-1}) were used as negative and positive controls, respectively.

Seed surface sterilisation and inoculation with *F. oxysporum* were conducted as described in section 3.3. Following inoculation, seeds were air-dried in a laminar flow cabinet before being soaked for 24 hours in the respective leaf extract solutions and controls. After 24 hours, the seeds were air-dried in a laminar flow cabinet.

For each treatment, four replicates of 25 seeds per roll (n = 100) were prepared. Three sheets of germination paper (53 × 25 cm) were moistened with sterile distilled water and stacked. Seeds were evenly arranged in a single horizontal line along the centre of the sheets and covered with a fourth moistened sheet. The sheets were rolled and sets of four rolls were placed inside plastic bags secured with elastic bands to maintain moisture. The rolls were positioned vertically and incubated in the dark at 21°C ± 2°C for nine days.

Seed germination, infection, and seedling development were recorded on day 5 and day 9. Measurements included the numbers of germinated, non-germinated, infected, normal, and abnormal seedlings. Shoot and root lengths of all normal seedlings were measured on day 9. Treatment effects and selection of the best performing plant extracts were based on germination percentage and infection rate.

An additional germination test was conducted to evaluate the effect of lower pathogen pressure. For this, seeds were inoculated with reduced spore concentrations of 2×10^4 and 1×10^5 spores mL^{-1}. The assay was performed under the same conditions as the main germination test. Seeds were treated with *L. camara* on 1.25 and 2.5 mg mL^{-1}, with sterile distilled water and Celest® XL (9 µg fludioxonil mL^{-1}, 3.6 µg mefenoxam mL^{-1}) included as controls.

3.7 Pot trial

The two best-performing plant extracts and concentrations identified from the poisoned food test and seed germination test were used as seed treatment in a six-week pot trial. *Lantana camara* at 1.25 mg mL^{-1} and 2.5 mg mL^{-1} were selected as the most effective treatments.

Seed surface sterilisation and inoculation with *F. oxysporum* were conducted as described in section 3.3. For each treatment, eight pots (20 cm diameter) were used, each containing five seeds sown in steam-sterilised soil (Gromor Potting Medium) to eliminate soil-borne pathogens. Non-inoculated seeds treated with sterile distilled water and the fungicide Celest® XL served as negative controls, while inoculated seeds treated with sterile distilled water and Celest® XL served as positive controls.

The pots were arranged in a completely randomised block design under open-air conditions with shade netting and were watered every two days. During the third week of growth, a minor infestation of aphids and cutworms was observed. To manage these pests, the plants were treated with BioNeem at 17 days after sowing (DAS) and Cypermethrin at 20 DAS, which effectively prevented further damage.

The germination rate was recorded at 7- and 9-day DAS. From 14 DAS onwards, the average chlorophyll content per pot was weekly assessed. Chlorophyll content was measured using a SPAD meter (Apogee MC-100), which provided the relative chlorophyll content of the leaflets as SPAD values (on a scale of -9.9 to 199.9). For each plant, four SPAD readings were taken, with approximately two plants measured per pot, resulting in a total of 64 chlorophyll measurements per treatment.

At 36 DAS, all plants were harvested, and the following parameters were determined: shoot length, root length, number of leaves, number of infected leaves, number of fallen leaves, total leaf area per plant (measured using the application 'Easy Leaf Area'), fresh and dry shoot and root mass. Additionally, the overall disease severity was determined on a scale from 1 to 5:

1) No visible infection;
2) Mild infection with small necrotic spots on one or two (typically older) leaves;
3) Moderate infection with necrotic spots on three or more leaves;
4) Severe infection with extensive necrosis on three or more leaves and/or loss of one older leaf;
5) Very severe infection with extensive necrosis and loss of two leaves.

3.8 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 30.0. Prior to analysis, data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Treatment effects were evaluated using one-way analysis of variance (ANOVA), and mean separations were determined with Fisher's Least Significant Difference (LSD) test ($p < 0.05$).

For the poisoned food assay, differences in mean colony diameter and colony area among treatments were analysed using the Single-Factor One-Way ANOVA ($p < 0.05$). Percentage inhibition of fungal growth (calculated as described in Section 3.5.1) was also compared among treatments. Extracts exhibiting more than 30% inhibition of fungal growth were selected for subsequent seed germination testing.

In the seed germination assay, treatment effects on germination percentage, infection rate, number of abnormal seedlings, and mean shoot and root length (day 9) were analysed using ANOVA ($p < 0.05$) followed by the LSD post-hoc test. Treatments were selected for the pot trial based on germination performance.

For the pot trial, differences in chlorophyll content among treatments over the growth period were analysed using a repeated measures general linear model with univariate analysis. Disease severity, scored on a 1 to 5 scale, was compared among treatments using the Kruskal-Wallis test. At harvest, final vegetative growth parameters (shoot length, root length, leaf area, number of (fallen/infected) leaves, fresh and dry biomass) were compared among treatments and the control using ANOVA and the LSD post-hoc test ($p < 0.05$).

In the Results, data are presented in graphs and tables. Significant differences among the treatments are shown using Compact Letter Display (CLD). Treatments sharing at least one common letter are not significantly different, whereas treatments with no shared letters differ significantly.

4. Results

The results are presented in the order in which the experiments were conducted. First, the antifungal activity of the plant extracts was assessed *in vitro* to identify the most promising extracts that inhibit *F. oxysporum* growth. These extracts were then evaluated for their effects on seed germination, infection levels and early seedling development. Finally, the selected treatments were tested on their performance under more realistic growing conditions in a pot trial.

4.1 *In vitro* antifungal assays

4.1.1 Disk diffusion assay

The disk diffusion method using *L. camara* extract against *F. oxysporum* showed no observable inhibition zones around the disks at either of the tested extract concentrations (10 mg mL^{-1} and 25 mg mL^{-1}) or spore concentrations (6×10^4 spores mL^{-1} and 5×10^5 spores mL^{-1}) (Appendix 1, Figure S1 and Figure S2).

4.1.2 Poisoned food assay

All concentrations of *L. camara* and *S. pinnata* extracts resulted in significantly smaller colony diameters and areas compared with the unamended PDA control ($p < 0.001$) (Appendix 2, Table S1). *Ceratonia siliqua* significantly reduced colony growth at 5.0 ($p < 0.001$) and 7.5 mg mL^{-1} ($p < 0.001$), while *C. dentata* showed a significant effect only at 7.5 mg mL^{-1} ($p = 0.037$). The smallest colonies were observed in plates treated with *L. camara* at 5.0 mg mL^{-1}, followed by *L. camara* at 7.5 mg mL^{-1} and *C. siliqua* at 7.5 mg mL^{-1}.

Inhibition of fungal growth was calculated relative to the unamended PDA control plates (see 3.5.1). *Lantana camara* at 5.0 mg mL^{-1} showed the strongest inhibition, reducing mycelial growth by 30% based on colony diameter (Figure 9 and Figure 10). *Curtisia dentata* exhibited minimal inhibition across all tested concentrations, and its inhibitory effect was significantly lower than that of *L. camara* at all concentrations, *C. siliqua* at 5.0 and 7.5 mg mL^{-1} and *S. pinnata* at 7.5 mg mL^{-1} ($p < 0.001$). For both *S. pinnata* and *C. siliqua*, inhibition increased linearly with extract concentration. Both extracts showed strongest inhibitory effects at the highest concentration of 7.5 mg mL^{-1}.

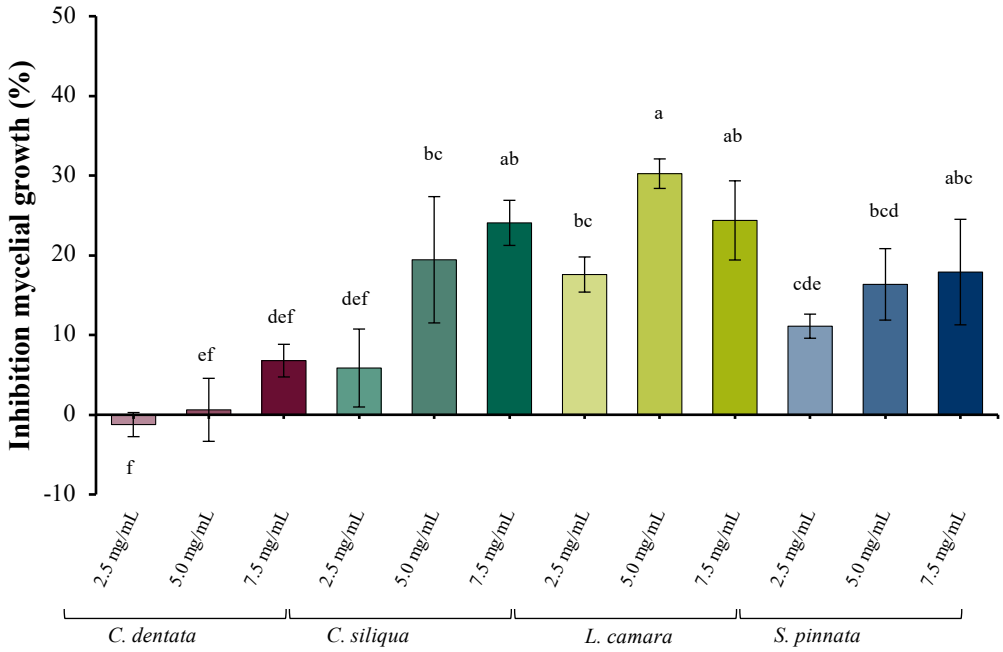

Figure 10: Percentage inhibition of F. oxysporum mycelial growth by agar infusion, based on colony diameter. Bars represent mean + error bars (SD) (n = 4). Different letters indicate significant differences among treatments ($p < 0.05$).

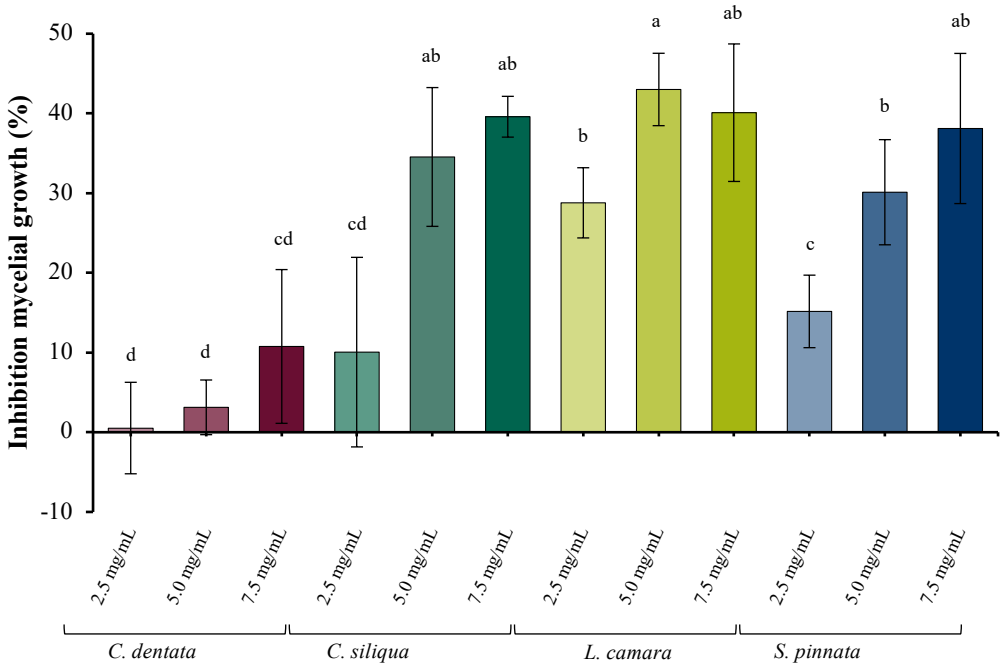

Figure 9: Percentage inhibition of F. oxysporum mycelial growth by agar infusion, based on colony area. Bars represent mean + error bars (SD) (n = 4). Different letters indicate significant differences among treatments ($p < 0.05$).

4.2 Detached leaf assay

No distinct lesions or visible infection symptoms developed on the cowpea leaves during incubation (Appendix 3, Figure S3-S4). The inoculation did not result in a clear infection, and the detached leaf assay could therefore not be used to assess treatment effects.

4.3 Seed germination trial

The plant extracts selected from the poisoned food assay were evaluated to assess the effect on germination percentage and infection by *F. oxysporum*. Three additional concentrations of *L. camara* extract (1.25, 2.5 and 10 mg mL^{-1}) were included to examine whether further dilution or higher concentration influenced seed germination and infection outcomes.

For *L. camara*, significantly higher germination percentages were observed at 1.25 ($p = 0.003$) and 2.5 mg mL^{-1} ($p = 0.010$) compared with the water control (Figure 11). The two concentrations also showed higher germination than *L. camara* at higher concentrations of 5.0 mg mL^{-1} to 10.0 mg mL^{-1}. However, germination at 1.25 and 2.5 mg mL^{-1} remained significantly lower than the positive Celest XL® control ($p = 0.016$; $p = 0.028$). *Schkuria pinnata* at 7.5 mg mL^{-1} completely inhibited germination. *Ceratonia siliqua* at 7.5 mg mL^{-1} did not show a significant difference in germination when compared to the water control.

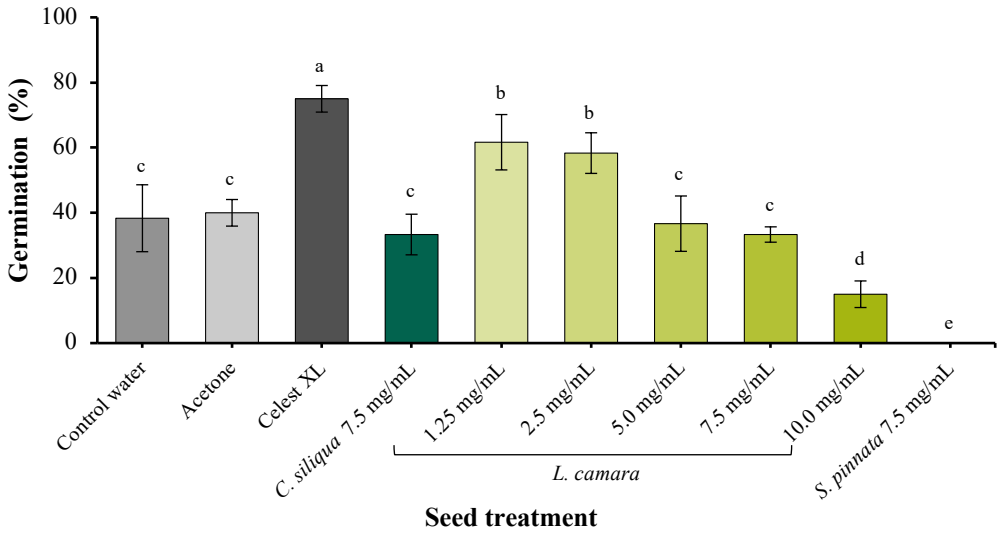

Figure 11: Percentage seed germination of F. oxysporum-inoculated seeds treated with different plant extracts and concentrations. Bars represent mean ± error bars (SD) (n = 100). Different letters indicate significant differences among treatments ($p < 0.05$)

The infection rate of the seeds was high, exceeding >75% across all treatments, including the positive control (Celest XL®) (Figure 12). Among the plant extract treatments, only seeds treated with *L. camara* at 2.5 and 7.5 mg mL^{-1} showed a significant reduction in infection relative to the control ($p = 0.045$; $p = 0.031$), although infection levels in these treatments also remained above 75%. In contrast, 100% infection was observed in seeds treated with *S. pinnata* at 7.5 mg mL^{-1} and *L. camara* at 10.0 mg mL^{-1}.

Because the infection levels in the main germination experiment exceeded 75% for all treatments, an additional germination test was conducted using lower spore concentrations of 2×10^4 and 1×10^5 spores mL^{-1} (Appendix 4, Figure S5-S6). These lower concentrations resulted in lower infection levels, while germination percentages remained similar to those observed in the first germination trial.

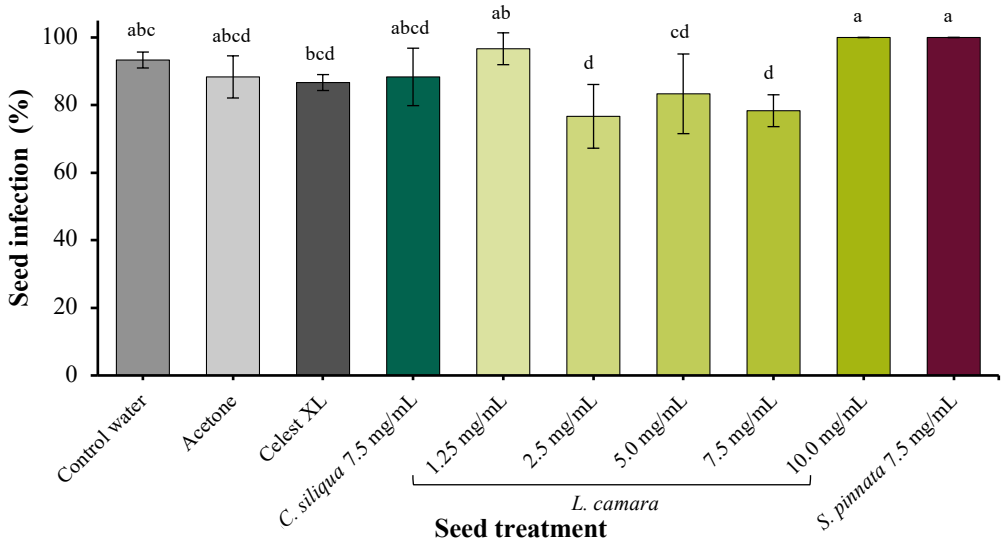

Figure 12: Percentage seed infection on F. oxysporum-inoculated seeds treated with different plant extracts and concentrations. Bars represent mean ± error bars (SD) (n =100). Different letters indicate significant differences among treatments ($p < 0.05$).

Given the overall low germination rate and high infection levels, the number of normal seedlings and thereby available replicates for shoot and root measures was limited. No normal seedlings were obtained for *S. pinnata* (7.5 mg mL^{-1}) or *L. camara* (10.0 mg mL^{-1}) and therefore could not be evaluated for seedling growth.

No significant differences in shoot length were observed between the treated seedlings and the water control (Table 2). However, seedlings treated with *C. siliqua* at 7.5 mg mL^{-1} developed significantly longer roots ($p = 0.043$), while those treated with *L. camara* at 1.25 mg mL^{-1} and Celest XL® showed significantly shorter roots compared with the control ($p < 0.001$; $p = 0.037$). The other treatments did not show consistent patterns in shoot or root length relative to the water control.

Table 1: Shoot and root length of normal seedlings after nine days of growth. Values represent mean ± SD. n indicates the number of replicates. Different letters within a column indicate significant differences among treatments ($p < 0.05$). "-" indicates that no normal seedlings could be measured.

Seed treatment	Concentration	Shoot length (cm)	Root length (cm)	*n*
Control		5.1 ± 3.6^{b}	5.7 ± 3.2^{bc}	*21*
Acetone (5%)		7.8 ± 3.0^{a}	4.0 ± 2.4^{cd}	*12*
Celest XL		6.4 ± 2.8^{b}	4.1 ± 1.3^{d}	*18*
Ceratonia siliqua	7.5 mg mL^{-1}	7.5 ± 2.7^{ab}	8.6 ± 3.2^{a}	*9*
Lantana camara	1.25 mg mL^{-1}	5.4 ± 1.0^{ab}	2.7 ± 1.2^{d}	*5*
	2.5 mg mL^{-1}	6.7 ± 2.9^{ab}	6.7 ± 2.0^{ab}	*15*
	5.0 mg mL^{-1}	6.4 ± 2.6^{ab}	4.7 ± 2.1^{bcd}	*8*
	7.5 mg mL^{-1}	7.1 ± 1.9^{ab}	6.3 ± 3.3^{ab}	*12*
	10.0 mg mL^{-1}	-	-	
Schkuria pinnata	7.5 mg mL^{-1}	-	-	

As infection rates were high across all treatments, selection of treatments for the pot trial was based solely on seed germination performance. Among the tested treatments, *L. camara* at 1.25 and 2.5 mg mL^{-1} exhibited higher germination percentages than the water control and were selected for further evaluation.

4.4 Pot trial

Weather conditions at the pot trial site were recorded daily (Appendix 5, Table S1). Rainfall was higher than usually expected for this time of year, and relative humidity remained high for an extended period. No significant differences in germination percentage were found among the treatments in the pot trial (Appendix 6, Figure S7).

Chlorophyll content was measured weekly from 14 DAS to assess plant physiological status and leaf yellowing associated with *F. oxysporum* infection (Figure 13). Across the entire experimental period, chlorophyll content declined in all treatments. No significant treatment effects on the rate or magnitude of this decline were seen.

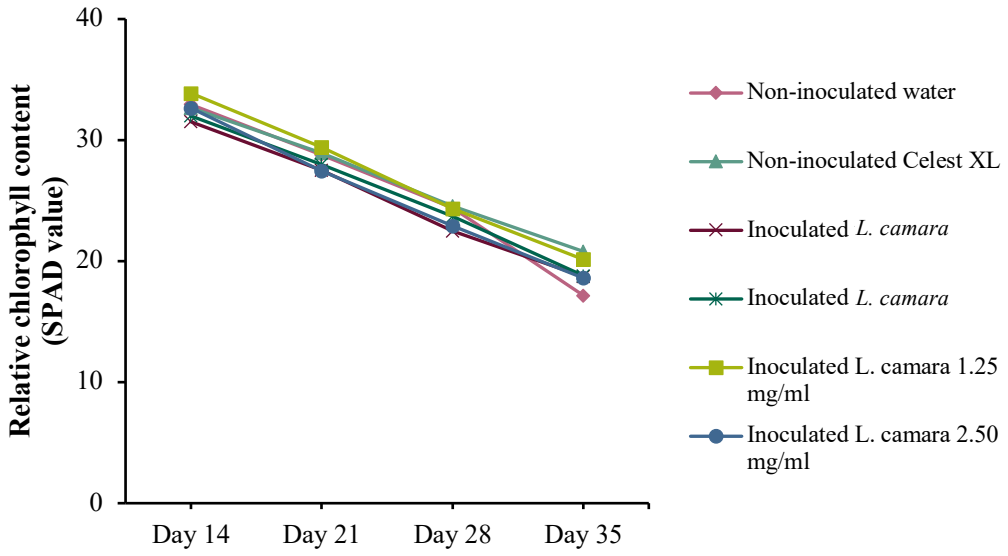

Figure 13: Chlorophyll content of the seedlings in the pot trial, measured weekly from 14 DAS. No significant differences in chlorophyll content throughout the growth period were seen ($p > 0.05$).

After harvest (36 DAS), disease severity on the seedlings was assessed using a visual score from 1 to 5 (Figure 14). No significant differences in disease severity were detected between the treatments. The non-inoculated Celest XL® treatment exhibited the lowest severity scores, followed by the inoculated Celest XL® seedlings. Highest disease severity was observed at the inoculated water treatment. Seeds treated with *L. camara* at 1.25 and 2.5 mg mL^{-1} both resulted in lower disease severity compared to the inoculated water control.

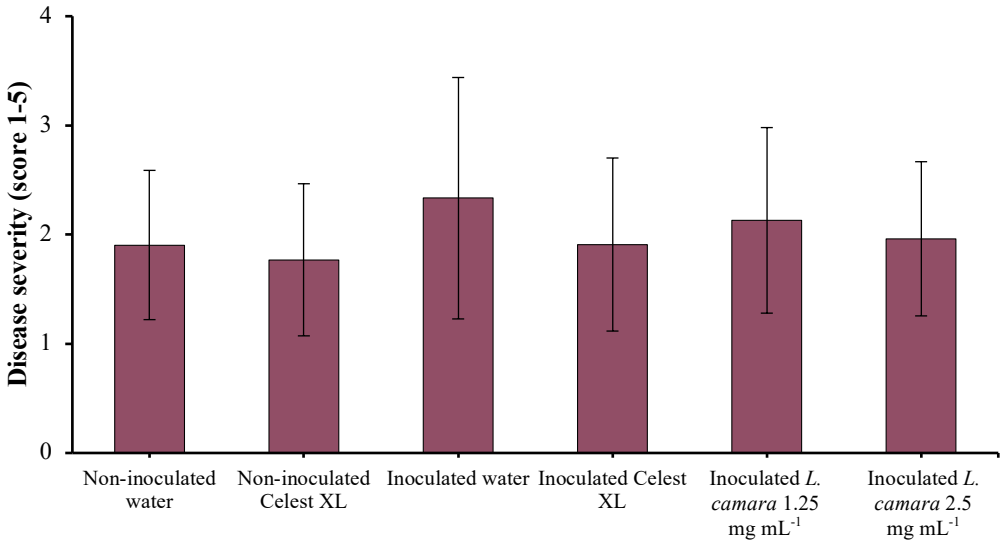

Figure 14: Average disease severity among seedlings in the pot trial. No significant differences among the treatments were seen ($p > 0.05$).

No significant differences in shoot length, root length or leaf area were observed among the treatments (Table 3). Additionally, no significant differences in the number of leaves, number of fallen leaves and number of infected leaves were observed (Appendix 6, Table S2).

From the inoculated seeds, the water control had the longest roots and largest leaf areas compared with both the Celest XL and plant extract treatments (Table 3). In contrast, the inoculated Celest XL® treatment with Celest XL® exhibited both the smallest shoot and root lengths.

Table 2: Average shoot length (cm), root length (cm) and leaf area (cm^2) of the cowpea seedlings in the pot trial. No significant differences among the treatments were seen ($p > 0.05$).

Seed treatment	Shoot length (cm)	Root length (cm)	Leaf area (cm^2)
Non-inoculated water	9.9 ± 2.0	33.0 ± 10.5	14.9 ± 8.3
Non-inoculated Celest XL	11.0 ± 2.8	33.3 ± 9.3	20.9 ± 10.6
Inoculated water	10.4 ±1.9	33.4 ± 12.3	20.2 ± 6.8
Inoculated Celest XL	9.3 ± 3.0	29.7 ± 10.0	19.0 ± 8.9
Inoculated *L. camara* 1.25 mg/ml	10.5 ± 2.4	30.2 ± 7.6	19.2 ± 11.1
Inoculated *L. camara* 2.50 mg/ml	10.3 ± 1.7	33.4 ± 7.8	18.6 ± 8.4

No significant differences in shoot fresh and dry mass were observed among the treatments (Figure 15; Appendix 6, Figure S8). The non-inoculated seeds treated with Celest XL® produced the highest dry shoot mass, while inoculated seeds treated with *L. camara* at 1.25 mg mL^{-1} had the lowest values.

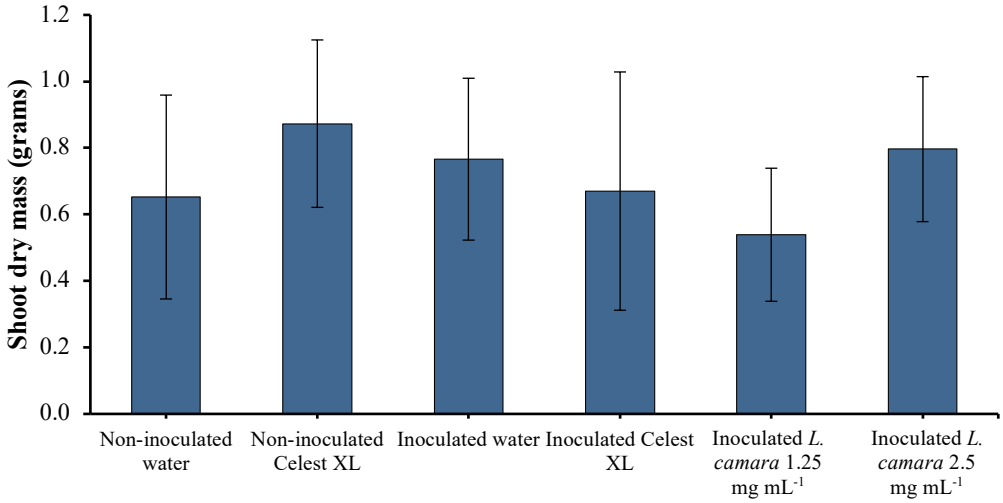

Figure 15: Average shoot dry mass (grams) of the cowpea seedlings in the pot trial. No significant differences among the treatments were seen (p > 0.05).

Similarly, no significant differences in root fresh and dry mass were observed among the treatments (Figure 16; Appendix 6, Figure S9). The inoculated water control showed root dry mass comparable to that of the non-inoculated Celest XL® treatment. Among the inoculated treatments, the water control produced higher root dry mass than both the Celest XL® treatment and the *L. camara* treatments at 1.25 and 2.5 mg mL^{-1}.

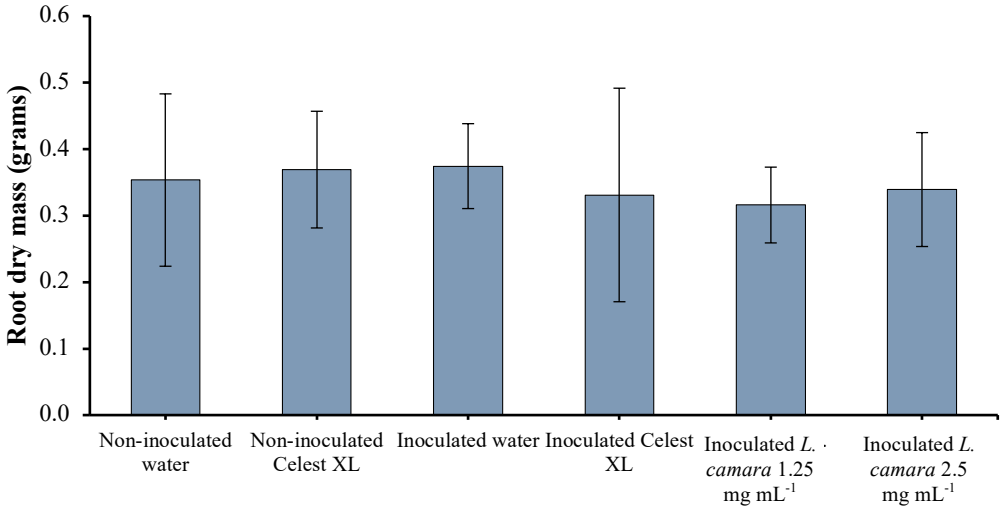

Figure 16: Average root dry mass (grams) of the seedlings in the pot trial. No significant differences among the treatments were seen (p > 0.05).

5. Discussion

This study examined the antifungal activity of four plant extracts against *F. oxysporum* and evaluated their effects on seed germination and early seedling development in cowpea. Among the tested extracts, *L. camara* consistently showed the strongest inhibitory effect on fungal growth in the poisoned food test, whereby the concentration of 7.5 mg mL^{-1} exhibited the greatest reduction in fungal growth. Nonetheless, *L. camara* at 10.0 mg mL^{-1} resulted in very poor seed germination, indicating that higher concentrations of the extracts can negatively affect seed viability. In contrast, lower concentrations of *L. camara* allowed better germination than the water control, suggesting a concentration-dependent balance between antifungal efficacy and phytotoxicity.

The pot trial showed no significant differences between the water control and seeds treated with *L. camara*. These findings suggest that while low concentration of *L. camara* may have potential as an effective plant-based seed treatment, further studies to optimise its efficacy are required.

5.1 Antifungal activity of plant extracts

The absence of inhibition in the disk diffusion assay is most likely explained by the limited ability of the plant compounds to diffuse through the agar medium. This limitation is attributed to the presence of nonpolar or waxy components within the extract. Key antifungal metabolites reported in *L. camara*, such as lantadene A and boswellic acid, are triterpenoids that are lipophilic and nonpolar (Passos et al., 2012; Seepe et al., 2020). Such compounds are known to diffuse slowly in aqueous agar (Bubonja-Sonje et al., 2020). For this reason, the well diffusion method or the poisoned food technique is considered more suitable for assessing antimicrobial activity of plant-derived extracts (Valgas et al., 2007). The poisoned food technique was selected for the *in vitro* antifungal experiment as the *L. camara* extract showed no clear inhibition using the disc diffusion method.

The poisoned food test demonstrated that all extracts, except for *C. dentata*, significantly reduced the growth of *F. oxysporum*. For *C. siliqua* and *S. pinnata*, the inhibition increased with increasing extract concentration, which indicates a clear dose-dependent antifungal effect. Similar concentration related responses have been widely reported in studies that used the poisoned food method to evaluate antifungal activity against phytopathogens (Uma et al., 2017; Asmaa El-Nagar et al., 2025). The observed inhibition is related to the presence of bioactive secondary

metabolites in the extracts, such as lantadene A and boswellic acid in *L. camara* (Seepe et al., 2020), alkaloids and flavonoids in *S. pinnata* (Masoko & Masiphephethu, 2019) and methyl esters and siloxanes in *C. siliqua* (Najem & Alhamdani, 2025). These compounds are known to eliminate or reduce pathogen growth through their natural antifungal properties (Sales et al., 2016).

Although *C. dentata* has been reported to contain bioactive compounds with strong antifungal activity against several fungal pathogens (Shai et al., 2008), the results showed inhibitory effect only at the highest concentration (7.5 mg mL^{-1}). The absence of activity at lower concentrations may indicate species-specific differences in the sensitivity of *F. oxysporum* to the compounds in *C. dentata*. This interpretation is supported by Wens and Geuens (2022), who investigated the antifungal activity of nine plant extracts against four phytopathogens (*Botrytis cinerea*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia minor*). The authors results showed that no single extract was effective against all four phytopathogens, demonstrating clear species-specific variation in susceptibility.

The strong inhibitory activity of *L. camara* against *F. oxysporum* corresponds with earlier research that reported activity against several *Fusarium* spp.. Previous studies have shown that the extract of *L. camara* can suppress both mycelial growth and spore germination of *Fusarium* spp., as it contains multiple bioactive compounds with broad antifungal potential (Seepe et al., 2020; Unah et al., 2024). These findings indicate that extracts of *L. camara* may offer a valuable natural source of antifungal activity against Fusarium wilt and could contribute to reducing the need for synthetic fungicides in agriculture. However, translating *in vitro* inhibition into an effective seed treatment can be challenging, as seed-treatment conditions differ from controlled laboratory experiments.

5.2 Detached leaf assay

The inoculation of cowpea leaves did not result in any lesion development in this study. *Fusarium oxysporum* is a soil- and seed-borne pathogen that typically enters the plant through the roots and colonises the vascular tissue (Di Pietro et al., 2003). Direct inoculation onto detached leaves may not initiate disease development in cowpea because foliar tissue is not a natural infection site.

However, some studies have reported successful use of detached leaf assays with other *F. oxysporum* formae specialis. For example, Priya et al. (2025) demonstrated that the detached leaf method provided a reliable and rapid pathogenicity test for *F. oxysporum* f. sp. *cubense* in banana, with symptoms occurring within 5 to 7 days.

Similarly, Ayele et al. (2021) found that the detached leaf method was a good indicator of pathogenicity of *F. oxysporum* in tomato.

In the present study, *F. oxysporum* did not cause visible symptoms on cowpea leaf tissue, indicating that the detached leaf assay is not appropriate for this host-pathogen system. Therefore, the detached leaf assay was considered unsuitable for further evaluation and was not used in this study.

5.3 Effects of plant extracts on seed germination

5.3.1 Infection rates

A notable outcome of the seed germination test was the consistently high level of seed infection across all treatments, including the positive control with Celest® XL. The seeds were inoculated with a suspension of 2×10^5 spores mL^{-1} prior to the relative seed treatments. Since even the fungicide treated seeds showed high infection rates above 80%, the method used for seed inoculation and germination testing seems unsuitable for evaluating antifungal seed treatments.

No studies were found in which seeds were artificially inoculated before being tested in the standard ISTA paper-roll germination test. For instance, Masangwa et al. (2017) did inoculate cowpea seeds prior to greenhouse evaluation but did not inoculate the seeds in the paper-roll germination test. The conditions of the paper-roll test (room temperature, darkness and constant moisture) are known to strongly favour rapid *F. oxysporum* growth (Mohsen et al., 2016). These conditions may have suppressed the antifungal capacity of both the plant extracts and the fungicide Celest® XL and led to uniformly high infection levels regardless of treatment.

An additional germination test was done to determine whether reducing the spore concentration would influence infection levels. The results showed that infection did not decrease at a lower inoculum dose. This indicates that a lower inoculum density does not overcome the limitations of the paper-roll germination test, as the test conditions still strongly favour fungal development.

A more relevant setup is described by Adeniji et al. (2020), who tested the bioprotective effects of *Pseudomonas* strains on maize seeds against *Fusarium* spp.. In their experiment, sterile seeds were first treated with the biological agent, air-dried and subsequently placed in a row along the centre of PDA plates. Agar plugs of *Fusarium* culture were placed on opposite sides of the seeds before incubation.

Afterwards, suppression of mycelial growth and seed germination was evaluated. A similar plate-based experiment may provide a more suitable method for

evaluating seed-pathogen interactions when testing plant extracts on cowpea seeds. It allows the infection to develop in a more controlled way and avoids the high moisture conditions of the paper-roll method.

5.3.2 Phytotoxicity

Seed germination was strongly influenced by the concentrations of the applied extracts. Germination decreased with increasing levels of *L. camara*, with less than 20% germination observed at 10.0 mg mL^{-1}. Similarly, seed treatment with *S. pinnata* at 7.5 mg mL^{-1} completely inhibited germination. These results indicate that both *L. camara* and *S. pinnata* may exhibit phytotoxic effects on cowpea seeds when applied at higher concentrations.

Extracts of *S. pinnata* have not previously been investigated as seed treatments and therefore limited/no information on its phytotoxicity is readily available. In contrast, the phytotoxic effects of *L. camara* have been widely studied and inhibitory effects of *L. camara* at increasing concentrations have been reported. For example, El-Kenany and El-Darier (2013) found that extracts of *L. camara* reduced germination of *Sorghum bicolour* seeds, with germination dropping below 20% at concentrations of 25 mg mL^{-1}.

The phytotoxicity of *L. camara* can be linked to its allelopathic activity. This species is known to contain allelochemicals in its leaves, stems, flowers and roots which interfere with the germination and early growth of many plant species (Kato-Noguchi & Kurniadie, 2021). Joshi et al. (2024) similarly reported that aqueous leachates from *L. camara* leaf tissue significantly suppressed the germination and seedling growth of wheat. Aromatic alkaloids and phenolic compounds have been identified as the main allelopathic substances in *L. camara*, both associated as inhibitors of seed germination (El-Kenany & El-Darier, 2013). Although these allelopathic compounds may contribute to antifungal activity, their broad toxicity means that concentration must be carefully controlled.

Given these findings, the use of high concentrations of *L. camara* extracts is not suitable for seed treatment in cowpea, as the inhibitory effects on germination outweigh any potential antifungal benefits. Additionally, the soaking duration may also have contributed to the phytotoxicity. Seeds were exposed to the extracts for 24 hours, during which phytotoxic compounds could accumulate within the seed tissues. Shorter soaking durations might reduce the negative effects on germination.

Future studies should evaluate different concentrations and exposure times to determine whether less intensive treatments can maintain antifungal activity while

minimising phytotoxic effects. In addition, efforts should be made to isolate and identify the active compounds within the extracts. By separating the antifungal compounds from those that are phytotoxic, purified fractions could be tested individually, which may help develop safer and more effective seed treatments.

In contrast, *C. siliqua* at 7.5 mg mL^{-1} did not significantly reduce seed germination, but it also failed to limit *F. oxysporum* infection. This suggests that the concentration used in this study remained safe for the seed, while it was insufficient to affect the pathogen on the seed coat. Given that *C. siliqua* did show inhibitory activity in the poisoned food test, it would be valuable to test higher extract concentrations to further evaluate its potential as a seed treatment. A seed germination test could be used to determine the threshold concentration at which antifungal activity is achieved without compromising germination.

5.3.3 Growth promoting effects

Shoot and root length were measured for all normal seedlings in the germination test. However, the number of normal seedlings within each treatment was low and measurements showed high variability. This was most likely due to the high infection rates observed across all treatments. *Fusarium oxysporum* is known to reduce germination in cowpea by causing seed rot (Fawole et al., 2006). The pathogen can also impair early seedling vigour and disrupt shoot-root balance (Wang et al., 2023), which aligns with the poor growth responses observed in this study.

Although *C. siliqua* at 7.5 mg mL^{-1} resulted in longer roots and *L. camara* at 1.25 mg mL^{-1} resulted in shorter roots compared with the control, replication was limited. These results may therefore simply be caused by stressful growing conditions rather than true treatment effects. Further experiments under reduced infection pressure are required to determine whether these treatments truly influence early seedling growth.

5.4 Performance of plant extracts in the pot trial

5.4.1 Disease expression

The inoculation and seed treatments used in the pot trial followed the same procedure as in the seed germination test. In the *in vitro* germination test, the inoculated water control exhibited higher infection rates and significantly lower germination compared to seeds treated with Celest® XL or *L. camara* at 1.25 and 2.5 mg mL^{-1}. In contrast, the pot trial showed no significant differences in germination between the water control and treated seeds, nor between non-inoculated and inoculated seeds. All treatments in the pot trial showed disease symptoms (score > 1). However, the overall level of infection was lower than observed in the seed germination test.

No significant differences in chlorophyll content were observed among the treatments in the pot trail. A reduction in chlorophyll levels was expected in infected plants with wilt symptoms, as *F. oxysporum* interfere in the vascular tissues causing yellowing and wilting of the leaves (Omoigui et al., 2018). Chlorophyll fluorescence parameters have been reported as useful tools for the early detection of wilt disease, for example in pine (*Pinus thunbergii*) (Liu et al., 2023). No significant differences in chlorophyll content were observed, which is consistent with the absence of significant differences in disease severity.

5.4.2 Effectiveness of the extract treatments

The concentrations of L. camara used in the pot trial (1.25 and 2.5 mg mL^{-1}) were not sufficient to reduce disease severity. This outcome was consistent with the seed germination test, where these concentrations also showed no inhibition of infection rate on the seeds.

Pawar et al. (2012) evaluated the effectiveness of *L. camara* extract against seed-borne mycoflora of soybean. Their extract was prepared from 5% dried plant material in hot sterile distilled water (equivalent to 50 mg mL^{-1}), and soybean seeds were soaked in the extract for 5, 15 and 30 minutes. After incubation on glucose nitrate agar (GNA) for 4-6 days, the 30-minute treatment completely inhibited all seed-borne fungi, while no negative effects on seed germination were seen.

The concentration used in this hot-water extract cannot be directly compared with the concentrations tested in present study, as prolonged soaking in acetone for three days is expected to extract higher levels and a broader spectrum of phytochemicals (Kumar et al., 2023). Nevertheless, the findings demonstrate that *L. camara* has the

potential to suppress seed-borne fungi on the seed coat without compromising germination under certain extraction and treatment conditions.

These results suggests that the effective concentration range for *L. camara* as a seed treatment may be narrow. Concentrations high enough to suppress *F. oxysporum* are likely to negatively affect seed germination, whereas non-phytotoxic concentrations fail to provide sufficient protection under *in vivo* conditions.

5.4.3 Environmental effects

The differences between treatments may have been masked by environmental factors during the pot trial. Heavy rainfall during the growth period led to prolonged soil saturation. Constant moisture and the inability of the soil to dry favour the spread and activity of *F. oxysporum* (Mohsen et al., 2016). For example, conditions with wet and compacted soil are known to promote Fusarium root rot in soybean (Yan & Nelson, 2022). Additionally, waterlogged conditions can weaken host root systems, making it more difficult to detect treatment effectiveness. Cowpea is known to be sensitive to waterlogging stress, especially when exposed to excess moisture during early growth stages (Olorunwa et al., 2022).

These environmental conditions likely contributed to the relatively uniform disease severity observed across the treatments. It is therefore recommended that future *in vivo* pot trials with plant extract treatments on cowpea should be conducted under more favourable and controlled conditions. Once the most promising extract treatments have been identified, it will also be important to test them further under natural open-field conditions.

5.4.4 Application method

The lack of significant treatment differences observed in the pot trial may also be attributed to limitations in the application method. The plant extracts used in this study were crude extracts and lacked adhesive agents. A binding compound is typically used in fungicides to secure the antifungal compounds to the seed coat (Pedrini et al., 2016). Under outdoor conditions with regular watering and rainfall, treatments that lack adhesion may dilute or leach from the seed surface. Although it is difficult to measure wash-off directly, prolonged soil moisture may have increased the solubility of the compounds.

Any reduction or loss of bioactive compounds from the fungicide or plant extracts shortly after germination may have left the seeds with little to no protection. It is therefore recommended that future studies evaluate the antifungal activity of the extracts as seed treatment with an appropriate binder to improve adhesion to the seed coat and enhance durability in field-like moisture conditions.

5.4.5 Functionality of the chemical control

Celest® XL did not perform as expected and failed to significantly reduce disease severity in cowpea. Seedlings from Celest® XL treated seeds also produced slightly shorter shoot and root lengths compared with the inoculated water control. Celest® XL is designed to be applied as a quick slurry coating, forming a thin layer on the seed surface (Syngenta, 2024). For the treatment of legumes against *Fusarium* spp., 125 mL of Celest® XL per 100 kg seeds is recommended, resulting in an approximately slurry concentration of 30 µg fludioxonil mL^{-1} and 10 µg mefenoxam mL^{-1}.

In this study, however, seeds were soaked in a diluted fungicide solution (9 µg fludioxonil mL^{-1}, 3.6 µg mefenoxam mL^{-1}) for 24 hours, rather than being coated and dried. 24 hours was chosen to mimic the same soaking times as the plant extracts. Extended soaking may have weakened the seed coat due to prolonged water exposure, allowing the fungicide solution to penetrate the seed rather than remaining on the surface where it is intended to act. However, no published studies were found that documented the harmful effects of prolonged exposure to fludioxonil or mefenoxam on seed tissue.

It can however be concluded that the Celest® XL treatment did not function as a reliable positive chemical control. This limits the ability to interpret the performance of the plant extracts, since there was no effective chemical comparison available.

6. Conclusions

This study showed that plant extracts, particularly *L. camara*, had clear antifungal activity against *F. oxysporum* under *in vitro* conditions. However, these effects did not translate into effective seed protection in either the germination test or the pot trial. High extract concentrations strongly inhibited fungal growth in the poisoned food test but caused severe phytotoxicity and reduced germination. None of the tested extracts achieved both suppression of *F. oxysporum* and acceptable germination. Although treatment with *L. camara* at 1.25 and 2.5 $mg \cdot mL^{-1}$ resulted in higher germination than the water control, these concentrations did not have any antifungal effect.

The pot trial did not show significant differences among treatments, which was largely influenced because of heavy rainfall and prolonged soil saturation that created conditions highly favourable for *F. oxysporum*. Furthermore, the Celest® XL positive control was ineffective due to the non-standard soaking application, preventing a reliable comparison. Overall, these factors limited the ability to evaluate the true effectiveness of the plant extracts as seed treatments.

The findings indicate that while plant extracts show promise as biological antifungal agents under *in vitro* conditions, their suitability as cowpea seed treatments remains limited. Achieving a balance between antifungal activity and seed safety for plant extracts as an alternative seed treatment against *F. oxysporum* requires further optimisation.

7. Future Prospects

The findings of this study highlight several important steps for future research aimed at developing plant-derived seed treatments against Fusarium wilt in cowpea.

- Use PDA-plate based seed germination tests: The ISTA paper-roll method creates unfavourable conditions for inoculated seeds and is therefore unsuitable for evaluation the antifungal efficacy of plant extracts. Plate-based experiments provide a more appropriate environment for both pathogen growth and seed performance examination.

- Optimisation of extract concentration and exposure time: Testing a wider range of concentrations and shorter treatment durations may help identify better conditions that maintain antifungal efficacy while minimising the phytotoxic effects on cowpea seeds.

- Identification of bioactive compounds: Isolation and characterisation of specific bioactive compounds, particularly those from *L. camara*, could allow the separation of antifungal compounds from unwanted phytotoxic compounds and provide insights into their modes of action.

- Alternative seed treatment methods: The use of suitable binding agents may improve adhesion of bioactive compounds to the seed coat. This could reduce leaching of the antifungal compounds under *in vivo* conditions.

- Evaluation under controlled and field conditions: Once optimal concentrations and application methods are determined, seed treatments should be tested in germination tests, controlled greenhouse experiments and field trials. The practicality and usability for smallholder farmers should be kept in mind.

By addressing these points, future studies could help the development of safe and effective plant-derived seed treatments for managing Fusarium wilt in cowpea.

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Popular science summary

Cowpea is one of the most important food crops in Sub-Saharan Africa. It provides rural families with an affordable source of protein, as the plant can grow well in poor soils and hot climates. Farmers are reliable on their cowpea harvest, so losing a large part of it can be devastating. Several factors influence the size of the harvest, including weather conditions such as rainfall and sunlight.

A large part of the cowpea harvest can also be lost to plant diseases, which can spread quickly throughout farmland. One such disease that is responsible for large losses every year is the disease called Fusarium wilt. This disease is caused by the fungus *Fusarium oxysporum*, which can live on seed coats and in the soil. Once the fungus infects a cowpea plant, it blocks the water transport inside the stem, causing the plant to turn yellow, wilt, and eventually die. The fungus can survive in the soil for many years, making it extremely difficult to get rid of.

Most farmers use chemical fungicides to control the disease, but these are often expensive, harmful to the environment and not always available to smallholder farmers in rural areas. For this reason, many farmers and scientists are exploring safer, cheaper and more natural ways to protect crops.

In this project, four common medicinal plants were tested to see whether their leaf extracts could slow down the fungal infection and help protect cowpea seeds. The idea is simple: many plants naturally produce substances that fight microbes, so could these plants help defend crops too?

The study was carried out in three stages. First, the four plant extracts were tested in the lab to see if they could stop the fungus from growing. The best-performing extracts were then applied to cowpea seeds to evaluate whether they could suppress fungal infection on the seed coat and whether the seeds could still germinate. Finally, the most promising treatments from the germination test were evaluated in a pot trial to determine whether they could help young cowpea plants survive the disease.

The results were mixed. Some extracts, especially from *L. camara*, were very good at slowing down the fungus in the lab. But when applied to seeds, things became more complicated. High concentrations damaged the seeds and prevented germination. Lower concentrations were safer for the seeds but not strong enough to protect them from infection. In the pot trial, the extracts did not show a clear ability to suppress the disease in cowpea.

So, what does this mean? The study shows that natural plant extracts do have potential, but they are not yet ready for use on crops. They need to be applied in the right amounts, using the right methods, and tested under realistic growing conditions. More research is needed before they can become a reliable, farmer-friendly tool to protect crops like cowpea.

Still, the message is hopeful: plants may one day help protect important food crops from devastating diseases, providing smallholder farmers with safer and more sustainable options.

Appendix 1

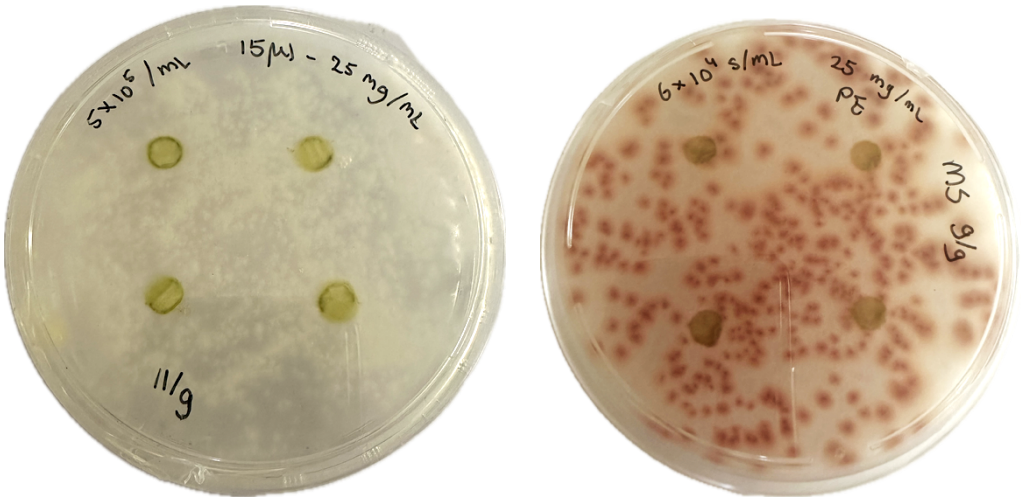

Figure S2: Inoculated Petri dish with F. oxysporum spore suspension (5 x 10^5 spores mL^{-1} & 6 x 10^4 spores mL^{-1}), four disks drenched in 25 mg mL^{-1} L. camara extract.

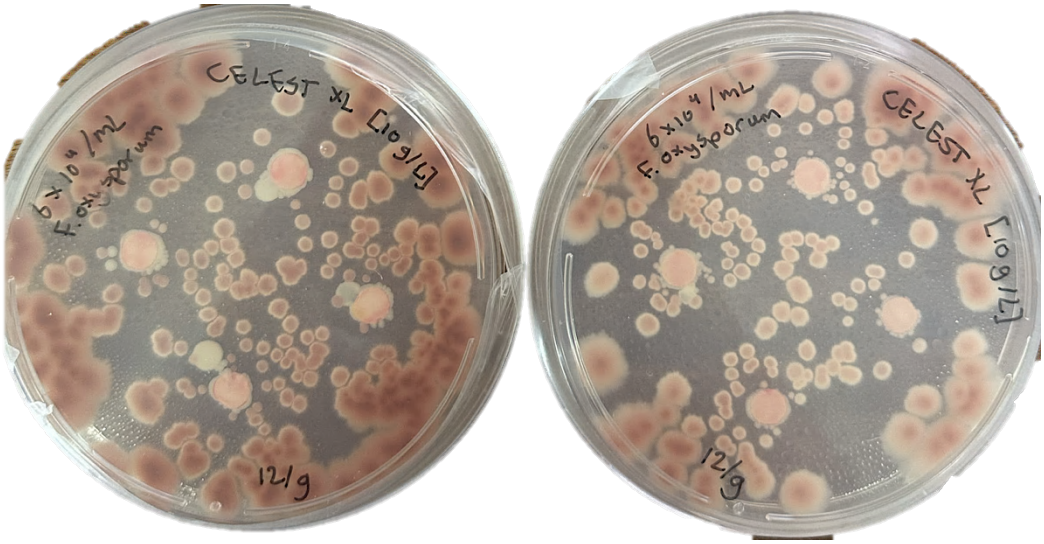

Figure S1: Inoculated Petri dishes with F. oxysporum spore suspension (6 x 104 spores mL-1), four disks drenched in Celest XL® (25 g L-1 fludioxinil, 10 g L-1 mefenoxam).

Appendix 2

Table S1: Average fungal colony diameter and area of F. oxysporum after five days of incubation on treatment-infused agar. Values represent mean ± SD (n = 4). Different letters within a column indicate significant differences among treatments ($p < 0.05$).

Agar infusion	Concentration	Colony diameter (mm)	Colony area (mm^2)
Control (unamended PDA)		40.5 ± 0.7^{ab}	1222 ± 67^{a}
Acetone (5%)		39.4 ± 0.6^{abc}	1213 ± 75^{a}
Celest XL		8.8 ± 0.8^{j}	57 ± 5^{f}
Curtisia dentata	2.5 mg mL^{-1}	41.0 ± 0.6^{a}	1216 ± 70^{a}
	5.0 mg mL^{-1}	40.3 ± 1.6^{abc}	1184 ± 42^{a}
	7.5 mg mL^{-1}	37.8 ± 0.8^{cd}	1090 ± 118^{ab}
Ceratonia siliqua	2.5 mg mL^{-1}	38.1 ± 2.0^{bd}	1099 ± 145^{ab}
	5.0 mg mL^{-1}	32.6 ± 3.2^{fgh}	800 ± 106^{ce}
	7.5 mg mL^{-1}	30.8 ± 1.1^{f}	738 ± 31^{c}
Lantana camara	2.5 mg mL^{-1}	33.4 ± 0.9^{f}	870 ± 54^{c}
	5.0 mg mL^{-1}	28.3 ± 0.8^{i}	696 ± 55^{e}
	7.5 mg mL^{-1}	30.6 ± 2.0^{hi}	732 ± 105^{de}
Schkuria pinnata	2.5 mg mL^{-1}	36.0 ± 0.6^{de}	1037 ± 56^{b}
	5.0 mg mL^{-1}	33.9 ± 1.8^{ef}	854 ± 81^{cd}
	7.5 mg mL^{-1}	33.3 ± 2.7^{gi}	756 ± 115^{ce}

Appendix 3

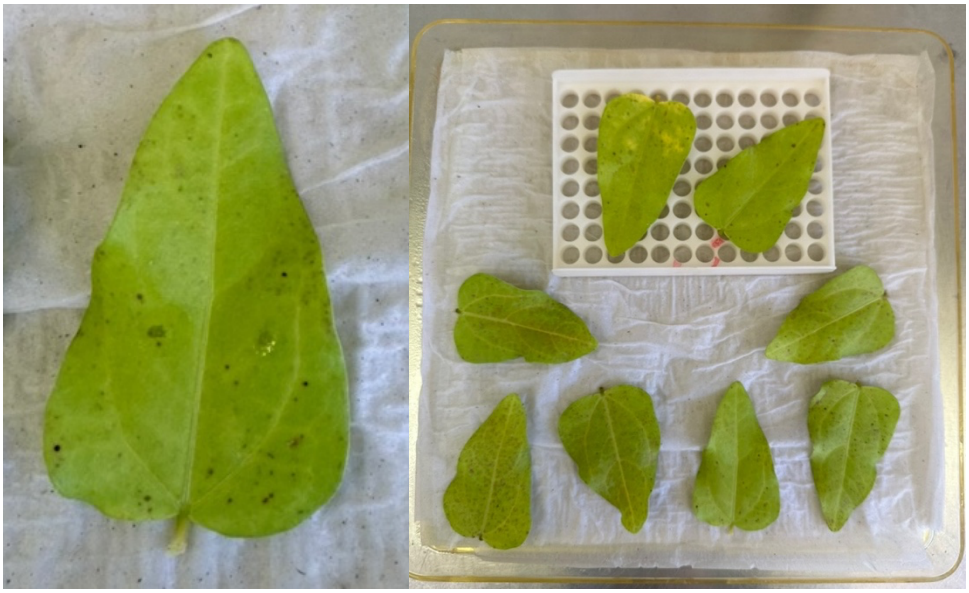

Figure S4: Cowpea leaves, directly after inoculation with F. oxysporum spore suspension.

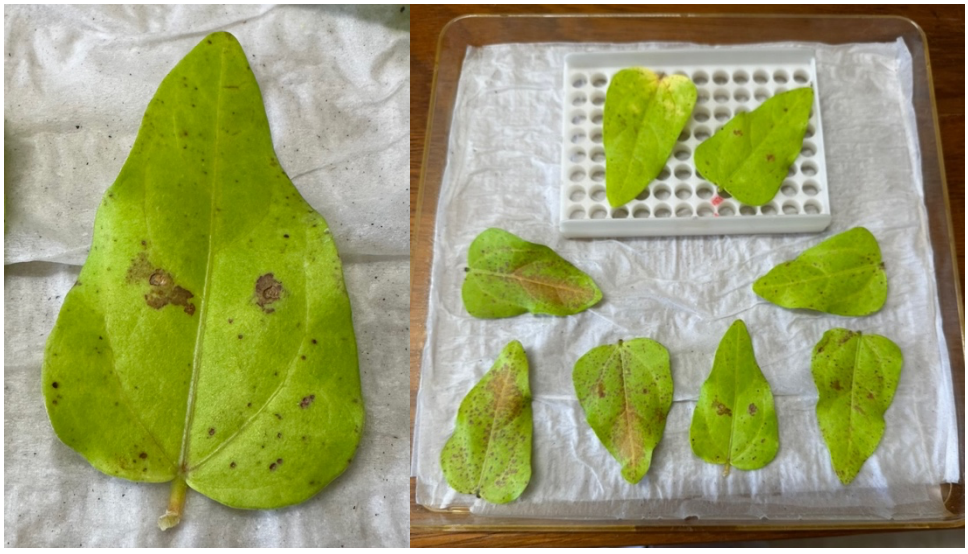

Figure S3: Cowpea leaves, four days after inoculation with F. oxysporum spore suspension.

Appendix 4

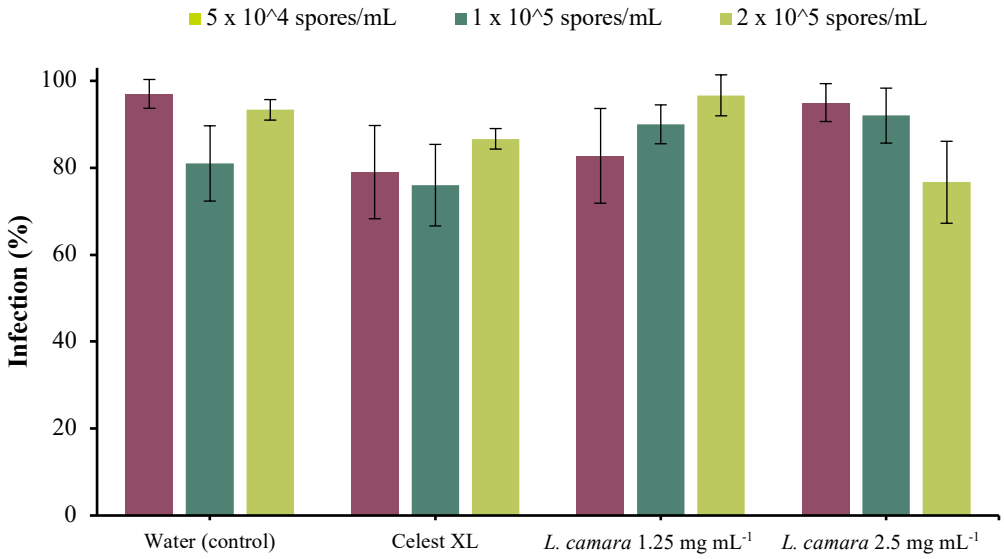

Figure S5: Infection percentage of seeds treated with different spore concentrations (2 × 10⁴, 1 × 10⁵, and 2 × 105 spores mL⁻¹). Bars represent mean ± SD (n = 100).

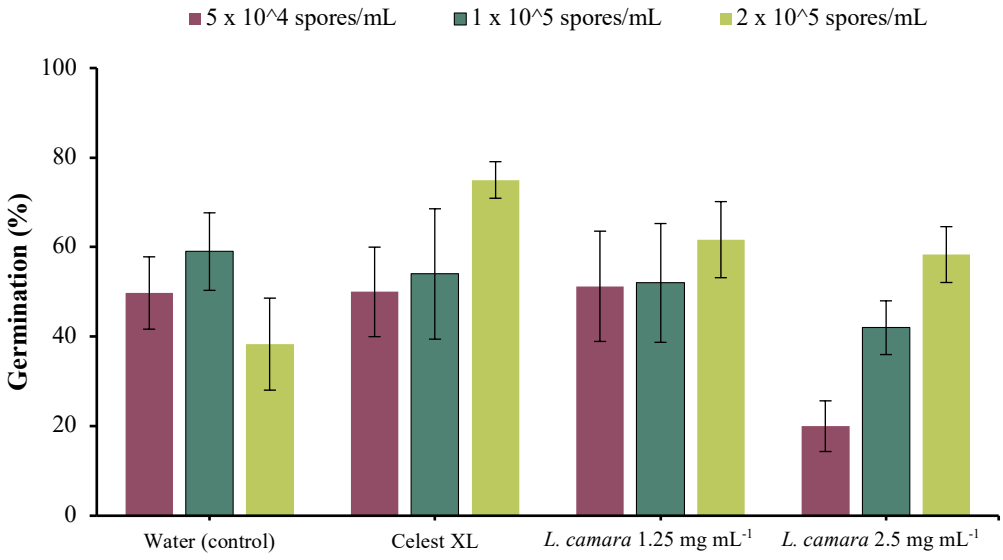

Figure S6: Germination percentage of seeds treated with different spore concentrations (2 × 10⁴, 1 × 10⁵, and 2 × 105 spores mL⁻¹). Bars represent mean ± SD (n = 100).

Appendix 5

Table S2: Weather data recorded at the Hatfield weather station (Field Trial Section 1, Experimental Farm). Minimum and maximum air temperature (°C), maximum relative humidity (RH) (%), and total rainfall (mm) are presented for Days 1–36 of the pot trial.

Date	Maximum Air Temperature (°C)	Minimum Air Temperature (°C)	Maximum RH (%)	Total Rainfall (mm)
2025-10-21	22,47	10,22	90,52	0,0
2025-10-22	24,34	10,12	95,51	0,0
2025-10-23	27,39	9,60	79,73	0,0
2025-10-24	29,53	9,77	70,43	0,0
2025-10-25	30,48	14,18	57,88	0,0
2025-10-26	32,37	13,77	63,08	0,0
2025-10-27	27,92	17,64	71,73	0,0
2025-10-28	29,71	14,33	94,87	1,9
2025-10-29	20,76	14,83	100,00	3,1
2025-10-30	28,96	14,59	100,00	24,3
2025-10-31	25,19	14,41	100,00	0,8
2025-11-01	27,82	14,06	100,00	0,0
2025-11-02	28,01	14,83	100,00	7,2
2025-11-03	22,00	14,15	100,00	0,0
2025-11-04	18,90	14,07	100,00	1,0
2025-11-05	29,24	12,34	100,00	0,1
2025-11-06	23,93	13,98	100,00	3,2
2025-11-07	22,40	13,03	100,00	15,7
2025-11-08	23,12	11,85	100,00	0,1
2025-11-09	25,25	12,19	99,19	0,0
2025-11-10	23,02	14,38	100,00	2,6
2025-11-11	24,32	13,85	100,00	9,7
2025-11-12	27,73	14,53	98,98	0,0
2025-11-13	30,77	15,05	92,81	0,0
2025-11-14	23,88	16,33	93,75	0,0
2025-11-15	23,09	14,09	100,00	2,6

2025-11-16	17,37	10,65	100,00	29,9
2025-11-17	15,28	9,86	100,00	3,8
2025-11-18	22,32	12,42	100,00	2,4
2025-11-19	25,95	13,10	97,13	12,2
2025-11-20	23,73	13,52	100,00	5,7
2025-11-21	26,16	13,86	98,12	0,1
2025-11-22	27,28	12,67	91,89	0,2
2025-11-23	26,26	13,29	94,76	0,0
2025-11-24	22,79	13,31	99,70	2,7
2025-11-25	23,18	12,60	100,00	24,4
2025-11-26	24,80	13,91	100,00	0,2
2025-11-27	26,88	14,82	100,00	5,3

Appendix 6

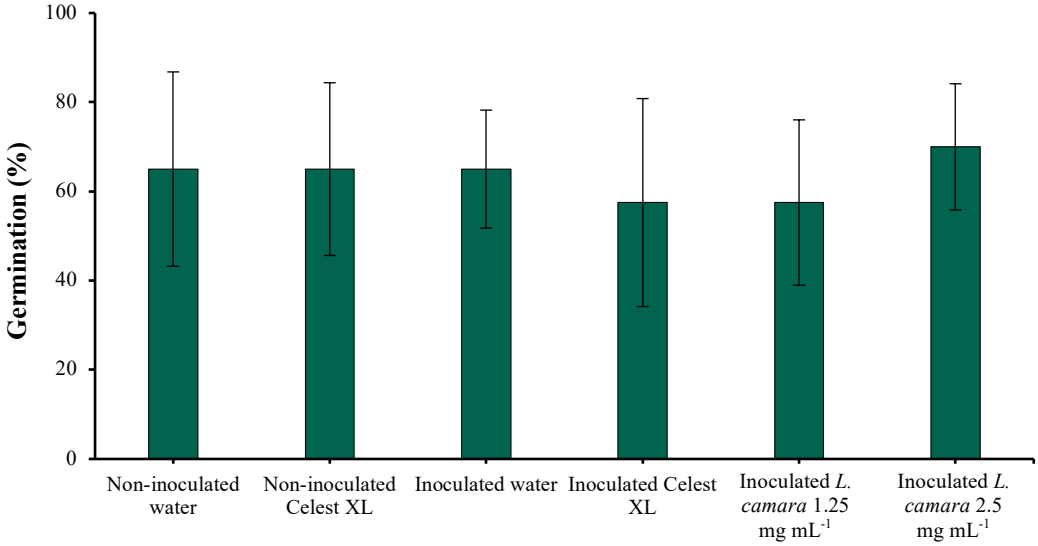

Figure S7: Percentage seed germination in the pot trial of seeds treated with different plant extracts and concentrations. Bars represent mean + error bars (SD). No significant differences between the treatments were seen ($p > 0.05$).

Table S3: Average number of leaves, number of fallen leaves and number infected leaves from the seedlings in the pot trial. Values represent mean ± SD. No significant differences between the treatments were seen ($p > 0.05$).

Seed treatment	Nr of leaves	Nr of fallen leaves	Nr of infected leaves
Non-inoculated water	5.2 ± 2.2	0.4 ± 0.6	1.0 ± 1.0
Non-inoculated Celest® XL	5.5 ± 1.4	0.5 ± 0.6	1.1 ± 1.0
Inoculated water	5.6 ± 1.8	0.3 ± 0.6	1.2 ± 1.0
Inoculated Celest® XL	6.0 ± 1.3	0.5 ± 0.7	0.7 ± 0.9
Inoculated *L. camara* 1.25 mg/ml	5.7 ± 1.7	0.7 ± 0.8	1.1 ± 0.9
Inoculated *L. camara* 2.50 mg/ml	6.0 ± 1.2	0.5 ± 0.7	1.6 ± 1.2

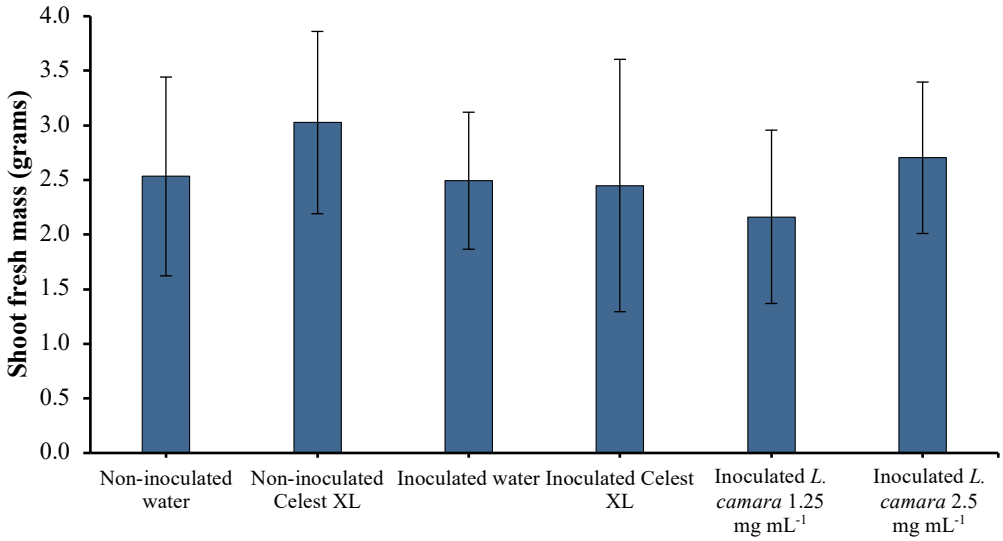

Figure S8: Average shoot fresh mass (grams) of the seedlings in the pot trial. No significant differences among the treatments were seen ($p > 0.05$).

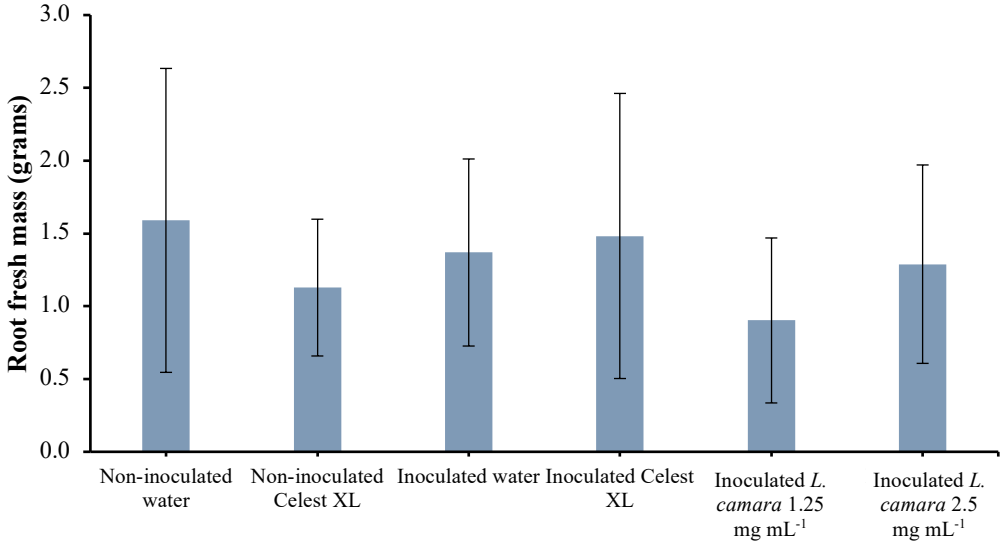

Figure S9: Average root fresh mass (grams) of the seedlings in the pot trial. No significant differences among the treatments were seen ($p > 0.05$).

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