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Research Article

Management of Fusarium Wilt of Cucumber Using Plant Extracts under *In Vitro* and High Plastic Tunnel Conditions in Balochistan, Pakistan

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ABSTRACT

Fusarium wilt of cucumber, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (*FOC*), poses a major threat to cucumber production, especially under protected cultivation systems such as high plastic tunnels. This study aimed to evaluate the antifungal efficacy of selected plant extracts against *FOC* under both *in vitro* and high tunnel conditions in Balochistan. *In vitro* assays demonstrated that all tested plant extracts significantly suppressed the radial growth of *FOC* compared to the untreated control. Turmeric and eucalyptus extracts exhibited the highest antifungal activity, reducing fungal colony diameters to 11 mm and 14 mm, respectively, at 12% concentration, compared to 85 mm in the control. Ginger and red radish showed moderate inhibition, while mint, black pepper, and garlic were the least effective. Based on *in vitro* results, turmeric, eucalyptus, and ginger were selected for further evaluation under high plastic tunnel conditions. Cucumber plants treated with these extracts exhibited a significant reduction in disease incidence (18.45-48.82%), plant mortality (16.65-36.80%), and root infection (20.32-52.42%) compared to untreated control plants (77.82%, 72.15%, and 75.50%, respectively). Eucalyptus was the most effective treatment across all parameters. Moreover, treated plants showed enhanced vegetative growth, with significant increases in shoot and root length, fresh biomass, and dry matter accumulation. The highest values for all growth parameters were recorded in eucalyptus-treated plants, followed by turmeric and ginger. In conclusion, turmeric and especially eucalyptus extracts show strong potential as eco-friendly solutions for managing *Fusarium* wilt in cucumber under protected cultivation, warranting further field-scale validation.

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Introduction

Cucumber (*Cucumis sativus* L.) is a significant and widely cultivated crop belonging to the family Cucurbitaceae. It is extensively grown across various continents and is originally native to South Asia. Globally, cucumber is cultivated on approximately 2.27 million hectares, yielding around 83.7 million tons with an average productivity of 37 tons per hectare (Tariq et al., 2020). China leads in global cucumber production, while Pakistan ranks 50th, with cultivation spread over 3.57 thousand hectares, producing 112.23 thousand tons at an average yield of 31.43 tons per hectare, mainly in Punjab, followed by Balochistan (GoP, 2024).

Cucumber is commonly consumed as a vegetable and,

due to its high water content (over 90%), is highly valued for its hydrating properties. It is a popular component of fresh salads and is also processed into pickles and relishes. Its high moisture content and low-calorie profile make it ideal for hydration and weight management (Sharma et al., 2020). Moreover, cucumber juice possesses antacid and carminative properties, helping regulate stomach acidity, relieve indigestion, and maintain pH balance in the digestive tract. It is also a rich source of essential vitamins such as vitamin C and K, and various antioxidants that contribute to overall health. Besides its nutritional benefits, cucumber also exhibits several medicinal properties (Sotiroudis et al., 2010; Nema et al., 2011; Sharma et al., 2012; Sharma et al., 2020). Cucumbers are often preserved as pickles, typically through fermentation in salt or vinegar, which not only enhances flavor and texture but also improves their nutritional profile (Uthpala et al., 2020).

The cultivation of cucumber in high plastic tunnels has gained popularity in Balochistan due to increasing demand, limited agricultural land, and water scarcity. These tunnels extend the growing season and enhance productivity by providing a controlled environment (Kumar et al., 2020). However, the enclosed conditions also create favorable environments for soil-borne diseases such as Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum*, which is transmitted through soil and water (Shen et al., 2008; Wang et al., 2021). Fusarium wilt can cause substantial yield losses ranging from 40% to 70% in affected areas (Sharma and Shukla, 2021). Continuous monocropping often leads to the accumulation of soil-borne pathogens and secondary salinization, exacerbating the challenge. It also increases the diversity and virulence of pathogenic fungi, reducing plant vigor and making crops more susceptible to pests and diseases (Meng et al., 2018; Cheng et al., 2020). The microclimate inside tunnels, characterized by elevated temperatures and humidity, provides optimal conditions for disease development (Agrios, 2005; Leslie and Summerell, 2006; Meyer et al., 2013; Abro et al., 2022). The pathogen typically invades the plant through the roots, leading to systemic wilt symptoms including leaf yellowing, wilting, stunted growth, and eventual plant death (Okungbowa and Shittu, 2012; Katan and Katan, 2019).

Managing Fusarium wilt is particularly challenging under environmental conditions that favor the development of the pathogen. Prolonged use of synthetic fungicides has led to the development of resistance in pathogen populations and contributed to environmental contamination, residual toxicity, and health concerns. Consequently, there is an urgent need to explore alternative, eco-friendly management strategies such as biopesticides, including plant-based fungicides (Yoon et al., 2013). Several studies have reported the antifungal efficacy of plant extracts such as turmeric (*Curcuma longa*), eucalyptus (*Eucalyptus globulus*), and ginger (*Zingiber officinale*) against soil-borne fungal phytopathogens (Cherkupally et al., 2017; Chen et al., 2018; Hassan, 2020; Xi et al., 2022).

Therefore, the present study was conducted to evaluate the antifungal potential of various plant extracts against *F. oxysporum* f. sp. *cucumerinum* (*FOC*), the causal agent of Fusarium wilt in cucumber, through *in vitro* assays and to further assess the most effective extracts under high plastic tunnels.

Materials and Methods

Isolation and identification of the pathogen

Root and stem samples from infected cucumber plants exhibiting characteristic symptoms of *F. oxysporum* were collected in paper envelopes from various tunnels in Balochistan. The samples were properly labeled, kept airtight, and transported to the Laboratory of Plant Pathology, Department of Plant Pathology, Sindh Agriculture University, Tandojam, for pathogen isolation and identification.

To expose the xylem tissue, plant samples were longitudinally sectioned (approximately 5 mm thick) near the stem base, including the xylem vessels. These sections were surface sterilized by immersion in 1% sodium hypochlorite solution for 1 min, followed by rinsing twice with sterile distilled water. Three to five tissue segments were placed on autoclaved Petri plates containing potato dextrose agar (PDA) medium. The plates were incubated at 27 °C for seven days.

Once fungal mycelial growth was observed, subculturing was performed on fresh PDA plates to obtain pure cultures. For morphological identification, PDA plates with robust fungal growth were examined macroscopically (colony characteristics) and microscopically. Colony morphology was assessed using a stereomicroscope, while microscopic features such as the shape and size of microconidia, macroconidia, and chlamydospores were observed under a compound microscope, following the protocol described by Leslie and Summerell (2006). The confirmed pure cultures were stored at 4°C for further studies.

Preparation of fungal conidial suspension

A conidial suspension of the test fungus was prepared from 10-day-old PDA cultures. Sterile distilled water (10 ml per plate) was added, and the mycelial mat was gently scraped using a sterile glass rod to release conidia. The resulting suspension was filtered through a double-layered muslin cloth into sterile tubes. The spore concentration was adjusted to 1×10^6 spores ml^{-1} using a hemocytometer (Abro et al., 2014; Vargas-Inciarte et al., 2019; Poussio et al., 2022).

Pathogenicity test

The pathogenicity of the fungus was assessed using a soil infestation method. Inoculations were carried out in 30 cm diameter earthen pots, each filled with 2 Kg of a sterilized soil mixture (sand:compost in a 2:1 ratio, w/w). Before inoculation, pots were rinsed with distilled water, air-dried, and surface-sterilized by spraying with ethanol to reduce the risk of contamination.

Each pot (except negative controls) was inoculated with 20 ml of the prepared spore suspension (1×10^6 spores ml^{-1}), which was thoroughly mixed into the upper 10 cm of soil using a sterile spoon. Pots were watered and incubated for one week to allow fungal establishment.

Seeds of the susceptible cucumber cultivar 'Mawasem' were surface sterilized by soaking in 2% sodium hypochlorite for 3 min, followed by three rinses with sterile distilled water. Seeds were sown in the prepared pots, and the development of wilt symptoms, seedling mortality, and root infection were monitored post-germination.

Preparation of plant extracts for phytoconstituent assessment

Plant materials used for extract preparation included ginger (rhizome), eucalyptus (leaves), turmeric (rhizome), mint (leaves), red radish (tubers), black pepper (seeds), and garlic (cloves) (Table 1). All samples were washed thoroughly with tap water followed by sterile distilled water, and then air-dried in the shade.

For extract preparation, 100 g of each plant material was ground separately in 100 ml of sterile distilled water using an electric blender. The homogenates were filtered through a double-layered muslin cloth and centrifuged at 4000 × g for 30 min. The clear supernatants were collected and stored at 4°C in sterilized glass bottles as stock solutions for subsequent studies (Cherkupally et al., 2017).

Table 1. Plant extracts used in the study.

English Name	Local Name	Scientific Name	Family	Plant Parts
Ginger	Adrak	*Zingiber officinale*	*Zingiberaceae*	Rhizome
Eucalyptus	Safedo	*Eucalyptus globulus*	*Myrtaceae*	Leaves
Turmeric	Haldhi	*Curcuma longa*	*Zingiberaceae*	Rhizome
Mint	Podena	*Mentha piperita*	*Lamiaceae*	Leaves
Red radish	Surkh moli	*Raphanus sativus*	*Brassiceae*	Tubers
Black pepper	Kali mirch	*Piper nigrum*	*Piperaceae*	Seeds
Garlic	Lehsan/Thoom	*Allium sativum*	*Alliaceae*	Cloves

In vitro* evaluation of plant extracts against *FOC

Plant extracts were evaluated at three different concentrations (4%, 8%, and 12%) using the Poisoned Food Technique. PDA medium was amended with the respective concentrations of each extract and poured into 90 mm sterile Petri plates. Each plate was inoculated with a 5 mm mycelial disc taken from the actively growing margin of a seven-day-old FOC culture. In control treatments, sterilized distilled water was used instead of plant extracts. The plates were incubated at 27°C, and each treatment was replicated three times. The experiment was arranged in a Completely Randomized Design (CRD). Radial fungal growth (in mm) was measured at 48-h intervals up to seven days post-inoculation. Based on their inhibitory effect, the most effective plant extracts were selected for further evaluation.

The percentage inhibition of fungal radial growth was calculated using the formula provided by Yelmame et al. (2010):

$$\text{Inhibition } \% = \frac{C - T}{C} \times 100$$

Where:

C = Radial growth of the pathogen in control,

T = Radial growth of the pathogen in treatment

Evaluation of selected plant extracts against Fusarium Wilt under high plastic tunnel conditions

Based on the *in vitro* results, the three most effective plant extracts, turmeric, eucalyptus, and ginger, were selected

for further evaluation under high plastic tunnel conditions at the Directorate of Vegetable Seed Production, Agriculture Research Institute, Quetta, Balochistan, from March to April.

The selected high tunnel had been under continuous cucumber monocropping for over nine years, resulting in natural soil infestation with *FOC*. Seeds of cucumber cultivar "Mawasem" were surface-sterilized using 2% sodium hypochlorite for 3 min, followed by three rinses with sterile distilled water. The seeds were then air-dried and sown on flat beds within the tunnel. Irrigation and fertigation were provided as per crop requirements via a drip irrigation system.

The experiment was laid out in a CRD with four replications per treatment. A total of 16 plots were maintained, each measuring 25 ft^2, with row-to-row and plant-to-plant spacings of 75 cm and 45 cm, respectively. One week after sowing, once the seeds had sprouted, the root zones were drenched with the most effective concentration (12%) of each selected plant extract. The control plots received no extract treatment.

Plants were regularly monitored for the appearance of symptoms associated with *FOC* infection. One month after extract application, plants were uprooted and assessed for disease incidence, plant mortality, and root infection percentages using the following formulas:

$$\text{Disease Incidence \%} = \frac{\text{Number of infected cucumber plants}}{\text{Total number of cucumber plant}} \times 100$$

$$\text{Plant Mortality \%} = \frac{\text{Number of dead plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{Root infection \%} = \frac{\text{Number of root pieces colonized by the fungus}}{\text{Total number of root pieces observed}} \times 100$$

Furthermore, plant growth parameters including shoot length, root length, shoot weight, and root weight were recorded.

Statistical analysis

All experiments were conducted in a CRD. The data were analyzed using analysis of variance (ANOVA) to determine significant differences among treatments. Means were compared using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$. Statistical analyses were performed using the Statistix 8.1 software package. Results are presented in graphical form, with standard errors indicated on the respective bars.

Results

In vitro* evaluation of plant extracts against *FOC

The experimental results demonstrated that all tested plant extracts inhibited the radial growth of *FOC* compared to the untreated control. Among the extracts, turmeric and eucalyptus were the most effective, significantly suppressing fungal growth at all tested concentrations. At the highest concentration (12%), turmeric and eucalyptus reduced colony diameter to 11.00 mm and 14.00 mm, respectively, compared to the control (85.00 mm). Even at the lowest concentration (4%), these extracts maintained considerable antifungal activity, with colony growth measuring 18.00 mm and 34.33 mm, respectively.

Ginger and red radish also exhibited antifungal activity, with colony diameters of 21.00 mm and 22.66 mm, respectively, at the 12% concentration. In contrast, mint, black pepper, and garlic were the least effective, showing colony growth of 65.33 mm, 57.33 mm, and 50.16 mm, respectively, at 12%. Furthermore, at lower concentrations (8% and 4%), these extracts showed limited inhibitory effects on fungal growth (Figure 1, 2).

Evaluation of selected plant extracts against *Fusarium* wilt in high plastic tunnel

The findings of the *in vitro* study provided valuable understandings for selecting the most effective plant extracts, turmeric, eucalyptus, and ginger, which were further evaluated against *Fusarium* wilt in cucumber plants grown under high plastic tunnels. The results from the tunnel trials demonstrated a significantly lower disease incidence (18.45-48.82%) in plants treated with plant extracts compared to untreated control plants (77.82%). Among all tested extracts, eucalyptus showed the lowest disease incidence (18.45%), followed by turmeric (28.82%), while the highest incidence among the treatments was recorded in plants treated with ginger (48.82%) (Figure 3A).

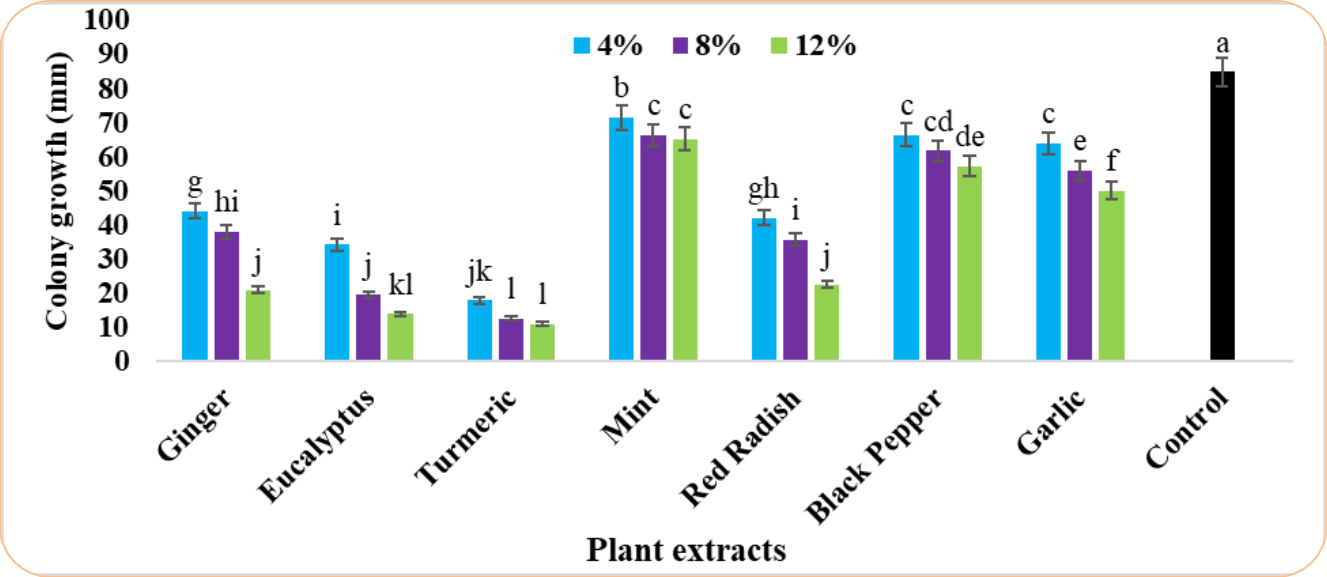


Figure 1. *In vitro* effect of various plant extracts on the colony growth of *F. oxysporum*. Different letters above the bars indicate statistically significant differences between means at P ≤ 0.05.

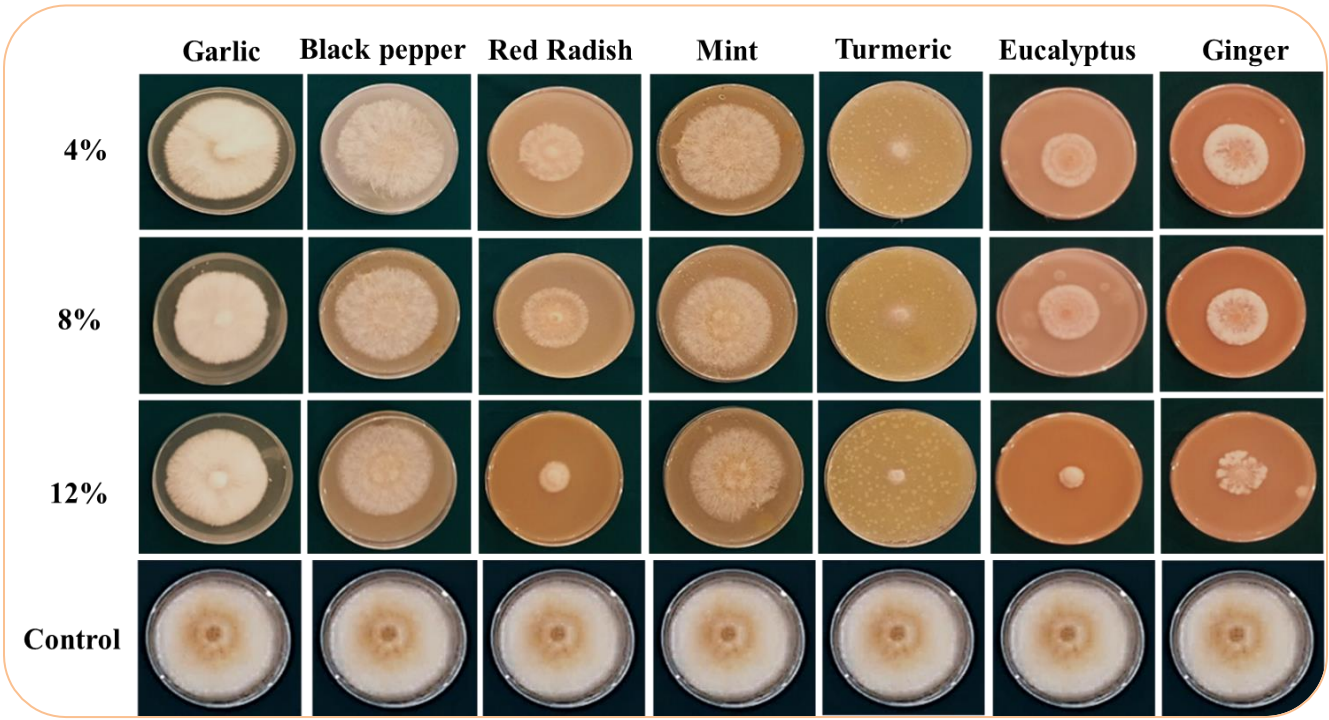


Figure 2. *In vitro* effect of various plant extracts on the mycelial growth of *F. oxysporum* compared to the control.

Similarly, plant mortality was significantly reduced in the treated plants, ranging from 16.65% to 36.80%, compared to 72.15% in the control. Eucalyptus again exhibited the lowest plant mortality (16.65%), followed by turmeric (22.60%), while ginger treatment resulted in the highest mortality among the treated plants (36.80%) (Figure 3B).

Root infection levels also decreased significantly in treated plants (20.32-52.42%) compared to the control (75.50%). The lowest root infection was observed in eucalyptus-treated plants (20.32%), followed by turmeric (31.25%) and ginger (52.42%) (Figure 3C). These reductions in disease incidence, plant mortality, and root infection were statistically significant (P ≤ 0.05), as determined by ANOVA followed by LSD tests.

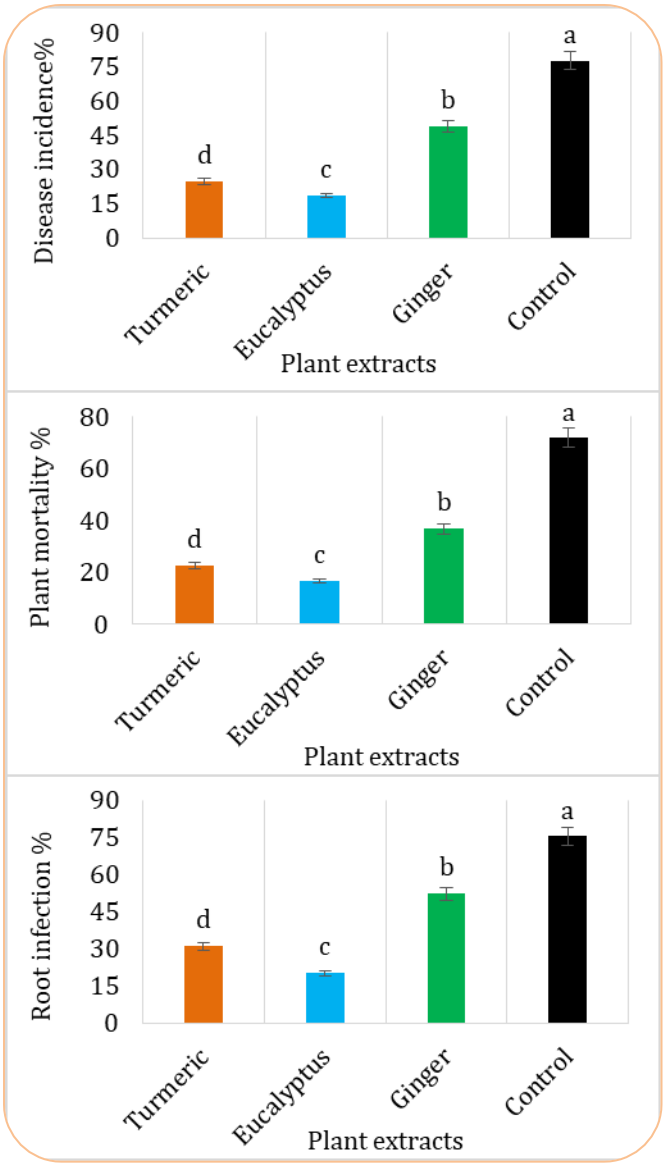


Figure 3. Effects of different plant extracts on (A) disease incidence, (B) plant mortality, and (C) root infection (%) in cucumber plants. Different letters above the bars indicate statistically significant differences at P ≤ 0.05.

Shoot and root length

Application of plant extracts significantly (P ≤ 0.05) suppressed *Fusarium* wilt and enhanced both shoot and root length in cucumber plants. Shoot lengths ranged from 36.65 to 49.27 cm and root lengths from 8.82 to 14.42 cm among treated plants. The maximum shoot (49.27 cm) and root (14.42 cm) lengths were recorded in eucalyptus-treated plants, followed by turmeric (47.62 cm shoot and 13.90 cm root). The minimum shoot and root lengths were observed in ginger-treated plants (36.65 cm and 8.82 cm, respectively) (Figure 4A, B).

Fresh shoot and root weight

All plant extracts significantly (P ≤ 0.05) increased the fresh biomass of cucumber plants. The highest fresh shoot (155.00 g) and root (10.80 g) weights were obtained from eucalyptus-treated plants, followed by turmeric (140.80 g shoot and 9.35 g root). The lowest fresh weights were recorded in ginger-treated plants, with 109.85 g shoot and 6.62 g root weight, respectively (Figure 4C, D).

Dry shoot and root weight

The dry shoot and root weights were also significantly (P ≤ 0.05) enhanced by the application of plant extracts. Eucalyptus-treated plants recorded the highest dry shoot (60.45 g) and root (2.50 g) weights, followed by turmeric (57.70 g shoot and 2.02 g root). Ginger-treated plants showed the lowest dry shoot and root weights (45.40 g and 1.25 g, respectively) (Figure 4E, F).

Discussion

High plastic tunnels are widely adopted in Balochistan due to their ability to provide a controlled environment, enhance cucumber production, and extend the growing season. However, the unique microclimatic conditions inside these tunnels favor the proliferation of soil-borne pathogens, including *F. oxysporum*, the causal agent of *Fusarium* wilt. Continuous monocropping further exacerbates the pathogen's virulence (Shen et al., 2008; Meng et al., 2018; Cheng et al., 2020; Wang et al., 2021).

In the present *in vitro* evaluation, plant extracts of turmeric, followed by eucalyptus and ginger, were found to be the most effective against the test fungus. The antifungal efficacy of these extracts increased with concentration. Conversely, extracts of mint, black pepper, and garlic showed comparatively lower antifungal activity. These results align with previous findings by Chen et al. (2018), Koondhar et al. (2023), and Abro et al. (2019), who reported that turmeric extract possesses potent antifungal compounds effective against multiple fungal species, including *Fusarium* spp. Similarly, Xi et al. (2022) demonstrated that ginger rhizome extracts completely inhibited the growth of *Fusarium* spp. by interfering with key enzymatic activities related to cell wall degradation and respiration, thereby impairing the pathogen's infectivity. Cherkupally et al. (2017) also reported that eucalyptus extracts inhibited the mycelial growth of *F. oxysporum* by 61.8%. Supporting this, Ramaiah and Garampalli (2015) screened extracts of fifteen plant species and found several effective in suppressing *F. oxysporum* growth, indicating the potential of plant-based antifungal agents in sustainable disease management.

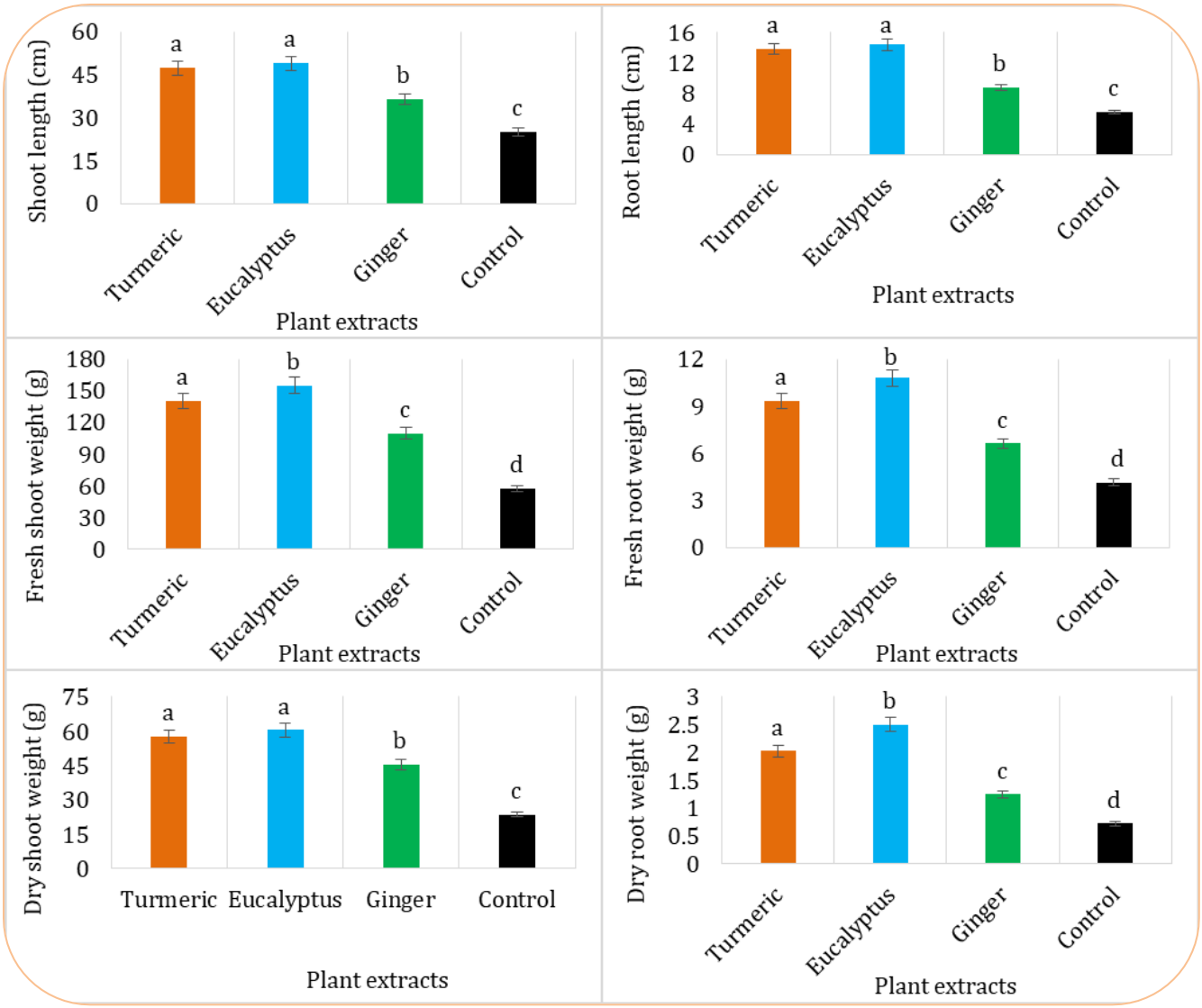


Figure 4. Effects of different plant extracts on (A) shoot length, (B) root length, (C) fresh shoot weight, (D) fresh root weight, (E) dry shoot weight, and (F) dry root weight of cucumber plants. Different letters above the bars indicate statistically significant differences between means at $P \leq 0.05$.

In the high plastic tunnel trials, the same three plant extracts also exhibited significant effectiveness against *Fusarium* wilt in cucumber. Eucalyptus extract was the most effective, followed by turmeric, showing a substantial reduction in disease incidence, plant mortality, and root infection. These treatments also improved plant growth parameters, including shoot and root length and fresh and dry biomass. Ginger, while effective, exhibited relatively lower efficacy compared to eucalyptus and turmeric. These findings indicate not only antifungal potential but also a positive impact on plant vigor under tunnel cultivation.

The results are consistent with the findings of Fradi (2022), who reported that eucalyptol in eucalyptus extract at a 12% concentration completely inhibited *F. oxysporum* growth. Eucalyptus-derived compounds are known to disrupt fungal cell walls and inhibit spore germination. Moreover, studies by Moghadamtousi et al. (2014), Chowdhury et al. (2008), Xi et al. (2022), and Nizamani et al. (2020) have confirmed the antimicrobial properties of bioactive compounds such as curcumin in turmeric and gingerols in ginger, which are capable of inhibiting or killing phytopathogenic fungi.

Yoon et al. (2013) emphasized that prolonged use of synthetic fungicides has led to resistance development in pathogens, environmental contamination, and residual

toxicity. These concerns underscore the need for sustainable alternatives, such as plant-derived biopesticides, including botanical fungicides, for integrated disease management.

Conclusion

The findings of this study suggest that certain plant extracts, particularly those from eucalyptus and turmeric, offer effective alternatives to synthetic fungicides for the management of *Fusarium* wilt caused by *F. oxysporum*. These extracts demonstrated significant antifungal activity both *in vitro* and under high plastic tunnel conditions, also contributing to improved plant health and growth. Future research should focus on understanding the environmental interactions, plant physiological responses, and the identification and standardization of the active constituents within these plant extracts for incorporation into broader, sustainable disease management programs.

Authors' Contributions

Adnan and MAA conceptualized and designed the study, developed the experimental framework, and created the figures; Adnan collected samples, conducted the experiments, analysed the data, and drafted the manuscript. JDH and RNS critically reviewed, edited, and proofread the manuscript for clarity, accuracy, and scientific rigor.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

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