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In-vitro Antifungal Activity of Medicinal Plant Extracts against *Fusarium oxysporum* Causing Wilt in Okra

Ameer Jan[1], Muhammad Hamayun[2], Muhammad Ahsan[3], Mufeed Hadi[3], Aatiq u Rehman[2], Tariq Ali[4], Shaikh Saddam[5], Sami Ullah[6], Ghulam Yasin Shaikh[7], Mushtaq Ahmed[2]

[1]Department of Botany, University of Makran, Panjgur, Balochistan, Pakistan.
[2]Department of Plant Pathology, Muhammad Nawaz Shareef University of Agriculture, Multan, Punjab, Pakistan.
[3]Department of Plant Pathology, University of Agriculture, Faisalabad, Punjab, Pakistan.
[4]Department Entomology, University of Sargodha, Punjab, Pakistan.
[5]Department of Plant Pathology, Faculty of Agriculture, Lasbela University of Agriculture Water and Marine Sciences, Uthal, Balochistan, Pakistan.
[6]Department of Horticulture, Muhammad Nawaz Shareef University of Agriculture, Multan, Punjab, Pakistan.
[7]National Nematological Research Centre, University of Karachi, Sindh, Pakistan.

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Correspondence to: Ameer Jan, Department of Botany, University of Makran, Panjgur, Balochistan, Pakistan.
Email: ameerjan@uomp.edu.pk

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ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum*, is a devastating disease of okra (*Abelmoschus esculentus*) that can significantly reduce yield and quality. Chemical fungicides have been the primary means of control but pose environmental and health risks. This study evaluated the *in-vitro* antifungal activity of crude ethanolic extracts from *Withania coagulans* leaves, *Zataria multiflora* leaves, and *Cuminum cyminum* seeds against *F. oxysporum* f. sp. *vasinfectum*. Extracts at concentrations of 2%, 4%, and 8% were incorporated into potato dextrose agar (PDA) and compared to the fungicide Antracol (propineb, 0.5%) and control. Mycelial growth was measured daily for 7 days, and the percentage of growth inhibition was calculated. *Z. multiflora* exhibited the greatest inhibitory activity, reducing mycelial growth by 70%, 75%, and 82% at 2%, 4%, and 8% concentrations, respectively. *C. cyminum* showed significant antifungal activity, with inhibitions of 63%, 74%, and 77% at the same concentrations. *W. coagulans* was the least effective, producing 34%, 44%, and 59% reductions. Antracol outperformed all plant extracts, reducing growth by 94%. The results indicate the potential of *Z. multiflora* and *C. cyminum* extracts as environmentally friendly alternatives for managing Fusarium wilt in okra. However, higher concentrations and optimization of extraction methods are needed to compete with conventional fungicides. Future studies should focus on in vivo and field evaluations, identification of active compounds, and integration with other disease management strategies for sustainable okra production.

INTRODUCTION

Okra (*Abelmoschus esculentus*), also known as lady finger or bhindi, is a warm season vegetable crop that is grown in large quantities in tropical and subtropical parts of the world and warm temperate zones (Gemede *et al*., 2015). Okra belongs to the Malvaceous group where its value lies in the green edible pods that contain Vitamin, A, C and K, dietary fibers and antioxidants. Its culinary applications, including its usage in soups, stews, fried or pickled foods, make it very much a dietary staple in many parts of the world, especially Africa, Asia and in the southern United States (Singh *et al*., 2014). Okra is of high agricultural and food security value besides its nutrition value due to its adaptability to various agro ecological conditions, low input ratio and its economic value to smallholder farmers (Jafari *et al*., 2025). Nevertheless, the work on okra is associated with significant difficulties, in particular due to diseases, and one of them is Fusarium wilt, which can badly traumatize both the productive volume and the quality component of okra (Ounis *et al*., 2024).

One of the diseases that have adversely impacted production of okra globally is Fusarium wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* fungus found in the soil (Kumar *et al*., 2014). The vascular wilt disease affects the xylem of the plants hence interposes the flow of water and nutrients leading to the wilting and yenning of the plants which subsequently ends up dying (Agrios, 2005). This pathogen presents a great difficulty due to its capacity to survive in the soil over long durations even without a host, which complexifies the crop rotation and soil

management solutions (Ounis *et al*., 2024). The disease causes leaf chlorosis, stunted growth, and early pod drop in okra and all these have the capacity to significantly reduce the marketable harvest (Jayasimha *et al*., 2014). The consequences of Fusarium wilt on okra farming are quite remarkable especially in areas where monoculture is common. Field losses as a result of severe infestation are recorded between 20-80% with small holder farmers recorded to be the most hit especially in developing countries due to poor access to resistant varieties or chemicals (Eziashi *et al*., 2006). The disease does not only affect crop yield but also affects the quality of pod which makes them not marketable due to discoloration or deformation. This is directly becoming detrimental to food security because low production worsens the food crisis and loss to the farmers due to the dependence of the farmers on okra as the main source of their income (Aguiar *et al*., 2014). Control of Fusarium wilt is also not easy due to the stubbornness of the pathogen. Cultural methods to be applied include crop rotation with non-host plants and soil solarization as well as resistant varieties, which are, however, under-utilised due to their affordability and scarcity (Shah *et al*., 2015). The use of chemical fungicides has a mixed record fighting *Fusarium oxysporum* at the appropriate time or when they are already mature in the answer, and environmental and health issues have to be accepted. Some potentially useful but still under investigation biocontrol approaches are the introduction of antagonistic microorganisms, including *Trichoderma* spp. (Shehzadi *et al*., 2025). Resistance to okra breeding or genetic engineering can be done, which is very important research that will reduce the effects of Fusarium wilt of okra and promote sustainable production (Yaseen *et al*., 2024).

Chemical fungicides have a long history of being the main pillars of disease control in crop production like the management of Fusarium wilt in okra. Nevertheless, their application comes with enormous drawbacks that undermine their effectiveness, sustainability to the environment and sustainability (Sharma *et al*., 2007). To begin with, most of the soil borne pathogens like the *Fusarium oxysporum* are resilient to the use of chemical fungicides when they take root in the ground. The fungus becomes resistant by developing structures that survive adverse conditions like chlamydospores that make it insensitive to fungicides (Agrios, 2005). Second, excessive chemical fungicide has led to the rise of the resistant strains of pathogens. The constant use of fungicides puts a selection force that causes a genetic alteration in the fungi, all of which have diminished or lost functionality of the chemicals (Mahmood *et al*., 2021). Resistance of some *Fusarium* species to popular fungicides like benomyl has been reported to hinder the control of the diseases (Shah *et al*., 2014). Third, methodological risks to the environment and massive health hazards of utilizing chemical fungicides also manage environmental risks to human health. Treated fields have the potential to be sources of runoff that can enter other water bodies to toxicify the aquatic environment and degrade biodiversity (Halstead *et al*., 2014). There is also the problem of non-target impacts on the beneficial microorganisms in the soil, nitrogen-fixing bacterium, further impairing the quality of soil, thus the long-term productivity of agriculture (Ray & Shaju, 2023; Anjaria & Vaghela, 2024). The risks to human health are also high since exposure to pesticides causes respiratory problems, nerve disorders, and higher risks of cancer in farmers and consumers due to food contamination (Mehmood *et al*., 2020; Omeje *et al*., 2022).

Lastly, the availability and costs reduce the application of chemical fungicides in poor-resource environments. The fact that there are high costs and low supply of quality fungicides inhibits their usage by the small holder farmer in places like sub-Saharan Africa where symptoms like Fusarium wilt have been prevalent ways of it (Muzari *et al*., 2012). Environmental and health concerns result in regulation of use of some fungicides in certain countries which further curtail the supply of some fungicides resulting in the need to develop alternative solutions. (Ajilogba and Babalolan, 2013). The short comings of chemical fungicides have triggered the exploration of alternative forms of disease control as well as the vaccine against fungi especially in the use of medicinal plants and their extracts to check fungal diseases based on antifungal properties. There is abundant in secondary metabolism of medicinal plants like the alkaloids, flavonoids, terpenoids, and phenolics that have effective antimicrobial activities (Thembo *et al*., 2010; Rongai *et al*., 2015). These natural products provide a good direction towards sustainable, eco-sustainable and affordable solutions of controlling plant diseases Fusarium wilt. The experiment was performed in a confined space (Cardenas-Laverde *et al*., 2021).

One of the key reasons of investigating the extracts is the possibility to break the pathogen resistance. Plant extracts are also bioactive since they harbor a variety of compounds that may have multiple mechanisms of action and may be acting synergistically, which decreases the potential of resistance (Mustafa *et al*., 2024; Teixeira *et al*., 2023). Other strong reasons are environmental and human health safety as plant extracts neem (*Azadirachta indica*), garlic (*Allium sativum*) showed antifungal effect on *Fusarium* species by destroying fungal cell membrane and preventing spores' germination (Talpur *et al*., 2023). The use of plant based antifungals would be less harmful to the environment with limited persistence on the environment unlike the manmade chemicals which could be very harmful to the environment (Bajpai & Kang, 2009). They are also less likely to be toxic to people and non-target species as they are of a natural origin, which makes them fall into the circle of demands of organic and sustainable agricultural methods. This will especially be applicable in areas where okra is an important crop since most consumers now favor residue-free fruits and vegetables (Mohapatra *et al*., 2024).

Plant based solutions are needed due to economic accessibility. Medicinal plants could be found locally and could be processed in simple low-cost ways; therefore, such medicine plants are acceptable to smallholder farmers when they do not allow cost inflation due to high chemicals (Nwafor *et al*., 2021; Mulugeta *et al*., 2024). With a low-infrastructure input, extracts of widely available plants can be made, turmeric (*Curcuma longa*) offering a cost-effective option to manage diseases in resource-limited environments (Seepe *et al*., 2020; Drinkwater &

Snapp, 2022). Lastly, the investigation into the potency of medicinal plant extracts also corresponds with the international desire to facilitate a more sustainable food production utilizing the least amount of non-organic chemicals. As regulators and consumers take greater interest in product chemical-residue load, plant-derived antifungals present an avenue to achieve both commercial and environmental objectives (Gitahi *et al*., 2021). The study of optimization of extraction methods, determination of the active compounds, and scaling up the use of such compounds are essential steps toward the achievement of their maximum potential (El Tannir *et al*., 2024; Hasan *et al*., 2020). The antifungal activity of *Withania coagulans*, *Zataria multiflora* and *Cuminum cyminum* had been previously investigated owing to their potentials as natural sources of antifungal activity, as they are rich in phytochemicals (Nosratabadi *et al*., 2023).

Withania coagulans commonly referred to as paneer dodi or Indian rennet, it is an Indian and Pakistani medicinal shrub used in traditional medicine to treat various diseases, including diabetes and infections (Maher *et al*., 2020). The antifungal effect of *Withania coagulans* the is explained by the presence of withanolides which are steroidal lactones possessing antimicrobial properties. Its fruits and leaf extracts have been shown to have potent antifungal properties against Candida albicans and *Fusarium* species among others. Methanolic and ethanolic leaf extracts of *W. coagulans* have shown in vitro inhibition of *C. albicans* with a minimum inhibitory concentration (MIC) of 5 mg/ml, on par with other medicinal plants *Curcuma longa* (Cherkupally *et al*., 2017) (Goel *et al*., 2011).

Zataria multiflora A thyme like plant, belongs to the Lamiaceae family and is native to Iran, Pakistan and Afghanistan and its oil is well researched containing high thymol (25.2%) and carvacrol (61.3%) (Mahmoudabadi *et al*., 2006). These terpenoids are what makes it have very strong antifungal property against various fungi, *Candida* species, Aspergillus species, and Malassezia species. A systematic study involving 33 research studies revealed that *Z. multiflora* EO successfully worked to decrease or prevent the proliferation of Candida in vitro, in vivo, and in dental biofilm models, but there was no significant distinction in activity between susceptible and resistant Candida strains (Kavoosi & Rabiei, 2015). A second study showed its exceptional anti-Malassezia activity with inhibition zones of 28.1 mm of *Z. multiflora* EO against *Malassezia* spp isolated in pityriasis versicolor patient, and thus, it can be used in clinical application. Moreover, the herb is also known to provide relief to the mind, which is in line with the therapy used in the management of the disease (Kavoosi & Rabiei, 2015; Mahmoudabadi *et al*., 2006). Meanwhile, *Z. multiflora* EO was found to restrain the growth of *Aspergillus flavus* colony and production of aflatoxin through transcriptional-level reversing fungal plasma membrane and cell wall structure (Gandomi *et al*., 2011; Fatemi *et al*., 2022). This is because it has broad-spectrum antifungal efficacy and low toxicity, making it a potential fungicide in the natural product category (Neamati*et al*., 2016; Khatibi *et al*., 2016).

Cuminum cyminum Known as cumin, Apiaceae family) is a spice whose EO has proven antimicrobial properties as it contains alpha-pinene (30%), and limonene (21%) (Romagnoli *et al*., 2010). Research has also indicated that the treatment of *C. cyminum* EO is better than that of *Z. multiflora* and other herbs against some fungi. In a report on the efficacies of Malassezia species, *C. cyminum* EO demonstrated the most significant inhibition zones (48.3 mm) in comparison with those of *Z. multiflora* (28.1 mm) and P. graveolens (26.1 mm) (Kavoosi & Rabiei, 2015; Freires *et al*., 2014). It has been proved by another research to be effective in the case of Candida albicans with inhibition zones of 16-55mm, as well as an MIC range of 150-2300 g/ml, but at least, the effectiveness of candidate to treat candidiasis by its usage has been proved (Lee *et al*., 2007). *C. cyminum* EO also preserved *Aspergillus flavus* even when encapsulated in the chitosan-caffeic acid nanogels, allowing better preservation of the oil and increasing its performance (Ziaee *et al*., 2015). Its antifungal nature is further evidenced by the fact that it was used in active packaging material and it was observed that it suppressed foodborne fungus and bacteria there (Lee *et al*., 2007; Devecioglu *et al*., 2022). Taken together, these reports reveal that among *W. coagulans*, *Z. multiflora*, and *C. cyminum*, there are indeed relevant antifungal effects due to the presence of varied types of bioactive compounds. The research of *Z. multiflora* and *C. cyminum* has been done more comprehensively, especially on their EOs, but the research of *W. coagulans* is still less significant, but there is still some potential, particularly on Candida and soil-borne fungi. In the mentioned substances, the effects of the same reaction under analysis are expressed differently in the case of 5-methylcytosine and 5-hydroxycytosine, where 5-methylcytosine has the same effect that 5-hydroxycytosine has on one reaction and the opposite effect on the other (Kumar *et al*., 2016). So, the aim of the study was to estimate the antifungal action of crude ethanolic extracts of *Withania coagulans*, *Zataria multiflora* and *Cuminum cyminum* on *Fusarium oxysporum* f. sp. *vasinfectum* which is causing Fusarium wilt disease in okra and to compare the *in-vitro* efficacy of these remedies with a conventional chemical fungicide under laboratory conditions.

Table 1

Figure of Chemical constituents and antimicrobial importance of Withania coagulans, Zataria multiflora, and Cuminum cyminum

Plant Name	Major Chemical Constituents	Antimicrobial Importance	References
Withania coagulans	Withanolides (withaferin A, withanolide A) Alkaloids Flavonoids Steroidal lactones	Inhibits *E. coli*, *S. aureus*, *Bacillus subtilis* Antifungal against *Aspergillus niger*, *Candida albicans*	Yasmin *et al*., 2025 Khan *et al*., 2021 Maurya *et al*., 2010
Zataria multiflora	Thymol Carvacrol Linalool p-Cymene Flavonoids	Strong antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa* Effective against *Candida spp.*	Ghorani *et al*., 2022 Khazdair *et al*., 2018 Shokri *et al*., 2017
Cuminum cyminum	Cuminaldehyde β-Pinene γ-Terpinene	Antibacterial against *Listeria monocytogenes*, *E. coli*, *Salmonella*	Mohammed *et al*., 2024 Srinivasan *et al*., 2018

p-Cymene Limonene	*typhi* Antifungal against *A. flavus*	Nadeem *et al.*, 2012

METHODS AND MATERIALS

Study Location

The experimentations were done at Plant Pathology Laboratory, Faculty of Agriculture, Lasbela University of Agriculture, Water and Marine Sciences (LUAWMS), Uthal, Balochistan, Pakistan. The lab was very well furnished with controlled climatic conditions like temperature and humidity to obtain accurate and reproducible results in the entire study.

Diseased Sample Collection

Samples of Fusarium wilt infected Okra (*Abelmoschus esculentus* L.) plants showing typical symptoms on wilting, chlorosis, vascular discoloration, and developing symptoms of stem necrosis were obtained from the Uthal district, Lasbela, Balochistan during the 2025 growing season. A stratified sampling procedure wwe used to provide a representative sample under various field conditions. At least 30 symptomatic plants/field were sampled and the samples consisted of roots, stems and leaves to capture the entire outlook of the disease symptoms. All the samples were packed separately in sterile polyethylene bags to avoid cross-contamination, and delivered at the Plant Pathology Laboratory of the LUAWMS within 24 hours of collection. Samples were kept refrigerated at 4 o C to conserve the viability of the pathogens during the process of sample storage.

Isolation and Identification of the Pathogen

Infected okra tissues (roots and stems) were used to isolate the pathogen after maintaining aseptic conditions as would normally occur in phytopathology. The samples were first washed through running tap water to remove soil and other debris then sectioned into 5-10 mm pieces using sterile scalpel. The surface sterilization involved immersion of the segments in 1% sodium hypochlorite (NaOCl) for 1 minute after which they were rinsed thrice in sterile distilled water to get rid of the sterilant. The sterilized segments were blotted dry on Whatman No. 1 filter paper under laminar flow hood to make them sterile.

The sterilized segments of the tissues were aseptically picked over the Potato Dextrose Agar (PDA; Difco, USA) medium with streptomycin sulfate, 50 mg/L, to prevent bacterial growth. The Petri plates were seeded at 28 +/- 2 C with 12 passing light conditions which were incubated towards 7 days to allow the fungal growth. Incubation colonies showing early growth of fungi were sub-passaged on to PDA plates to provide pure cultures. The single pathogen was identified as *Fusarium oxysporum* fsp. *vasinfectum* because of morphological appearance, microconidia, macroconidia, chlamydospore and colony appearance (Skovgaard *et al.*, 2001). The identification was performed with the aid of a compound microscope (Olympus BX51, Japan) at x400 to make microscopic examination. Pure cultures were stored in PDA slant at 4oc where they would be ready to use in further experiments.

Medicinal Plant Selection and Extract Preparation

The three medicinal plants were selected based on availability and literatures on their antifungal properties and they include work *Withania coagulans* (Dunal) leaves, *Zataria multiflora* Boiss. leaves and *Cuminum cyminum* L. seeds. Healthy, grown-up plants were harvested in the growing season of 2025. The identity of all the plant species was verified by a botanist at LUAWMS and voucher specimens are deposited in the university herbarium to be used in case of referencing.

Fresh leaves of *W. coagulans* and *Z. multiflora* were washed with tap water to remove the dust and debris and then dried in air at room temperature for 24 hours. The dried specimens were ground into fine powder by means of a sterile mortar and pestle. To extract, 50 gm powder of each of the plant species was taken and added to 500 mL of 95% ethanol in a 1 L conical flask. This mixture was shaken in an orbital incubator at 150 rpm overnight, 24 hours at 25°C in order to extract the bio active compounds. Slurry obtained was then filtered under vacuum as filter papers (Whatman No. 1) and the filtrates concentrated at 75°Cand 20 rpm using the rotary evaporator to evaporate the ethanol completely. The crude extracts were then dried on hot plate at 50°C to get a solvent free residue. The pure extracts were placed in sealed airtight containers and were kept at 4°C till the time of use. Prior to their working, 2 g of each crude extract was dissolved in 10 mL of ethanol, after which 90 mL of sterile distilled water was added to obtain a 2% (w/v) solution. Serial dilutions were then made to get final concentrations of 2%, 4%, and 8% which were used to perform antifungal assays.

Antifungal Bioassay

The antifungal effect of the medicinal plant extracts was also determined against a fungus *Fusarium oxysporum* f.sp. *vasinfectum* via the food poisoning method. The plant extracts were added in 2%, 4% and 8% to PDA medium. A 5-mm agar plug of a 7-day old pure culture of *F. oxysporum* f.sp. *vasinfectum* was placed at the center of each Petri plate with amended PDA. PDA was contained in control plates Treatments were replicated five times to provide statistical credence, and plates were incubated at 28 +/- 2C in a 12-hour photoperiod and incubated at room temperature of 7 days. Linear colony growth measurements were performed daily with the aid of a digital caliper and mycelial growth was expressed as percentage reduction in relation to the control with the following formula:

$$\textbf{Reduction= (\%)=} \frac{\text{Control growth} - \text{Treatment Growth}}{\text{Control growth}} \times 100$$

A standard fungicide, Antracol (0.5% concentration), was included as a positive control for comparison. The fungicide solution was prepared by dissolving 0.5 g of Antracol in 100 mL of distilled water, from which 0.5 mL was added to 100 mL of PDA medium. The amended PDA was autoclaved at 121°C for 15–20 minutes and poured into sterile Petri plates under aseptic conditions.

Treatment Formulations

The antifungal efficacy of the plant extracts was evaluated by incorporating them into Potato Dextrose Agar (PDA) medium using the food poisoning technique. The following treatments were applied:

1. **T1**: 2% plant extract (2 mL of 2% stock solution per 100 mL PDA).

2. **T2**: 4% plant extract (4 mL of 4% stock solution per 100 mL PDA).
3. **T3**: 8% plant extract (8 mL of 8% stock solution per 100 mL PDA).
4. **T4**: Standard fungicide (Antracol, 0.5% concentration; 0.5 mL of 0.5 g/100 mL solution per 100 mL PDA).
5. **T5**: Control (0% extract; PDA).

The positive control used was Antracol (propineb 70 percent active ingredient) fungicide. In order to accomplish the fungicide therapy, 0.5 g of Antracol was dispersed in 100 mL of sterile distilled water, and 0.5 mL of this preparation was added to 100 mL of molten PDA. Any amendments of PDA media were autoclaved at 121°C, 15 -20 minutes to sterilise them after which they were poured into clean 90-mm sterile Petri dishes in the presence of laminar flow hood. All the treatments were repeated five times to guarantee statistical soundness and duplicated plates were inoculated with 5-mm agar plug of a 7-day-old pure culture of *Fusarium oxysporum* f.sp. *vasinfectum*.

Preparation of Fungicide

Antracol (propineb, 70 per cent active ingredient) fungicide was included as a positive control to determine how effective the medicinal plant extracts were in antifungal effect against *Fusarium oxysporum* f.sp.. *vasinfectum*. The fungicide treatment was prepared by dissolving 0.5 g of Antracol in a sterile distilled water of 100 mL in a sterile conical flask to give a 0.5 percent (w/v) stock solution. Dissolution was done using a magnetic stirrer and the mixture was thoroughly stirred until complete dissolution at room temperature (25±2°C for 10 minutes. Depending on the stock solution, 0.5 mL accurate quantity of the stock solution using a calibrated micropipette was added to 100 mL of molten Potato Dextrose Agar (PDA; Difco, USA) at 45-0°C to prevent thermal degradation of the active ingredient. The water to which the fungicide had been added to the PDA was then thoroughly agitated. Zinc nitrate as well as sodium citrate have adolescence where the entrance of the stem cell into puberty is offered (Van Der Weerden *et al*., 2008).

The PDA medium was sterilized through autoclaving at 121°C and 15 psig, at least 15 minutes to 20 minutes. Once autoclaved, the medium was cooled to between 45-50°C under laminar flow hood to help it remain sterile, after which it was poured into sterile petri plates (90 mm) and allowed to set. Each plate would have 20 mL of medium or so as to allow same level of fungal growth as assessed. The plates were wrapped up and kept at 4C at max 24 hours before they were used in order to preserve the integrity of the fungicide and medium.

Statistical Analysis

This experiment was done under a Completely Randomized Design (CRD) and five replications were taken per treatment to add power and incorporate the experimental variation. Three concentration levels (2%, 4%, and 8% of crude extracts of *Zataria multiflora*, *Withania coagulans* and *Cuminum cyminum*, 0.5% of a standard fungicide Antracol and control) were used in the treatments. Data on the linear growth of colonies and % reduction in mycelial growth of *Fusarium oxysporum* f.sp. *vasinfectum* was recorded after each 24 hours of incubation period for a total of 7 days.

The statistical analysis was carried out in the R software (version 4.2.1). Tests were done to assess whether the mean value of the data are normally distributed in relation to ANOVA requirements by use of Shapiro-Wilk test and homogeneity of the variances in relation to ANOVA assumptions using Levene test. Data on colony growth in a linear manner passed through one Mach analysis of variance (ANOVA) to be able to ascertain the existence of any significant differences among treatments. The post-hoc comparisons of treatment means were carried out after significant effects were indicated (p 0.05) using the Tukey HSD test to determine how certain treatments differed. The percentage growth inhibition of mycelia was also determined against the control values and analyzed accordingly. Statistical procedures were done in accordance with tips given by Gomez and Gomez (1984). Data was reported as mean values ± standard error and the level of significance was taken at the 5 percent probability (p = 0.05).

RESULTS

In-vitro Efficacy of Medicinal Plant Extracts against Linear Colony Growth of *Fusarium oxysporum f.sp. vasinfectum* Causing Fusarium wilt Disease of Okra

Balochistan, Pakistan, is known to have a rich diversity of native medicinally important plants most of which have strong antifungal activity against phytopathogens. The *in-vitro* action of crude ethanolic extracts of three medicinal plants leaves of *Withania coagulans* (Dunal), leaves of *Zataria multiflora* Boiss., and seeds of *Cuminum cyminum* L. against *Fusarium oxysporum* f.sp. *vasinfectum*, was evaluated in the present study as the causal agent of the Fusarium wilt disease in okra (*Abelmoschus esculentus* L.). The anti-fungal properties were evaluated using food poisoning method where extracts of different concentrations of 2, 4 and 8 percent were added to Potato Dextrose Agar (PDA) to determine their presence. Antracol (propineb, 0.5 percent concentration) was used as standard fungicide and unamended PDA was used as negative control. Mycelial growth was determined in culture media about 0.1 cm of the colony length was measured manually each day up to 8 days incubation period at 28°C and percentage of the colony growth inhibition was calculated as compared to the control.

The results as shown in **Table 3.1** show that there is a marked difference in the antifungal activity of the tested plant extracts. Within all the concentrations tested, *Zataria multiflora* exhibited the greatest inhibitory activity, with 70% (2.59 ± 0.12 mm) 75% (2.15 +/- 0.10 mm) and 82 (1.50 +/- 0.08 mm) using 2%, 4% and 8% concentrations, respectively, as compared to the control (8.70 +/- 0.15 mm). The effectiveness of *Z. multiflora* can be explained by a significant concentration of thymol and carvacrol compounds that can ruin the membranes of fungal cells and prevent spore germination. *Cuminum cyminum* also showed significant antifungal ability with reduction of mycelial growth by 63 (3.14 +/- 0.14 mm), 74 (2.28+/- 0.11 mm) and 77 (1.94 +/- 0.09mm) percentages at 2%, 4 and 8 percentages of concentration, respectively.

Antifungal activity of *C. cyminum* could be attributed to cuminaldehyde and other volatile oils as this disturbs fungal metabolism.

Conversely, *Withania coagulans* exhibited the lowermost efficacy out of all the tested extracts where their inhibitory effects were 34% (5.71 + 0.18 mm), 44% (4.85 + 0.16 mm), and 59% (3.56 + 0.13 mm) at concentration levels of 2%, 4%, and 8%, respectively. *W. coagulans* has the antimicrobial potential of withanolides and alkaloids; nonetheless, it may not be as effective because it may not have enough concentrations of these metabolites or because they might not be effective against *F. oxysporum* f.sp. *vasinfectum*. The typical fungicide, Antracol, was even more effective and inhibited mycelial growth by 94% (0.56 0.03 mm), which accentuates not only its antibiotic potency but also the adverse environmental and health effects of such chemical substances.

Z. multiflora at the concentration of 8 percent proved to be more effective than any other plant extract, but less effective compared to Antracol. The results indicate that *Z. multiflora* and *C. cyminum* extracts, especially at high concentrations, have potential in using them as eco-friendly solutions to managing Fusarium wilt in okra.

Table 3.1

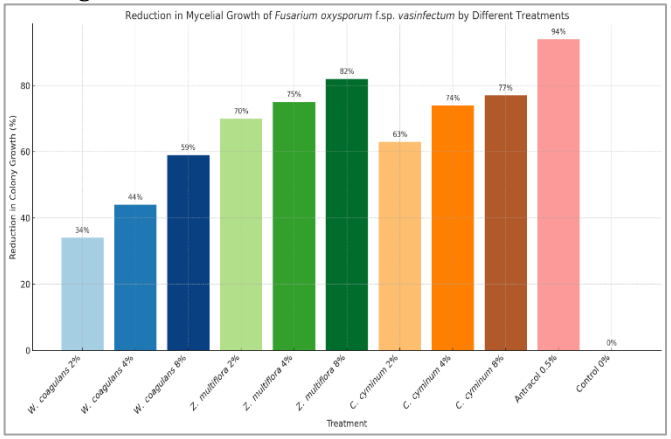
In-vitro Efficacy of Medicinal Plant Extracts against Linear Colony Growth of Fusarium oxysporum f.sp. vasinfectum Causing Fusarium wilt Disease of Okra

Treatments	Dose (%)	Linear Colony Growth (cm)							Reduction in Colony Growth (RCG) (cm)	Reduction (%)
		1st Day	2nd Day	3rd Day	5th Day	7th Day	8th Day	Total Average Growth	C - TCG	RCG (100)/C
Withania coagulans	2%	0.10 ± 0.01	0.45 ± 0.02	0.70 ± 0.03	1.61 ± 0.05	3.94 ± 0.10	5.71 ± 0.18	5.71 ± 0.18	2.99	34%
	4%	0.50 ± 0.03	0.85 ± 0.04	1.16 ± 0.05	2.56 ± 0.08	3.46 ± 0.12	4.85 ± 0.16	4.85 ± 0.16	3.85	44%
	8%	0.05 ± 0.01	0.26 ± 0.02	0.50 ± 0.03	1.16 ± 0.04	2.55 ± 0.09	3.56 ± 0.13	3.56 ± 0.13	5.14	59%
Zataria multiflora	2%	0.05 ± 0.01	0.18 ± 0.01	0.31 ± 0.02	0.69 ± 0.03	1.74 ± 0.06	2.59 ± 0.12	2.59 ± 0.12	6.10	70%
	4%	0.08 ± 0.01	0.11 ± 0.01	0.26 ± 0.02	0.96 ± 0.04	1.72 ± 0.07	2.15 ± 0.10	2.15 ± 0.10	6.55	75%
	8%	0.01 ± 0.00	0.05 ± 0.01	0.15 ± 0.01	0.45 ± 0.02	0.95 ± 0.05	1.50 ± 0.08	1.50 ± 0.08	7.20	82%
Cuminum cyminum	2%	0.05 ± 0.01	0.30 ± 0.02	0.48 ± 0.03	1.27 ± 0.05	2.73 ± 0.09	3.14 ± 0.14	3.14 ± 0.14	5.56	63%
	4%	0.03 ± 0.01	0.16 ± 0.01	0.32 ± 0.02	0.85 ± 0.04	1.53 ± 0.06	2.28 ± 0.11	2.28 ± 0.11	6.42	74%
	8%	0.03 ± 0.01	0.21 ± 0.01	0.29 ± 0.02	0.85 ± 0.03	1.48 ± 0.06	1.94 ± 0.09	1.94 ± 0.09	6.75	77%
Standard Fungicide	0.5%	0.00 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.13 ± 0.01	0.32 ± 0.02	0.56 ± 0.03	0.56 ± 0.03	8.14	94%
Control (C)	0%	0.25 ± 0.02	0.73 ± 0.03	1.08 ± 0.04	2.43 ± 0.08	5.73 ± 0.12	8.70 ± 0.15	8.70 ± 0.15	0.00	0%
LSD (0.05 level)									0.10	

Note: Values represent means ± standard error of five replications. Linear colony growth was measured daily over 8 days. Reduction in colony growth (RCG) was calculated as the difference between control growth (C) and treatment growth (TCG). Percentage reduction was calculated as (RCG × 100)/C. LSD = Least Significant Difference at p ≤ 0.05.

Figure 3.1

In-vitro Efficacy of Medicinal Plant Extracts against Linear Colony Growth of *Fusarium oxysporum* f.sp. *vasinfectum* Causing *Fusarium wilt* Disease of Okra



The **Figure 3.1** shows that the extracts of *Zataria multiflora* and *Cuminum cyminum* are among the complete and significantly eco-friendly alternatives to using eco-unfriendly chemicals in controlling the prevalence of Fusarium wilt in okra and specifically that of *Z. multiflora* is the most efficient plant extract to use in the management of the disease in the okra plant. The chemicals however outperformed the plant extracts with the chemical fungicide, Antracol, being the most active. The moderate efficacy of *Withania coagulans* also entailed the possibility of a further increase in its antifungal activity with the increase of its concentrations or improvement of the extraction process. These findings could support the development of *Z. multiflora* and *C. cyminum* as possible alternatives to disease management techniques because they have sustainable potential as alternatives to chemical fungicides; however, optimization is required to compete with conventional chemical fungicides.

RECOMMENDATIONS AND CONCLUSION

The *in-vitro* antifungal activity of *Withania coagulans* leaf crude ethanol and *Zataria multiflora* and *Cuminum cyminum* crude ethanol extracts were examined and compared against *Fusarium oxysporum* f. sp. *vasinfectum*, the fungus pathogen of Fusarium wilt of okra. Incorporation of extracts was done at 2%, 4 and 8 percent rate of PDA which was compared to the fungicide Antracol (0.5) and a control which was not amended. The concentration that produced the greatest inhibitory effect was on *Z. multiflora*, which suppressed the mycelial growth by 70, 75 and 82 percentages at 2, 4 and 8 percent concentrations respectively. *C. cyminum* showed a lot of antifungal activity, with inhibitions of 63, 74, and 77 % at the same concentrations as well. *W. coagulans* was least effective, and it produced 34, 44 and 59 percent reductions. Antracol seemed to do better than any plant extract (it cut growth by 94%). The results indicate the potential of *Z. multiflora* and *C. cyminum* extract in managing Fusarium wilt in okra since they are environmentally friendly according to the results obtained but the stronger concentrations of these extracts appear to be better ecofriendly alternatives to requesting further research to make them more effective and also to be applied as integrated disease management agents.

According to the results of this research, the antifungal potential of *Zataria multiflora* and *Cuminum cyminum* extracts should be tested in future in vivo and field tests to confirm the results in the natural environment. Despite such valuable effects drawn from laboratory assays, evaluation of these extracts in the greenhouse and open field will assist in establishing their homogeneity, feasibility, and a possibility of their inclusion within the disease management frameworks. Elaborate phytochemical examination ought to be conducted to isolate and identify the dynamic elements bringing about antifungal impacts of the extracts. It will enable standard formulations to be developed in concentrated forms so that their performance is optimized and that it could be reproducible. The productivity of the active compounds could be enhanced by optimized extraction parameters, alternative solvents or advanced extraction, Soxhlet extraction, or microwave-assisted extraction. A toxicology should be done to determine the phytotoxicity it produces on okra plants, the effects it has on the environment with regard to the harm it may cause/disrupt to good bacteria and pollinators. To make it useable, user-friendly form relatives, which may be a spray or emulsion, have to be developed that can be imbibed by farmers easily. Lastly, these botanical extracts can be applied in wider Integrated Disease Management (IDM) strategies along with other methods, including crop rotation, resistant variety and biocontrol agents, as this would be more sustainable and effective in the management of Fusarium wilt. Education and extension among farmers are crucial in making sure that farmers are aware and use environmentally-friendly alternatives especially in economically constrained farms.

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