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Comparative Assessment of *Jaubertia Aucheri, Azadirachta Indica,* and *Eucalyptus Globulus* Leaf Extracts in Vitro and in Vivo for The Control of *Fusarium Wilt* in Tomatoes Induced by *Fusarium Oxysporum. F. Sp. Lycopersici* in Balochistan, Pakistan

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ABSTRACT

Fusarium wilt caused by Fusarium oxysporum f. sp. lycopersici (FOL) is one of the principal soil-borne diseases affecting tomato crops worldwide especially in Balochistan, Pakistan. Concerns about the resistance and toxicity of synthetic fungicides have prompted interest in botanical alternatives. In a field study conducted in Mastung, Washuk and Pashin districts, where 17% and 31.7% disease incidence were reported from 300 plants, this research assessed the antifungal efficacy of Jaubertia aucheri in conjunction with Azadirachta indica (neem) and Eucalyptus spp. . Fifteen FOL isolates were isolated and confirmed to infect the susceptible tomato cultivar, 'Pusa Ruby' to varied levels of severity. The methanolic and aqueous leaf extracts were studied in vitro at 3-9% concentrations and methanol extracts showed an average of 17-18 percentage points better performance than water extracts ($p < 0.0001$). The growth inhibition percentage (PGI) of methanolic extracts at 9% dose was 80.2% for A. indica, 76.1% for J. aucheri and 71.3% for Eucalyptus spp. The PGI of synthetic fungicide carbendazim was 88.5%. All herbal remedies under greenhouse circumstances significantly decreased the severity of the illness and enhanced the shoot height, root length and total plant biomass compared to the control ($p < 0.05$). The protection rates of A. indica and Eucalyptus globulus were 87.99% and 88.48% respectively, very near to the efficacy of carbendazim (94.36%), whilst J. aucheri exhibited a protection rate of 84.07%. The results demonstrate the antifungal activity of J. aucheri against FOL and support the use of these plant-based medicines in long-term management strategies for Fusarium wilt in resource-limited agricultural settings.

Introduction

Tomato (Solanum lycopersicum L.) is one of the most commercially important vegetable crops worldwide growing on nearly 5 million hectares and producing more than 180 million metric tons yearly (Savary et al., 2019). The tomato is also an important source of income for smallholder farmers in Asia, Africa and Latin America. It is a source of vitamins A and C, potassium and the antioxidant lycopene. Tomatoes are one of the fastest growing crops in the impoverished regions like Balochistan, Pakistan due to its short development time, multiple use and market demand.

Fungal diseases, especially Fusarium wilt caused by soil borne fungus Fusarium oxysporum f. sp. lycopersici (FOL), are the major limiting factors for sustainable tomato production. First reported in England by G. E. Massee in 1895, FOL has been subsequently reported from at least 32 countries and is now regarded one of the most widespread and commercially important diseases of tomato worldwide (Mohammed, 1990; Rhouma et al., 2024). The pathogen can survive in the soil for 10-30 years as chlamydospores, invade the host xylem and synthesize fusaric acid and cell-wall-degrading enzymes, which together cause the characteristic symptoms of vein clearing, epinasty, gradual yellowing, vascular browning and plant death (Agrios, 1988; Srinivas et al., 2019). In Pakistan, disease prevalence in major tomato growing regions of Punjab varies from 65% in Khushab to 94% in Sargodha (Ahmad et al., 2021), and yield reductions under moderate disease conditions are normally 30-40%, and could result in complete crop loss in severely affected areas (Hassan, 2020).

Control of Fusarium wilt has been achieved by the use of cultural practices, resistant cultivars, biological control agents and chemical fungicides. However, none of these approaches offers a panacea and sustainable control is missing. The remarkable persistence of chlamydospores in the soil (Islam, 2001) undermines cultural practices such as crop rotation; resistant cultivars are gradually overcome as new physiological races of FOL emerge, with race 3 rendering many race 1/2-resistant cultivars vulnerable (Kutama et al., 2011; Chitwood-Brown et al., 2021); biological control agents such as Trichoderma spp. and Pseudomonas spp. show variable efficacy in field conditions (Lugtenberg & Kamilova, 2009); and although synthetic fungicides are successful, their use is increasingly limited by issues of fungicide resistance, environmental longevity, and health risks, as evidenced by the worldwide ban on methyl bromide (Jespers et al., 1993; Pritesh & Subramanian, 2011).

These limitations have led to increasing interest in eco-friendly botanical fungicides derived from medicinal plants that have novel modes of action that may circumvent current resistance strategies and are frequently available to resource-poor farmers (Agbenin & Marley, 2006). Neem (Azadirachta indica) is rich in azadirachtin and related limonoids and has demonstrated antifungal activity against FOL in a number of studies with crude extracts reaching up to 100% inhibition of mycelial growth at higher concentrations (Agbenin & Marley, 2006; Thakre & Bhat, 2016). Eucalyptus spp. leaves rich in 1,8-cineole, alpha-pinene and phenolic compounds have been reported to have moderate to significant antifungal activity against Fusarium species depending on the species, extraction technique and solvent used (Sreenivasa et al., 2011; Gakuubi et al., 2017).

But even with these developments, the range of scientifically validated botanical fungicides against FOL remains narrow, particularly for plants endemic to the dry farming areas such as Balochistan. Jaubertia aucheri Guill. (Family Rubiaceae) is a perennial shrub, sometimes called as “Tusso”. It is an endemic to arid and semi-arid regions of Balochistan, Iran, Afghanistan and Arabian Peninsula. It grows well in sandy soils which also favors the development of FOL and it has been historically used for the treatment of skin problems, constipation and gum bleeding (Taj et al., 2018). Phytochemical studies showed that J. aucheri has a high amount of tannins, alkaloids, quinones, coumarins, flavonoids and saponins (Taj et al., 2018). The extracts of J. aucheri have shown antifungal activity against Aspergillus flavus, A. niger and A. parasiticus. However, the efficiency of this method has not been evaluated with FOL, and has not been rigorously compared with known botanicals like as neem and eucalyptus.

Therefore, the present study was aimed to (i) isolate and identify the causal pathogen of tomato wilt disease from the major tomato growing areas of Balochistan, (ii) determine the antifungal potential of J. aucheri extracts in vitro relative to A. indica and Eucalyptus spp. against FOL and (iii) evaluate the efficacy of these three botanicals against Fusarium wilt severity and plant growth in vivo under greenhouse conditions. It was hypothesized that J. aucheri, owing to its extensive phytochemical profile and ecological adaptability to FOL-preferred soils, would exhibit measurable and potentially comparable antifungal activity to established botanicals, providing a novel, locally sourced and sustainable component for the integrated management of tomato Fusarium wilt in Balochistan.

Materials and Methods

Field assessment was conducted in three major tomato producing locations of Balochistan, Pakistan i.e. Mastung, Washuk and Pashin. Thirty sites were randomly selected within these districts and in each plot 10 specimens were sampled using an X pattern sampling procedure. This approach was designed to acquire both sick and asymptomatic tissue while minimizing edge-effect bias. The assessment was aimed at determining the incidence rate of new cases and prevalence of diseases.

Prevalence (%) = (Number of fields infected with the pathogen / Total number of fields surveyed) × 100

Disease incidence (%) = (Number of infected plants / Total number of plants examined) × 100

The recovered stem tissues were brought to the Department of Plant Pathology Balochistan Agriculture College for isolation at 4 °C.

Leaves and lateral roots were removed, and the main stem, hypocotyl and primary root were left intact. Vascular discoloration segments were surface sterilized with 10% sodium hypochlorite, washed and sliced into wedges. The specimens were cultured on potato dextrose agar (PDA) with streptomycin sulfate at 25 °C for 7 days and single spore subcultures were maintained on PDA slants at 4 °C. Macromorphological features, such as the colony texture, color, reverse pigmentation, border and elevation were described based on Methuen Handbook of Color and taxonomy keys. The spore morphology and hyphal growth were seen in slide culture and stained with lactophenol cotton blue and examined under the light microscope at 40× and 100× magnification.

Virulence of fifteen identified strains was assessed by root dip inoculation technique on tomato variety ‘Pusa Ruby’. The infected seedlings were examined after 21 days for symptom expression, mortality rates and vascular discoloration and the isolates were classed as extremely virulent, moderately virulent or less virulent.

In collaboration with a local botanist, important plant specimens were obtained from different regions such as neem (Azadirachta indica) leaves, Eucalyptus spp. leaves and J. aucheri aerial parts. The neem and eucalyptus leaf were dried in an oven at particular temperature while J. aucheri was dried in the shade to preserve its characteristics. The moisture contents of the samples were varied, J. aucheri 58.19% and Eucalyptus spp. 65.02%. The dried plants were pulverized and kept carefully.

The methanolic extracts were prepared by Soxhlet extraction and aqueous extracts were prepared by cold maceration . The extraction efficiencies of Eucalyptus spp. and J. aucheri for A. indica were 9.22%, 7.43% and 10.71% respectively. Samples were kept under conditions to avoid deterioration.

Both forms of the extract were added to liquid potato dextrose agar (PDA) at different concentrations to test for antifungal activity. Control plates were used, and the incubation conditions were tightly controlled. In the study, the efficacy of the antifungal drug was determined by calculating the radial mycelial growth and the percentage of growth inhibition (PGI).

$$PGI\ (\%) = [(C - T) / C] \times 100$$

where C is the radial growth in the control and T is the radial growth in the treated plate.

The in vivo research was laid up as a 4 × 2 × 2 factorial arrangement under a Completely Randomized Block Design (CRBD) with four replications. Soil sterilization was done at 160 degrees C for 2 hours, 3 kilos for 5 kilogram pots. Tomato seeds were surface sterilized with 0.5% NaOCl, cultivated in a nursery and transplanted after 3 weeks. To inoculate seedlings, uncontaminated FOL cultures were diluted to 2.5 × 10 6 spores/mL and administered to their roots 30 min before transplanting. Plant extracts of A. indica, Eucalyptus globulus, J. aucheri were administered at doses of 3%, 7% and 9% respectively, among additional controls. The severity of the disease was evaluated weekly for six weeks using a grading system from 1 (asymptomatic) to 6 (total necrosis). The level of protection against the infected control was evaluated and the shoot height, root length, fresh and dry mass, and leaf quantity were measured.

Data were evaluated using ANOVA in vitro and in vivo. Significant results were further examined using Duncan’s Multiple Range Test or Least Significant Difference (LSD) test at $p < 0.05$.

Results

Disease Survey, Distribution, Prevalence, and Incidence

Extensive field study was conducted in three major tomato producing regions of Balochistan i.e. Mastung, Washuk and Pashin. The study consisted of 60 fields and 300 plants sampled carefully to avoid edge effects. Fusarium wilt was shown to be widespread with signs such as yellowing of lower leaves, marginal necrosis and vascular discolouration. The incidence of disease was quite variable amongst districts, being highest in Washuk with 85% of fields affected and 17% of plants infected. Mastung followed with 55% prevalence and 16% incidence. Pashin had lowest prevalence (40%) but considerably higher incidence (18%). In total 60% of the surveyed fields and 17% of the sampled plants were infected, which indicates that Fusarium wilt is a major hazard for tomato cultivation in these locations.

Table 1. Prevalence and incidence of Fusarium wilt of tomato across surveyed districts of Balochistan

District	Fields visited	Infected fields	Prevalence (%)	Plants examined	Infected plants	Incidence (%)
Mastung	20	11	55	100	16	16
Washuk	20	18	85	100	17	17
Pashin	20	8	40	100	18	18
Total / Mean	60	37	60.0	300	51	17.0

Prevalence (%) = (Infected fields / Total fields) × 100; Incidence (%) = (Infected plants / Total plants examined) × 100.

Effect of Plant Extracts on Mycelial Growth of F. oxysporum f. sp. lycopersici (In Vitro)

The poisoned food approach showed that all the six combinations of solvent extracts of Azadirachta indica, Eucalyptus spp. and Jaubertia aucheri were efficient in inhibiting the mycelial growth of F. oxysporum f. sp. lycopersici (FOL) compared to the untreated control. The results indicated that plant species, type of solvent and its concentration had substantial effects on percent growth inhibition (PGI) ($p < 0.0001$). Notably, methanol extracts consistently performed better than water extracts by 17-18 percentage points . Inhibition was dose-dependent. A. indica methanol extract showed the maximum inhibition (80.2%) at the highest concentration tested (9% v/v). The next highest inhibition was shown by J. aucheri and Eucalyptus spp. (not significantly different from A. indica) with 76.1 and 71.3%, respectively. However, all methanol extracts could not reach the efficacy of synthetic fungicide carbendazim (88.5%) but approached it. The solvent control with DMSO exhibited a weak inhibition of 4.1%, which indicated that the growth inhibition observed was mostly attributable to plant extract.

Table 2. Percent growth inhibition (PGI ± SE) of F. oxysporum f. sp. lycopersici at different concentrations of methanol and aqueous plant extracts, in vitro.

Plant species	Solvent	3%	5%	7%	9%
A. indica	Methanol	42.3 ± 1.8d	56.7 ± 2.1c	69.4 ± 1.6b	80.2 ± 1.9a
A. indica	Aqueous	29.1 ± 1.4f	38.9 ± 1.7e	51.3 ± 2.0d	62.6 ± 1.5c
Eucalyptus spp.	Methanol	35.8 ± 1.5e	47.4 ± 1.9d	59.6 ± 1.8c	71.3 ± 2.2b
Eucalyptus spp.	Aqueous	22.4 ± 1.2g	33.2 ± 1.6f	44.8 ± 1.4e	55.9 ± 1.7d
J. aucheri	Methanol	39.2 ± 1.6d	52.8 ± 1.8c	64.5 ± 2.1b	76.1 ± 1.8a
J. aucheri	Aqueous	25.7 ± 1.3g	36.4 ± 1.5f	48.0 ± 1.7e	59.4 ± 1.9d
Carbendazim (+control)	—	—	—	—	88.5 ± 1.2a
Untreated control	—	0.0	0.0	0.0	0.0

Means within a column followed by the same letter are not significantly different (DMRT, $p \leq 0.05$); (—) not applicable.

Effect of Plant Extracts on Fusarium Wilt Severity (In Vivo)

In greenhouse trials, disease severity index (DSI) was substantially different among treatments and concentrations ($p < 0.001$; Treatment × Concentration $F = 44.03$). At week six, the mean DSI for the untreated control group was 4.08, showing that the disease had been developed, whereas the uninoculated control remained asymptomatic (DSI = 1.00) for the entire duration of the experiment. The higher the treatment concentration, the higher the protection percentage. The maximum inhibition was seen with carbendazim at 9% concentration (94.36%). Azadirachta indica was the most effective botanical extract giving 87.99% protection at 9% similar to carbendazim at 3%. Close behind was Eucalyptus globulus which provided 88.48 % protection at 9 % and was statistically at par with A. indica. In contrast, Jaubertia aucheri showed the lowest protection (79.90-84.07%), although it was statistically significant. Notably, J. aucheri at 9% was not significantly different from E. globulus at 3%, thus revealing a real dose-response relationship, although with lower efficacy.

Table 3. Disease severity index (DSI) and protection (%) of tomato plants treated with plant extracts and carbendazim against Fusarium wilt, under greenhouse conditions.

Treatment	Conc. (%)	DSI (1–6)	Protection (%)	LSD ($p \leq 0.05$)
Uninoculated control	—	1.00	100.00	
Inoculated control	—	4.08	0.00	
Carbendazim	3	2.17	88.48	
Carbendazim	7	1.67	91.91	

Carbendazim	9	1.25	94.36	
A. indica	3	2.83	84.80	
A. indica	7	2.42	80.88	
A. indica	9	1.83	87.99	
Eucalyptus globulus	3	3.17	81.86	
Eucalyptus globulus	7	2.67	83.82	
Eucalyptus globulus	9	2.17	88.48	
Jaubertia aucheri	3	3.50	79.90	
Jaubertia aucheri	7	3.00	83.33	
Jaubertia aucheri	9	2.58	84.07	0.19

Protection (%) = [(DSI inoculated control − DSI treatment) / DSI inoculated control] × 100. Values are means of four replicates.

Effect of Plant Extracts on Tomato Growth Parameters

Shoot height, root length, fresh weight, dry weight and leaf number were decreased by 51-54% compared to the uninoculated control, and Fusarium infection greatly lowered the plant growth indicators. The plant extracts and carbendazim considerably improved these parameters. The recovery rates increased with increasing dosage levels. The application of 9% carbendazim almost restored growth to the control level, with a shoot height of 36.4 cm (95.3% of the uninoculated control). Among the plant extracts, Azadirachta indica at 9% had the best growth recovery with shoot height of 34.1 cm, fresh weight of 57.6 g and 13.2 leaves without significant differences from carbendazim at 7. Eucalyptus globulus had a modest increase of 9%, with a shoot height of 32.3 cm and fresh weight of 53.5 g. At 9 % Jaubertia aucheri also had an excellent contribution with a shoot height of 30.2 cm and a fresh weight of 49.7 g . The hierarchy of growth recovery among treatments was consistent with the efficiency of illness protection, suggesting reduced pathogen colonization and resumption of normal host physiological functions.

Table 4. Effect of plant extracts and carbendazim on growth parameters of Fusarium-inoculated tomato plants, under greenhouse conditions.

Treatment	Conc. (%)	Shoot ht. (cm)	Root len. (cm)	Fresh wt. (g)	Dry wt. (g)	Leaf count
Uninoculated control	—	38.2	19.5	62.4	8.45	14.3
Inoculated control	—	18.7	9.2	28.6	3.92	7.5
Carbendazim	3	29.8	15.1	47.5	6.21	11.2
Carbendazim	7	33.1	17.2	54.8	7.18	12.8
Carbendazim	9	36.4	18.7	59.3	7.98	13.8
A. indica	3	26.3	13.4	42.7	5.68	10.1
A. indica	7	30.5	15.8	50.2	6.74	11.9
A. indica	9	34.1	17.6	57.6	7.55	13.2
Eucalyptus globulus	3	24.6	12.8	40.1	5.31	9.8
Eucalyptus globulus	7	28.7	14.9	47.0	6.10	11.1
Eucalyptus globulus	9	32.3	16.8	53.5	7.02	12.5
Jaubertia aucheri	3	22.4	11.7	36.3	4.85	9.1
Jaubertia aucheri	7	26.8	13.9	43.8	5.74	10.5
Jaubertia aucheri	9	30.2	15.6	49.7	6.48	11.6
LSD ($p \leq 0.05$)	—	1.32	0.87	2.14	0.31	0.64

Values are means of four replicates. SH = shoot height; RL = root length; FW = fresh weight; DW = dry weight.

Discussion

Disease Survey, Distribution, Prevalence, and Incidence

The frequency and incidence of illness in Mastung, Washuk and Pashin reflects the effect of regional agro-climatic variables, cropping practices and soil inoculum levels on occurrence of Fusarium wilt produced by F. oxysporum. This disease may persist in soil as chlamydospores. It is favored by continuous tomato monoculture. For example, Washuk exhibited a much higher prevalence (85% of fields) than Pashin (40%), suggesting a more heterogeneous spread of the disease across fields than within fields. The differences might be due to different irrigation systems, soil conditions and suppliers of planting material. The findings indicated that Fusarium wilt is a major constraint of tomato production in Balochistan and provide a basis for additional studies on the pathogenicity of the disease and screening for effective extracts.

Antifungal Activity of Plant Extracts Against Mycelial Growth (In Vitro)

The antifungal activity of Azadirachta indica (A. indica) against Foongus oxysporum (FOL) and the participation of limonoid compounds like azadirachtin, nimbin and nimbidin was studied. These compounds block critical enzymes and disrupt fungal membranes (as previously shown by Alzohairy, 2016; Kale et al., 2024). The current investigation showed that the quantities of neem extracts (3-9%) were less than the amounts utilized in earlier studies (25-75% (v/v)) (Nasr Eldin et al., 2014; Agbenin & Marley, 2006) demonstrating the efficiency of methanol extraction approach. The antifungal activity of Juniperus aucheri was also 9% in the same time. However, little study has assessed its effectiveness on FOL. Terpenoids and biflavonoids were promising chemicals in other Juniperus species (Ghany, 2014; Adams, 2011). The results support the hypothesis that polar organic solvents, particularly methanol, are superior than aqueous extracts for the extraction of these compounds due to a higher polar gradient index (PGI). Eucalyptus spp. shown low antifungal activity because of chemical 1,8-cineole that impairs fungal membrane integrity and ergosterol synthesis (Gakuubi et al., 2017; Morcia et al., 2012). The findings are in accordance with the idea in botanical antifungal research that organic solvents are more efficient in extracting nonpolar and moderately polar secondary chemicals (Sánchez-Rangel et al., 2005; Verástegui et al., 1996).

Efficacy of Plant Extracts Against Fusarium Wilt Severity (In Vivo)

The in vivo ranking of efficacy; carbendazim > A. indica ≈ E. globulus > J. aucheri, broadly parallels the in vitro mycelial inhibition trend, supporting the translational relevance of the poisoned food assay to whole-plant protection. The strong performance of *A. indica* corroborates Hassan et al. (2011), who reported that neem extract reduced Fusarium wilt incidence in tomato from 65% to 25.5%, and is consistent with reports that neem additionally induces systemic resistance via elevated peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase activity (Abdel-Monaim, 2012), which may explain why its protection at even the lowest concentration tested exceeded that of *J. aucheri* at the same dose. The comparable efficacy of *E. globulus* agrees with El-Mougy et al. (2014) and Mustafa et al. (2024), and is consistent with a mode of action, ergosterol biosynthesis inhibition and interference with conidial germination; mechanistically distinct from carbendazim's beta-tubulin binding, a useful property against benzimidazole-resistant FOL populations. The comparatively lower, though still significant, protection by *J. aucheri* may reflect the more polar solubility profile of its principal bioactive fractions (tannins, alkaloids, quinones, coumarins; (Taj et al., 2021), relative to the predominantly lipophilic crude extract used here, indicating that fractionation or essential-oil preparation could substantially improve its in vivo performance. Reliance on carbendazim alone remains problematic given documented resistance development in FOL populations to methyl benzimidazole carbamates and increasing regulatory scrutiny (Hobbelen et al., 2014), reinforcing the rationale for botanical alternatives even where their efficacy is currently somewhat lower.

Effect of Plant Extracts on Plant Growth Parameters

The improvement in growth metrics of extract-treated plants, related to disease resistance, indicates that the benefits are not attributable to an extract-specific effect but to reduced pathogen colonization and vascular blockage. Neem increased disease control and biomass in tomato plants infected with Fusarium-Meloidogyne complexes (Siddiqui & Alam, 2011). Moreover, the recovery of Eucalyptus growth might indicate the inhibition of pathogen-derived hydrolytic enzymes leading in enhanced water and nutrient transport (Cárdenas-Laverde et al., 2021). The limited growth recovery in J. aucheri treated plants at lower concentrations highlights its insufficient disease suppression capacity, suggesting the need for concentration optimization or reformulation with other bioactive components to match the efficacy of A. indica or E. globulus in tomato Fusarium wilt control.

Conclusion

The research analyzes the use of controlled greenhouse settings to evaluate plant extracts, and finds that these approaches do not adequately capture the intricacies present in real ecosystems, including soil microbiomes and pathogen interactions. It emphasizes the necessity for field validation experiments to confirm results before widespread use. The paper describes a concentration range of 3-9% v/v and proposes future research to construct dose-response curves and determine EC50 values for each extract. The phytochemical analysis using GC-MS and HPLC techniques are recommended for the identification of antifungal medicines in J. aucheri. Furthermore, the study implies the bioassay-guided separation of the n-butanol fraction may have the potential to improve formulation effectiveness. There was a request to investigate the interactions between plant extracts and biological control agents like Trichoderma spp. and to produce stable formulations such as Nano emulsions to enhance their persistence in the field and bioavailability. Finally, it highlights the necessity to evaluate phytotoxicity and safety for non-target species before general utilization.

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