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LABORATORY AND FIELD EVALUATION OF TOMATO EARLY BLIGHT (ALTERNARIA SOLANI)

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Abstract

Alternaria solani *is a highly destructive fungal pathogen responsible for early blight in tomato (Solanum lycopersicum L.), posing a significant threat to tomato production in Pakistan. Various management approaches–including chemical fungicides, biological control agents, and plant-based extracts–have been explored to mitigate this disease. The present study evaluated the effectiveness of five plant extracts–Citrullus colocynthis, Azadirachta indica, Moringa oleifera, Calotropis procera, and Prosopis cineraria–for controlling early blight, as well as the host response of different tomato germplasm against* ***A. solani****. Aqueous extracts were prepared at 2.5%, 5%, and 7.5% concentrations. The in vivo experiment was conducted under a Completely Randomized Design (CRD) at the Faculty of Agriculture and Environment, The Islamia University of Bahawalpur.Results revealed that foliar application of Citrullus colocynthis extract significantly reduced disease severity and incidence, achieving the lowest disease incidence at 11.57%. These findings indicate that Citrullus colocynthis possesses strong antifungal activity against* ***A. solani*** *while also promoting plant growth. This dual effect underscores its potential as an eco-friendly alternative to conventional chemical fungicides. However, further research is required to clarify its mode of action, environmental safety, and wider applicability in sustainable disease management strategies.*

INTRODUCTION

Tomato belongs to Solanaceae family and has been called the "poor man's apple" (KALIA, 2024). They are recognized as both an economically significant and nutritionally vital crop globally (Panno et al., 2021). Their
cultivation supports livelihoods, enhances food security, and provides essential nutrients that contribute to human health (Sekaran et al., 2021). Tomato farming is a key source of income for many farmers, particularly in regions like Pakistan and India, where it serves as a profitable cash crop (Khan et al., 2023). The production of tomatoes contributes to food security by providing a reliable source of fresh produce, which is crucial in areas with limited vegetable consumption (Ebert, 2020). Tomatoes are used both raw in a salad, and cooked in various savory sauces, stews, and soups (Montanari et al.,2021). Economically significant processed goods include dried and canned tomatoes (Shankara et al., 2015). Tomatoes are a rich source of vitamins A and C, potassium, and antioxidants, which are essential for maintaining health and preventing diseases (Kumar et al., 2020). Regular consumption of tomatoes is linked to reduced risks of chronic diseases such as cardiovascular disease, cancer, and

diabetes (Collins et al., 2022). It is a rich source of amino acids, dietary fibers, carbohydrates, minerals like P, Ca & Fe, and vitamins (C, A & K) (Kumar et al., 2020). Tomato production holds significant global importance as a staple food crop, contributing to food security and economic growth (Idama et al., 2021). Tomatoes are among the most widely cultivated crops globally, essential for both local economies and international trade (Chanda et al., 2021). As a key component of various cuisines, tomatoes are critical for food security, especially in developing countries (Joya et al., 2022). An estimated 124.4 million tons of fresh tomato fruits are produced annually in the world, making tomatoes the most significant fruit and vegetable crop in consumption (Maurya et al., 2022). Its extensive application and nutritional value have boosted the demand for fresh markets and processed tomato types, and larger production of tomatoes is necessary to supply the ever-increasing demand (Adhikari and Panthee, 2019). However, it faces substantial challenges from microbial diseases. The most important issues for the low production of tomatoes are fungal diseases. Early blight is one of the tomato's most damaging foliar diseases caused by *Alternaria solani*. Potatoes and tomatoes are the primary hosts for the fungus *A. solani* (Schultz and French, 2009).

A. solani is a significant fungal pathogen responsible for early blight, primarily affecting tomato crops at any stage of development (Abada et al., 2008). It is found worldwide, including in tropical, temperate, and subtropical climates, where it reduces crop yield (Hijmans et al., 2000). The economic losses and yield reductions associated with this disease are substantial, with reported yield losses ranging from 5% to as high as 79% depending on the crop and environmental conditions. *A. solani* produces appressoria that facilitates infection. Early blight on older tomato leaves manifests as small to irregular brown dots with a "bull eye" look. *A. solani* also causes symptoms including seedling wilt, stem rot, flower blight, and fruit drop (Agrios, 2005). If there are several lesions, the tissue around the initial lesions may turn yellow, and the entire leaf may become necrotic and chlorotic (Kouyoumjian, 2007). The disease thrives at temperatures between 25-30°C and high humidity levels ranging from 80 to 90% (Solankey et al., 2021). Maximum conidial germination occurs at 30°C and 100% relative humidity, indicating the importance of environmental monitoring (Solankey et al., 2021). The disease is polycyclic, allowing multiple epidemics within a single cropping season, complicating management efforts. Leaves, fruit, and stem are all susceptible to be diseased by *A. solani* and it can harm at any time throughout the plant's growth cycle. It causes fruit spots, leaf spots, and collar rot on stems (Abada et al., 2008). Small, dark brown patches, most noticeable on the oldest and lowest leaves, are the most prominent symptoms. The entire leaf can get necrotic and chlorotic. The spots become larger, gaining the appearance of a bull's eye due to the development of concentric rings. Tomato fruits are damaged by the spread of disease, the proliferation of lesions, and the defoliation of the plants in good climatic circumstances (Kouyoumjian, 2007). As determined by morphological and physiological research, the early blight pathogen produces big, long- beaked, concatenated spores (Simmsons, 2000).

For centuries, people have been able to lessen the severity of diseases caused by a wide range of pathogens by employing cultural practices (Gatahi et al.,2020). Changing farming techniques that support the establishment of disease pathogens is one method of cultural management. Crop rotation refers to alternating between various types of crops grown in the same region throughout successive growing seasons. This method is among the most efficient in altering early blight in tomatoes. It has been observed that the first inoculum of early blight can be decreased by rotating crops from different families (Madden et al., 1978).

To boost tomato production in terms of quality and quantity, it is necessary to create new effective environmentally friendly strategies that are beneficial for plants, soil and beneficial microbes. In this study, five medicinal plant extracts (*Citrullus colocynthis, Azadirachta indica, Moringa oleifera, Calotropis procera* and *Prosopis cineraria*) were tested against early blight of tomato caused by *A. solani*.

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1. Materials and Methods

1.1. Collection of Pathogens from Diseased Samples

Fifty samples of tomato were collected with typical symptoms produced by A. solani. Once the samples had been collected, they were placed in a clean and sterile plastic bag or container. The container was labeled with the plant species, the plant part, and the location of the sample. The samples were transported to the laboratory as soon as possible for further analysis. After being cleaned well, sample fragments were placed aseptically on Petri dishes with Potato Dextrose Agar medium. Petri dishes with the inoculated fungus were kept at 25±2°C for three to five days and checked at regular intervals to track the spread of the fungus. To confirm the identity of the isolated fungus, it was examined under a microscope. A small amount of fungal culture was placed on a glass slide and observed under a microscope. *A. solani* was identified by its characteristics and morphology, such as the shape and size of the conidia, and the presence of septate hyphae.

2.2 Preparation of Plant Extracts with standard concentrations

Fresh fruits, flower buds and leaves of *Citrullus colocynthis*, *Calotropis procera*, *Azadirachta indica*, *Moringa oleifera* and *Prosopis cineraria* were collected and dried under shade for 10 days, respectively. The shade-dried fruits, buds and leaves were further dried in an oven. These dried fruits, buds and leaves were grinded into fine powder in an electric grinder. The fine powder was passed through muslin cloth to obtain a very fine powder. A series of concentrations, i.e., 7.5%, 5%, and 2.5% were prepared. 75 g, 50 g, and 25 g fine powder dissolved in 1000 ml distilled water separately to obtain 7.5%, 5%, and 2.5% concentrations, respectively.

Table 1. Disease rating scale

Sr No.	Grade	Infection
1.	0	No spots on the leaves
2.	1	0-10% leaf area covered
3.	2	11-25% leaf area covered
4.	3	26-50% leaf area covered
5.	4	51-75% leaf area covered
6.	5	76-100 leaf area covered

2.5 Statistical analyses

2.3 *In-Vitro* Evaluation of Plant Extracts against *A. solani*

The efficacy of five plantextractsviz., *Citrullus colocynthis*, *Calotropisprocera*,*Azadirachta indica*,*Moringa oleifera* and *Prosopis cineraria* were tested against *A. solani* on potato dextrose agar medium using poisoned food technique and dual culture technique using Potato Dextrose Agar (PDA) as a basal medium under controlled condition.

2.4 *In-Vivo* Evaluation of Plant Extracts against *A. solani*

In October 2022, nursery-grown seedlings 30–35 days old were planted in a gross area of 39.5 m x 11 m = 434.5 m^2 with a spacing of 60 cm x 4 cm. Five different plant extracts were tested, each at a different concentration (according to treatments), and compared to an untreated control group. Two sprays of the extract at the determined concentration were sprayed 15 days apart after the sickness first appeared. Each plot's percentage of disease incidence was recorded and compared to that of the control plot following two sprays of extracts 15 days apart. Early blight of tomato is caused by *A. solani*, which was confirmed by observing symptoms and doing microscopic analyses. On a scale from 0 to 5 that is given below (Table 1), early blight disease was documented in 5 randomly chosen plants from each plot (Din et al., 2020).

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Two-factor factorial ANOVA with CRD (complete randomized design) was used for the analysis of data.

Tukey's LSD at P = 0.05 was used to examine the differences between treatments after the data were subjected using Statistix statistical package (ver. 10). Graph Pad Prism software (Ver. 5) was used for the designing of graphs (Din et al., 2020).

2. Results

3.1 Inhibition Zone (mm)

For the prevention and treatment of early blight, plant extracts have antifungal action. The analysis of the variance table showed that there is a significant relationship between treatments and concentrations as shown in table 2. Results showed that *Citrullus colocynthis* and *Azadrachta indica* are more effective against early blight disease. Maximum inhibition zone (mm) was observed by plant extract of *Azadrachta indica* (32.840 mm) at 7.5% concentration followed by *Citrullus colocynthis* (25.977mm), *Moringa oleifera* (25.077 mm), *Calotropis procera* (24.653 mm), *Prosopis cineraria* (16.813 mm) as compared to control. In the same way, *Azadrachta indica* also showed maximum inhibition zone (24.653mm and 19.733mm) at 5% and 2.5% concentration respectively while *Prosopis cineraria* showed minimum inhibition zone (11.360 mm and 9.370 mm) at both 5% and 2.5% concentrations as compared to control as shown in Figure 1.

Table 2. Analysis of variance for inhibition zone (mm) of tomato under different treatments

Source of	DF	SS	MS	F	P Value
Replications	2	9.79	4.89		
Concentratio	2	323.5	161.7	188.03	0.0001
Treatments	5	1737	347.4	403.84	0.0002
Concentratio	10	114.2	11.4	13.28	0.0014
Error	28	24.09	0.86		
Total	47				

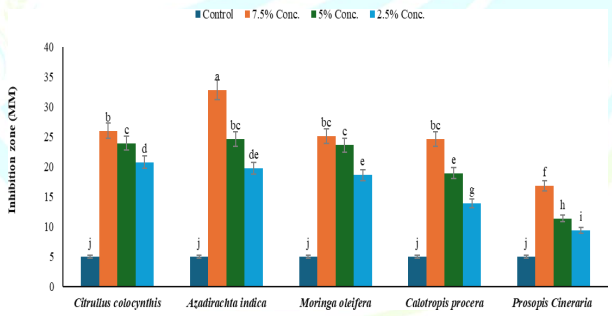

Figure 1. Effect of different treatments with different concentrations on the inhibition zone (mm) of tomato

3.2 Disease Incidence (%)

For the prevention and treatment of early blight, plant extracts have antifungal action. The analysis of the variance table showed that there is a significant relationship between treatments and concentrations as shown in table 4.

Table 4 Analysis of variance for disease incidence (%) of tomato under different treatment

Results showed that *Citrullus colocynthis* and *Azadrachta indica* are more effective against early blight disease. Minimum disease incidence (%) was observed by plant extract of *Citrullus colocynthis* (11.573%) at 7.5% concentration followed by *Azadrachta indica* (15.547%), *Moringa oleifera* (18.410%), *Calotropis procera* (21.533%), *Prosopis*

cineraria (22.597%) as compared to control. In the same way, *Citrullus colocynthis* also showed minimum disease incidence (12.070% and 14.603%) at 5% and 2.5% concentration respectively while *Prosopis cineraria* showed maximum disease incidence (23.843% and 30%) at both 5% and 2.5% concentrations as compared to control as shown in Figure 2.

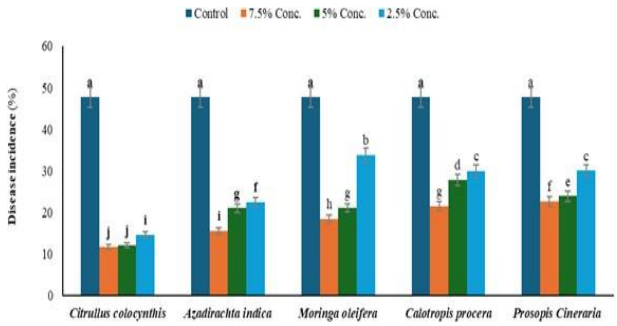

Figure 2. Effect of different treatments with different concentrations on disease incidence (%) of tomato

3. Discussion

Citrullus colocynthis, is recognized for its wide range of therapeutic properties and traditional medicinal uses across various cultures. This plant is primarily found in arid regions of Africa, the Middle East, and parts of Asia. It is notably used for treating gastrointestinal disorders, including indigestion, gastroenteritis, and intestinal parasites, as well as for its purgative and laxative effects. Due to its bioactive compounds like essential oils, glycosides, alkaloids, and flavonoids. *Citrullus colocynthis* is also studied for its antioxidant, anti-inflammatory, anti-diabetic, anti-cancer, and antimicrobial activities (Cheng et al., 2023; Li et al., 2022).

Bio-friendly plant-based extracts i.e. (*Citrullus colocynthis, Azadirachta indica, Moringa oleifera, Calotropis procera* and *Prosopis cineraria*) have shown potential in managing early blight in tomatoes, primarily through their antifungal properties and integration into broader disease management strategies. These plant extracts can also be utilized as integrated pest management systems to enhance tomato crop resilience against *A. solani* (Tortorici et al., 2022).

Source of Variance	DF	SS	MS	F Value	P Value
Replications	2	3.06	1.532		
Concentrations	2	312.4	156.21	317.7	0.0000
Treatments	5	2985.7	597.15	1214.5	0.0002
Concentrations	10	224.9	22.49	45.74	0.0011
Error	28	13.7	0.49		
Total	47				

Tomato plant development features improved considerably after being treated with *Citrullus colocynthis*. Leaf count, stem height and root length of tomato were all greatly improved. Among all these plant extracts *Citrullus colocynthis* has been shown maximum increase in shoots and roots weight as it contains bioactive chemicals that play important functions in plant growth, such as cell division, nutrient availability, translocation, photosynthesis, and yield.

Citrullus colocynthis has an active biological compound which exerts direct antimicrobial effects and induces systemic resistance in plants (Hemeg et al., 2020). Genes are present in plants that provide protection in large number. These defense genes are triggered by environmental cues, leading to systemic resistance to pathogens. Disease severity and lesion size reduction show that *Citrullus colocynthis* significantly reduces *A. solani* growth.

These findings are consistent with those of (Nashwa and Abo-Elyousr, 2013). Who found that *Citrullus colocynthis* offered substantial protection against early blight pathogens in tomato leaves. According to their findings, the presence of active components invaded early blight infestation in tomato leaves. In particular, 6-gingerol found in the ginger extract may have induced a short-lived effect on the tomato plant's transcriptome.

Citrullus colocynthis significantly improved the growth parameters of tomato plants and produced the driest root and shoot weight of any plant examined. (Yanar et al., 2011) supported these findings, stating that components and compounds of *Citrullus colocynthis* play critical roles in plant development by controlling cell division, nutrient availability and translocation, photosynthesis, and other aspects of plant growth and development that influence yield.

Plant treated with *Citrullus colocynthis* was found to contain the highest root and shoot length. Even though the exact mechanism by which PE's application decreases plant length is not fully understood, it may be due to the presence of phytochemicals, in particular bioactive compounds (Mohanty et al., 2015), which may trigger defence mechanisms against *A. solani*. (Salih et al. 2022) corroborated our findings when they reported that plants boost their defence system with help from a wide variety of enzymes. A fungal pathogen's infection in tomatoes triggers a cascade of defense mechanisms, one of which is increased yield production and accumulation (Ahmad et al., 2017). Plants that are exposed to biotic or abiotic stress have been shown to benefit greatly from the action of the polyphenol oxidase (PPO) enzyme (Mohamed et al., 2021).

4. Conclusion

Tomatoes are among the most extensively farmed and consumed vegetables in the world. Early blight of tomato, caused by *Alternaria solani*, is one of the most dangerous and common diseases of cultivated tomatoes, yet it is only one of several fungal infections that can harm tomato plants. Cultural, biological, chemical, and plant-extract management techniques are all used in the fight against tomato early blight. Regular use of chemical fungicides is detrimental to both human health and the environment. The use of plant extracts as a therapy technique is a key focus of this research due to their relative safety. In conclusion, both *vitro* and *in-vivo* testing of the plant extracts indicated that they were effective against the early tomato blight caused by the fungus Alternaria. Contrarily, *Citrullus colocynthis*, among all plant extracts, has demonstrated significant suppressive effects against *A. solani* in both laboratory and field conditions. In future combining these botanicals with biocontrol agents like *Trichoderma spp.* could show improved disease control and increased fruit yield. The integration of these plants into a comprehensive management plan, including cultural practices and chemical treatments, enhances overall effectiveness.

5. Conflict of Interest

The authors declare no conflict of interest.

6. Funding

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