



Research Article

Morphological Assessment of Resistance Potential in Tomato Against Early Blight Disease and Its Management

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Abstract | Tomato (*Lycopersicon esculentum* Mill.); a vital member of *Solanaceae* family and commonly consumed vegetable worldwide. Early blight caused by the fungus *Alternaria solani*, with salient feature of target board effect or concentric rings is the most common foliar disease of tomato that reduces fruit yield. The present study was aimed at avoidance of fungicides and management of early blight through resistance source and nutrients. Tomato germplasm (Nadir F₁, Naqeeb F₁, Johir, Gohar, Walter Gaint FM₉ and Money Maker) were screened against *Alternaria solani* to find out the resistance potential against early blight under field conditions. Data of disease severity and disease incidence was recorded fortnightly that showed only a single variety “Johir” expressed resistant (R) response out of 6 entries. The response of 2 varieties “Nadir F₁ and Gohar” was tolerant, “Walter Gaint FM₉” was susceptible (S) while “Naqeeb F₁ and Money Maker” were highly susceptible (HS) against early blight. The varieties “Nadir F₁, Naqeeb F₁, Johir and Gohar” were used for management purpose. The plants were treated with zinc sulphate (ZnSO₄), copper sulphate (CuSO₄), manganese sulphate (MnSO₄) and boric acid were used in different combinations i.e., T₁ (ZnSO₄+CuSO₄), T₂ (MnSO₄+CuSO₄), T₃ (boric acid+CuSO₄) and T₄ (ZnSO₄+MnSO₄+CuSO₄+BA). The combination of ZnSO₄+MnSO₄+CuSO₄+BA showed minimum disease severity (5.85%) and disease incidence (23.15%) as compared to control (28.59%). The outcomes of present study would be the way forward for getting rid of irrational use of synthetic chemicals for disease management.

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Introduction

Tomato is a major fruit also consumed as a vegetable globally which provides important antioxidants, fibers, vitamins and minerals (Gautier *et al.*, 2008). Tomato is susceptible to an array of diseases, among them early blight is the most destructive incited by *Alternaria solani*, that affects foliage and fruits resulting in 80% yield losses (Shamurailatpam and Kumar, 2020). The pathogen affects leaves, fruits and stems can be severely limiting the yield of tomato when weather is favorable and susceptible cultivars are used. Extreme defoliation can occur and result in sunscald appears on fruit (Roy *et al.*, 2019). *A. solani* releases enzymes like cellulase which break host cell wall and comprises pectin methyl galacturonase which help in the colonization of host (Shahbazi *et al.*, 2011). *A. solani* is a soil inhabiting and airborne pathogen found mostly in tropical and subtropical regions. *A. solani* made the concentric rings with brown to black spots appear on the lower side of leaves in bull eye pattern (Ghazanfar *et al.*, 2016). Infected plant leaves become pale yellow and dry afterward (Gleason and Edmunds, 2005).

During the last few years, it has been noticed that early blight found most widely due to favorable environmental conditions and its management is done excellently through different fungicides (Verma and Verma, 2010). There was considerable reduction in early blight disease severity under field and *in-vivo* conditions by using mancozeb and propiconazole. Integrated disease management strategies were compared for the management of early blight disease among which chemicals gave the most significant control (Jindo *et al.*, 2021). The development of early blight diseases was interrupted by manipulation of different cultural practices (Maurya *et al.*, 2022). Beneficial microbes have the ability to control *A. solani* causing early blight disease. Different *Trichoderma*, *Bacillus* and *Pseudomonas* species were used that inhibited the pathogen growth (Kulimushi *et al.*, 2021). Early blight disease of tomato was managed by using compost, biochar and plant growth promoting rhizobacteria (PGPR) that focused upon increasing the plant defense responses rather than directly killing or inhibiting the pathogen growth (Rasool *et al.*, 2021). Inappropriate uses of chemicals cause severe health hazards problems for humans thus right dose of fungicides at proper time interval should be compulsory (Krik *et al.*, 2005).

Early blight disease can be controlled by cultural practices, fungicides and biological control. Fungicide application is a major way to manage the early blight disease of tomato in both field experiments and greenhouse (Zafar and Shaukat, 2018). Besides disease control, some fungicides shows beneficial growth promoting and physiological effects on plants, involving delaying of senescence of leaf (Bertelsen *et al.*, 2001) and enhancing the contents of chlorophyll (Butkute *et al.*, 2008).

Excessive uses of chemical fungicides direct to increase fungicides resistance in pathogens and contamination of environment (Chaerani and Voorrips, 2006). Therefore, a cheaper, balanced and eco-friendly approach should be implemented and adopted by farmers to manage the disease (El-Shennawy and Abd El-Ail, 2018). Resistant cultivars development can be very sustainable and effective control measure for the control of early blight disease and minimize the yield losses in tomato (Adhikari *et al.*, 2017).

Micronutrients are the most effective in regulating plant growth due to enzymatic action and they are needed by plants in very small quantities (Sathya *et al.*, 2009). The growth of the plant is restrained due to deficiency of micronutrients, as a result of which the plant becomes susceptible to diseases, thereby causing a deleterious effect on its yield (Mushtaq *et al.*, 2016). Micronutrients, particularly zinc and boron, are very much necessary for the reproduction, growth and development of plants. These micronutrients are needed by plants in minimum quantities, yet they are best in regulating the growth, development and reproduction of plant, due to enzymatic action (Singh and Prakash, 2013).

In recent study different micronutrients were used against the disease and tomato germplasm were evaluated to find out the source of resistant against early blight of tomato. S these may be the sustainable management tactics against early blight disease.

Materials and Methods

The experiment was conducted in research area, Department of Plant Pathology, University of Agriculture, Faisalabad (UAF) (Pakistan).

Collection of tomato germplasm

Six commercially grown tomato varieties (Nadir F1,

Naqeeb F1, Johir, Gohar, Walter Gaint FM9 and Money Maker) were collected from Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan).

Collection of diseased samples

The infected leaves of tomato showing the symptoms of early blight disease were collected from the field. All the collected samples were brought to the Plant Mycology Lab, Department of Plant Pathology (UAF) and stored in a refrigerator for isolation and identification of pathogenic fungus.

Preparation of culture media

Potato dextrose agar medium: PDA medium was prepared for the isolation of fungal pathogen from the diseased samples of tomato. Sliced Potato 250.00g; Agar-agar 20.00g; Glucose 20.00g; Distilled Water 1000ml.

Potatoes were peeled first then slice into the small pieces and stewed in 500 ml of condensed water for starch extraction. The boiled material was filtered through muslin cloth. 20g of agar-agar and 20g glucose were added, and volume of solution was made up to 1000ml by adding more distilled water. In autoclave, the solution was sterilized at 121°C and 15 psi pressure for 15 to 20 minutes.

*Isolation and purification of *Alternaria solani**

Fungal pathogen was isolated from the infected leaves of tomato on PDA plates. Infected leaves were rinsed with tap water and surface sterilized with 70% ethanol followed by distilled water thrice. Samples were blotted dry and aseptically placed in an autoclaved plates having PDA medium. Petri plates were incubated at 25°C. After 7 days, a whitish growth of mycelium was observed and a portion from the periphery having single hyphal tip was separated and transferred to the other petri plates having medium to get pure culture and plates were stored in incubator at 25°C.

Morphological identification of fungus

For morphological identification, isolated fungus was examined under stereoscope in order to study the colony appearance including its texture, color, shape of the hyphae and spores. From the isolated plates, mycelium was picked with sterilized needle and placed on the slide with one drop of water and covered with the cover slip then examined under microscope (Figure 1).



Figure 1: *Morphological identification of *Alternaria solani*.*

Identification of pathogen was confirmed by observing the morphological features of mycelium. Characteristic features of genus are the production of beaked, pigmented conidia with thin transverse and longitudinal septa (muriform). *Alternaria* has septate, dark coloured mycelium and germinates short simple, erect conidiophores that contain single and branched chains of conidia in acropetal manner. Pure culture of isolated fungus was maintained in PDA petri plates kept in refrigerator (Singh, 2009).

Screening of tomato germplasm against early blight of tomato

For screening, 6 varieties (Nadir F1, Naqeeb F1, Johir, Gohar, Walter Gaint FM9 and Money Maker) of tomato were transplanted in the field. Twelve plants were transplanted in each row. For the better growth of the crop agronomic practices such as hoeing, fertilizer dose, irrigation and weeding were done.

Management of early blight through nutrients

Five treatments (Zinc Sulphate + Copper Sulphate, Manganese Sulphate + Copper Sulphate, Boric Acid + Copper Sulphate, Zinc Sulphate + Manganese Sulphate + Boric Acid + Copper Sulphate and water as T₁, T₂, T₃, T₄, T₅, respectively). In T₁, T₂ and T₃ each nutrient at the concentration of 0.25g/500ml water was used for the management of disease. In T₄ each nutrient at the concentration of 0.13g/500ml water was used. Four different varieties of tomato (Nadir F1, Naqeeb F1, Johir and Gohar) were grown in 3 replications with randomized complete block design (RCBD). Early in the morning, foliar spray was applied at the regular interval of 15 days.

Data collection

Data was collected at 15 days intervals. Disease severity and disease incidence was calculated by using formulas given below:

$$\text{Disease severity} = \frac{\text{Number of infected leaves}}{\text{Number of total leaves}} \times 100$$

$$\text{Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Number of total plants}} \times 100$$

Disease severity of early blight of tomato was determined on the basis of following disease rating scale (Akhtar *et al.*, 2019) (Table 1).

Statistical analysis

The collected data was analyzed by using Fisher's analysis of variance (ANOVA) and 5% level of significance was used to compare the treatment means with the help of Statistix 8.1.

Results and Discussion*Response of tomatoes germplasm against early blight disease of tomato*

Out of six varieties, 1 variety expressed resistant (R) response i.e. Johir (23.59%) disease severity and ranked 2nd in disease rating scale. Two varieties

showed moderately susceptible (MS) response i.e. Nadir F₁ (32.96%) and Gohar (32.69%) disease severity with disease rating scale 3 and only one variety showed susceptible (S) response with disease rating scale 4 i.e. Walter Gaint FM₉ (49.48%). While 2 varieties showed highly susceptible (HS) response with disease rating scale 5 i.e. Naqeeb F₁ (62.83%) and Money Maker (65.81%) disease severity and expressed maximum disease development (Table 2).

Effect of different treatments on disease severity of early blight of tomato

In management trial, varieties with varied resistance level (Gohar, Johir, Nadir F₁ and Naqeeb F₁) were sown under field conditions and data was recorded fortnightly. Analysis of variance showed that the effect of treatments on disease severity was significant i.e. all the treatments contributed in disease suppression. The 2 way interaction of treatments with varieties was also significant (Table 3). The combination of boric acid and copper sulphate gave best results and showed (2.28%) disease severity followed by combination of manganese sulphate and Copper sulphate showed (3.86%), combination of zinc sulphate and copper sulphate showed (4.28%) disease severity as compared to the control (4.93%) (Table 4).

Table 1: *Disease rating scale.*

Rating of disease	Severity symptoms for whole plant assay	Percent disease index (PDI)	Reaction of disease
0	No visible symptoms apparent	0	Immune
1	A few minute lesions to about 10% of the total leaf area is blighted and usually confined to the 2 bottom leaves	0.01-10	Highly resistant
2	Leaves on about 25% of the total plant area are infected	10.01-25	Resistant
3	Leaves on about 50% of the total plant area are infected	25.01-40	Moderately susceptible
4	Leaves on about 75% of the total plant area are infected	40.01-60	Susceptible
5	Leaves on whole plant are blighted and plant is dead	>60.01	Highly susceptible

Table 2: *Response of tomato germplasm against early blight disease.*

Disease severity categories	Varieties	Disease severity (%)	Response	Rating of disease
0	-	-	Immune (I)	0
0.01-10.00	-	-	Highly Resistant (HR)	1
10.01-25.00	Johir	23.59	Resistant (R)	2
25.01-40.00	Nadir F ₁	32.96	Moderately susceptible (MS)	3
	Gohar	32.69		
40.01-60.00	Walter Gaint FM ₉	49.48	Susceptible (S)	4
≥ 60.01	Naqeeb F ₁	62.83	Highly susceptible (HS)	5
	Money Maker	65.81		

Table 3: Analysis of variance for effect of nutrients on early blight disease severity.

Source	DF	SS	MS	F	P
Replications	2	0.84	0.42		
Treatments	4	45.71	11.43	48.53	0.0000**
Varieties	3	29.92	9.97	42.35	0.0000**
Treatments x Varieties	12	144.94	12.08	51.29	0.0000**
Error	38	8.95	0.24		
Total	59	230.35			

**Highly Significant; p -value < 0.01; * Significant p -Value < 0.05; ^{NS} Non-significant; p -Value > 0.05.

Table 4: All-Pairwise comparisons test of disease severity for treatments.

Treatments	Disease severity (%)
Control	4.93 a
BA+CS	2.28 c
MnS+CS	3.86 b
ZS+CS	4.28 b
ZS+CS+MnS+BA	3.99 b

$\alpha = 0.05$; Tukey HSD Value = 0.57

Interactive effect of treatments and varieties on disease severity of early blight of tomato

The effect of 5 different treatments combinations were evaluated against 4 varieties of tomato and the results revealed that the combination of boric acid and copper sulphate on Nadir F1 gave best results and showed (1.28%) severity of disease followed by zinc sulphate and copper sulphate on Naqeeb F1 (6.77%) while maximum severity of disease (8.16%) was shown in combination of manganese sulphate and copper sulphate on Nadir F1 as represented by Table 5.

Table 5: All-pairwise comparisons test of disease severity for treatments x varieties.

Varieties	Disease severity (%)				
	Control	BA+CS	MnS+CS	ZS+CS	ZS+CS+MnS+BA
Gohar	4.82de	2.95ghi	1.53ij	3.77efg	2.85ghi
Johir	4.89cde	4.34defg	4.10defg	1.79hij	3.25fgh
Nadir F1	4.66def	1.28j	8.16a	4.78de	6.36bc
Naqeeb F1	5.32bcd	0.56j	1.66ij	6.77Ab	3.52efg

$\alpha = 0.05$; Tukey HSD value = 1.51

Tomato (*Lycopersicon esculentum* Mill.) is very important vegetable crops around the world as well as in Pakistan and it belongs to family *Solanaceae*. Tomato

contains carotene, lycopene, β -carotene, vitamins (A, B, C), water (93-94%) and several phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives which are good for human health (Gautier *et al.*, 2008). Early blight of tomato infects all parts of the plant and incites more than 55% yield losses (Nashwa and Abo-Elyousr, 2013). The durable strategy for minimizing quantitative and qualitative losses due to early blight disease is the use of resistant genotypes, and many researchers are striving to search for resistant source in adverse environment by field evaluation (Gondal *et al.*, 2012). None of the varieties screened against early blight was immune and only a single variety was resistant. These results are in line with that of Kumar and Sarivatava (2013), who screened 44 genotypes of tomato, and recorded 1 resistant, 2 highly resistant, 15 moderately resistant, 20 susceptible and 5 highly susceptible response.

Nagesh *et al.* (2020) sown 50 genotypes for phenotypic characterization against early blight; out of 50 only 10 genotypes were regarded as resistant. The level of disease incidence and severity varied in different geographic location. The variation in disease severity may be attributed to too many factors like relative humidity, temperature, diverse cultivars used, rainfall, sowing date and it could be pathogenic variability. Akhtar *et al.* (2019) evaluated 400 cultivars in net house and found only "21396" advance line which showed resistant response. Mostly, in maximum of the previous screening experiments planned for early blight disease resistance in tomato, none tomato variety from wild or domesticated was regarded as resistant (Foolad and Ashrafi, 2015). Rex *et al.* (2023) stated that percent disease index varies with reference to genetic resistance potential of the germplasm; as it was ranged from 20-60% when screened on the basis of symptomology. There were similar results in the field evaluation studies conducted by (Shoaib *et al.*, 2020). Anderson *et al.* (2021) suggested that there is need to find the resistance against early blight from wild tomato hosts and its introgression in domesticated tomato lines as a sustainable management strategy. Hashemi *et al.* (2019) is of the opinion that searching for resistance source and its introgression into high yielding and good qualitative traits cultivars is the suitable, long lasting and environmentally safe option rather to use the synthetic chemicals for disease management.

Kumar *et al.* (2015) summarized his screening

experiments with the statement that wild accessions in which resistance source was identified could be used for breeding programs for resistance development. Chohan *et al.* (2015) used the different local and exotic germplasm for morphological characterization based upon salient symptom target board effect and described “Roma” and “Nagina” as highly susceptible genotypes. Yadav *et al.* (2015) categorized 15 advanced lines as resistant out of more than 50; those were screened under natural inoculum pressure.

Pandey *et al.* (2023) necessitates the breeding resistance in tomato genotypes based upon omics approaches because many of the cultivars are highly susceptible to early blight disease. It was observed that bio-control agents used for the control of pathogens act slowly and they are difficult to apply under field conditions which pave the way for need of finding the resistant source for disease management (Stangarlin *et al.*, 2015). *A. solani* interrupts with normal plant physiology by encoding cell wall degrading enzymes and toxins (Ramezani *et al.*, 2019). Singh *et al.* (2023) recorded an altered metabolite profile in tomato plants stressed with *Alternaria* infection. Hernandez-Aparicio *et al.* (2021) observed the activation of salicylic acid mediated defense pathway in early blight infected plants. There was increased expression of genes encoding for resistance and volatiles against the pathogen attack. Up-regulation of defense related genes and secondary metabolites were also observed by (Moghaddam *et al.*, 2019).

In present study, all the treatments were effective in managing the disease, the mechanism does not rely on killing the pathogen but it was due to boost and resume the interrupted functions of the plant. *A. solani* deters plant defense system by the production of toxins alternaric acid, altersolanol and zinniol that affect the protoplasm. It invades the host cells and extracts nutrients that weakens the plant and plant shows characteristics symptoms due to interruption with normal physiology (Mugao, 2023). Early blight pathogen creates pore in the cell wall of the plants, while boron strengthens the cell wall. Thus, the disease severity was minimum in boric acid treated plants (Sulus and Leblebici, 2022). The combined use of zinc sulphate and boric acid enhanced protein and oil contents in the plants that was depleted in invaded situation (Rahman *et al.*, 2020). Ahmad *et al.* (2012) reported that nutrient is very important for the development and growth of both the microorganisms

and plants and they act as a major factor to manage the diseases. Disease severity can be affected by essential nutrients. As there is no general rule that a specific nutrient can reduce the disease severity, but it may enhance the severity and incidence of different diseases or it may have a different effect in different environment. Effect of micronutrients on decreasing the severity of disease can be attributed to the involvement in biochemistry and physiology of plant, as various processes that can affect the response of plant to pathogens (Dordas, 2008). The concentric rings on leaves reduce the photosynthetic area and frequency of photosynthesis in potato plants leading to reduced growth and yield. Application of magnesium sulphate increases the chlorophyll production by repairing the chloroplast cells that has been destructed due to the interruption of fungal attack (Ahmed *et al.*, 2023).

Conclusions and Recommendations

It can be concluded that “Johir” is the most resistant variety and combination of zinc sulphate, magnesium sulphate, boric acid and copper sulphate gave the best control against early blight disease of tomato. It is recommended to incorporate the resistant germplasm in future breeding programs so that varieties with high yield, good qualitative traits can be developed. It is also recommended to use nutrient combinations against fungal diseases to discourage the use of fungicides.

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Novelty Statement

Early blight disease of tomato is usually managed by using different fungicides like mancozeb, chlorothalonil etc., very negligible work was focused to manage this disease through resistant source and non-chemical means. This study was planned to find out the environmentally safe management of early blight in Pakistan.

Author's Contribution

Aysha Fatima: Formal analysis and wrote original

draft.

Safdar Ali: Conceptualization and supervision.

Saira Azmat: Review and editing

Luqman Amrao: Investigation.

Muhammad Usman Ghani: Visualization.

Yasir Iftikhar: Methodology.

Muhammad Ahmad Zeshan: Data curation.

Adeel Ahmad: Fund Acquisition.

Humaira Kalsoom: Sample Collection.

Conflict of interest

The authors have declared no conflict of interest.

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