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# EVALUATION OF CHILI GERMPLASM AGAINST LEAF SPOT AND ITS MANAGEMENT BY NUTRIENTS AND FUNGICIDES

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**ABSTRACT**

Chili (*Capsicum annum* L.) is a widely grown vegetable in tropical and subtropical areas that is attacked by a diverse range of bacterial and fungal pathogens, causing severe yield and quality losses. *Alternaria* leaf spot, caused by *Alternaria alternata*, is the major fungal disease of chili that deteriorates the vitality of the crop. The objective of the current research is to identify the most effective nutrient combination and fungicide against *Alternaria* leaf spot disease and to evaluate resistant sources. The current research was conducted using six varieties (CH-121, Chilli-900 F1, Red Wing, SAYBAN, AAHP-1, and Diamond CH-121) collected from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Seeds were sown in the Plant Pathology field area, University of Agriculture, Faisalabad, to screen against leaf spot disease. The effect of different fungicides and nutrients to overcome chili leaf spot was studied, and three treatment combinations were prepared: ($ZnSO_4$ (ZS) + $MnSO_4$ (MS) + boric acid (BA) + Topsin M), (ZS + MS + BA + Mancozeb), and (ZS + MS + BA + Vitavax). Among these, the combination of (ZS + MS + BA + Vitavax) was the most effective against leaf spot disease. Minimum disease severity was observed with ZS + MS + BA + Vitavax in CH-121 (15.00), while maximum disease severity was observed with the control in Red Wing (93.67). Environmental data were collected and statistically analyzed. There was a significant ($p < 0.05$) but positive correlation between maximum temperature, minimum temperature, wind speed, rainfall, and disease severity. There was a negative correlation between relative humidity and disease severity. In conclusion, micronutrients were found to enhance *Alternaria* leaf spot tolerance in chili plants, providing a cost-effective and easily obtainable solution. The combined use of nutrients and fungicide is effective in preventing the development of resistance in pathogens against fungicides.

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## INTRODUCTION

Chili (*Capsicum annuum* L.) is cultivated on approximately 16% of the total spice cultivation area worldwide (Shoaib et al., 2021). Due to its crucial taste and flavor, coupled with a high medicinal and nutritional profile, chilli is grown in all provinces of Pakistan, ranking 3rd after potato and onion (Iqbal et al., 2017; Iqbal et al., 2021). In Pakistan, an area of 64.2 thousand hectares is dedicated to chilli cultivation, with an annual production of around 143.1 thousand tons, contributing 1.5% to the GDP (Rais et al., 2021).

Chilies are perhaps the most sensitive crop to various pathogens (Shahbaz et al., 2015; Asghar et al., 2020; Tariq-Khan et al., 2017, 2020; Saba et al., 2022; Aslam and Mukhtar, 2023a,b, 2024) especially fungal infections, which are a major constraint in achieving better production. The resultant effects of many mycotoxins in food decrease the quality of the final harvested products (Sarkar et al., 2021). The most devastating and widespread fungal disease of chilli is leaf spot/blight caused by *Alternaria alternata* (Fr.) Keissler, responsible for 60% of economic losses (Soomro et al., 2019). The fungus can invade crops both before and after harvest, posing a significant threat to seed quality (Costa et al., 2019). Characteristic symptoms of leaf blight development initially appear as small, circular, brown spots, progressing into irregular shapes that cover large areas of the leaves, resulting in drying, withering, and defoliation (Sarkar et al., 2022). Affected chilli fruit also become susceptible to rot, leading to the production of toxins. The quality of the seed is greatly affected, and the use of this seed for the next crop may increase the threat of rapid dissemination of the pathogen in the whole field (Matić et al., 2020; Nguyen et al., 2021; Saeed et al., 2022).

Leaf spot of chilli is commonly managed using different chemical fungicides, which do not align with sustainable agricultural practices. Micronutrients are applied for better growth and to boost the host plants. Zinc has an eco-friendly impact on host plants against many fungal diseases and acts as the gatekeeper of the plant immune system, boosting the antioxidant machinery against disease stress and regulating intracellular signaling pathways (Awan et al., 2019; Hossain et al., 2021). Zinc sulfate ($ZnSO_4$) is frequently used against fungal pathogens due to its high solubility and mobility, as well as easy access to host plants through spraying and drenching methods (Gomes et al., 2020).

The presented literature review emphasizes the need for the current study, indicating that micronutrients have a significant toxic effect on the fungus and can help manage leaf spot problems in chilli by increasing the bioactivity of the chilli plants. The present study was planned to assess the potential of foliar spray of micronutrients and chemicals against the fungal pathogen *Alternaria alternata*, the causal organism of chilli leaf spot disease.

## MATERIALS AND METHODS

### Nursery establishment

The nursery for chilli plants was established by collecting seeds from six varieties, namely CH-121, SAYBAN, AAHP-1, Chilli-900 F1, Red Wing, and Diamond CH-121, obtained from Ayub Agricultural Research Institute, Faisalabad. The seeds were sown in the research area of Plant Pathology at the University of Agriculture Faisalabad (UAF), Pakistan. Initially, the seeds were sown in a tray, and later they were transplanted into the field where augmented and randomized complete block design were employed for screening and management trials, respectively.

### Screening of chilli varieties

The evaluation of chilli varieties (CH-121, SAYBAN, AAHP-1, Chilli-900 $F_1$, Red Wing, and Diamond CH-121) for resistance to *Alternaria* leaf spot was done by using rating scale given in Table 1 proposed by .

Table 1: Rating scale used for categorization of chili cultivar for their relative resistace or suscetibility or leaf spot.

| Response/Category | Disease (%) |
|---|---|
| Immune (I) | 0% |
| Highly Resistant (HR) | 1-10% |
| Resistant (R) | 11-20% |
| Moderately Resistant (MR) | 21-30% |
| Susceptible (S) | 31-50% |
| Highly Susceptibe (HS) | >50% |

**Isolation identification and purification of pathogen**

The infected leaves and stems of chili plants were observed within the experimental area, and the samples were collected and brought to the Mycology Lab for the isolation and identification of the causal organism. The general fungal isolation medium, potato dextrose agar (PDA), composed of potato starch (20 g/L), dextrose (20 g/L), and agar agar (16 g/L), was used. A 2% aqueous solution of hydrogen peroxide ($H_2O_2$) was employed to disinfect the collected samples, which were then placed on PDA solidified plates. The inoculated plates were incubated for 10-12 days at 25°C in the dark and under light. Spore and mycelial growth were observed under the microscope.

The pathogen was purified using mycelium transfer and hyphal tip procedures and identified using available literature, based on parameters such as plate color, colony pattern, presence of micro and macroconidia, spore form, size, and structure.

**Management of disease through nutrients and fungicide combinations**

In the management experiment, three chili varieties were sown based on a moderately resistant to susceptible response. The crop received three treatment combinations of nutrients + fungicides, along with a control, to manage chili leaf spot (Table 2). All standard agronomic practices were performed to maintain the crop in good condition. The rationale behind the three treatments was to evaluate the effect of different nutrients on boosting plant growth and defense mechanisms in combination with fungicides.

The data were collected on the basis of the disease severity by using below mentioned formula:

$$\text{Disease Severity (\%)} = \frac{\text{No. of infected leaves}}{\text{No.of total leaves}} \times 100$$

**Data recording of agronomic parameters and role of environmental parameters in disease progression**

Three plants were randomly selected from each row of every replication, and the shoot and root lengths of the chili germplasm were measured using the scale. The average length was calculated by taking the mean of the measurements from the three plants and expressed in centimeters (cm). Additionally, the fresh and dry weights of three randomly chosen plants from each replication of the germplasm were measured after uprooting, using a weighing balance. The average weight was calculated by taking the mean of the weights from the three plants and expressed in grams (g).

Table 2: Treatment details applied against *Alternatria* leaf spot of chili.

| Treatments | Combinations | Dose |
|---|---|---|
| Treatment 1 | $ZnSO_4$+ $MnSO_4$+Boric acid +Topsin M | 1 g/L |
| Treatment 2 | $ZnSO_4$+ $MnSO_4$+Boric acid +Mancozeb | 1 g/L |
| Treatment 3 | $ZnSO_4$+ $MnSO_4$+Boric acid +Vitavax | 1 g/L |

The data on environmental variables, including maximum temperature, minimum temperature, relative humidity, wind velocity, and rainfall, were collected from the meteorological department of UAF. Correlation analysis was applied to the data of environmental variables and disease severity, and the role of the environment in disease severity was observed based on the correlation coefficient values.

**Statistical analysis**

The mean values calculated from each observation were subjected to analysis of variance using Statistix 8.1 software. The correlation analysis of environmental conditions was conducted using SPSS software. The time consumption (in seconds) for counting the adults was also analyzed using analysis of variance (ANOVA). Tukey's HSD test was employed to compare the means of observations from different experiments.

**RESULTS**

**Response of chilli germplasm against *Alternaria* leaf spot under natural conditions**

There was no disease immunity observed in any of the varieties/cultures examined in the current investigation (Table 3). The two lines of chili, CH-121 (23.67%) and Chilli-900-F1 (28.33%), exhibited moderate resistance to the disease. The susceptible genotypes were Diamond CH-121 (39.33%) and Red Wing (48%). The highly susceptible genotypes were SAYBAN (53.67%) and AAHP-1 (57.33%).

**Effect of micronutrients and chemicals on disease severity**

CH-121 showed the maximum reduction percentage

(58.92%) in disease severity with the treatment of Zinc Sulphate + Manganese Sulphate + Boric Acid + Vitavax. Red Wing and Chili-900-F1 exhibited the minimum reduction percentages of 25.56% and 25.54%, respectively, with the treatment of Zinc Sulphate + Manganese Sulphate + Boric Acid + Topsin M (Table 4).

Table 3. Response of different chili varieties against *Alternaria* leaf spot disease.

| Scale | Disease Category | Varieties | Disease Severity (%) | Response |
|---|---|---|---|---|
| 1 | 0 % | – | – | Immune |
| 2 | 1-10 % | – | – | Highly Resistant |
| 3 | 11-20 % | – | – | Resistant |
| 4 | 21-30 % | CH-121 | 23.67 | Moderately Resistant |
| | | Chilli-900-F1 | 28.33 | |
| 5 | 31-50 % | Diamond CH-121 | 39.33 | Susceptible |
| | | Red wing | 48.00 | |
| 6 | >50 % | SAYBAN | 53.67 | Highly Susceptible |
| | | AAHP-1 | 57.33 | |

Table 4. All-pairwise comparisons test of disease severity for treatments x varieties.

| Mean Disease Severity (%) | | | | | |
|---|---|---|---|---|---|
| Variety | Intervals 15 Days | Treatments | | | |
| | | Control | Micronutrients + Mancozeb | Micronutrients + Topsin M | Micronutrients + Vitavax |
| CH-121 | $X_1$ | 28.20 B | 29.20 Ab | 25.86 B | 26.53 B |
| | $X_2$ | 58.66 B | 35.46 Ef | 36.53 Ef | 32.00 F |
| | $X_3$ | 67.00 a | 40.66 c | 42.66 c | 25.66 d |
| | $X_4$ | 87.66 A | 34.33 E | 49.00 Cd | 15.00 F |
| Chili-900-F1 | $X_1$ | 31.00 ab | 35.66 ab | 34.66 ab | 30.33 ab |
| | $X_2$ | 61.66 b | 36.46 ef | 45.53 d | 33.66 f |
| | $X_3$ | 69.66 a | 41.00 c | 53.33 b | 28.00 d |
| | $X_4$ | 90.00 a | 40.33 de | 54.33 bc | 22.00 f |
| Red Wing | $X_1$ | 42.66 a | 35.00 ab | 38.33 ab | 38.00 ab |
| | $X_2$ | 67.00 a | 39.46 e | 51.53 c | 36.00 ef |
| | $X_3$ | 72.33 a | 50.00 b | 55.00 b | 31.33 d |
| | $X_4$ | 93.66 A | 50.33 C | 60.33 B | 38.33 E |

Micronutrients = Zinc Sulphate ($ZnSO_4$) + Manganese Sulphate ($MnSO_4$) + Boric acid, Alpha = 0.05, Tukey HSD Values for; X1 = 14.224, X2 = 5.1230, X3 = 7.1484, X4 = 8.6933

**Effect of micronutrients and chemicals on plant parameters**

Treatments and varieties interactions indicated that the maximum shoot length (78.133 cm) was observed in CH-121 with Zinc Sulphate + Manganese Sulphate + Boric Acid + Vitavax, while the minimum shoot length (41.900 cm) was recorded with the control in Red Wing. Conversely, the maximum root length (18.490 cm) was measured in CH-121 with Zinc Sulphate + Manganese Sulphate + Boric Acid + Vitavax, and the minimum length (4.667 cm) was recorded with the control in Red Wing. Zinc Sulphate + Manganese Sulphate + Boric Acid + Vitavax demonstrated the maximum fresh weight (383.60 g) in CH-121, while the minimum weight (115.58 g) was recorded with the control in Red Wing. The maximum dry weight (160.19 g) was measured with Zinc Sulphate + Manganese Sulphate + Boric Acid + Vitavax in CH-121, and the minimum weight (45.79 g) was recorded with the control in Red Wing (Table 5).

**Role of environmental factors in disease developments**

Temperature is one of the most important components that impact plant health in many ways and also help the initiation and progression of many diseases. There was a significant ($p < 0.05$) but positive correlation between the maximum temperature and disease severity of all three varieties, with the values being CH-121 (0.97), followed by Red Wing (0.97), and Chilli-900-F1 (0.98) (Table 6). Similarly, there was a significant ($p < 0.05$) but positive correlation between the minimum temperature and disease severity of all three varieties, with the values being CH-121 (0.94), followed by Red Wing (0.94), and Chilli-900-F1 (0.95).

Table 5. All-pairwise comparisons test of plant parameters recorded for treatments x varieties.

| Effect of Micronutrients and Chemicals on Plant Parameters | | | | | |
|---|---|---|---|---|---|
| Plant Parameters | Varieties | Treatments | | | |
| | | Control | Micronutrients + Mancozeb | Micronutrients + Topsin M | Micronutrients + Vitavax |
| Shoot Length (cm) | CH-121 | 46.467 c | 74.800 a | 61.600 b | 78.133 a |
| | Chilli-900-F1 | 44.100 c | 62.400 b | 60.333 b | 75.300 a |
| | Red Wing | 41.900 c | 61.267 b | 46.700 c | 74.600 a |
| Root Length (cm) | CH-121 | 7.867 D | 14.800 B | 8.320 D | 18.490 A |
| | Chilli-900-F1 | 5.300 e | 11.267 c | 7.887 d | 17.470 a |
| | Red Wing | 4.667 e | 8.900 d | 7.793 d | 14.360 b |
| Fresh Weight (g) | CH-121 | 128.25 f | 329.10 b | 256.80 d | 383.60 a |
| | Chilli-900-F1 | 117.80 f | 283.67 cd | 222.00 e | 364.92 a |
| | Red Wing | 115.58 f | 270.13 d | 195.00 e | 308.87 bc |
| Dry Weight (g) | CH-121 | 57.80 de | 111.78 bc | 96.33 c | 160.19 a |
| | Chilli-900-F1 | 51.68 de | 101.98 bc | 69.67 d | 158.90 a |
| | Red Wing | 45.79 e | 97.98 c | 60.33 de | 121.59 b |

Alpha = 0.05, Tukey HSD Values for; Shoot Length = 8.8387cm, Root Length = 2.2576cm, Fresh Weight = 34.019g, Dry Weight = 23.418g

Table 6. Effect of Environmental factors on disease development.

| Variables | CH-121 | Chili-900-F1 | Red Wing |
|---|---|---|---|
| Max. Temperature p-value | 0.9756<br>0.0000** | 0.9784<br>0.0000** | 0.9747<br>0.0000** |
| Min. Temperature p-value | 0.9497<br>0.0000** | 0.9522<br>0.0000** | 0.9401<br>0.0000** |
| Relative Humidity p-value | -0.9511<br>0.0000** | -0.9550<br>0.0000** | -0.9310<br>0.0000** |
| Rain Fall p-value | 0.2275<br>$0.4771^{NS}$ | 0.2352<br>$0.4619^{NS}$ | 0.2292<br>$0.4736^{NS}$ |
| Wind Speed p-value | 0.5825<br>0.0469* | 0.5782<br>0.0489* | 0.6210<br>0.0312* |

Humidity accelerates disease progression by modifying the infection process, sporulation, and spore spread. The invasion of upper plant parts by pathogens is triggered by rain and excessive humidity. Infection and disease progression are more likely to occur when atmospheric humidity is significantly greater (>85%). There was a negative correlation between relative humidity and disease incidence in all three varieties; as relative humidity increases, disease incidence decreases. This relationship was found to be significant at $p < 0.05$, with correlation coefficients for the varieties as follows: Red Wing (-0.93), CH-121 (-0.95), and Chilli-900-F1 (-0.96).

The quantification of spore removal from the host and escape from the source site is crucial for successfully simulating spore transport across any distance. There was a significant ($p < 0.05$) but positive correlation between wind speed and disease severity of all three varieties, with the values being Chilli-900-F1 (0.57), followed by CH-121 (0.58), and Red Wing (0.62), as recorded.

**DISCUSSION**

Preventing plant diseases with resistant cultivars is the most suitable, practical, competitive, and cost-effective strategy (Ashfaq et al., 2014; Aslam et al., 2017). The use of resistant cultivars not only provides disease resistance but also saves time, attention, and money compared to other disease control methods (Thakur et al., 2018; Garibaldi et al., 2019; Amrao et al., 2021; Khruengsai et al., 2021; Moya-Elizondo et al., 2021; Sarkar et al., 2021; Shoaib et al., 2021; Kumar et al., 2022). The decline in chili production and the deterioration of fruit quality have heightened the necessity for a long-term strategy to stop the disease's spread (Geddes-McAlister and Shapiro, 2019; Pickel et al., 2020; Bibi et al., 2023; Mukhtar et al., 2023; Shahbaz et al., 2023).

The current research aimed to determine the source of resilience to *Alternaria* leaf spot of chili induced by *A. alternata*. The genotypic response to disease revealed that cultivars CH-121 and Chilli-900-F1 showed moderately resistant, Diamond CH-121 and Red Wing susceptible, and SAYBAN and AAHP-1 highly susceptible responses. Parey et al. (2013) conducted a similar study, stating that the goal of eco-friendly management of chili leaf spot was to identify primary resistant sources against the pathogens. *A. alternata* and *Aspergillus flavus* were found to be linked with the disease in samples of chili leaves obtained from various places.

In the present study, the minimum value of disease severity and the maximum values of shoot length, root length, fresh weight, and dry weight were observed with the treatment of ZS + MS + BA + Vitavax. Prasad and Naik (2003) found that Mancozeb, as a seed dresser, was efficient against *Alternaria* infections, followed by Bavistin, Iprodione, and Thiram. Yadav et al. (2015) stated that fungicidal seed treatments with Indofill Z 78, Indofill Z 45, Captol, Ziram, Captofol, and Thiram are used to inhibit *A. alternata* development in the laboratory. Captol provided the highest degree of inhibition (100%), followed by Captofol (89.5%) at a dosage of 0.15%.

This article examines the efficiency of fungicides for controlling *Alternaria* leaf spot in tropical areas under field conditions and the use of an implementation approach that could significantly increase disease control efficacy. Shoaib et al. (2021) stated that Zinc, a vital mineral for plant growth, also enhances disease resistance and functions as an antifungal agent in plants. In this study, the effects of $ZnSO_4$ on *A. alternata* propagation were explored *in vitro*, as well as the effect of foliar $ZnSO_4$ administration in disease-stricken chili pepper plants *in vivo*. *In vitro*, $ZnSO_4$ produced 100% suppression occurring at 8.55 mM, as well as abnormal antioxidant enzyme activity. Changes occurred in the protein structure of the fungal biomass after Zn accumulation. *In vivo*, pathogen infection generated the highest severity of leaf spot disease, resulting in a considerable drop in the plant's growth rate.

Based on physicochemical factors and disease severity analysis, useful information about the optimal treatment for disease management is provided. In chili pepper plants, foliar Zn (0.038 mM) increased the activity of antioxidants and defense chemicals while controlling 77% of illness. Foliar $ZnSO_4$ has been found to be a successful and long-term agricultural practice for controlling *Alternaria* leaf spot disease in chili pepper plants in the study.

The correlation of environmental variables, i.e., maximum temperature, minimum temperature, wind speed, relative humidity, and rainfall, with *Alternaria* leaf spot was observed. There was a significant ($p<0.05$) but positive correlation between maximum temperature, minimum temperature, wind speed, rainfall, and disease severity. There was a negative correlation between relative humidity and disease severity.

Many researchers have demonstrated that increased or decreased temperature has a direct impact on chili yield. Bell peppers grown at high temperatures (33°C) had less fruit set and flower malformation than bell peppers cultivated at low temperatures (below 18°C), resulting in reduced fruit set (Hernández-Carrión et al., 2014). In the case of chili, temperature also has a role in the development of diseases. Higher temperatures resulted in a considerable increase in disease incidence. High temperatures aided in the infection of necrotic spots in chilies (Roggero et al., 1999).

Uneven rainfall distribution causes significant production loss due to a number of physiological and biochemical hurdles, as well as infestations by insect pests and diseases. Soil acidity and salinity are two of the most damaging abiotic factors of crop yield, causing major crop loss around the world (Sarada et al., 2015). About 40% of the world's agricultural land is acidic, and over 100 million hectares of land in South and Southeast Asia are unsuitable due to soil acidity and the issues that come with it (Ismail et al., 2007).

It is suggested that micronutrients be used to boost *Alternaria* leaf spot tolerance in chili plants because it is a cost-effective and easily obtainable solution. A field study could be useful in evaluating the impact of micronutrients on *Alternaria* leaf spot. Furthermore, the impact of micronutrients on the pathogenicity and virulence of *A. alternata* demands a more in-depth and thorough investigation to take advantage of existing understanding of host-pathogen associations.

The effectiveness of foliar application of $ZnSO_4$ compared to ZnO, ZnO + nanoparticles, and Zn-EDTA in terms of translocation, accumulation, and mobility has also been proven by previous studies (AbdElAziz et al., 2021; Rehman et al., 2021). $ZnSO_4$ plays a significant role in the amelioration of the host defense system against fungal diseases *in vivo* conditions and also has growth inhibitory effects *in vitro* on different fungal pathogens such as *A. flavus*, *Fusarium graminearum*, and *Penicillium citrinum* (Savi et al., 2013; Du et al., 2019; Zeeshan et al., 2023).

In particular, different manageable doses of $ZnSO_4$ are used against fungal pathogens, managing up to 60% of diseases, including peach gummosis, charcoal rot of mung bean, powdery mildew of eucalyptus, and tomato early blight, either by inhibiting fungal growth or by inducing resistance against these diseases in host plants (Li et al., 2016; Silva et al., 2016; Shoaib et al., 2020). Furthermore, against biotic stresses, Zn increases the enzymatic activity of the host, including peroxidase (POX), catalase (CAT), and polyphenol oxidase (PPO), having beneficial effects on the chili immune system (Shoaib and Awan, 2021). According to Cabot et al. (2019), Zn has played a significant role in disease resistance by inducing the host defense system.

## CONCLUSIONS

The current study concludes that the combination of nutrients and fungicides i.e. ZS + MS + BA + Vitavax proved to be the most effective in minimizing *Alternaria* leaf spot severity. The study describes the importance of environmental factors, indicating a significant positive correlation between temperature, relative humidity and disease severity.

## AUTHORS' CONTRIBUTIONS

BZ wrote manuscript and conducted research; SA conceived idea and supervised research; MAZ edited manuscript; GMS analyzed data; YA provided technical assistance in lab work; RB helped in environmental conditions data; MUG helped in data collection; AA proof read the manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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