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Research Article

Epidemiological Characterization of Turnip Leaf Spot Disease and its Management

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ABSTRACT

Turnip crop is badly affected by *Alternaria* leaf spot (ALS) that cause huge qualitative and quantitative losses. The objectives of present study were to evaluate the resistance of turnip germplasm against ALS, effect of environmental variables on ALS disease development, management of ALS disease through nutrients and to compare the yield parameters of both healthy and infected turnip plants. Turnip germplasm consisting of 4 varieties (Nankana Red, Golden Ball, PTWG and Purple Red) were sown for screening against ALS under natural conditions using augmented design. Similar varieties were sown for the management of ALS through nutrients which were applied weekly and data of disease severity was recorded after each application. The data of environmental variables were obtained from weather observatory and subjected to correlation analysis to check its effect on disease development. Yield parameters i.e. plant height, shoot length, root length, fresh and dry weight were recorded from both healthy and diseased plants. None of the 4 varieties were found to be immune or highly resistant according to disease rating scale. Nankana Red and Purple Red were resistant and highly susceptible against ALS disease, respectively. There was significant reduction of yield parameters in diseased plants which was boosted after the application of treatments. The combination of 4 treatments (sulphates of manganese, zinc, copper and boric acid) gave the highest yield reduction with 18.45% disease severity. The effect of temperature and wind speed was positive while of relative humidity and rainfall was negative with ALS disease development. This research provides valuable insights into the management of leaf spot disease and highlights the need for sustainable agricultural practices to ensure food security.

Keywords: Nutrients, Leaf spot, Turnip, Weather variables, Germplasm screening.

INTRODUCTION

Turnip (*Brassica rapa* L.) is the member of *Brassica* genus and family *Brassicaceae* (mustard family) (Cao et al., 2021). It is grown in different parts of the world for tuberous roots and green leaves that are important for food production to feed the population and support the economic facet of horticulture (Yang et al., 2023). Emerging in relevance as a major biotic menace to turnip production, Alternaria leaf spot (ALS) is inclined towards the pathogen caused by *Alternaria brassicicola*. In turnip plants, ALS breaks down the foliage, which prevents photosynthetic ability, defoliation and yield decrease (Patel et al., 2018). *A. brassicicola* is a necrotrophic fugal pathogen which infest the turnip and other members of the *Brassicaceae* family lose up to 60% yield (Madhu-Kiran et al., 2018). An infected plant by turnip is ingested by the pathogen either through conidia or infected plant residue are produced the specific pathogenic spores and toxins that are required to advance the disease (Xu et al., 2021). ALS disease is a factor in turnip production based on environmental factors, regional climates and seasonal weather which show the necessity of an intergraded disease management (IDM) (Fagodiya et al., 2022).

An earlier research has demonstrated that turnip's Alternaria leaf spot is more challenging to manage than other fungal diseases (Matić et al., 2020). Fungicides have been considered regarding their effectiveness on the management of ALS of turnip (Nira et al., 2022). Fungicides have low efficacy toward ALS disease because of resistance of *A. brassicicola,* although application of multiple strategies of IDM are recommended (Shoaib et al., 2021).

The present experiment was planned for following aims:

Assessment of resistance and yield potential of different cultivars against *Alternaria* leaf spot of Turnip in relation to environmental factors and to evaluate the effectiveness of nutrients against ALS.

MATERIALS AND METHODS

Field preparation and collection of Turnip Seeds

Field area was prepared according to the recommended agronomic practices for turnip in the experimental area of Department of Plant Pathology, University of Agriculture, Faisalabad (UAF-Pakistan). The seeds of 4 turnip varieties (Nankana red, Purple red, Golden Ball and PTWG) were collected from the Vegetable Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan).

Experimental design and layout

The germplasm for screening was sown in augmented design while for management in randomized complete block design (RCBD) with 3 replications. Seed potato was sown on ridges with row to row distance 45 cm and plant to plant was 30 cm. To find out the most effective management strategy, 6 treatments were applied for foliar spray.

Use of nutrients for the management of ALS

The combination of nutrients used treatments of ALS consisted of Boric acid (BA), Manganese sulphate (MS), Zinc sulphate (ZS) and Copper sulphate (CS).

Table 1. Abbreviations of Treatments

Sr. No	Treatment Name	Abbreviation	Dose
1	$ZnSO_4$+$CuSO_4$	ZS+CS	0.25g /L each
2	Boric acid+$CuSO_4$	BA+CS	0.25g /L each
3	$MnSO_4$+$ZnSO_4$+$CuSO_4$	MS+ZS+CS	0.17,0.16, 0.17g /L, respectively
4	$CuSO_4$+$ZnSO_4$+Boric acid	CS+ZS+BA	0.17,0.16, 0.17g /L, respectively
5	$MnSO_4$+$ZnSO_4$+Boric acid+$CuSO_4$	MS+BA+CS+ZS	0.12,0.12, 0.13, 0.13g /L, respectively
6	$MnSO_4$+Boric acid+$CuSO_4$	MS+BA+CS	0.17,0.16, 0.17g /L, respectively

Disease severity data recording from screening and management trials

The results of the treatments were recorded on the basis of disease severity formula and disease rating scale was used for categorization (Mishra et al*.,* 2024).

Table 2. Disease rating scale for ALS disease severity

Scale	Disease incidence %	Response
0	0%	Immune
1	1-10%	Highly resistant
3	11-20%	Resistance
5	21-30%	Moderately resistance
7	31-50	Susceptible
9	<50%	Highly susceptible

$$\text{Disease Severity }\% = \frac{\text{No.of infected leaves}}{\text{Total no.of leaves}} \times 100$$

Data of yield parameters

Randomly 3 plants were selected from each row of each replication and shoot and root length was measured with the help of scale. By taking the mean of 3 plants, average length was calculated and expressed in centimeters (cm). Fresh and dry weight was recorded by using weighing balance and expressed in grams (g) from 3 plants and then taking the average.

Isolation and identification of pathogen

Plant tissues infected with ALS disease, were taken from the field area of Plant Pathology for isolation of *Alternaria brassicicola*. The leaves were chopped into 1cm pieces and sterilized by immersing them in 2% aqueous hydrogen peroxide solution for 30 seconds, followed by a 1 minute dip in sterile distilled water. The water on the surface was removed by soaking it in sterile filter paper. Three to four samples per plate were placed on the media plates. The samples were incubated for 4-5 days at 25°C in the incubator.

A. brassicicola colonies, were identified based on plate color, colony pattern, presence of micro and macro conidia, spore form, size, and structure.

Effect of environmental factors on *Alternaria* leaf spot disease development

The data of weather variables (minimum temperature, maximum temperature, relative humidity and wind speed) was collected from weather observatory installed at UAF (Pakistan).

Statistical analysis

Analysis of variance (ANOVA) was performed to determine the significance of differences among genotypes, nutrient treatments, and their interactions. Mean values were compared by least significant difference (LSD) test at 5% significance level. Statistical analysis was performed by using statistics 8.1 and environmental data was correlated by Pearson's correlation (Steel et al., 1997).

RESULTS

Screening of turnip varieties for resistance level to ALS disease *in-vivo*

It was found that genotype "Nankana red" showed resistance against the *A. brassicicola* with 18.67% ALS disease severity followed by "Golden Ball" (29.33%) that was categorized as moderately resistant. The genotype "PTWG" was susceptible with 45.67% disease severity followed by "Purple red" with 72% disease severity and regarded as highly susceptible genotype (Table 3).

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

Scales	Disease Categories	Varieties	Disease Severity (%)	Response
1	0 %	–	–	Immune
2	1-10 %	–	–	Highly Resistant
3	11-20 %	Nankana Red	18.67 d	Resistant
4	21-30 %	Golden Ball	29.33 c	Moderately Resistant
5	31-50 %	PTWG	45.67 b	Susceptible
6	>50 %	Purple Red	72.00 a	Highly Susceptible

Different letters are indicating significant difference in column

Evaluation of nutrients against ALS disease of turnip

The 2 ways interaction between treatments and varieties was significant; it means that the comparison of nutritional treatments for the management of ALS disease severity was different in turnip germplasm (Table 4). The individual effect of treatments and varieties was also significant meaning that all the treatments reduced disease severity in all varieties.

Table 4. Analysis of variance for disease severity (%) against ALS of turnip

Source	Df	SS	MS	F	P
Replications	2	0.6	0.29		
Treatment	6	26593.8	4432.30	812.04	0.0000**

Varieties	3	975.1	325.05	59.55	0.0000**
Treatment x Varieties	18	290.7	16.15	2.96	0.0011**
Error	54	294.7	5.46		
Total	83	28154.9			

** Highly Significant p-value < 0.01
* Significant p-Value < 0.05
NS Non-significant p-Value > 0.05

Among treatments, minimum disease severity was observed by treatment of MS+BA+CS+ZS (18.75%) followed by BA+CS+MS(30.16%), CS+ZS+MS (35.75%), BA+CS+ZS (40.04%), BA+CS (45.29%), ZS+CS (51.28%) (Table 5).

Table 5. Evaluation of nutritional treatments on ALS disease severity

Treatment	**Mean Disease severity (%)**
Control	79.25 a
BA+CS	45.29 c
BA+CS+MS	30.16 f
BA+CS+ZS	40.04 d
CS+ZS+MS	35.75 e
MS+BA+CS+ZS	18.45 g
ZS+CS	51.28 b
Alpha = 0.05	
Tukey HSD-Value = 2.9198	

Different letters are indicating significant difference in column

The response of varieties against treatments indicated that minimum disease severity were shown by Nankana red (38.51%) followed by Golden Ball (41.24%), PTWG (44.16%) and Purple red (47.67%) respectively (Table 6).

Table 6. Response of varieties towards treatments for ALS disease management

Varieties	**Mean Disease severity (%)**
Golden Ball	41.24 c
Nankana Red	38.51 d
PTWG	44.16 b
Purple Red	47.67 a
Alpha = 0.05	
Tukey HSD-Value = 1.9114	

Different letters are indicating significant difference in column

The 2 ways interaction between treatments and varieties indicated that the lowest severity (15.67%) of ALS was showed by MS+BA+CS+ZS in “Nankana red” variety and highest disease severity (85.83%) was in Control of “Purple red” (Table 7).

Table 7. Two ways interaction between Treatment x Varieties

Treatment	**Mean Disease severity (%)**			
	Golden Ball	**Nankana Red**	**PTWG**	**Purple Red**
Control	74.67 b	73.51 b	83.01 a	85.83 a
BA+CS	42.51 de	40.83 defg	44.01 d	53.83 c
BA+CS+MS	28.51 j	28.33 j	29.51 ij	34.333 fghij
BA+CS+ZS	38.33 defgh	36.51 efghi	41.51 def	43.83 de
CS+ZS+MS	33.51 ghij	31.33 hij	37.33 defgh	40.83 defg
MS+BA+CS+ZS	17.83 k	15.67 k	19.67 k	20.67 k

ZS+CS	53.33 c	43.33 de	54.13 c	54.33 c
Alpha = 0.05				
Tukey HSD-Value = 7.4626				

Different letters are indicating significant difference in column

Effect of nutrients on yield parameters

The maximum height (82.97cm) was measured in the plants treated with MS+BA+CS+ZS followed by BA+CS+MS (73.71cm) while minimum plant height (57.62 cm) was recorded by ZS+CS. The maximum shoot length (75.41 cm) was measured in plants treated with MS+BA+CS+ZS followed by BA+CS+MS (70.08 cm); while minimum shoot length was recorded by ZS+CS (50.15 cm). The maximum root length (18.01 cm) was measured by the treatment MS+BA+CS+ZS and minimum root length (14.92 cm) in ZS+CS treated plants. The highest fresh weight (24.99 g) was recorded in MS+BA+CS+ZS treated plants and minimum (15.19) in ZS+CS treatment. The dry weight was highest (16.822 g) in MS+BA+CS+ZS treatment while lowest (8.985g) in ZS+CS (Table 8).

Table 8. Effect of nutrients on yield parameters of turnip

Treatment	Plant height (cm)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
BA+CS	60.98 d	55.62 e	15.17 c	17.12 e	10.32 e
BA+CS+MS	73.71 b	70.08 b	17.42 a	22.22 e	14.75 b
BA+CS+ZS	66.58 c	60.27 d	15.75 bc	18.64 d	11.51 d
CS+ZS+MS	71.32 b	64.31 c	16.33 b	20.49 c	13.57 c
MS+BA+CS+ZS	82.97 a	75.41 a	18.00 a	24.99 a	16.82 a
ZS+CS	57.62 e	50.16 f	14.92 a	15.19 f	8.99 f
Control	41.75 f	37.33 g	11.08 a	10.03 g	5.21 g
Alpha = 0.05					
Tukey HSD-Value = 2.97					

Different letters are indicating significant difference in column

Effect of environmental factors on ALS disease development

Temperature (both maximum and minimum) were directly proportional to the disease as increase in temperature resulted in increased ALS disease severity in all 4 varieties. In case of maximum temperature, correlation coefficient (r) is very near to +1 indicating strongly positive relationship with ALS disease severity in all varieties with maximum (0.94) in PTWG and minimum (0.90) in purple red. A similar increasing trend was recorded in minimum temperature with Purple red (0.92) followed by Golden Ball (0.93), Nankana red (0.93) and PTWG (0.95) correlation coefficient values. The relationship of relative humidity and rainfall was significantly negative with ALS disease severity i.e. increase in these parameters decreased disease severity. The correlation coefficients were (-0.74) PTWG, (-0.77) Golden Ball, (-0.77) Purple red and (-0.80) Nankana red. Wind speed affected ALS positively in all the 4 varieties and the values were PTWG (0.93) followed by, Golden Ball (0.95), Purple red (0.95) and Nankana red (0.96) (Table 9).

Table 9. Effect of environmental factors on ALS disease development

	Golden Ball	Nankana Red	PTWG	Purple Red	Maximum Temp.	Minimum Temp.	Relative Humidity
Nankana Red p-Value	0.99 0.0000**						
PTWG p-Value	0.98 0.0000**	0.99 0.0000**					
Purple Red p-Value	0.99 0.0000**	0.99 0.0000**	0.99 0.0000**				

Max. Temperature p-Value	0.91 0.0000**	0.91 0.0000**	0.93 0.0000**	0.89 0.0001**			
Min. Temperature p-Value	0.93 0.0000**	0.93 0.0000**	0.95 0.0000**	0.92 0.0000**	0.98 0.0000**		
Relative Humidity p-Value	-0.77 0.0031**	-0.79 0.0019**	-0.74 0.0057**	-0.77 0.0030**	-0.57 0.0483*	-0.69 0.0124*	
RainFall p-Value	-0.17 0.5909NS	-0.15 0.6375NS	-0.24 0.4453NS	-0.15 0.6329NS	-0.52 0.0766NS	-0.41 0.1857NS	-0.36 0.2486NS
WindSpeed P	0.94 0.0000**	0.95 0.0000**	0.92 0.0000**	0.94 0.0000**	0.78 0.0028**	0.84 0.0005**	-0.93 0.0000**

** Highly Significant p-value < 0.01
* Significant p-Value < 0.05
NS Non-significant p-Value > 0.05

DISCUSSION

Screening of turnip varieties indicated that none of the 4 turnip cultivars was immune or highly resistant and were found as resistant, moderately resistant, susceptible and highly susceptible against ALS disease, respectively. The varying resistance potential is due to the varied genetic makeup of each cultivar that confers defense against pathogens through physical barriers, biochemical responses and pathogen recognition (Saharan et al., 2021). Al-Lami et al., (2023) evaluated different varieties from *Brassicaceae* family for resistance against *Alternaria brassicicola;* which showed varying degrees of resistance potential. Mishra et al., (2024) conducted the biochemical characterization of genotypes which are under attack of *A. brassicicola*; there was enhanced accumulation of methionine and tryptophan in resistant cultivars. Li and Wu (2023) performed repeated trials of mustard genotypes for finding the resistance potential against *A. brassicicola* based upon phenotypic characterization. The production antioxidant enzymes are accomplished against pathogen invasion leading to initiation of defense signals (Narware et al., 2023). It has also been noted that glucosinolates in the members of *Brassicaceae* family, are originated from methionine and tryptophan that have a significant role in increasing resistance (Choudhury et al., 2022). Diksha et al., (2023) established a field to look for resistance sources in *Brassica rapa* (turnip) cultivars against leaf spot disease at various stages of growth and found all levels of resistance in various genotypes. Doullah *et al.* (2006) tested 52 lines for *Alternaria brassicicola* resistance and found 2 varieties, Saori and Edononatsu, were resistant and 5 varieties, Norin-F1-Bekana, Tokinashi Taisai,Yajima Kabu,Purara and Tateiwa Kabu, moderately resistant.

In current study, the combination of $MnSO_4$+Boric acid+ $CuSO_4$+ $ZnSO_4$ reduced the disease intensity and increased yield in all genotypes. These results can be supported by the findings of Jha et al., (2016); who investigated the effects of ZnSO4, Borax, and $CaSO_4$ against ALS infections. Dordas (2008) stated that manganese can control plant diseases by taking part in in phenol biosynthesis, photosynthesis, lignin and numerous other functions. $MnSO_4$ after absorption in plant foliage stimulates the photosynthetic processes and regulates the production of antioxidant enzymes (Salehi et al., 2023). The increase in yield due to the application of treatments can be attributed to the increase in antioxidant enzymes and photosynthetic activity which has significant role in plant growth and yield increment (Munir et al., 2020). According to the result outcome, ALS increased with increase in temperature while decreased with enhanced humidity. The relationship of wind speed and ALS was directly proportional. The weather variables in areas with heavy rainfall and long wet seasons significantly increase the growth of several *Alternaria* species on *Brassicae* (Mamgain and Biswas, 2020). The spore production capacity of *A. brassicicola* increases with increase in temperature; however it grows best at 25°C (Sinha et al., 2021). High temperature reduces the plant's ability to boost the defense mechanism against stresses and also hinders its metabolic and enzymatic activities due to which disease intensity increased at high temperature (Cardoso et al., 2020).

CONCLUSION

The study aimed to evaluate the resistance of turnip germplasm against Alternaria leaf spot (ALS) and the impact of environmental variables on disease development. Four turnip germplasm varieties were sown for screening and management. Results showed that none of the four varieties were immune or highly resistant. Nankana Red and Purple Red were resistant and highly susceptible. The combination of four treatments led to the highest yield reduction with 18.45% disease severity. The research underscores the need for sustainable agricultural practices to ensure food security.

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Not applicable.

AUTHOR CONTRIBUTIONS

SS conceived and conducted the study, as well as drafted the manuscript. SA supervised research. HK contributed to manuscript editing. MNS helped in treatment formulations. MUG Statistical analysis. YI reviewed and assisted in editing and refining the manuscript. MAZ helped in epidemiological studies and discussion.

COMPETING OF INTEREST

The authors declare that the research was carried without any commercial or financial relationships that could be construed as a potential conflict of interest.

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