



Official publication of Pakistan Phytopathological Society

Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)

https://pjp.pakps.com



RESEARCH ARTICLE

Resistance of Wheat Germplasm Against Spot Blotch and *in Vitro* Management Through Different Plant Extracts

^aMuhammad Junaid*, ^aAbid Riaz, ^aArsalan Hussain, ^bMuhammad Fayyaz, ^cMuhammad N. Sajid, ^aMuhammad A. Hayat

^a Department of Plant Pathology, Faculty of Agriculture, PMAS Arid Agriculture University, Rawalpindi, Pakistan.

^b Crop Diseases Research Institute (CDRI), National Agricultural Research Center (NARC), Islamabad, Pakistan.

^c Potato Research Station, Sialkot, Department of Agriculture, Punjab, Pakistan.

Corresponding Author:

Abid Riaz, Email: abidriaz@uaar.edu.pk

Article History:

Submitted: May 05, 2024; Revised: July 17, 2024; Accepted for Publication: August 25, 2024.

ABSTRACT

Wheat serves as Pakistan's main source of dietary energy and has the title of being the country's most important grain crop. Spot blotch, a fungal disease caused by *Bipolaris sorokiniana*, is significantly impacting wheat yields in the wake of rising temperature coupled with humid conditions during the crop season in Pakistan. This study investigated wheat germplasm resistance against the disease in NARC field conditions and *in vitro* botanical control measures. Out of 100 wheat lines, none was found to be highly resistant, 6 were found resistant and 5 showed moderate resistance. These six promising lines viz., TRB-95-KAC, TRB-62-127, BARS-KT-DNL-95, BARS-KT-DNL-102, BARS-KT-DNL-103, BARS-KT-DNL-105 are recommended for breeding program. Further research is needed to confirm these findings at other field locations. Pertaining to *in vitro* pathogen management, growth inhibition was found source and concentration dependent and had a positive correlation with the increase in concentrations within the same source/plant. Amongst three plant extracts used at various concentrations, highest antifungal activity against the pathogen in terms of growth inhibition was found @ 10% of garlic and *Alstonia* in PDA after 7 days' incubation as compared to eucalyptus at the same concentration.

Keywords: Resistance; Spot blotch, *Bipolaris sorokiniana*, Wheat, *in vitro*.

INTRODUCTION

Spot blotch (SB), a devastating disease of wheat in warm, humid growing locations worldwide, is caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*). *B. sorokiniana* is capable of producing toxins like helminthosporol and sorokinianin and can infect leaves, stems, roots, rachis, and seeds. Since SB can affect any type of wheat, disease-prone areas must have an integrated disease control plan. Several fungicides, particularly those in the triazole group, have

demonstrated efficaciousness in mitigating disease; other beneficial cultural management techniques include crop rotation, tillage, and early planting (Bereset *et al.*, 2020; Roy *et al.*, 2021; Roy *et al.*, 2023). The production of wheat is confronted with several issues in South Asian nations such as India. One such challenge is spot blotch which is caused by *B. sorokiniana* and can result in a loss of 25–43% of grain yield, depending on the infection stage (Roy *et al.*, 2024). Although contaminated seed is

the main source of inoculum for spot blotch, the fungus can also live in soil and plant debris (Kumar *et al.*, 2022). Numerous agronomic and cultural strategies have been put forth to address spot blotch, but none of them have proven to be entirely successful (Tahir *et al.*, 2022).

In 2023–24, 9.6 million hectares of wheat were sown, compared to 9.0 million ha the previous year, indicating a 6.6% increase (Kamble *et al.*, 2024). They reported 11.6 percent increase in yield *i.e.*, 31.4 million tonnes as compared to 28.2 million tonnes of the previous year. However, abiotic and biotic stressors prevented wheat production from reaching the objective of 32.2 million tonnes (Rauf *et al.*, 2022). Management methods for spot blotch include disease-free seed, seed treatment with fungicides, cultural practices in order to reduce inoculum sources, fertilization, crop rotation, use of chemicals and the research on disease resistance (Kamble *et al.*, 2024). Disease resistance is the best long-term control at no cost for the farmer and has been ecologically safe. Therefore, in the current study, wheat germplasm has been tested against the disease. Additionally, an aspect of in vitro management of the Pathogen has also been undertaken to test the efficacy of local plant extracts.

MATERIALS AND METHODS

Screening of Wheat Germplasm Against Spot Blotch:

Wheat germplasm was screened at National Agricultural Research Centre (NARC) Islamabad to investigate the sources of resistance against spot blotch. In the experiment, the Morocco was utilized as a spreader and was planted after every 20 test entries. The study was conducted using randomized completely block design. Each variety/line was sowed in two rows of 1 meter each, with a 30 cm row to row distance. All agronomic activities, such as ploughing, irrigation and weeding were the same for all varieties/lines.

Field Inoculation: Before heading, each variety was sprayed with inoculum using an atomizer, derived from a pure culture prepared in distilled water at a concentration of 3.4×10^4 conidia/ml. Immediately following inoculation, the field was irrigated. Upon maturity, data was collected using a two-digit scale (00–99). This scale, adapted from Saari and Prescott (1975) for foliar diseases (Pandey *et al.*, 2018), assigns the first digit (D1) to disease progression in height and the second digit (D2) to severity based on the extent of diseased leaf area.

The categorization of reaction is as follows:

0=HR

1-10% =R

11-30% =MR

31-50% =MS

51-60% =S

61% or above =HS

The data was recorded by using following formula

Disease response= $\frac{(D1 \div 9) \times (D2 \div 9) \times 100}{100}$

D1= Disease progression in height

D2= Severity based on the extent of diseased leaf area

9= Highest value of disease rating scale

100= Remains constant

In Vitro Management Through Plant Extracts:

Selection of Plant Extracts: Three plant extracts *i.e.*, Euclyptus, garlic and Alstonia were used to minimize growth of *B. sorokiniana*. These were commonly known and easily available for antibacterial and antifungal purpose. The extracts were taken from the shaded dried leaves of Eucalyptus, Alstonia tree and underground stem of garlic.

Preparation of Plant Extract: Methanolic extract method was used for the preparation of botanical extracts following the protocol of (Sattar *et al.*, 2018). By using this method, plant materials were cleaned with tap water and dried in shade and then powder was made by grinding plant parts. Plant components (powder) and methanol were combined in a 1:3 ratios, meaning that 50 grams of plant powder was added to 150 milliliters of methanol. Following a thorough mixing of the methanol and plant powders, the solutions were shaken on a shaker for 48 hours before putting in glass bottles to homogenize. The solutions were then given the opportunity to go through improved extraction. After 48 hours, the extracts were filtered using Whatman filter paper no.1 and then put in glass pans to allow the methanol to dry completely (Atiq *et al.*, 2024; Yaqoob *et al.*, 2024). After 24 hours of continuous methanol evaporation, the extracts were collected. Stock solutions were made by adding 1:1 concentration. After harvesting, each of the extract was weighed and mixed in sterilized distilled water equal to amount of extract. This stock solution was filtered through Whatman no. 1 and 100% solution (stock) was prepared for further use @ 2.5%, 5%, and 10% concentrations.

Topsin M was used against *B. sorokiniana* under the controlled conditions. Stock solution was prepared by

adding 0.6 g Topsin M in 250 ml sterilized distilled water (recommended dose 2.4 g/1000 ml water) as reported by Tiwari *et al.* (2022).

***In Vitro* Application of Plant Extracts and Fungicides:**

Under *in vitro* conditions, the plant extracts and fungicide were prepared at 3 varying concentrations *i.e.*, 2.5%, 5%, and 10% concentrations in potato dextrose agar media. Every concentration of plant extract solution and fungicide under went for more analysis through 3 replications. A negative group was used as well, treating the *B. sorokiniana* in media plates without mixing of any plant extract or fungicide. Subsequently, the PDA was tested against the fungal cultures using the poisoned food technique. The mycelial growth of *B. sorokiniana* was observed from 3-7 days with 2day interval to confirm the impact of the plant extracts and fungicide on *B. sorokiniana*. The effectiveness of plant-based substances and antifungal agents was documented in terms of the percentage of inhibition of mycelial growth, calculated according to the formula given below:

$$\text{MGI}\% = \frac{C - T}{C} \times 100$$

Where, MGI = mycelial growth inhibition.

C = Mycelial growth (cm) of the test pathogen in control.

T = Mycelial growth (cm) of the test pathogen in treatment.

RESULTS AND DISCUSSION

Screening of Wheat Germplasm Against Spot Blotch:

The spot blotch is reported to be both soil (Reis and

Santos, 1987) and seed borne in nature (Reis, 1982). Therefore, resistant varieties and cultivars were produced by breeding to manage spot blotch for sustainable agriculture. One hundred entries were screened with inoculums suspension spray under field conditions. Out of 100, none of the candidate line was observed highly resistant to spot blotch. Six lines were observed resistant, 5 moderately resistant, 4 moderately susceptible, 17 susceptible and 69 were observed highly susceptible including the checks. (Table1). The entries that exhibited resistant to moderately resistant are strongly recommended to be used and can further be exploited in breeding program. Development of resistant varieties has helped in combatting the diseases like spot blotch in other countries like Bangladesh and India. In a study by Siddique *et al.* (2006), an assessment was carried out to evaluate the impact of habitat-induced resistance and loss in new varieties in Bangladesh following the introduction of the iconic 'Kanchan' varieties.

Siddique *et al.* (2006) observed that disease severity and yield loss rates were lower among wheat varieties developed since 1983, indicating a consistent trend towards higher yields in new varieties. Furthermore, the level of resistance to leaf blight in many new varieties and growth lines signified successful progress in breeding protection over the past two years.

Table 1. Details of response of wheat germplasm against spot blotch under NARC field conditions

Entry No	Name	Disease Response	Reaction	Response
1	Local White	99	100	HS
2	22BT008	78	69	HS
3	22BT010	87	69	HS
4	22BT017	88	79	HS
5	22BT015	67	52	S
6	22BT016	78	69	HS
7	21BT005	78	60	S
8	20BT005	87	69	HS
9	19BT022	99	100	HS
10	TRB-15-CHU	99	100	HS
11	TRB-25-BEC	88	79	HS
12	TRB-26-KAU	23	7	HS
13	TRB-37-F7	78	60	S
14	TRB-46-BEC	67	52	S
15	TRB-48-VOR	78	69	HS
16	TRB-52-QUA	88	79	HS
17	TRB-53-MAY	56	37	MS
18	TRB-61-MEL	78	69	HS

19	TRB-63-CIR	88	79	HS
20	Morroco	99	100	HS
21	TRB-65-MAY	67	52	S
22	TRB-69-MUN	78	69	HS
23	TRB-70-WAD	99	100	HS
24	TRB-71-WAX	89	889	HS
25	TRB-83-MAY	89	89	HS
26	TRB-88 W	67	52	S
27	TRB-89-VIL	56	37	MS
28	TRB-90-ATT	78	69	HS
29	TRB-940-N1	88	79	HS
30	TRB-95-KAC	12	2	R
31	TRB-100-MUC	56	37	MS
32	TRB-102-MUL	34	15	MR
33	TRB-3-SUP	67	52	S
34	TRB-5-WBL	78	69	HS
35	TRB-8-NEX	87	69	HS
36	TRB-18-WBL	56	37	MS
37	TRB-21-SER	78	69	HS
38	TRB-31-WBL	88	79	HS
39	TRB-60-124	56	37	MS
40	Morroco	99	100	HS
41	TRB-62-127	23	7	R
42	TRB-63-129	67	52	S
43	TRB-66-145	78	69	HS
44	TRB-78-324	88	79	HS
45	TRB-83-334	67	52	S
46	TRB-84-336	88	79	HS
47	TRB-107-73	78	69	HS
48	TRB-144-25	67	52	S
49	TRB-S1-14	78	69	HS
50	Local White	89	89	HS
51	TRB-S6-3	78	69	HS
52	TRB-S6-11	88	79	HS
53	TRB-S6-12	67	52	S
54	TRB-S6-13	78	69	HS
55	TRB-S6-16	88	79	HS
56	TRB-S6-26	67	52	S
57	TRB-S9-1	78	69	HS
58	TRB-S9-42	78	69	HS
59	NR-636	88	79	HS
60	Morroco	88	79	HS
61	NR-637	78	69	HS
62	NR-638	67	52	S
63	NR-639	78	69	HS
64	NR-640	56	37	MS
65	NR-641	88	79	HS
66	NR-642	45	25	MR
67	NR-643	78	69	HS
68	NR-644	67	52	S
69	NR-645	45	25	MR
70	NR-646	88	79	HS

71	NR-647	88	79	HS
72	NR-648	89	89	HS
73	NR-649	78	69	HS
74	NR-650	89	89	HS
75	NR-651	88	79	HS
76	BARS-KT-DNL-93	45	25	MR
77	BARS-KT-DNL-94	67	52	S
78	BARS-KT-DNL-95	23	7	R
79	BARS-KT-DNL-96	78	69	HS
80	Morocco	99	100	HS
81	BARS-KT-DNL-97	78	69	HS
82	BARS-KT-DNL-98	67	52	S
83	BARS-KT-DNL-99	78	69	HS
84	BARS-KT-DNL-100	88	79	HS
85	BARS-KT-DNL-101	45	25	MR
86	BARS-KT-DNL-102	23	7	R
87	BARS-KT-DNL-103	12	2	R
88	BARS-KT-DNL-104	78	69	HS
89	BARS-KT-DNL-105	23	7	R
90	BARS-KT-DNL-106	67	52	S
91	BARS-KT-DNL-107	78	69	HS
92	BARS-KT-DNL-108	78	69	HS
93	BARS-KT-DNL-109	88	79	HS
94	BARS-KT-DNL-110	67	52	S
95	BARS-KT-DNL-111	78	69	HS
96	BARS-KT-DNL-112	78	69	HS
97	Sehar	99	100	HS
98	Galaxy	99	100	HS
99	Local White	99	100	HS
100	Morocco	99	100	HS

In Vitro Management Through Plant Extracts: In this study, three plant extracts *i.e.*, Alstonia, garlic, and eucalyptus extracts were examined to check the growth of *B. sorokiniana* under in vitro conditions. Each plant extract was further divided into three different concentrations: 2.5%, 5.0%, and 10%. Pathogen was inhibited at highest (10 %) concentration as shown in Table 2. Three replicates were used for each plant extract concentration in the analyses. These concentrations were mixed in media plates and incubated at 25+2 °C for seven days. The mean of pathogen growth (cm) was observed after 3,5 and 7 days after inoculation.

Garlic (*Allium sativum*) was very effective plant extract under in-vitro conditions, at all 3 concentrations (2.5%, 5.0% and 10%). The fungal growth was inhibited highest *i.e.* 1.5 cm at 10% concentration and observed 2.08 cm against 5% concentration while 3 cm against 2.5 % concentration as compared to control 4.2 cm after 7 days of incubation. This finding aligns with previous studies which

have highlighted the antifungal properties of garlic, attributed to compounds like allicin. Tiwari *et al.*, (2023) reported that garlic extract inhibited the growth of various fungal pathogens, including *Aspergillus* and *Penicillium* species.

Alstonia (*Alstonia scholaris*) was also found effective control measure against the pathogen. Growth of fungus was examined 2.9 cm against 2.5% concentration while that was effectively inhibited at 10% concentration as compared to control 4.2 cm after 7 days of incubation. This is consistent with earlier research that has identified various bioactive compounds in Alstonia species with antifungal activity. Azhar *et al.* (2016) demonstrated that Alstonia extracts inhibited the growth of *Fusarium oxysporum*, a common soil-borne fungal pathogen. Its efficacy was statistically at par with garlic at final reading.

Eucalyptus (*Eucalyptus globules*) was found less effective against *B. sorokiniana* after than former two extracts Alstonia under *in vitro* conditions. The growth of fungus

was observed 3.06 cm at 2.5% concentration and 2.86 cm at 5% concentration while 2.55 at 10% concentration of eucalyptus extract as compared to untreated petri plate with *B. sorokiniana* measured 4.2 cm after seven days of incubation. Gakuubiet *al.* (2017) presented that Eucalyptus extract having inhibitory activity against *Penicillium*. This could be attributed to the presence of essential oils in eucalyptus, which have been reported to possess antifungal properties.

Fungicide Topsin M (positive control) was the moderately effective under at our given dilutions/concentrations. Under *in vitro* condition growth of *B. sorokiniana* was examined 3.4 cm at 2.5%, 2.85 cm at 5% concentration while 2.1 cm at 10 % concentration of fungicide as compared to control measured 4.2 cm after 7 days of incubation. The readings of all given extracts and fungicide are mentioned below in the Table 2.

Table 2. Mean growth (cm) of *B. sorokiniana* after treating media with different plant extracts at 3 different concentrations and Topsin M at 3, 5 and 7 days after inoculation.

Garlic						
Days	2.5%	M.G.I	5%	M.G.I	10%	M.G.I
3	2.4	31%	1.5	55%	0.88	74%
5	2.7	30%	1.9	50%	1.19	68%
7	3	28%	2.08	50%	1.5	64%
Alstonia						
Days	2.5%	M.G.I	5%	M.G.I	10%	M.G.I
3	1.13	33	1.3	48%	0.41	86%
5	1.55	26	1.79	40%	0.7	80%
7	2.9	31	2.2	48%	1.1	73%
Eucalyptus						
Days	2.5%	M.G.I	5%	M.G.I	10%	M.G.I
3	2.26	19%	2.1	35%	1.63	19%
5	2.64	11%	2.4	33%	1.9	11%
7	3.6	23%	2.85	32%	2.1	23%
Topsin M						
Days	2.5%	M.G.I	5%	M.G.I	10%	M.G.I
3	2.6	19%	2.1	35%	1.63	49%
5	3.2	11%	2.4	33%	1.9	47%
7	3.4	23%	2.85	32%	2.1	50%

CONCLUSION

Present studies report none of the lines as highly resistant. However, 6 resistant lines found in our experiment may be further tested at other locations in next years for breeding. *In vitro* data demonstrates the potential of plant extracts as natural alternatives to chemical fungicides for controlling *B. sorokiniana*. Among the three plant extracts, garlic and Alstonia emerged as more effective, significantly inhibiting more fungal growth at 10% concentrations. The fungicide Topsin M, used as a positive control, showed moderate

efficacy, comparable to Alstonia and garlic extracts. However, it is important to note that chemical fungicides often have environmental and health concerns. Therefore, exploring natural alternatives like plant extracts is crucial for sustainable agriculture. Our studies report that SB can be serious disease in Pakistan and elsewhere if warm and humid conditions prevail simultaneously during grain filling and later stages. Therefore, the disease need more attention of breeders to develop disease resistant varieties in future as being done in case of wheat rusts.

REFERENCES

- Atiq, M., F.A. Muhammad, N.A. Rajput, H. Ahmad, S. Ahmad, M. Usman, A. Husnain, A. Nawaz, S. Iqbal H. Ahmad. 2024. Surveillance and management of brown spot of potato. *Achieves of Phytopathology and Plant Protection*, 57 (5): 387-404.
- Azhar, W., K. Jabeen and S. Iqbal. 2016. Appraisal of *Alstonia scholaris* anti fungal activity against Fusarium wilt. *Journal of Agricultural Research*, 54(2): 120-124.
- Beres, B. L., J.L. Hatfield, J.A. Kirkegaard, S.D. Eigenbrode,

- W.L. Pan, R.P. Lollato and J. Wiersma. 2020. Toward a better understanding of genotype × environment × management interactions—a global wheat initiative agronomic research strategy. *Frontiers in Plant Science*, 11: 515450.
- Gakuubi, M. M., A.W. Maina and J.M. Wagacha. 2017. Antifungal activity of essential oil of *Eucalyptus camaldulensis* Dehnh against selected *Fusarium* spp. *International journal of microbiology*, 2017(1): 8761610.
- Kamble, U., X. He, S. Navathe, M. Kumar, M. Patial, M.R. Kabir and P.K. Singh. 2024. Genome wide association mapping for field spot blotch resistance in South Asian spring wheat genotypes. *The Plant Genome*, 17(1): e20425.
- Kumar, S., S.R. Jacob, R.R. Mir, V.K. Vikas, P. Kulwal, T. Chandra and K. Singh. 2022. Indian wheat genomics initiative for harnessing the potential of wheat germplasm resources for breeding disease-resistant, nutrient-dense, and climate-resilient cultivars. *Frontiers in genetics*, 13: 834366.
- Li, W. R., Q.S. Shi, H.Q. Dai, Q. Liang, X.B. Xie, X.M. Huang and L.X. Zhang. 2016. Antifungal activity, kinetics and molecular mechanism of action of garlic oil against *Candida albicans*. *Scientific reports*, 6(1): 22805.
- Pandey, A., R. Paudel, K. Kafle, Sharma and R. Basnet. 2018. Varietal screening of wheat genotypes against spot blotch disease (*Bipolaris sorokiniana*) under field condition at Bhairahawa, Nepal. *Jour. of the Institute of Agriculture and Animal Sciences*, 35(1): 267–276.
- Roy, C., P. Juliana, M.R. Kabir, K.K. Roy, N.C. Gahtyari, F. Marza and P.K. Singh. 2021. New genotypes and genomic regions for resistance to wheat blast in south Asian germplasm. *Plants*, 10(12): 2693.
- Roy, C., X. He, N.C. Gahtyari, S. Mahapatra and P.K. Singh. 2023. Managing spot blotch disease in wheat: Conventional to molecular aspects. *Frontiers in Plant Science*, 14: e1098648.
- Saari, E. E. and J.M. Prescott. 1975. A scale for appraising the foliar intensity of wheat diseases. *Plant disease reporter*, 59(5): 377-380.
- Sattar, A, A. Riaz, M.Inam-ul-haq and M. K. N. Shah. 2018. In vitro antifungal activity of selected indigenous plant extracts against *colletotrichum capsici*. *International Journal of Biosciences*, 12(4):145-150.
- Siddique, A. B., M.H. Hossain, F. Duveiller and R.C. Sharma. 2006. Progress in wheat resistance to spot blotch in Bangladesh. *Journal of Phytopathology*, 154(1): 16-22.
- Tahir, O., S.A.K. Bangash, M. Ibrahim, S. Shahab, S.H. Khattak, I. Ud Din and S. Harakeh. 2022. Evaluation of agronomic performance and genetic diversity analysis using simple sequence repeats markers in selected wheat lines. *Sustainability*, 15(1): 293. doi. Org/10.3390/su15010293.
- Tiwari, P., Singh and P.K. Gupta. 2023. In vivo and in vitro assessment of plant extracts against *Bipolaris sorokiniana* disease causing spot blotch of wheat. *Annals of Plant Protection Sciences*, 31(1):13-21.
- Yaqoob, F., M. Atiq, N.A. Rajput, A. Nawaz, M. Kashif, M.J. Matloob, A. Jabbar, W. Din, F. Ali and A. Ullah. 2024. Appraisal of chemotherapy, plant defense activators, and genetic resistance against eyespot disease in sugarcane. *Plant protection*, 08(02): 325-340.

Contribution of Authors:

Muhammad Junaid	: Carried out research, including planning, experimentation and writing the manuscript
Abid Riaz	: Provided consistent guidance and advice as MS degree supervisor throughout the research.
Arsalan Hussain	: Played an essential role in screening wheat varieties and the manuscript's formatting
Muhammad Fayyaz	: Supported the screening process for the wheat varieties providing expertise.
Muhammad N. Sajid	: Guided the lead author in the extraction process from the plant parts and helped in obtaining in vitro data and its analysis
Muhammad A. Hayat	: Assisted with laboratory experiments and provided technical support for critical aspects of the research