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Research Article

EFFECTIVENESS OF ONION GERMPLASM, FUNGICIDES, AND PLANT EXTRACTS IN MANAGING PURPLE BLOTCH DISEASE OF ONION

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ABSTRACT

In the present study conducted at Ayub Agricultural Research Institute, Faisalabad, ten specific onion lines were evaluated for disease resistance against purple leaf blotch. The variety Phulkara emerged as the top performer with the lowest disease incidence of 6.57% and an AUDPC value of 55.67, indicating resistance to this disease. Various fungicides demonstrated differing effects on disease management. Copper hydroxide proved to be the most effective at 0.10%, reducing disease incidence from 17.59% in the first spray to 12.70% in the third. The second-best fungicide was trifloxystrobin 25% + tebuconazole, which reduced disease incidence from 67.51% in the first spray to 61.67% in the third compared to the control. Among all plant extracts, three sprays of *Nicotiana tabacum* extract at S/50, S/25, and S/15 proved to be most effective, reducing disease incidence from 43.51% to 35.59%. Similarly, *Moringa oleifera* extract reduced disease incidence from 42.61% to 35.70% compared to the control. These findings suggest that the onion variety Phulkara, copper hydroxide fungicide at a concentration of 0.10%, and extracts of *N. tabacum* and *M. oleifera* exhibit promising potential in managing purple leaf blotch disease in onions. However, further studies are necessary to corroborate these results and to fully understand the biological and chemical implications of their usage.

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INTRODUCTION

The onion (*Allium cepa* L.) is an important staple crop that significantly impacts human nutrition globally. However, the cultivation of superior onions faces many challenges. A prominent threat to onions is the purple leaf blotch disease, a well-known disease resulting in substantial annual crop productivity reductions (Stewart and Franck, 2015). *Alternaria porri*, a fungal infection, appears as purple blotches on onion leaves, resulting in significant leaf damage and ultimately affecting the quantity and quality of onion bulbs (Kritzman et al., 2005). Currently, fungicides are commonly used against this disease. However, increasing concerns about the sustainability of the environment, the development of resistance to fungicides, and a need for organic products make it necessary to find other methods (Amar, 2015). Selecting and adopting onion varieties resistant to diseases could be a viable and sustainable solution. Within this particular framework, this study aims to evaluate various onion cultivars for their ability to withstand purple leaf blotch disease.

Purple leaf blotch, a disease caused by the fungal pathogen *A. porri*, poses a substantial challenge to managing onions, a globally important crop (Kumar and Verma, 2017). It is worth mentioning that the use of several fungicides has been proven effective in reducing the spread of this widespread disease and improving the amount of crops produced (Stewart and Viaene, 2016).

Several effective fungicides have emerged as promising therapies (Iqbal and Mukhtar, 2020a). Trifloxystrobin 25 + tebuconazole, for example, has been proven to effectively hinder the growth of the fungus, hence decreasing the harmful impact of the disease (Bains, 2019). Furthermore, copper hydroxide has been employed for its antifungal characteristics, hence reducing the damage inflicted by the purple blotch (Varma and Mishra, 2020). Propiconazole, iprobenfos, and mancozeb are additional fungicidal drugs used for managing this condition. Their use has contributed to reducing disease occurrence and enhancing onion productivity (Kumar and Verma, 2017; Garg and Exner, 2018).

Additionally, the synergistic effect of azoxystrobin + difenoconazole has been acknowledged for its substantial efficacy in impeding the advancement of the disease (Bains, 2019). Tebuconazole and difenoconazole have been used for their antifungal solid properties, providing efficient control against the destructive disease (Stewart and Viaene, 2016). While there has been some improvement in utilizing fungicides to combat purple leaf blotch, further study is necessary to enhance existing interventions and uncover more potent therapies.

Several alternative tactics have been implemented to control this disease, with an increasing focus on the potential utilization of botanical extracts (Iqbal et al., 2014; Shahzaman et al., 2015; Iqbal and Mukhtar, 2020b; Shahbaz et al., 2023). For example, *Nicotiana tabacum*, which has evident antibacterial characteristics, has demonstrated the potential to reduce the harmful consequences of this fungal infection (Varma and Mishra, 2020).

Furthermore, the extract of *Allium sativum*, which has high levels of organosulfur substances known for their ability to inhibit the growth of microbes, has been observed as a promising biocontrol agent for this particular disease (Stewart and Viaene, 2019). *Moringa olifera*, a powerful botanical source known for its proven antifungal characteristics, has been used to reduce the adverse effects of the purple blotch disease on onion crops (Kumar et al., 2017). *Azadirachta indica*, commonly used in herbal remedies for its antifungal properties, has demonstrated potential in managing the disease. This suggests that it should be taken into account for sustainable agriculture (Ainsworth and Sumner, 2021). However, conducting further research is essential to fully exploit these plant extracts' potential in managing the purple blotch condition.

The purple leaf blotch is a widespread onion disease (Smith and Anderson, 2021). The presence of the disease may reduce onion yields, posing a significant problem if it remains under control. A recent study on the management of purple leaf blotch has demonstrated the need for a combination of cultural, biological, and chemical control approaches (Smith and Anderson, 2021). Despite the emergence of resistance and environmental concerns, the investigation of plant-derived extracts has been performed in the interest of disease management. However, traditional fungicide applications remain essential (Rocky et al., 2023). It is necessary to compare different management strategies to establish effective and lasting control programs for purple leaf blotch in onion production. Therefore, considering all the facts mentioned above, the main goal of this study was to evaluate the effectiveness of different onion varieties or lines, fungicides, and plant extracts as sustainable management methods for controlling leaf blotch disease in onion fields.

MATERIALS AND METHODS

Establishment of disease screening nursery

In the Ayub Agricultural Research Institute, Faisalabad study area, ten different varieties/lines of onions (Table 1) were planted on September 25th, 2022, during the Rabi crop growing season. For screening, a range of onion varieties/lines with varying levels of resistance, from moderately resistant to very sensitive, were utilized as reference entries for a purple blotch of onion. A total of ten onion varieties/lines were cultivated in a randomized complete block pattern in the experimental area of the Vegetable Research Institute, Ayub Agricultural Research Institute, Faisalabad. The incidence of purple blotch disease was assessed using the disease scale developed by Sharma (1997). The disease incidence data were recorded two and a half months after transplanting for each cultivar, using the formula provided by Kumar (2007).

$$\text{Disease Incidence (\%)} = \frac{\text{Total infected plants observed}}{\text{Total plants observed}} \times 100$$

Table 1. Detail of onion varieties/lines used for evaluation against purple leaf blotch.

Sr. No.	Varieties/ Lines
1	VRIO-3
2	Faisalabad Red
3	VRIO-6
4	Phulkara
5	Pak-10321
6	Dark Red
7	Pusa Red
8	Mirpurkhas
9	Red Imposta
10	VRIO-9

Calculation of AUDPC

The calculation of the Area under Disease Progress Curve (AUDPC) was performed using the method proposed by CIMMYT.

$$\text{AUDPC} = \sum_{i=1}^{n-1} [xi + Xi1]/2](ti + 1 - t1)$$

Where Xi represents the disease's incidence on the date I, it means the time in days between I and date I + 1, and n represents the number of dates on which disease incidence was recorded (Shaner and Finney, 1980).

Management of purple blotch disease of onion through fungicides

The efficacy of eight fungicides, each at three varying concentrations (Table 2), was assessed against purple blotch disease of onion in a natural field setting. A control group was also included in the evaluation. The field experiment used a randomized complete block design in the Vegetable Research Area of Ayub Agricultural Research Institute at Faisalabad. The field was cultivated with healthy onion bulbs of the Phulkara variety, using the conventional row to row distance of 30-45 cm and plant to plant spacing of 10-15 cm. All additional cultural traditions were implemented to maintain the field in optimal conditions. A total of three applications were made, with a fifteen-day gap between each application. Disease incidence data were collected seven and fourteen days following each application. The initial spray application was conducted promptly upon the manifestation of disease symptoms.

Table 2. Detail of fungicides used for the management of onion purple leaf blotch.

Sr. No.	Fungicides	Formulation	Concentration(%)
1	Trifloxystrobin 25 + tebuconazole	50WG	0.05
2	Tebuconazole	25 EC	0.10
3	Mancozeb	75 WP	0.30
4	Iprobenfos	48 EC	0.10
5	Propiconazole	25 EC	0.10
6	Difenoconazole	25 EC	0.10
7	Copper hydroxide	77 WP	0.25
8	Azoxystrobin 18.2 + difenoconazole	11.4 SC	0.05

Management of purple blotch on onion through plantextracts

Four botanical extracts (*Nicotiana tabacum, Allium sativum, Moringa olifera*, and *Azadirachta indica*) were assessed in outdoor settings using three different concentrations (S/50, S/25, and S/15). Three applications of all botanical extracts were made. The initial application of plant extracts was administered immediately at the onset of the first disease sign in the field, followed by further sprays at 15-day intervals. To prepare plant extracts, 75 g of fresh leaves were soaked in 25 ml of sterilized water using a sterilized pestle and mortar. The solution was filtered using four layers of sterilized muslin cloth and Whatman filter paper No. 14, resulting in the standard (S) plant extract (Ilyas et al., 1996). Similarly, S/50 and S/25 were created by combining 100 ml of a standard concentration with 100 ml of sterilized water and 25 ml of a standard concentration with 100 ml of sterilized water, respectively. Additionally, a solution with a concentration of S/15 was produced.

Statistical analysis

The data were statistically analyzed using the Fisher analysis of variance approach. Treatment means were compared using the Least Significance Difference (LSD) test at a 5% probability level employing M Stat C software.

RESULTS

Evaluation of onion germplasm for relative resistance to purple blotch disease of onion

The Table 3 presents a comprehensive evaluation of various varieties/lines, namely Phulkara, Dark Red, Mirpurkhas, VRIO-6, VRIO-9, Pak-10321, Faisalabad Red, Pusa Red, Red Imposta, and VRIO-3. Each of the lines was analyzed based on disease rating, disease incidence (D.I.), status, and area under disease progress curve (AUDPC), providing valuable insights for disease management against purple leaf blotch of onion in these specific varieties.

It was observed that, Phulkara appeared to have the lowest disease incidence of only 6.57% against purple leaf blotch of onion earning a disease rating of 1 and a relatively low AUDPC of 55.67 indicating resistance to disease. On the contrary, VRIO-3 displayed the highest level of disease incidence of 61.41%, earning a disease rating of 5 and significantly high AUDPC of 1153.45 indicating higher susceptibility to the disease (Table 3). Between these extremes, the other strains such as Dark Red and Mirpurkhas exhibited moderate resistance (MR) against purple leaf blotch of onion, with D.I. of 18.08%, and 16.59% respectively. Their AUDPCs were 131.35 and 363.45 respectively. Varieties such as VRIO-6 and VRIO-9 exhibited moderate susceptibility (MS) and susceptibility (S) respectively with higher D.I. of 26.76% and 42.34% and AUDPC values of 332.75 and 538.95. The remaining varieties viz. Pak-10321, Faisalabad Red, Pusa Red, and Red Imposta showed clear susceptibility (S) to disease with high disease incidences ranging from 47.04% to 55.74% and elevated AUDPC values above 800 (Table 3).

This information provides useful insights into the resilience and susceptibility of different varieties against purple leaf blotch of onion disease and can help researchers in their quest to develop disease-resistant strains and effective disease management strategies.

Table 3. Disease rating, disease intensity, status and accumulated disease progress curve of different varieties/lines

Sr. No.	Name of varieties/lines	Disease rating	D.I. (%)	Status	AUDPC
1	Phulkara	1	6.57	R	55.67
2	Dark Red	2	18.08	MR	131.35
3	Mirpurkhas	2	16.59	MR	363.45
4	VRIO-6	3	26.76	MS	332.75
5	VRIO-9	4	42.34	S	538.95
6	Pak-10321	4	50.37	S	856.8
7	Faisalabad Red	4	52.74	S	1032.35
8	Pusa Red	4	47.04	S	1077.9
9	Red Imposta	4	55.74	S	957.35
10	VRIO-3	5	61.41	HS	1153.45

Evaluation of fungicides for the management of purple blotch disease of onion

Various fungicides were evaluated for their efficacy in controlling the purple leaf blotch disease in onions. The fungicides were tested at specific concentrations, and their effectiveness was assessed by assessing the percentage of disease incidence. The efficacy of these fungicides was evaluated and documented using three applications for each treatment.

Initially, a solution of Trifloxystrobin 25 + tebuconazole fungicide was prepared with a concentration of 0.05%. The data showed a progressive decline in disease occurrence across the three applications, decreasing from 67.51% to 61.67%. At a concentration of 0.10%, copper hydroxide exhibited a notable reduction in disease occurrence. The incidence rates decreased from 17.59% to 12.70% throughout the three sprays, as indicated in Table 4.

At a concentration of 0.30%, the application of Propiconazole initially reduced the disease frequency from 46.44% to 40.56% throughout three sprays. Curiously, when Iprobenfos was applied at a concentration of 0.10%, it showed higher rates of disease occurrence at first, but these rates decreased gradually in later sprays. The application of Azoxystrobin 18.2 + difenoconazole at a concentration of 0.05% demonstrated favorable outcomes by reducing the occurrence of the disease from an initial rate of 42.59% to 34.73% after the final spray (Table 4).

The fungicides Tebuconazole and Difenoconazole were tested at 0.25% and 0.10%, respectively. Both studies demonstrated a significant decrease in disease rates from the first to the final spray application. Mancozeb, a fungicide tested at a concentration of 0.10%, initially had a disease incidence of 59.41% and decreased to 50.59% after the third application. In addition, the control group, which did not receive any fungicide treatment, consistently maintained a disease incidence rate of 85.67% throughout all sprays, demonstrating the effectiveness of fungicides in managing the disease.

Each fungicide exhibited its distinct influence on the levels of disease incidence. Over time, each of them made significant contributions to reducing the disease rate, highlighting their essential role in effectively managing the purple leaf blotch in onions.

Table 4. Effectiveness of various fungicides for the management of purple leaf blotch of onion.

Sr. No.	Fungicides	Concentration (%)	Disease Incidence (%)		
			Spray-1	Spray-2	Spray-3
1	Trifloxystrobin 25 + tebuconazole	0.05	67.51 A	64.52 B	61.67 C
2	Copper hydroxide	0.10	17.59 A	15.52 B	12.70 C
3	Propiconazole	0.30	46.44 A	43.19 B	40.56 C
4	Iprobenfos	0.10	83.31 A	78.08 B	75.85 C
5	Azoxystrobin 18.2 + difenoconazole	0.05	42.59 A	39.07 B	34.73 C
6	Tebuconazole	0.25	35.55 A	28.34 B	25.40 C
7	Mancozeb	0.10	59.41 A	54.46 B	50.59 C
8	Difenoconazole	0.10	35.42 A	30.52 B	27.70 C
9	Control	-	85.67	85.67	85.67

Means that do not share a letter are significantly different.

Evaluation of botanicals for the management of purple blotch disease of onion

In order to assess the efficacy of plant extracts in controlling the purple leaf blotch disease in onions, several plant species were employed, including *N. tabacum*, *A. sativum*, *M. oleifera*, and *A. indica*. The application of each plant extract was done at three different concentrations viz: S/50, S/25, and S/15. Subsequently, the disease incidence was recorded after each application or spray and documented in Table 5.

The examination of the *N. tabacum* extract revealed a gradual decrease in the occurrence of disease, decreasing from 43.51% to 35.59% over the course of three sprays. These findings suggest that *N. tabacum* has the potential to decrease the occurrence of disease, likely because of its natural antifungal properties. Similarly, the use of *M. oleifera* extract resulted in a decrease in disease occurrence from 42.61% to 35.70%, indicating its effectiveness in controlling the purple leaf blotch of onion (Table 5). However, the use of *A. sativum* demonstrated a slightly elevated occurrence of disease, with initial documented rates of 58.93%, which gradually reduced to 51.99% after the third application. While there were some decreases, the overall rate of disease occurrence was still higher compared to the previous two samples. Similarly, *A. indica* exhibited a marginal decrease in disease incidence from 54.41% to 51.61%, suggesting a comparatively less substantial influence on disease control compared to other plant extracts (Table 5).

Surprisingly, the control group without any plant extracts indicated a consistent disease incidence rate across all sprays. The reduction in disease incidence in other plant extracts suggests that they may have potential fungicidal activities which could help manage the purple leaf blotch of onion. However, further in-depth studies are required to substantiate these preliminary findings and understand the exact mechanisms behind their antifungal properties.

Table 5. Effectiveness of various botanicals for the management of purple leaf blotch of onion.

Sr. No.	Botanicals	Concentration(%)			Disease Incidence (%)		
					Spray-1	Spray-2	Spray-3
1	*Nicotiana tabacum*	S/50	S/25	S/15	43.51 A	39.78 B	35.59 C
2	*Allium sativum*	S/50	S/25	S/15	58.93 A	55.88 A	51.99 B
3	*Moringa olifera*	S/50	S/25	S/15	42.61 A	40.19 B	35.70 C
4	*Azadirachta indica*	S/50	S/25	S/15	54.41 A	52.55 A	51.61 B
9	Control	-	-	-	79.56	79.56	79.56

DISCUSSION

The evaluation of several onion varieties/lines for purple leaf blotch disease reveals substantial variations, indicating the existence of genetic variability in their tolerance to this pathogen among the tested types. The findings are consistent with prior studies, emphasizing that the susceptibility to purple leaf blotch disease varies significantly across different onion cultivars (Srinivas et al., 2016). Furthermore, it is crucial to analyze these variations considering each line or variety,s specific ecological and agronomical needs to develop practical and efficient methods for managing this disease.

Some cultivars exhibited strong resistance to the disease, providing a valuable resource for improving disease resistance in susceptible lines through breeding activities. Strong evidence indicates that using resistant plant varieties is crucial in strategies to manage diseases. This approach provides a sustainable and ecologically friendly alternative to using chemicals for control (Zewde et al., 2014). This further confirms the need for genetic resistance as a fundamental component of integrated disease management techniques against purple leaf blotch. On the other hand, the increased susceptibility of specific cultivars to the disease emphasizes the importance of continuous surveillance and preventative measures to avoid substantial reduction in crop production. These particular cultivars need additional protection, which can be achieved using chemical or biological management methods (Miah et al., 2018).

Present investigation has focused on the dissipation kinetics of trifloxystrobin and tebuconazole in onion and soil, highlighting the persistence and dissipation rates of these fungicides after application (Mohapatra, 2014). Some recent studies have also explored the degradation behavior, residue distribution, and dietary risk assessment of propiconazole in celery and onion under field conditions, emphasizing the importance of understanding the fate of fungicides in different crops (Yadav et al., 2017).

Additionally, investigations into the sensitivity of fungal isolates to propiconazole and its impact on disease control have been conducted, shedding light on the effectiveness of this fungicide in managing diseases like dollar spot (Chethana et al., 2011). Furthermore, the stereoselective analysis and dissipation of propiconazole in wheat, grapes, and soil have been studied to assess environmental risks and food safety associated with this fungicide (Aujla et al., 2013).

The research on fungicides used for onion purple blotch management underscores the need for comprehensive studies on the behavior, efficacy, and potential risks associated with these chemicals in agricultural settings. By evaluating the dissipation kinetics, residue distribution, and sensitivity of pathogens to fungicides, researchers aim to provide insights into optimizing fungicide applications for effective disease management while minimizing environmental impact and ensuring food safety.

The current study showed the relative efficacy of fungicides and plant extracts in onion purple blotch disease management. Recent concerns about fungicide resistance and the need for more sustainable disease management make the findings important (Sharma et al., 2021).

Previous studies have shown that copper compounds are effective against *Alternaria* species (Gupta et al., 2020). Copper-based fungicides like copper hydroxide reduce disease incidence. Copper-based fungicides have broad-spectrum effectiveness against many fungal diseases because they disrupt fungal cell membranes and enzymatic activities (Lamichhane et al., 2018). The present investigation found that some synthetic fungicides, such as iprobenfos, were less effective than others, which may indicate fungicide resistance in the local *A. porri* population, a rising concern in onion-producing countries. Resistance shows the importance of integrating multiple control strategies, including plant-derived extracts, to reduce resistance and maintain fungicide efficacy (Gupta et al., 2020).

In modern agro-pathology, natural plant extracts are an appropriate replacement for synthetic fungicides in controlling diseases such as the purple blotch of onion (Patel and Jog, 2017). The utilization of accessible natural resources is considered a sustainable disease prevention method mainly when these extracts are readily available and economically viable. Research conducted using extracts from *N. tabacum, A. sativum, M. olifera,* and *A. indica* has demonstrated positive results in lowering the occurrence of diseases (Kim and Pan, 2018).

The *N. tabacum* extract exhibited favorable characteristics against numerous fungal diseases, and its antifungal activity is mainly attributed to nicotine. This alkaloid demonstrates potent bioactivity against various pathogens (Muhammad et al., 2019). The observed decrease in the prevalence of onion purple blotch disease can be attributed to this application of the extract. Garlic, scientifically known as *A. sativum*, is widely recognized for its antibacterial characteristics linked to its sulfur compounds, specifically allicin (Mishra et al., 2012). The characteristics above may have played a role in the decrease in disease occurrence in the onions that were treated with the extract derived from *A. sativum*. *M. olifera* extracts, which contain a high concentration of bioactive chemicals such as flavonoids and phenolics, have gained significant recognition for their ability to inhibit the growth of fungi (Ul Haq et al., 2014). Utilizing this extract in agro-pathology could be a viable strategy for promoting sustainable farming. *A. indica*, commonly called Neem, includes azadirachtin, which possesses insecticidal and antifungal effects (Sharma et al., 2022). Its utilization can be a natural solution for controlling the onion purple blotch disease. In conclusion, using plant extracts to control onion purple blotch provides an eco-friendly and sustainable substitute for synthetic fungicides.

CONCLUSION

It is concluded that certain varieties of onions, such as Phulkara, demonstrated a higher resistance to the purple blotch disease. Fungicides like tebuconazole, copper hydroxide and trifloxystrobin+ tebuconazole, were effective in reducing disease incidence. Furthermore, plant extracts, especially *Moringa oleifera* and *Nicotiana tabacum*, showed potential as natural fungicides with noticeable reductions in blotch incidence. However, more in-depth research is required to further substantiate these findings and determine the exact antifungal mechanisms.

AUTHORS' CONTRIBUTIONS

AM, and SN designed, formulated and laid out the study; AM, SN, and SS conducted the experiments; SA and US collected, arranged and analyzed the data; AAK, and MU provided technical assistance; AM and RK supervised the work; SN and YA wrote the manuscript; YA proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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