AJAB

Asian J Agric & Biol. 2021(4).
DOI: 10.35495/ajab.2020.01.039

Original Article

Induction of resistance in onion against purple leaf blotch disease through chemicals

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Received:
January 14, 2020
Accepted:
May 03, 2021
Online First:
June 08, 2021
Published:
September 21, 2021

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Abstract

Onion is one of the world's most important vegetable crop cultivated in Pakistan and plays a significant role in human diet. Numerous diseases attack on onion crop, but purple leaf blotch is the most important one, because it causes *80 to 90% of* onion yield loss all over the world. In current experiment twenty-three fungicides at three concentrations (0.5, 1, and 1.5 g/L) were evaluated against *Alternaria porri* causing purple blotch under Randomized Complete Block Design (RCBD) on susceptible variety of onion (Pink Panther). Among all fungicides, chlorostrobin expressed prominent results causing 62.05% reduction in disease severity, followed by Nanok (61.55), Shincar (54.86), Cabrio Top (53.33), Thril (50.00), Jalwa (48.11), Success (45.00), Alliette (41.61), Rally (39.83), Copper oxychloride (36.66), Score (33.05), Topas (29.88), Melodydue (13.27), Dithane M (11.66), Sulphax (6.55), Ridomil Gold (3.38) % respectively as compared to control. Similar results were observed in case of interaction b/w treatments and their concentrations. Results of current study are helpful for farmers, scientist, and researchers for timely management of purple leaf blotch disease of onion.

Keywords: *Amaryllidaceae*, *Allium cepa*, Chlorostrobin, Nanok, Fosetyl Aluminium

How to cite this:

Younas M, Atiq M, Rajput NA, Abbas W, Bashir MR, Ahmad S, Ullah MS, Bhatti WA, Liaqat N and Ahmad I, 2021. Induction of resistance in onion against purple leaf blotch disease through chemicals. Asian J. Agric. Biol. 2021(4): 202001039. DOI: https://doi.org/10.35495/ajab.2020.01.039

Introduction

Onion (*Allium cepa* L.) is one of the most important bulb crop, among all vegetables (Teshika et al., 2019) that belongs to family *Amaryllidaceae* genus Allium (Akbar, 2020). The Mediterranean and South West Asia are the native regions for the growth of onion (Ekşi et al., 2020). It is well known due to its pungent taste and presence of sulfenic acid. Onion plays a crucial role in different industries including food and medicine. It contains carbohydrates (8.7g), soluble proteins (9.22-13.21g), vitamin C (45.07mg) and 88.65% water in 100gram sample of fresh onion (Sami et al., 2021), while onion contains natural beneficial

compounds including thiosulfinates, saponins, polyphenols (Golubkina and Gianluca, 2020) and flavonoids (especially in red onion) (Akbar, 2020). 99.9 million tonns worldwide production was recorded from an area of 5.19 million hectares (FAO, 2019) while in Pakistan 2.06 million tonnes production was recorded from an area of 146.1 thousand hectares (GOP, 2020).

Onion crop is susceptible to attack by various fungal, bacterial, and viral diseases. Among them, purple blotch is most destructive and damaging foliar disease of onion which is caused by a fungal pathogen *Alternaria porri* (Firdausi et al., 2020). The attack of *A. porri* is more sever in humid and warm environment. Purple blotch occurs in severe form on seed crop as compared to bulb crop and causes 30-100 percent seed loss (Sonawane et al., 2020). Disease risk can be minimized by crop rotation, fungicides and bio-control agents like *Trichodema viridi, Trichodema virens*, *Trichodema harzianum* and *Penicillium citrinum* which expressed inhibitory effect towards spore germination of *A. porri* (Hariprasad et al., 2021; Firdausi et al., 2020) but unfortunately these bio-control agents are slow acting and suppress the pest population rather than killing them. That's why these are not adopted by farmers.

Different cultural and agronomic practices are used to minimize the risk of diseases but these put heavy labor cost and require extra time & manpower. Use of plant extracts against purple blotch is another alternative method against this disease because plants are rich sources of bioactive compounds (Dar et al., 2020). Extracts of higher plants have insecticidal, antibacterial, and antifungal properties (Abdelkhalek et al., 2020; Ghasemi et al., 2020). Extracts of plants such as *Azadirchta indica, Adhatoda vasica, Datura metal, Ocimum sanctum, Calotropis procera, Annona reticulata, Spilenthis acmela,* and *Lawsonia inermis* are used against purple blotch disease (Islam et al., 2020; Rahman et al., 2015) but unfortunately plant extracts are not easily available and are slow action against diseases but can be control through the use of chemical fungicides (Islam et al., 2020) like Ridomil, mencozeb, hexaconazole, penconazole, difenoconazole, Azoxystobin (Paneru et al., 2020; Dar et al., 2020). Purple blotch can also be significantly reduced by following seed treatment with Vitavax Power @ of 5g/ Kg of seed along with foliar application of Tebuconazol 25 EC @ 1 ml^{-1}(Mandi et al., 2020). Management of disease by using fungicides is mostly preferred because resistant sources are not always desirable and effective. Among all methods, response chemical method is very quick, and farmer is forced to use fungicides when disease appears in the field in epidemic form. Therefore, the current study was designed to evaluate different fungicides at different concentrations against purple blotch under field conditions.

Material and Methods

Isolation, identification and purification of *Alternaria porri*

Potato dextrose agar (PDA) media was prepared for the isolation of *A. porri* by using boiled potato slices, dextrose, and agar. Sterilized Petri plates (100mm×15mm) were used during experiment to avoid contamination. Onion infected leaves exhibiting characteristic symptoms of purple leaf blotch were collected and brought to Plant Pathology laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad. Diseased portion of leaves along with some healthy portion were cut into small pieces (2mm) and sterilized with 70% ethanol for 60 sec and placed on the Petri plates containing media and put them in an incubator (GEN2 BOD) at 21℃ temperature under dark conditions and observed daily for fungal growth.

Evaluation of fungicides under field conditions

Present experiment was conducted in Vegetable Research Institute, AARI Faisalabad in 2019. Susceptible variety namely Pink Panther was collected from a Vegetable Research Institute, Ayyub Agricultural Research Institute (AARI), Faisalabad. Susceptible variety of onion was cultivated by following (R×R) and (P×P) distance of 30 cm and 10 cm respectively under Randomized Complete Block Design (RCBD) by following all horticultural and husbandry practices (weeding and hoeing etc.). Twenty three fungicides (Cabrio Top, Success 40 WSP, Antracol, Melody Due, Curzate M, Ridomil Gold, Rally, Alliette, Cytrol, Co Pride, Thrill, Shincar, Topas, Score, Tilt, Flu Max, Jalwa, Chlorostrobin, Nanok, Copper Oxychloride, Dithane M, Topsin M, Sulphax) were collected from market and were evaluated at three concentrations (0.5g, 1.5 and 1.0g/liter of water). Hand sprayer (l HT-401) was used for the application of fungicides on every row of varieties. First spray of fungicides was applied on the appearance of disease symptoms were appeared under natural conditions. While remaining two sprays were

carried out at the interval of 10 days by hand sprayer. Disease data was recorded by following visual observations and rating scale (Sharma, 1986).

According to this scale**:**

Description	Rating Scale	Response
Disease symptoms are not present	0	I
Few spots present on the tip, covers less than 10% leaf area	1	R
Dark purplish brown patches are present covering less than 20% leaf area	2	MR
Patches along with paler outer region, covering up to 40% leaf area	3	MS
long lines are present covering up to 75% leaf area	4	S
Complete leave dried or its breakdown occur from stalk	5	HS

I= Immune, R= Resistant, MR= Moderately Resistant, MS= Moderately Susceptible, S= Susceptible, HS= Highly Susceptible

Data of disease severity was recorded by adapting following formula (Wheeler, 1969)

$$\text{Disease severity (\%)} = \frac{\text{Sum of all individual disease rating}}{\text{Total No. of plant assessed} \times \text{Maximum rating}} \text{ X } 100$$

While reduction in disease severity was calculated by using following formula after application of fungicides against purple leaf blotch of onion

$$\text{Reduction in disease severity (DS)\%} = \frac{\text{DS in control plants} - \text{DS in treated plants}}{\text{DS in control plants}} \times 100$$

Statistical analysis

Data was examined through analysis of variance (ANOVA) and treatments were compared by using Fisher's Least Significant Difference (LSD) test. All the statistical tests were performed by using SAS statistical software.

Results

Identification of *Alternaria porri* on the basis microscopy

Light to dark olivacious and grayish white colonies with concentric rings were appeared. Colony margins were complete, irregular, and wavy with fluffy and velvety texture while conidial shape was straight to curve along with light to deep brown colour which confirms the existence of *A. porri*. The number of horizontal and vertical separation in the conidia ranged from 3.00 to 6 µmm as shown in fig.

Figure-1. Microscopic view of *Alteria porri* causing purple blotch of onion.

Evaluation of chemicals against purple blotch disease under field conditions

Pink panther exhibits susceptible response with 70% severity of purple blotch disease, So this variety was used for determining the efficacy of fungicides towards *A.porri;* Among all treatments, Chlorostrobin expressed (62.05)% reduction in disease severity followed by Nanok (61.55%), Shincar (54.86%), Cabrio Top (53.33%), Thrill (50.00%), Topsin M (49.83%), Jalwa (48.11%), Cytrol (45.00%), Copride (45%), Success 40 WSP (45%), Tilt (44.66%), Alliette (41.61%), Flu max (41.55%), Rally(39.83%), Copper oxychloride (36.66%),Score (33.05%), Antracol (30.%), Topas (29.88%), Melody due (13.27%), Dithane M (11.66%), Curzate (6.66%), Sulphax (6.55%) and Ridomil gold (3.38%) respectively as compared to control as shown in table1 and fig.2

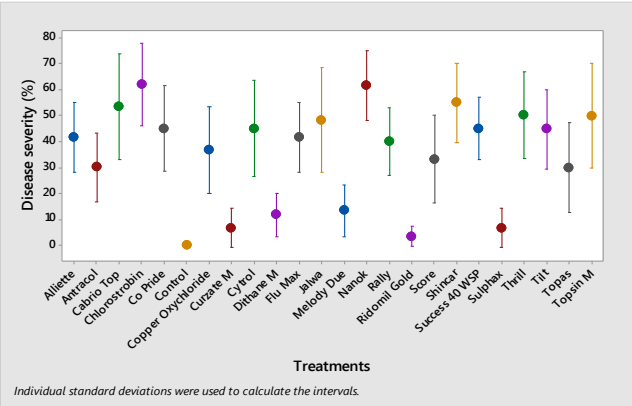

Figure-2. Evaluation of different fungicides against purple blotch disease under field conditions

Table-1. Reduction in severity of purple leaf blotch of onion under field conditions

Sr.#	Treatments	Active ingredients	Reduction in Disease Severity (%)
1	Cabrio Top	Pyraclostrobin	53.33c
2	Success 40 WSP	Chlorothalonil +Metalaxyl	45.00f
3	Antracol	Propineb	30.00k
4	Melody Due	Iprovalicarb +Propineb	13.27l
5	Curzate M	cymoxanil + mancozeb	6.66n
6	Ridomil Gold	Metalaxyl + Mancozeb	3.38o
7	Rally	Myclobutanil	39.83h
8	Aliette	Fosetyl Aluminium	41.61g
9	Cytrol	Thiophanate methyle+chlorothalonil	45.00f
10	Co Pride	Copper Oxychloride	45.00f
11	Thrill	Bismenthazole	50.00d
12	Shincar	Carbendazim	54.86b
13	Topas	Penconazole	29.88k
14	Score	Diafinaconazole	33.05j
15	Tilt	Propiconazole	44.66f
16	Flu Max	Metalaxyl-m+fluazinam	41.55g
17	Jalva	Deltamethrine +Triazophos	48.11e
18	Chlorostrobin	Azoxystrobin+chlorothalonil	62.05a
19	Nanok	Flutrifol + Azoxystrobin	61.55a
20	Copper oxychloride	Copper	36.66i
21	Dithane M	Mancozeb	11.66m
22	Topsin M	Thiophanate methyl	49.83d
23	Sulphax	Sulphur	6.55n
24	Control	Distilled water	0.00p

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).

Impact of interaction between treatments and concentrations on the development of onion purple leaf blotch under field conditions

In case of interaction between treatments and their concentrations on the development of purple blotch disease of onion under field conditions, Chlorostrobin expressed maximum disease reduction (39.50, 60, 86.66%) @ of 0.5,1,1.5 g/L of water, followed by Nanok (40, 65, 79.66%), Shincar (35.00, 49.66, 79.91%) Cabrio Top (20, 60, 80%), Cytrol (20.00, 40.00, 75.00%), Thrill (25, 50, 75%), Copride (20,45, 70%), Success40 WSP (25, 50. , 60%), Rally (20, 40 ,59.50%), Alliette (20, 44.83, 60%), Flu Max (20,44.66, 60%), Topsin M (19.66, 49.83, 80%), Jalwa (19.66,44.66, 80%), copperoxy chloride (10,40,60%) Antracol (10.33, 29.66, 50%), Score (9.66, 29.50, 60%)Topas (10, 20,59.66%), Melody due (0.00, 10, 29.83%), Dithane M (0.00, 10, 25%), Curzate M (0.00, 0.00, 20%), Sulphax (0.00, 0.00, 19.66%) Ridomil gold (0.00, 0.00, 10.16%) at all concentrations i.e. 0.5, 1 and 1.5 g/L of water respectively as shown in table 2 and fig.3.

Table-2. Effect of interaction between treatments and concentration on the development of purple blotch disease under field conditions

Treatments	Reduction in disease severity (%)		
	Concentrations		
	0.5g/liter of water	1.0g/liter of water	1.5g/liter of water
Cabrio Top	20.00m	60.00f	80.00b
Success 40 WSP	25.00 l	50.00g	60.00f
Antracol	10.33n	29.66k	50.00g
Melody Due	0.00o	10.00n	29.83k
Curzate M	0.00o	0.00o	20.00m
Ridomil Gold	0.00o	0.00o	10.16n
Rally	20.00m	40.00i	59.50f
Alliette	20.00m	44.83h	60.00f
Cytrol	20.00m	40.00i	75.00c
Co Pride	20.00m	45.00h	70.00d
Thrill	25.00 l	50.00g	75.00c
Shincar	35.00j	49.66g	79.91b
Topas	10.00n	20.00m	59.66f
Score	9.66n	29.50k	60.00f
Tilt	24.50 l	40.00i	69.50d
Flu Max	20.00m	44.66h	60.00f
Jalwa	19.66m	44.66h	80.00b
Chlorostrobin	39.50i	60.00f	86.66a
Nanok	40.00i	65.00e	79.66b
Copper oxychloride	10.00n	40.00i	60.00f
Dithane- M	0.00o	10.00n	25.00 l
Topsin- M	19.66m	49.83g	80.00b
Sulphax	0.00o	0.00o	19.66m
Control	0.00o	0.00o	0.00o

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P≤0.05).

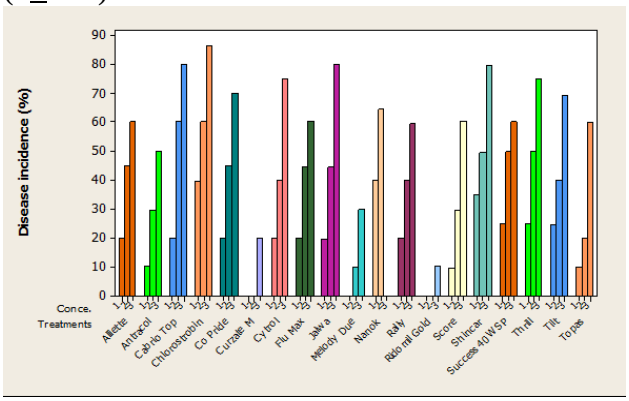

Figure-3. Assessment of interaction between treatments and concentrations against purple leaf blotch under field conditions

Discussion

Onion purple leaf blotch is the most disparaging disease caused by *Alternaria porri*. Among fungal diseases (Bal et al., 2019) which causes 50% yield losses (Jhala et al., 2017). Development of disease depends on the time of infection and host genotype. *A. porri* attacks on aerial plants parts. Presence of favorable environmental conditions (temperature, relative humidity, rainfall, and wind speed) susceptible germplasm and virulence strain of pathogen are responsible for the disease development. Most economical way for the management of purple blotch is the use of resistant varieties, but due to scarcity of resistant varieties, if the disease appeared in the field in epidemic form, then farmers have only one option, which is the use of chemical fungicides towards purple blotch of onion, as it is quick in action and easily available. Therefore, current study was designed for evaluation of different chemical fungicides against purple leaf blotch of onion under field conditions. So in in current study, twenty-three fungicides were evaluated against purple blotch of onion under field conditions at three concentrations. Among these fungicides, chlorostrobin (azoxystrobin + chlorothalonil) was proved most effective by causing 62.05 % reduction in disease severity followed by Nanok (azoxystrobin+ Flutriafol), Shincar (carbendazim), Cabrio Top (Metiram + Pyraclostrobin), Thrill (Bismirthazole), which expressed up to 50% reduction and nine fungicides namely Jalwa (Penthiopyrad), Success40 WSP (Chlorothalonil + Metalaxyl), Cytrol, Copride, Alliette (Fosetyl Aluminium), Flu Max, Tilt, Rally (Myclobutanil) and Topsin M causes up to 40 % reduction in disease while four fungicides Copper oxychloride (Copper) and Score (Difenaconazole), Topas (Penaconazole + Difenaconazole), Antracol, controlled more than 30% disease and remaining five fungicides Melody due (Propineb + iprovalicarb), Dithane M (Dithiocarbamates), Curzate M, Sulphax (sulphur) and Ridomil gold (Mancozeb + Metalaxyl) controlled less than 20% disease respectively as compared to control.

Paneru et al. (2020) worked on different six chemical fungicides for determining their efficacy against purple leaf blotch disease and found that Mencozeb + Cymoxanil expressed maximum reduction in disease severity followed by hexaconazole, Tebuconazole, Dimethomorph and carbendazim. Similarly, Mandi et al. (2020) used combinations of different fungicides along with seed treatment of vitavax power against purple bloch disease of onion. It was concluded that foliar application of Tubaconazole 25 EC @ 1 ml 1^{-1} and Azoxystrobin 23 SC @ 1 ml 1^{-1} was the most effective in controlling disease followed by Difenoconazole 25 EC @ 0.6 ml 1^{-1}. In contemporary studies, chlorostrobin expressed significant results by suppressing *A. porri* because it consists of azoxystrobin and chlorothalonil which inhibits multi sites of different enzymes. Nanok is effective fungicide against various diseases belonging to Ascomycetes, Basidiomycetes, Deuteromycetes, and oomycetes as, it has curative as well as protectant properties and is highly systemic which results in long term efficacy It inhibits the respiration, mycelial growth, spore germination and maintains normal green leaf area which results into high average potential yield. Cabrio Top caused reduction in disease severity up to 50% by blocking energy supply to fungus. Alliette causes 40% disease reduction due to its systemic nature as well as multiple modes of action as, it attacks on various growth stages of pathogens which results into rapidly disease reduction, Alliette not only controlled the plant pathogen but also stimulates the defense system of plant by creating a barrier in plant against pathogens and inhibited fungal spore germination by preventing disease transmission. Score causes more than 30 % reduction in disease severity by suppressing the biosynthesis of sterol in cell membrane.

Results of the present study are also supported by the work of Ali et al. (2016), who concluded that chemical fungicides showed best results by reducing and inhibiting fungal mycelial growth. Results of Ekabote (2020) and Ravikumar et al. (2020) are hand in line with the results of present experiment who determined that Tubeconazole and Trifloxystrobin (chlorostrobin) is best fungicide for the management of *A. porri*. They described that isolates are more sensitive to chlorothalonil as compared to mancozeb against purple blotch disease. Results of the contemporary study are helpful for the management of purple leaf blotch for farmers and researchers and scientists.

Conclusion

Chlorostrobin and Nanok expressed maximum reduction in disease severity under field conditions at the rate of 1.5g/L. So, it is recommended for farmers to use these fungicides should be used by farmers and researchers against purple leaf blotch of onion.

Acknowledgement

The authors are grateful to the Ayub Agricultural Research Institute (AARI) Faisalabad to allow carrying out field trials in experimental area of plant pathology section. The authors are highly thankful o Phytopathology Laboratory, University of Agriculture, Faisalabad.

Human and animal rights
This research does not include any animal and/or human trials.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: None.

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Contribution of Authors

Younas M: Data collection and manuscript writing
Atiq M: Conceived idea, data analysis and literature review
Rajput NA, Liaqat N & Ahmad I: Data analysis and interpretation
Abbas W & Bhatti WA: Manuscript writing and approval
Bashir MR: Designed research methodology and collected data
Ahmad S & Ullah MS: Literature review and manuscript write up