Studies on Survey of Onion for Purple Blotch Disease Caused by (Alternaria porri) in Major Growing District of Bihar

H.M. Singh¹ and J.K. Tiwari²

1. Sr. Tech. Officer (Horti.), 2. Asstt. Director (Horti.), National Horticultural Research and Development Foundation, Patna, Bihar

Corresponding author e-mail: hmsingh1983@gmail.com,

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ABSTRACT

Onion (Allium cepa L.) is one of the important bulb crops and most important commercial vegetable crops cultivated extensively in India and it accounts for 90 per cent of the exported vegetables from India in terms of value. The production of bulbs and seeds is limited by certain diseases. The most serious one is the purple blotch caused by Alternaria porri (Ellis). The disease causes extensive damage to bulbs as well as seed crop and also a major limiting factor in cultivation of onion. In view of the destructive nature of purple blotch of onion the present investigation was conducted through survey to know the disease incidence collection of infected samples. A survey was conducted during Rabi 2018-2019 in major onion growing areas of central part of Bihar viz. Nalanda, Muzaffarpur, Vaishali, Patna and Samastipur districts. The highest (37.17) per cent disease index was noticed in field of Pariaunna village of Nalanda district. While, the lowest (17.62) per cent disease index was recorded in Azampur village of Vaishali district. Among the districts, severity of disease was more in Nalanda (34.42) and less in Vaishali (23.98). Isolation was made from onion leaves showing typical purple blotch symptoms. Pure culture of Alternaria porri was obtained and its pathogen city to onion plants was proved. On the basis of isolation and morphological studies, the pathogen was identified as Alternaria porri (Ellis).

Key words: Onion; Purple blotch; Alternaria blight; PDI; Pathogen city;

Onion (*Allium cepa L.*) is one of the oldest known vegetable crops popularly called as "queen of Kitchen". The genus *Allium* is comprising of more than 700 spp and belongs to the family Alliaceae. Vavilov (1951) reported its primary centers of origin as Central Asia and the Near East and Mediterranean are the secondary centers of origin. Onion provides excellent taste to dishes along with a number of theraptic properties such as antibacterial, antifungal, antihelmintic, anti-inflammatory, antiseptic etc (Chethana, B. S. et al 2013). As per the world scenario of onion during 2015-16, China was the major producer of onion with 26.3 per cent followed by India with 22.6 per cent and USA occupied 3rd place with 3.8 per cent of production (Anonymous, 2015). In India onion occupied an area of about 1.22 m. ha with a production of 20.99 million tones and productivity of 21.2 tones per ha during the year 2016-17. The major onion growing states are Maharashtra (30%), Madhya

Pradesh (15%), Karnataka (11%), Gujarat (10%), Bihar (7%), Andhra Pradesh (5%), Rajasthan (4%), Haryana (3%) and others (15%) (Indian Horticulture Database 2015). Onion is susceptible for numerous pests and diseases throughout growing period under field conditions. Alternaria blight is one of the most devastating disease (Marmath et al. 2013). Several factors have been identified for the low productivity of onion in India. The most important factors responsible are the diseases like purple blotch, downy mildew, stemphylium blight, basal rot and storage rots etc., and non-availability of varieties resistant to biotic and a biotic stresses. Among the foliar diseases, purple blotch is one of the most destructive diseases, commonly prevailing in almost all onion growing pockets of the world, which causes heavy loss in onions under field conditions. Losses ranging from 30 to 80 per cent. The disease may reach epidemic states during the favorable conditions of high relative humidity (80-90%) and optimum temperature (24±10C) (*Yadav et al. 2013*).

The name "Purple blotch" for this disease was proposed by (Kushal, et al 2015). He named the causal organism as Alternaria which was later amended to Alternaria porri. The pathogen Alternaria porri destructs the leaf tissue which destroys the stimulus for bulb initiation and delays bulbing and maturation. Severe attack on flowering alliums can completely girdle flower stalks with necrotic tissue, causing their collapse and total loss of seed production capacity. Alternaria infection of onion is widespread particularly in rainy season or high moisture conditions. Survey and surveillance form the basis for any successful plant protection strategy. Successful plant protection depends upon early detection of the disease severity followed by timely adoption and application of preventive measures (Priya, R. U. et al 2013). However, systemic survey on the distribution and severity in central parts of Bihar is lacking. There is a need to undertake systemic survey to identify hot spots for the disease in 5 districts of central parts of Bihar. Keeping all these aspects in view, the present investigation was undertaken to know the disease severity in central parts of Bihar.

Table 1. Area and Production of onion for major producing districts in Bihar

2014-15		2015-16		
Areain	Prod.	Area	Prod.	
'000ha	'000MT	'000ha	'000MT	
6.00	160.00	6.06	161.60	
3.90	83.10	3.94	83.93	
2.60	67.00	2.63	67.67	
2.60	63.00	2.63	63.63	
2.40	62.00	2.43	62.62	
2.40	59.30	2.42	59.89	
1.80	44.00	1.82	44.44	
1.80	42.50	1.82	42.93	
2.00	42.40	2.02	42.82	
1.60	40.30	1.62	40.72	
1.30	32.50	1.31	32.83	
1.40	31.40	1.41	31.71	
1.40	30.40	1.41	30.70	
1.50	30.30	1.52	30.60	
1.30	30.00	1.31	30.30	
1.20	27.30	1.21	27.57	
1.20	26.00	1.21	26.26	
1.10	25.40	1.13	25.67	
	Area in '000ha 6.00 3.90 2.60 2.40 2.40 1.80 1.80 1.60 1.30 1.40 1.50 1.30 1.20 1.20	Area in Prod. '000ha '000MT 6.00 160.00 3.90 83.10 2.60 67.00 2.40 62.00 2.40 59.30 1.80 44.00 1.80 42.50 2.00 42.40 1.60 40.30 1.30 32.50 1.40 31.40 1.40 30.40 1.50 30.30 1.30 30.00 1.20 27.30 1.20 26.00	Area in Prod. Area (000ha) Area (000ha) Area (000ha) 6.00 160.00 6.06 3.94 2.60 67.00 2.63 2.60 63.00 2.63 2.40 62.00 2.43 2.40 59.30 2.42 1.80 44.00 1.82 1.80 42.50 1.82 2.00 42.40 2.02 1.60 40.30 1.62 1.30 32.50 1.31 1.40 31.40 1.41 1.50 30.30 1.52 1.30 30.00 1.31 1.20 27.30 1.21 1.20 26.00 1.21	

^{*}These district under survey for purple blotch disease of onion.

METHODOLOGY

Survey for onion purple blotch severity in central part of Bihar: A roving survey was conducted to know the per cent disease index of purple blotch disease in selected districts of Bihar during Rabi 2018-19 when the crop was at physiological maturity. The survey was carried out from onion growing districts viz., Nalanda, Muzaffarpur, Patna, Vaishali, and Samastipur. The purple blotch severity was scored by following 0-5 scale as given by Sharma (1986). The details of scales are as shown below.

- No disease symptoms.
- i.i A few spots towards tip covering 10 per cent leaf area.
- iii. Several dark purplish brown patch covering upto 20 per cent leaf area.
- iv. Several patches with paler outer zone covering upto 40 per cent leaf area.
- v. Leaf streaks covering upto 75 per cent leaf area or breaking of the leaves from center.
- vi. Complete drying of the leaves or breaking of the leaves from center.

Further, per cent disease index (PDI) was worked out by using following formula proposed by *Wheeler* (1969).

$$PDI = \frac{SIR}{TLO} \times 100$$

Where

SIR= Sum of individual ratings

TLO= Total no. of leaves observed Max. Grade

Isolation of the pathogen from purple blotch infected sample: The pathogen (Alternaria porri) from the purple blotch infected leaf samples collected from different areas of central part of Bihar were isolated separately by following tissue isolation technique. The infected leaves along with healthy portions were cut into small bits and were surface sterilized with 1:1000 mercuric chloride solutions for 30 seconds and washed three times in sterile distilled water before transferring them to potato dextrose agar. The plates were incubated at room temperature (28±10C) and observed periodically for fungal growth. The colonies which developed from the tissue bits were transferred to PDA slants.

Single spore isolation: Ten ml of clear sterilized water agar of two per cent strength was poured into Petri plates and was allowed to solidify. Dilute spore suspension was prepared using sterile distilled water from 12 days old culture. One ml of suspension was spread uniformly in Petri plates over which two per cent agar was poured aseptically and allowed to solidify. Then the plates were examined under low power objective (10 xs) of compound microscope to locate the conidia. Single isolated conidian was then marked under the microscope field with ink on the surface of the plate. Those marked agar areas were cut and transferred to PDA slants with the help of Cork borer (2 mm) under aseptic conditions and incubated at temperature of 28±10C.

Proving the pathogen city: Onion seedlings were raised in earthen pots, size 6" X 5", filled with sterilized soil. Plants were thoroughly cleaned with sterilized distilled water using moist cotton. Later, the plants were sprayed with distilled water. They were covered with polythene bags for 24 hr. The inoculums suspension from ten day old culture was prepared in sterile distilled water and sprayed on to the plants. Similarly control plants were sprayed with sterile distilled water for comparison. The seedlings were covered with polythene bags and were incubated for 120 hr. to ensure successful penetration of the pathogen into the tissue. The polythene bags were removed after five days and seedlings were kept under greenhouse conditions. Observations were made regularly for the appearance and development of symptoms. After appearance of disease symptoms, re-isolation was made from the diseased tissues of artificially infected plants. The isolate obtained was compared with the original culture for confirmation of fungus under study.

RESULTS AND DISCUSSION

During the present investigation a field survey was conducted to gather information on the severity of purple blotch of onion from major onion growing districts of Bihar and selected only 5 districts for survey & operations.

Survey for the severity of purple blotch of onion: A roving survey was carried out for recording the severity of purple blotch disease of onion during Rabi 2018-19 in five major onion growing districts of Bihar viz. Nalanda, Muzaffarpur, Patna, Vaishali and Samastipur. The survey for symptomatology, severity, distribution and spread was carried out at physiological maturity and the data pertaining to survey work is presented in Table 2.

The survey revealed that prevalence of the disease in all locations and disease severity ranged from 17.62

Table 2. Survey for purple blotch of onion in central part of Bihar

District	Block	Village	PDI
Nalanda	Chandi	Gauri	31.32
		Haheshpur	33.64
		Dayalpur	32.95
	Noorsarai	Pariaunna	37.17
		Charuvi Beldari	35.11
		Bara Khurd	36.71
	Bihar Sarif	Sohdih	33.16
		Pahadi	35.55
		Paroha	34.22
		Mean	34.42
Muzaffarpur	Sarka	Harpur Kishuni	27.33
		Gobardhanpur	24.67
		Mushari Ram	25.75
	Manipur	Harka Mansahi	26.87
	Mampai	Kodaria	23.45
		Rampur Hari	21.83
	Gaighat	Janta Tok	26.89
	Gaignat	Shakarwara Nur	28.85
		Dahila Patsawan	25.28
		Mean	25.65
Dotno	Down Athernal acla		
Patna	Barh-Athmalgola	Daulatpur	34.17
		Ramnagar Karari	30.19
	D'I 4	Dharampur	28.87
	Bihta	Tikaitpur	33.27
		Kelhanpur	25.74
	- · · · ·	Datiana	26.29
	Bakhitiarpur	Dedur	28.65
		Ghoswari	27.92
		Misi	25.88
		Mean	28.99
Vaishali	Desari	Azampur	17.62
		Phatikwara	21.87
		Khur Rampur	23.49
	Jandaha	Adalpur	25.58
		Basantpur	23.84
		Hasanpur Buzurg	24.36
	Mahua	Kushahar	26.38
		Paharpur	27.29
		Hakimpur	25.41
		Mean	23.98
Samastipur	Bibhutipur	Kerai	27.39
1	Ī	Belsandi Tara	25.13
		Patpara	26.53
	Dalsinghsarai	Basaria	23.48
		Pagra	21.42
		Mathurapur	20.78
	Kalyanpur	Madhurapur	32.17
	isaryanpui	Ladaura	35.76
		Govindbari	33.86
		Jovingoan	$_{\mathcal{I}}$

to 37.17 per cent disease index (PDI) in different parts of the districts surveyed. The highest severity (37.17 PDI) of purple blotch was recorded in fields of Pariaunna village in Nalanda district (Plate 2b), whereas least severity (17.62 PDI) of the disease was recorded at Azampur village in Vaishali district (Plate 2a). The average severity of 34.42 per cent disease index was recorded in Nalanda district followed by Patna (28.99 PDI) and Samastipur (27.39 PDI). The lowest disease severity of 23.98 per cent disease index was recorded in vaishali district. The Purple blotch of onion was severe in Nalanda district compared to vaishali district. This could be because of favorable environmental conditions and initial inoculums prevailed in this rejoin might have helped in the rapid development of the disease in Rabi. Working on survey of Alternaria leaf blight and other diseases of onion (Fillinger S. et al 2016) concluded that it is the most predominant and severe disease in the onion growing areas of Maharashtra. Kale, S. M.S. et al. (2014) in their report on status of field diseases and insect pest of onion in India also indicated that purple blotch incidence was high in both winter and post-winter seasons when high humidity prevailed. The present findings are in accordance with the results of *Chethana*, et al (2011) who conducted survey is also revealed that incidence of purple blotch of onion was noticed in all districts central part of Bihar. During Rabi 2018-19 survey various symptoms of the disease were noticed on leaves and also on bulbs. At initial stages, leaves were with circular to oval water-soaked areas which later on, as the disease progressed, became oblong and a fresh zone of discolored tissue was formed around the spots. Initially spots were white, but later turned pinkish or purple. The change in colour started from the center and gradually progressed towards the periphery, where it changed into light purplish. The transition of colour was marked by concentric rings clearly visible to the naked eye. The older leaves were more susceptible than younger leaves and were relatively more susceptible when they reach close to bulb maturity. The symptoms of the disease were photographed and are presented (Plate 3).

Isolation: Isolation of the pathogen was made from onion leaves showing typical symptoms of the disease. Leaves with such symptoms were collected for the isolation purpose. Standard tissue method was followed after surface disinfection as described in material and

methods and further isolation brought into pure culture by single spore isolation. The pure culture of the fungus was obtained after eight days of inoculation which showed whitish growth at initial stage turning later to ash gray color. Such pure culture obtained was again sub cultured on potato dextrose agar slants and kept in the refrigerator at 5°C for further studies (Plate 4). *Tripathy, et al (2013)* obtained pure culture of the fungus using tissue isolation method and described it as a new technique for inoculums preparation and concluded that spore or conidial suspension is the most effective inoculums.

Identification of pathogen: Identification of fungus was carried out based on the morphological characters of the fungus isolated. The fungus in the present study produced septate mycelium. Later it produced conidiophores arising singly or in small groups. The conidiophores were straight or flexuous, sometime geniculate, septets, pale or mid brown in color and measured upto 120 μm long and 6-10 μm thick, with one or several conidial scars. A mature conidiophores usually produced solitary conidian but occasionally it also produced conidia with very short chains, straight or curved, rostrate, beak generally equal to the length of the body of the conidian, pale brown to mid golden brown in colour.

Overall length of conidia ranged from 100- 300 μ m, 15 – 20 μ m thick in the broadest part with 7-12 transverse and zero to several longitudinal septa, beak flexuous, pale, 2-4 μ m thick and tapering. The typical conidian is photographed and is shown (Plate 4). All these characters agreed with those of *Alternaria porri* described by *Mishra et al* (2014) with minor variation in shape and dimension which may be either, due to host or environmental factor and hence were considered to fall within the limits for species. *Chethana, et al* (2011), who worked on purple blotch of onion, also indicated *A. porri* as the causal agent of the disease and the description is in line with the present investigation.

Pathogen city test: For proving pathogen city on host, the pathogens were artificially inoculated on the leaves of onion plants as described in material and methods. After ten days of inoculation, the leaves exhibited symptoms of infection. Earlier infection symptoms could be seen as a small, water soaked lesions which appeared on leaves. Later, these spots started to enlarge

and became sunken and purplish in color, with yellow halo. However, this complete expression of the disease symptoms was clearly noticed after 60 days of inoculation. The typical symptoms like purplish zonate spots were noticed on leaves of the artificially inoculated plants. The symptoms were photographed and are presented (Plate 4). In the present study, symptoms of the disease mentioned above and inoculation technique were found to be in agreement with the typical symptoms of the disease described earlier by many workers (Priya, et al, 2013 and Vinamrata Patilkulkarni, 2013) who proved pathogen city of onion by spraying conidial suspension on the host surface. The pathogen was re-isolated from such leaves and the morphological character of the re-isolated organism was compared with the original culture of the pathogen which was

similar in all respects. Hence, the causal agent of the disease was confirmed as *Alternaria porri* (Ellis).

CONCLUSION

The surveys of various symptoms of the disease were noticed on leaves and also on bulbs. At initial stages, leaves were with circular to oval water-soaked areas which later on, as the disease progressed, became oblong and a fresh zone of discolored tissue was formed around the spots. Initially spots were white, but later turned pinkish or purple. The Purple blotch of onion was severe in Nalanda district compared to vaishali district. This could be because of favorable environmental conditions and initial inoculums prevailed in this rejoin might have helped in the rapid development of the disease in *Rabi*.

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