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PLANT PHYSIOLOGY

Survey for purple blotch of onion (*Alternaria porri* (Ellis) Cif.) in northern parts of Karnataka

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Abstract

Onion (*Allium cepa* L.) is one of the important bulb crop 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 ofbulbs and seeds is limited by certain diseases. The most serious one is the purple blotch caused by *Alternariaporri* (Ellis) Cif. 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 or severity and collection of infected samples. A survey was conducted during *kharif* 2013-2014 in onion growing areas of Northern Karnataka *viz.*, Bijapur, Bagalkot, Gadag and Dharwad districts. The highest per cent disease index was noticed in Ilkal village of Bagalkot district. While, the lowest per cent disease index was noticed in kerur village of Bagalkot district. Among the districts, severity of disease was more in Bijapur and less in Gadag.Isolation was made from onion leaves showing typical purple blotch symptoms. Pure culture of *A. porri* was obtained and its pathogenicity to onion plants was proved. On the basis of isolation and morphological studies, the pathogen was identified as *Alternariaporri* (Ellis) Cif.

Highlights

- Based on survey, onion samples were collected and isolation has been carried out. Isolation and morphological studies helps in identifying *Alternaria porri*

Keywords: Onion, purple blotch, alternaria blight, PDI and pathogenicity

The onion (*Allium cepa* L.) (Latin '*cepa*' = onion), also known as the bulb onion or common onion and is rightly called as "*Queen of Kitchen*" and is one of the oldest known and important vegetable crops grown in India. According to Vavilov (1951) the primary center of origin lies in Central Asia. Onion is cultivated and used around the world. India is a traditional grower and assumes second position in onion production with 86.34 million tonnes from 4.36 million hectares area (FAOSTAT 2013). Onion is cultivated round the year throughout the country. The major onion growing states are Maharashtra (33%), Karnataka (17%), Gujarat (10%), Bihar (7%), Madhya Pradesh (7%), Andhra Pradesh (5%), Rajastan (3%), Haryana (3%) and others (15%) (Indian Horticulture Database 2011). 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 abiotic 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 100 per cent. The disease may reach epidemic states during the favourable 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 Nolla (1927). He named the causal organism as *Alternariaalli* which was later amended to *Alternariaporri.* The pathogen *Alternariaporri* 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 (Sudarshan Rao, 1975). However, systemic survey on the distribution and severity in Northern parts of Karnataka is lacking. There is a need to undertake systemic survey to identify hot spots for the disease in Northern parts of Karnataka. Keeping all these aspects in view, the present investigation was undertaken to know the disease severity in northern parts of Karnataka.

Material and Methods

Survey for onion purple blotch severity in Northern Karnataka

A roving survey was conducted to know the per cent disease index of purple blotch disease in districts of Northern Karnataka during*kharif*2013 when the crop was at physiological maturity. The survey was carried out from onion growing districts *viz.*, Bijapur, Bagalkot, Gadagand Dharwad. The purple blotch severity was scored by following 0-5 scale as given by Sharma (1986). The details of scales are as shown below.

1. No disease symptoms.
2. A few spots towards tip covering 10 per cent leaf area.
3. Several dark purplish brown patch covering upto 20 per cent leaf area.
4. Several patches with paler outer zone covering upto 40 per cent leaf area.
5. Leaf streaks covering upto 75 per cent leaf area or breaking of the leaves from center.
6. 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).

$$PDI = \frac{\text{Sum of individual ratings}}{\text{Total no. of leaves observed Max. Grade}} \times \frac{100}{}$$

Isolation of the pathogen from purple blotch infected sample

The pathogen (*Alternariaporri*) from the purple blotch infected leaf samples collected from different areas of Northern Karnataka 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±1^{0}C) 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 conidium 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±1^{0}C.

Proving the pathogenicity

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 inoculum 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 onion growing districts of Northern Karnataka.

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*kharif*2013 in four major onion growing districts of Northern Karnataka *viz.*, Bijapur, Bagalkot, Gadag and Dharwad. 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 1 and Plate 1.

The survey revealed that prevalence of the disease in all locations and disease severity ranged from 18.02 to 36.23 per cent disease index (PDI) in different parts of the districts surveyed. The highest severity (36.23 PDI) of purple blotch was noticed in fields of Ilkal village in Bagalkot district (Plate 2b), whereas least severity (18.02 PDI) of the disease was recorded at Kerur village in Bagalkot district (Plate 2a). The average severity of 28.58 per cent disease index was recorded in Bijapur district followed by Bagalkot (28.09 PDI) and Dharwad (27.38 PDI). The lowest disease severity of 25.83 per cent disease index was recorded in Gadag district. The Purple blotch of onion was severe in Bijapur district compared to Gadag district. This could be because of favorable environmental conditions and initial inoculum prevailed in this rejion might have helped in the rapid development of the disease in *Kharif*.

Working on survey of *Alternaria* leaf blight and other diseases of onion, Patil and Patil (1991) concluded that it is the most predominant and severe disease in the onion growing areas of Maharashtra. Srivastava *et al.* (1994) 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 rainy and post-rainy seasons when high humidity prevailed. The present findings are in accordance with the results of Chethana (2000) who conducted survey in Northern parts of Karnataka during *kharif* 1999 also revealed that incidence of purple blotch of onion was noticed in all districts of Northern Karnatakaand recorded highest per cent of disease incidence in Ronihal village (Basavanabagewaditaluk) of Bijapur district and lowest in Wadullur village of Raichurtaluk.

Survey carried during *kharif* 2006 revealed that purple blotch was severe in six districts of Northern Karnataka *viz.*, Dharwad, Bagalkot, Bijapur, Belgaum, Gadag and Haveri. Isolation and morphological studies revealed *A. porri* and *A. alternata* as pathogens (Pramod kumar, 2007). Survey during *kharif* 2012-13 revealed that purple blotch was found in all parts of Northern Karnataka and was severe in Haveri district (Vinamrata Patilkulkarni, 2013)

Table 1: Survey for purple blotch of onion in Northern parts of Karnataka

District	Taluka	Village name	Stage of the crop	Crop grown condition	Per cent Disease Index (PDI)
Bijapur	Bijapur	Hitnalli	Physiological maturity	Rainfed	25.89
		Jumnal	Physiological maturity	Irrigated	31.16
		Utnal	Physiological maturity	Rainfed	22.43
	Basavana Bagewadi	Telagi	Physiological maturity	Irrigated	33.03
		Golasangi	Physiological maturity	Irrigated	30.86
		Yatnal	Physiological maturity	Rainfed	28.16
			Mean 28.58		
Bagalkot	Hunagund	Hunagund	Physiological maturity	Rainfed	29.12
		Kudalasangam	Physiological maturity	Irrigated	32.76
		Ilkal	Physiological maturity	Irrigated	36.23
	Badami	Badami	Physiological maturity	Rainfed	19.38
		Kerur	Physiological maturity	Rainfed	18.02
		Kerakalmatti	Physiological maturity	Irrigated	33.07
			Mean 28.09		
Gadag	Naragund	Naragund	Physiological maturity	Rainfed	28.80
		Konnur	Physiological maturity	Rainfed	19.68
		Kelakeri	Physiological maturity	Rainfed	29.01
			Mean 25.83		
Dharwad	Navalgund	Navalgund	Physiological maturity	Irrigated	33.12
		Annigeri	Physiological maturity	Rainfed	22.21
		Timmapur	Physiological maturity	Rainfed	26.82
			Mean 27.38		

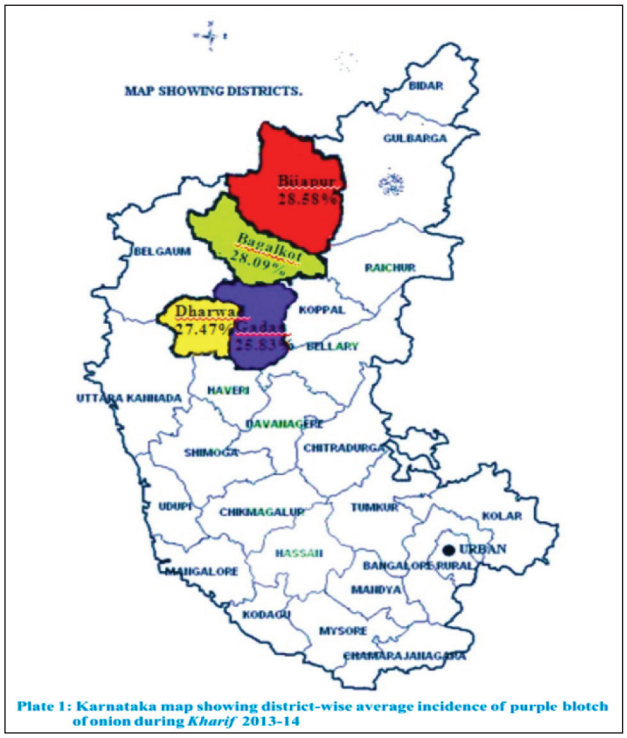

Plate 1: Karnataka map showing district-wise average incidence of purple blotch of onion during *Kharif* 2013-14

Plate 2a: Severity of purple blotch of onion at Kerur (Badami Taluk)

Plate 2b: Severity of purple blotch of onion at Ilkal (Hunagund Taluk)

During 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 discoloured 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).

Plate 3: Symptoms and disease grading of purple blotch of onion

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). Dhiman and Chadha (1986) obtained pure culture of the fungus using tissue isolation method and described it as a new technique for inoculum preparation and concluded that spore or conidial suspension is the most effective inoculum.

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, septate, pale or mid brown in color and measured upto 120 µm long and 6-10 µm thick, with one or several conidial scars.

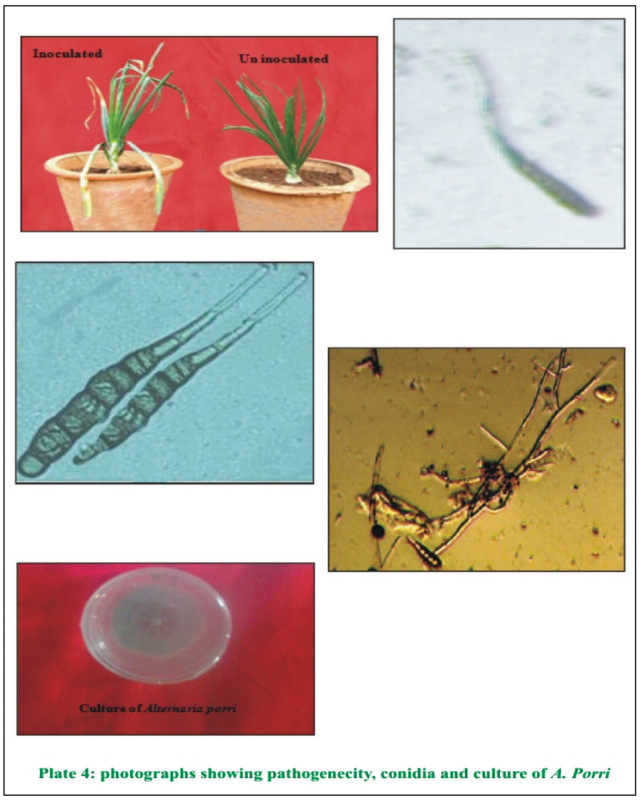

Plate 4: photographs showing pathogenecity, conidia and culture of *A. Porri*

A mature conidiophore usually produced solitary conidium 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 conidium, pale brown to mid golden brown in colour. Overall length of conidia ranged from 100 - 300 µm, 15 – 20µm thick inthe broadest part with 7-12 transverse and zero to several longitudinal septa, beak flexuous, pale, 2-4 µm thick and tapering. The typical conidium is photographed and is shown (Plate 4). All these characters agreed with those of *A. porri* described by Cifferi (1930) and Ellis (1971) 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 (2000), 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.

Pathogenicity test

For proving pathogenicity 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 (Ponnappa, 1974; Utikar and Padule, 1980; Patil and Patil, 1992; Chetana, 2000) who proved pathogenicity 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 *Alternariaporri* (Ellis) Cif.

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