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Alternaria Species on Brassicaceae in Eastern Zone in Nepal And Adjoining Area of North Bihar

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ABSTRACT

This paper deliberated to present future stance and strategy for variation of Alternaria brassicicola leaf spot of Broccoli (Brassica oleracea var. italica L.)was evaluated based on morphological, cultural and molecular parameters during Rabi season 2014 and 2015. Six isolates of Alternaria were cultured in vitro using different growth medium and the growth pattern of the fungi was studied. Maximum 86 leaf spots were recorded from different places followed and minimum 17 spots from CSA samples. The size of spots shown variations in different isolates collected from different places. The leaf spots were followed the reducing trend with the increasing number of spots. The maximum 0.5-1.9mm size of spot was noticed in CSA samples and minimum0.2-0.7mm from different places of northern Bihar and Biratnagar sub metropolitan city. The growth of A. brassicicola recorded maximum in host extract media followed by Potato Dextrose Agar while the growth observed minimum in Czapek's medium. The variations also exhibited in protein profiling by using SDS-PAGE.

Introduction

Alternaria is a genus of Deuteromycetinity and Alternaria species are and also known as major plant pathogens. They are also common allergensin humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes lead to asthma. They readily cause opportunistic infections in immuno compromised people such as AIDS patients. Broccoli (Brassica oleracea var.) is one of the important vegetable in Southern part of Nepal and Northern part of Bihar, India ranked second in global production[*Kirk PM,et.al]*[. It accounts 5MT production taking the place of cauliflower among medium to rich income families due to the presence of high nutrients and medicinal properties. Besides high nutritive values broccoli also contains some glucosinolet substances which activate some enzymes in our body that inhibits cancer causing agent. The productivity of this crop in India is about 260q ha-1 against the world average 275q ha-1. In comparison to cauliflower the productivity of Broccoli is very less though both belongs to the family Brassicaceae distinguished only by the presence of multiple flower buds in broccoli rather than a single curd in cauliflower. The production of broccoli may be increased up to 33 to 35 percent by the inclusion of integrated nutrient and pest management practices. One of the major causes is the leaf spot diseases caused by *Alternaria brassicicola[Kelman, MJet.al]* . It is cosmopolitan in their distribution reported in all continents and identified as most damaging fungal disease (Ghose et al. 2008). The yield losses have been reported in the range of 32-69 percent by this fungus [Surviliene et al. 2004; Shrestha et al.2005]. The disease decreases the nutritive value of this vegetable, their storability and also decreases the resistance of vegetable to rot[*Ran Yupinget.al.]* . The first appearance of the disease start on the leaves as necrotic lesions often describes as black and sooty with chlorotic yellow halos surrounding the lesions site in wet season. *A. brassicicola* is a necrotrophic plant pathogenic fungus secretes toxic secondary metabolites and proteins that cause cell death (Ravindra, 2013). In recent years the incidence of this disease reported very severe losses which pose a new threat to *Brassicaceous* vegetable cultivar. Due to fluctuating in environmental conditions the pathogen does not have a uniform growth rate. The pathogens greatly influenced by weather condition. Studies on pathogenic variability have the foundation for the development of pre-breeding populations as strategic defence mechanism (Vishwanath et al. 1999). Researches on *A. brassicicola* in Broccoli are still scarce, therefore field identification, bioformulations and disease forecasting module is not developed so far. At least 20% of agricultural spoilage is caused by *Alternaria* species; most severe losses may reach up to 80% of yield, though. Many human health disorders can be caused by these fungi, which grow on skin and mucous membranes, including on the eyeballs and within the respiratory tract. Allergies are common, but serious infections are rare, except in people with compromised immune systems. However, species of this fungal genus are often prolific producers of a variety of toxic compounds. The effects most of these compounds have on animal and plant health are not well known. Many species of alternaria modify their secondary metabolites by sulfoconjugation; however the role of this process is not yet understood.The terms *alternariosis* and *alternariatoxicosis* are used for disorders in humans and animals caused by a fungus in this genus. The present study was undertaken to characterize the infection behavior, pathogenic growth pattern caused by *A. brassicicola* under different growing medium for better understanding of the fungus.

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Materials and Methods

Collection, Isolation, Purification and Maintenance of Alternaria brassicicola

The large number of dark blighted leaf samplesof Broccoli were collected from different locations Northern Bihar and Biratnagar Metropolitan city . The spotted leaves showing disease symptoms were screened out for isolation of the pathogen. The pathogen was identified on the basis of its morphological and cultural characters with the help of key as per Chand et al. (2007) as well as the pathogenicity to the host were also assessed. The culture was purified using single spore technique and maintained at 2% Potato Dextrose Agar slant at 6-8 ^{o}Ċ[*Wiest, Peter]*.

Results and Discussion

Symptomatological variability

Symptom parameters of the leaf spots exhibited variations in growth pattern, size and even in number of spots collected from different places under the study. Maximum 86 numbers of spots were recorded in the samples collected from different palce of northern Bihar and Biratnagar sub-metropolitan and minimum 17 spots were recorded in the samples from CSA (Fig.1). The size of spots ranged 0.2-1.9mm with light olivaceous, brown colour along with concentric rings with black pin point at centre.

The size of spots observed maximum at CSA and minimum at SHIATS. The size of the spots observed larger with decreasing the number of spots (Table 1). Ellis (1973) reported the spot size of this fungi upto 1.8mm with light olivaceous, brown colour and profuse sooty spores. Number of disease spot and their size was observed variation among different study sites as also confirmed by[Chand et al. (2007)] due to variations in temperature, humidity and other environmental factors.

Morphological variability of the pathogen

Morphological characteristics of five representative isolates of Alternaria spp.including shape, size of conidia and conidiophores was measured at 40X magnification using calibrated filler micrometer in the microscope. Number of transverse and longitudinal septa was also counted.

Cultural variability of the pathogenThe variations in growth pattern, color of colony were recorded separately for Potato Dextrose Agar (PDA), PDA+CaCO3, Host Extract Agar, Czapek's Agar and Richard's Agar mediums[**Nowicki, Marcin]**.These isolates were incubated at 25.2oĊ with 95.5% humidity and data on parameters were recorded on 4th and 7th day after inoculation[Dewdney, M. M.]

Table . 1 Number,size and colour of the leaf spot samples in Brocoli.

Isolates	No.of spot	Colour of spot	Spot size in diameter (mm)	Yellow hollow
ND	48±5.30	Light brown	0.3 – 1.3	Absent
CSA	17±3.50	Olivaceous brown	0.5 – 1.9	Absent
INR	30±5.22	Dark brown	0.4 – 1.8	Present
BHU	74±4.60	Olivaceous brown	0.2 – 1.8	Present
NAINI	86±4.05	Dark brown	0.2 – 0.7	Present

Table 2. Morphological variations in different isolates of Alternaria brassicicola.

Morphological Chanracteristics	SAMPLE ISOLATES				
	ND	CSA	IIVR	BHU	NAINI
Conidiphore Colour	Olivaceous brown	Pale olivaceos brown	Olive brown	Olive brown	Pale olivaceos brown
Conidiphore Shape	Simple erect and curve septate	Simple erect	curve septate	Simple erect	Simpleer ect
Length (µm)	24 – 76	25 - 80	25 - 75	25 - 75	24 - 75
Width (µm)	4 – 8	5 - 10	3-8	4-8	5-10
Conida					
Conida in chain	2 – 3	4-6	4-8	5-8	4-6
Shape	Cylindrical to obclavate	Cylindrical to obclavate	Cylindrical to obclavate	Cylindrical to obclavate	Cylindrical to obclavate
Cross septa	2 – 5	2-6	2-8	4-6	2-7
Longitudinal septa	1 - 2	1-3	1-2	1-2	1-2
Length (µm)	15 - 120	20-120	20-110	13-108	15-110
Width (µm)	6 - 13	6-15	8-16	6-15	6-16
Beak	Non - existent	Non - existent	Non - existent	Non - existent	Non - existent

Table 3. The growth and colour of Alternaria brassicicola under different growing media after 4 days.

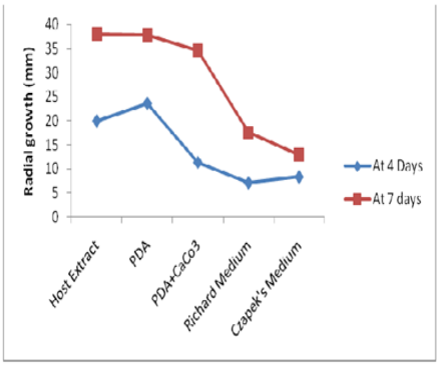
Isolates	Host extract		PDA		PDA+CaCo3		Richard medium		Czapek's Medium	
	Growth (mm)	Colour	Growth (mm)	Colour	Growth (mm)	Colour	Growth (mm)	Colour	Growth (mm)	Colour
ND	18 (2.11)	White	24 (3.33)	Dull white	10 (1.22)	Greenish black	7 (0.25)	Light brown	6 (0.15)	Gray brown
CSA	23 (3.50)	White	29 (1.55)	white	11 (0.88)	Light brown	8 (1.11)	Gray black	11(0.55)	Gray white
IIVR	24 (2.21)	White	20 (3.50)	Dull white	18 (1.76)	Pale brown	6 (0.55)	Light brown	8 (1.03)	Gray brown
BHU	14 (3.00)	White	20 (2.44)	Dull white	8 (0.50)	Brown	8 (1.20)	Black	7 (1.00)	Gray brown
NAINI	25 (3.68)	Pale black	25 (3.10)	Dull white	10 (1.00)	Light black	7 (0.87)	Light brown	10 (0.08)	Gray brown

Table 4. Effect of different media on radio growth and colour of Alternaria brassicicola after 7 days.

Isolates	Host extract		PDA		PDA+CaCo3		Richard medium		Czapek's Medium	
	Growth (mm)	Colour	Growth (mm)	Colour	Growth (mm)	Colour	Growth (mm)	Colour	Growth (mm)	Colour
ND	36 (3.57)	Black	38 (2.30)	Black	36 (2.20)	Gray white	14 (0.55)	Dark brown	12 (1.30)	Gray brown
CSA	40 (2.80)	Black	40 (3.50)	Black	34 (3.18)	Light brown	20 (2.03)	Dark brown	14 (0.55)	Gray brown
IIVR	38 (4.00)	Black	36 (2.600	Light brown	30 (2.55)	Brown	25 (0.77)	Dark brown	11 (0.88)	Gray brown
BHU	39 (3.05)	Black	37 (3.12)	Black	38 (1.50)	Gray white	13 (1.00)	Black	13 (0.55)	Gray brown
NAINI	37 (4.40)	Black	38 (2.50)	Black	35 (1.80)	Dark brown	16 (0.56)	Dark brown	15 (1.20)	Gray brown

Graph 1

Mean radial growth of A. brassicicola in different mediums isolated from Broccoli.



Molecular variability

The highest molecular weight of the fungus was recorded (22 kDa) in the samples collected from CSA followed by NDUAT (Fig. 3) andBiratnagar area (21kDa) indicated very high virulency of the fungusto break the resistance of the host and higher yield loss in broccoli. This was in confirmation of earlier report by [Chand and Chakrabarti (2003)].The minimum molecular weight of the protein was found in SHIATS sample might be due to the higher resistance of the host as well as the poor virulency of the fungus in this area.Similarly [Mora and Earle (2001)] also observed the high kDa due to higher endochitinase activity in transgenic Brassica varieties than control varieties. The variations in different isolates of Alternaria in various sample areas were might be due many factors such as the genetic variations of the fungus, variation in environment and also due to the different varieties of broccoli grown in sampled areas. It was concluded from the present study that different isolates of Alternaria may exhibits differential growth pattern and symptom in different areas even in same hosts. The fungus growth was highest and fastest in CSA sample and minimum in northern Bihar , sample. Similarly the colour, conidial structure and other morphological parameters of the leaf blight Alternaria of Broccoli showed variability. The variation was also found in molecular level of protein in which CSA isolates expressed highest kDa and virulence to host as compared to other isolates of the study.

Conclusions

According to the results observed in this study, we can say that different crops of brassicae can work as efficient source of inoculums of A. brassicae and A. brassicicola for new plantings. Studies of cross inoculation, in which all the isolates are inoculated in all the species of host, are necessary to confirm if the inoculums coming from the fields or cultural remains of species would also be suitable according to the pathogenicity and virulence in a distinct species. In another study, we observed that isolates of A. brassicae and A. brassicicola were able to infect and cause some level of symptoms in brassicae of european and oriental groups, as well as some brassicae weeds. However, different levels of quantitative resistance in some hosts were also observed and characterized, mainly for the presence of lesions in smaller sizes.As observed in other the results in this work reinforce the evidence that among the species of Alternaria which cause diseases in brassicae in Pernambuco, preference for host occurs, mainly in Chinese cabbage, broccoli and cabbage. This aspect should be considered in the development of strategy for management of Alternaria, mainly involving the use of resistant cultivars and crop rotation with different species of brassicae, considering the species of the pathogen prevailing in each species of brassicae in Biratnagar sub-metropolitan and adjacent part of Bihar.

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