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Studies on morphological and cultural variability of *Alternaria cucumerina* var. *cyamopsidis* in clusterbean

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Abstract
Clusterbean is an important legume cultivated mostly on marginal and sub marginal lands of arid and semi-arid regions. Overall, India produces around 80% of global cluster bean production. The diseased samples were collected from Northern Madhya Pradesh for determining variability among collected twenty five isolates of *Alternaria cucumerina* var. *cyamopsidis* causal agent of Alternaria blight in Clusterbean based on conidial morphology and cultural variability on Potato dextrose agar medium. Out of twenty five isolates, five isolates viz: isolate A showed white with light olive black centre, isolate B was dark olive grey colour with white centre, isolate C and E were dark black and brown in colour respectively whereas the isolate D showed dark olive black colony with brown fluffy edge. On the basis of mycelial growth the isolate were found different in each other. The maximum conidial length was occurred in the isolate B (114.72 µm) and the conidial width was in the isolate B (7.0 µm). The maximum number of horizontal and vertical septa was associated with the isolate of D (7.16 µm and 4.48 µm respectively). The maximum radial growth of the colony was found by the isolate B (88.4 mm). Variability studies are important to document the changes occurring in populations and individuals as variability in morphological and physiological traits to indicate the existence of different pathotypes.

Keywords: Alternaria blight, morphological and cultural variability, clusterbean

Introduction
Clusterbean is commonly known as *Guar, Chavli, kayi* and *Khutti*. Guar is grown in *Kharif* season in arid and semi-arid regions of India. It is drought hardy, deep rooted, summer annual legume. Guar is one of the most important and potential vegetable cum industrial crop grown for its tender pods for vegetable purpose and for endospermic gum (Kumar, 2005; Rai *et al*., 2012) [5, 11]. The gum is produced primarily from ground endosperm (Sabahelkheir *et al*., 2012) [13]
In India, Clusterbean occupies an area of 2, 20 million hectares with a production of 0.60 million tonnes from India. Clusterbean is mainly exported to USA, Germany, Netherland, Italy, UK, Japan, and France value at Rs. 200 million rupees annually (Singh *et al*., 2009) [14].
The production and productivity of Clusterbean in terms of grain and fodder is highly affected by a number of phytopathogenic fungal and bacterial diseases *viz*., bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*), *Alternaria* leaf spot (*Alternaria cyamopsidis*), anthracnose (*Colletotrichum capsici* f. sp. *cyamopsicola*), Curvularia leaf spot (*Curvularia lunata*), charcoal rot/damping off (*Macrophomina phaseolina*), dry root rot/leaf blight (*Fusarium solani* and *Rhizoctonia solani*), Myrothecium leaf spot (*Myrothecium roridum*), powdery mildew (*Oidiopsis taurica*), wilt (*Fusarium caeruleum*). *Alternaria* spp. are economically important pathogens widely distributed throughout the world and cause devastating disease on field crops. Alternaria leaf blight is a common disease caused by *A. cucumerina* var. *cyamopsidis* in guar-growing area of western India and Pakistan. Severe Alternaria blight of cluster was also reported from Pusan ad Madras (Ambesh *et al*., 2014) [1].
The low productivity of this crop is attributed to many factors, one of which is the loss due to diseases. There is a need to understand different aspects of the major fungal foliar pathogen of *Alternaria cucumeriana* var. *cyamopsidis* with respect to its morphological and cultural variability since not much work has been done on these aspects in the past. In addition, it will helps in comprehensive understanding of the causal organism. Keeping into view its importance as a vegetable and its adaptability to arid drought conditions, there is need for its improvement for yield.

Materials and Methods

Collection and isolation of pathogen

During survey, naturally infected guar leaves showing typical symptoms of Alternaria blight of guar were collected from the different guar growing areas of Northern Madhya Pradesh. *Alternaria* species were isolated from these infected leaves by standard tissue isolation technique in the laboratory.

Purification of the isolated fungus was carried out using hyphal tip techniques. The fungus was transferred aseptically on the PDA slants in culture tubes. Through frequent sub-culturing, the fungus was purified and pure culture was maintained on agar slants in culture tubes and stored in refrigerator for further studies. The growing mycelium was picked with an inoculation needle and transferred on to PDA slants and incubated at 28 ± 2°C for 7 days.

Variability of the pathogen

Morphological variability

Morphological characters such as length and width of conidia, number of horizontal and vertical septa and length of beak were measured under 40 x using Olympus Microscope (CHI 20) and the pathogen was cultured on PDA. All the above mentioned measurements were compared with the measurements given by Ellis (1971) [3] for the identification of *Alternaria* species.

Cultural variability

Twenty five isolates were grown on PDA to find the difference in colony characters among them. Single spore was placed in center of media to get pure colony. After 7 days of sub-culturing the mycelial growth, type of colony margin, colour of colony and mycelial width (µm) were observed. Each set of experiment replicated thrice and the plates were incubated at 27 ± 1^0 C in B.O.D. incubator.

Results and Discussion

Morphological and cultural Variability

The different isolates collected during survey from the Northern Madhya Pradesh were subjected to morphological variability tests and the results are presented in the Table 1. The study showed that conidia of different isolates were septated by 1-6 vertical and 3-9 horizontal septa. The isolate D showed maximum horizontal septa 7.16 whereas minimum was observed in most of the remaining isolates 6.2, 6.4, 6.84 and 6.94. The isolate D showed m0aximum of 4.48 vertical septa whereas remaining isolates showed minimum of 3.8, 4.76, 3.92 and 4.40 vertical septa. The maximum conidial length was found from the isolate B 114.72 µm which was followed by isolate C (90.2 µm), E (72.37 µm), and A (68.15 µm) respectively. The least conidial length was recorded from the isolate D (36.2 µm). Jackson and Weber (1959) [6] reported that *A. cucumerina* from musk melons produced the colonies which were amphigenous and described the conidiophores as, arising singly or in small groups, erect, straight or flexuous, sometimes geniculate, cylindrical, septate pale to midbrown, up to 110µ long, 6-10 µm thick, usually with several well developed conidial scars.

Table 1: Occurrence of *A cyamopsidis* in major Clusterbean growing districts of Madhya Pradesh

Districts	Isolate Code	Size of conidia (length x breadth) µm	Beak length (µm)	Overall length of conidia (µm)	Number of septa	
					horizontal	Vertical
Gwalior (A)	A1	24.32 x 6.5	45	69.32	6.5	3.2
	A2	22.41 x 5.1	43.2	65.61	5.4	3.2
	A3	23.40 x 4.2	41.7	65.1	5.4	3.1
	A4	25.41 x 7.2	46.2	71.61	7.2	5.4
	A5	24.42 x 6.2	44.71	69.13	6.5	4.1
	Mean	23.99 x5.8	44.16	68.15	6.2	3.8
Morena (B)	B1	34.24 x 7.2	81.2	115.44	8.4	5.2
	B2	33.22 x 6.5	80.52	113.74	7.1	4.1
	B3	36.44 x 8.5	82.4	118.84	8.6	5.2
	B4	32.44 x 5.4	78.5	110.64	8.2	4.2
	B5	35.14 x 7.4	80.11	115.25	8.4	5.1
	Mean	34.29 x 7	50.54	114.72	6.46	4.76
Bhind (C)	C1	27.32 x 4.1	64.24	91.56	6.2	3.3
	C2	25.22 x 3.8	61.2	86.42	5.2	2.4
	C3	28.77 x 5.2	59.5	88.27	7.2	4.4
	C4	26.84 x 6.5	70.1	96.94	8.2	5.3
	C5	25.55 x 4.8	62.4	87.95	7.4	4.2
	Mean	26.74 x 4.88	63.2	90.22	6.84	3.92
Datia (D)	D1	28.71 x 7.21	7.32	36.03	7.2	5.1
	D2	26.44 x 8.1	8.91	35.35	7.2	4.3
	D3	27.24 x 6.5	7.4	34.64	6.1	3.4
	D4	29.41 x 8.2	9.4	38.81	8.1	5.2
	D5	26.81 x 7.8	8.4	35.21	7.2	4.4
	Mean	27.72 x 7.56	8.1	36.2	7.16	4.48
Shivpuri (E)	E1	31.44 x 7.1	40.22	71.66	7.2	3.1
	E2	30.4 x 8.4	38.7	69.1	7.0	4.3
	E3	32.4 x 7.9	41.5	73.9	6.2	5.0
	E4	31.22 x 6.8	39.7	70.92	6.1	4.2
	E5	34.11 x 8.6	42.5	76.31	8.2	5.4
	Mean	31.9 x 7.7	40.52	72.37	6.94	4.40

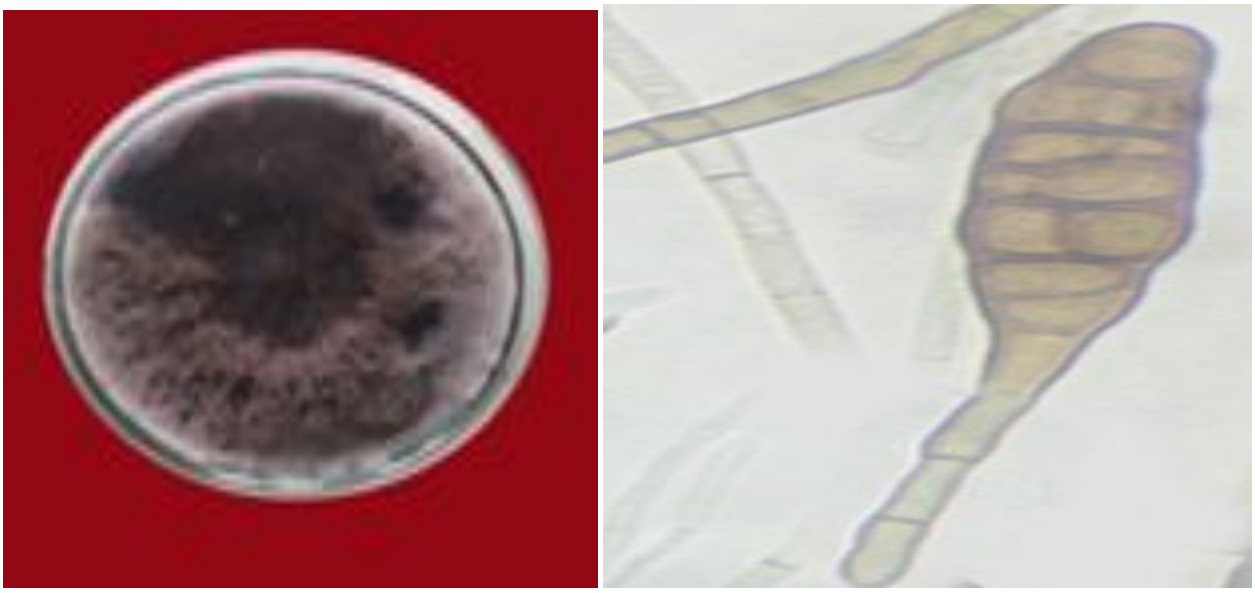
The colony characteristics and measurements were recorded after 7 days of incubation (Table 2). The findings showed that the mycelial width was larger in the isolate A (4.3 mm) followed by the isolate C (4.0 mm), E (3.8 mm) and D (3.6 mm) while the minimum mycelial width was found in isolate B (3.3 mm).

Table 2: Distribution and Cultural characteristic of *A. c.* var. *cyamopsidis* from different districts of Northern MP

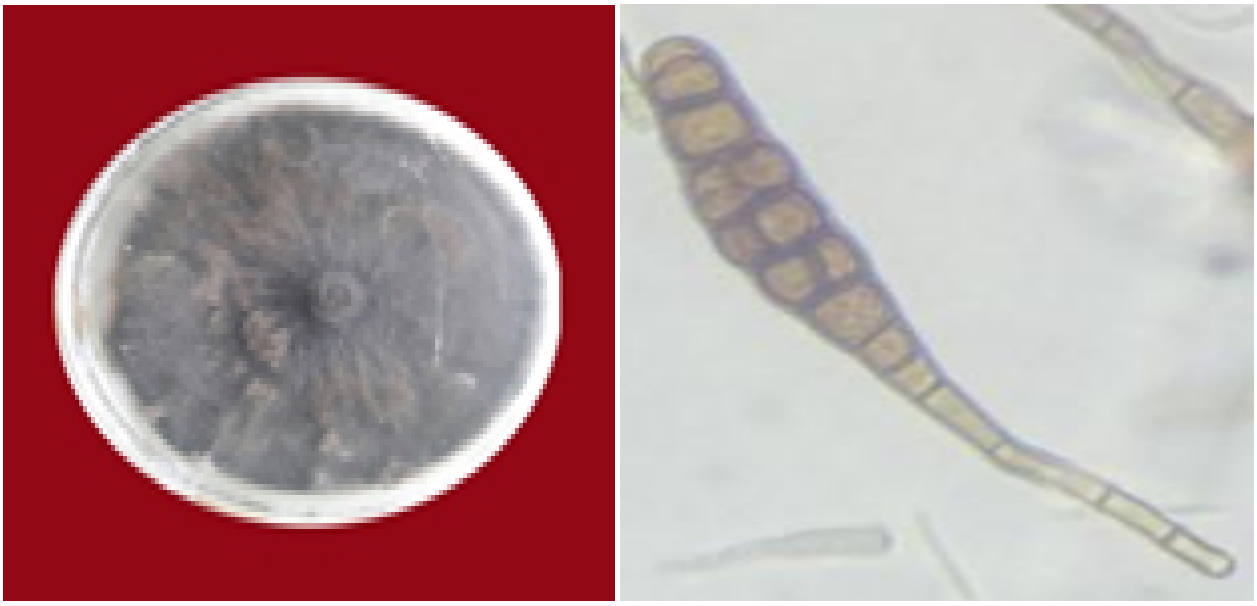
S.N.	Districts	Isolate code	Colony diameter (mm)	Culture colour	Mycelial width (mm)
1	Gwalior	A	85.2	White with light olive black centre	4.3
2	Morena	B	88.4	Dark olive grey colour with white centre	3.3
3	Bhind	C	80.6	Dark black colour	4.0
4	Datia	D	79.0	Dark olive black with brown fluffy edge	3.6
5	Shivpuri	E	79.2	Dark brown	3.8

The data showed that each isolate was different in their colour of the colony. The isolate A showed white with light olive black centre, the isolate B was dark olive grey colour with white centre. The isolate C and E were dark black and brown in colour respectively whereas the isolate D showed dark olive black colony with brown fluffy edge (Plate 1).

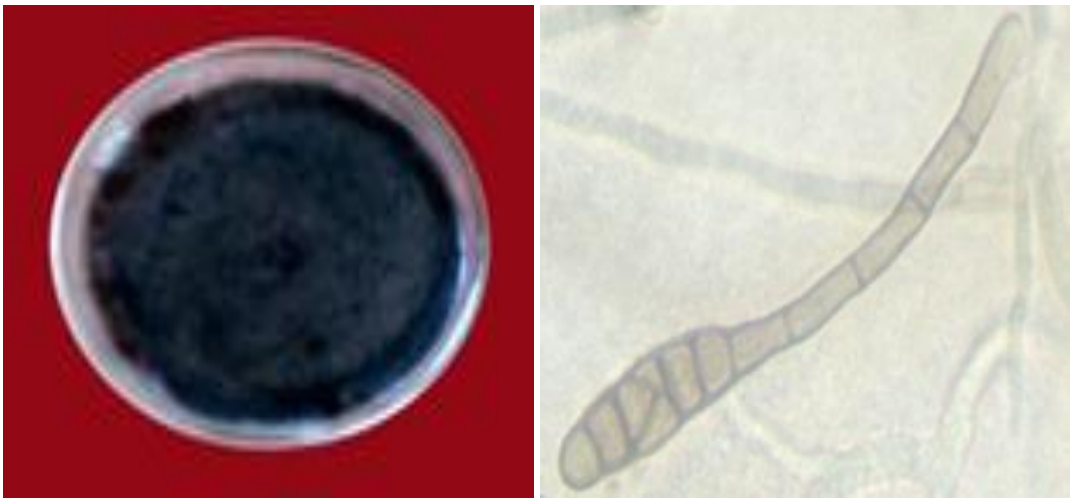
Meena *et al.*, (2014) [10] collected five isolates of leaf blight of isabgol caused by *A. alternata* from different agro climate zone of Rajasthan. They found all the five isolates differed in cultural characters i.e. dark black coloured and very fast mycelial growth with smooth margins, light black with white at centre and fast growing, dark brown and medium mycelium growth with smooth margins, black coloured, medium flat mycelial growth. Researchers also observed diversity in cultural characteristics such as growth rate, type of growth, colony colour and sporulation among different isolates of *Alternaria solani* (Babu *et al*., 2000) [2]. The conidial morphology of *A. solani* isolates are in accordance with those described by Ellis and Ellis (1985) [4]. However Kaul and Saxena (1889) [8] concluded that spore dimension was not useful in distinguishing *Alternaria* species. Gaur *et al*., (2012) showed variation in different isolates of A. alternate in symptoms, growth characteristics colony diameter, pattern of sporulation, size of conidia and number of septa. Similar results were also obtained by Jadhav *et al*., (2011) [7] and Ramegowda, (2007) [12] who reported the variability in cultural and morphological characteristics in *Alternaria* species.



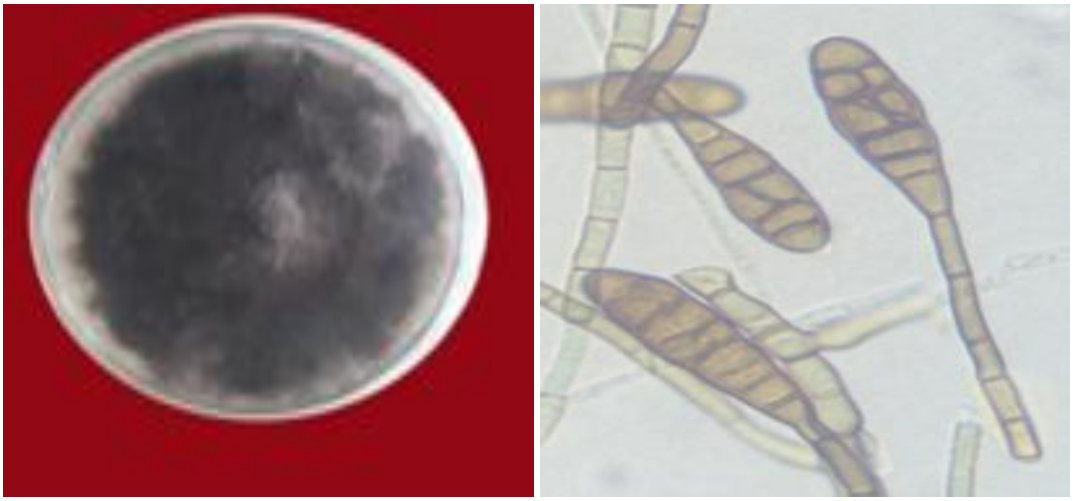
Gwalior



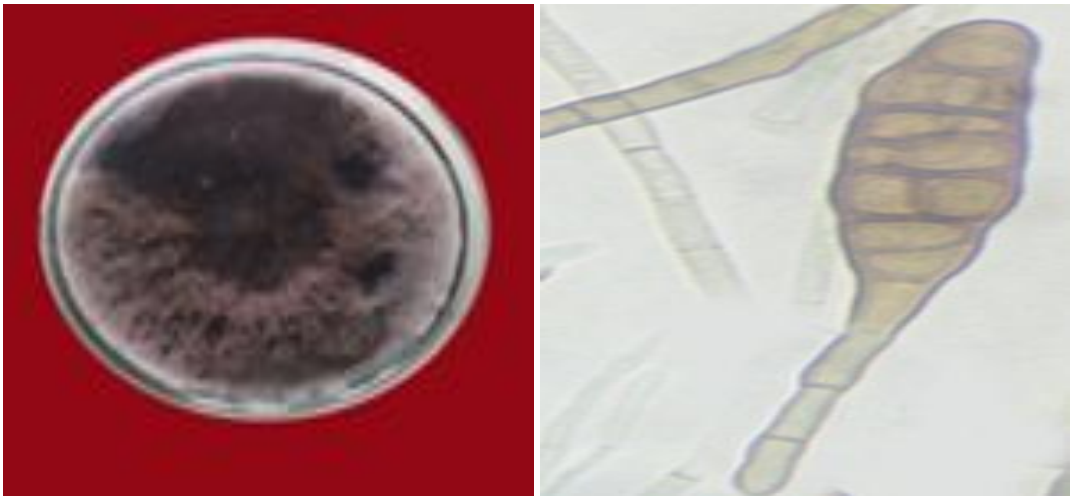
Morena



Bhind



Datia



Shivpuri

Plate 1: Comparative view of different pathogens isolated from different locations.

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