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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(8): 502-512

www.thepharmajournal.com
Received: 22-05-2023
Accepted: 26-07-2023

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Morphological characterization of *Alternaria* spp. derived from different naturally infected crops

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Abstract
The present investigation titled **"**Morphological characterization of *Alternaria* spp. derived from different naturally infected crops" was carried out at the Department of Plant Pathology, College of Agriculture, IGKV Raipur (C.G) during 2020-21. *Alternaria* spp. were isolated from nine different naturally infected host crop. The symptomatology of diseases was observed in field. The different isolates were used for studying pathogen charactracter. The study includes morphological characters i.e, length, width, beak of conidia, no of horizontal and vertical septa and cultural characteristic were observed. From this study, that *Alternaria* species was identifies with their unique necrotic lesions, which sometimes have a target like appearance due to growth interruptions and black sooty growth on the necrotic lesions. Microscopic preparations of *Alternaria* spp. conidia revealed unique conidial morphology respectively.

Keywords: Symptomatology, lessons, target appearance, morphological characterization

Introduction
Alternaria is an important fungal genus with a worldwide distribution. This Hyphomycetes ascomycete with phaeodictyospores includes saprophytic, endophytic and pathogenic species, which can be plant pathogens, post-harvest pathogens or human pathogens (Thomma 2003) [18]. *Alternaria* species are foliar pathogens that cause a relatively slow destruction of host tissues through the reduction of photosynthetic potential. An infection leads to the formation of necrotic lesions, which sometimes have a target like appearance due to growth interruptions caused by unfavorable conditions. The fungus resides in the centre of the lesion, which is surrounded by an un-invaded chlorotic halo, a symptom that is commonly observed for the infection process of necrotrophic pathogens.

Materials and Methods
Alternaria spp. derived from different naturally infected crops. The *Alternaria* spp. collected from nine different host *viz*, onion, brinjal, beans, cabbage, linseed, mustard, pea, potato, tomato. The symptomatology study was done and the spore character of the different spp. were observed, length, width, beak length and number of horizontal and vertical septation of conidia by using stage and ocular micrometer. These isolates were grown on PDA media, and after 15 days and their colony characters, were observed.

Collection of diseases samples
Diseased samples were collected from Horticulture farm of College of Agriculture Raipur, IGKV (C.G.).

Single spore isolation
Single spore isolation technique developed by Kotasthane & Singh (2000) [1] and Kotasthane and Agrawal (2010) [19] was followed. Plants tissues infected with *Alternaria* spp. were derived from naturally infected crops. Infected plant tissues were then kept in moist chamber to induce spores. Spores were then picked with a fine capillary glass needle up under a NICON stereo binocular microscope (Model SMZ 745T). They were then placed on the 4% water agar. With a fine capillary glass needle the individual spores was separated as far as possible from each other. The plates were then incubated at 27 °C for 4-6 h. to allow spores to germinate. The germ tube of germinating spores penetrated deep inside the water agar thus gripping the agar surface. Germinated spores were then lifted by removing the agar disk along with the germinated spore using a sterilized inoculation needle and aseptically transferred to the Potato Dextrose Agar slants and incubated at 27 °C.

Efficiency of isolation of single spore was almost 100% by this method. The growth appears in the Potato Dextrose Agar slants within 3-4 days at 27 °C.

Maintenance of cultures of *Alternaria* spp.

The pure culture obtained and maintained by repeated sub-culturing. The stock culture grown on potato dextrose agar (PDA) slants were stored at 10 °C in refrigerator.

Morphological characters of different *Alternaria* spp.

For morphological characterization of the fungus the infected leaves samples were collected in a sterilized polythene bag and brought to the lab. After surface sterilization by 0.1% sodium hypochloride solution, infected leaves was cut by sterilized sharp blade and kept in a moist chamber for 24–48 hrs. For sporulation. Cultures slide were made and observed under the compound microscope. The microscopic measurement were taken with the help of micrometry technique

Results and Discussion

Symptomatology: In Onion Disease symptoms appear at the leaf's tip, especially on older leaves, and subsequently spread to younger leaves, oval-shaped lesions observed, at centre dark brown to purple colour, these lesions quickly coalesced and spread throughout the leaf blade, and the leaves began to dry from the tip downward. (Agale RC., *et al* 2014) [1]. In Tomato crop diseases symptom first appeared as small dark brown spots on the lower older leaves. The tissue around the primary lesions may turns bright yellow, and later theses lesions Coalescence, the entire leaves may become necrosis and chlorotic. These spots get enlarged, they develop concentric rings. (Najibullah Rahmatzai., *et al* 2016) [26] In Potato crop first symptoms of early blight appear as small, circular or irregular, dark-brown to black spots on the older leaves, Multiple lesions on the same leaf also may coalesce, or grow together, to form one mass. As lesions coalesce, yellowing of plant tissue observed. And also dark concentric rings alternating with bands of light-tan tissue observed, giving them a distinctive target spot appearance. (Bauske., *et al* 2018) [2]. *Alternaria* leaf spot caused by *Alternaria alternata* is one of the important foliar diseases of brinjal *(Solanum melongena* L.). In brinjal crop the symptoms observed as, little dark brown and sunken patches in lower leaves which turns into concentric rings, and the tissue surrounding the patches becomes yellow. These lesions later Coalescence and the leave becomes necrotic dry and fall off. In bean crop the lesions first appear on the lower leaves as small, brown circular spots which slowly increase in size and develop concentric brown rings with dark margins. These spots Coalescence and form large necrotic lesion and becomes dark black in colour due to spore production. *Alternaria* leaf spot generally occurs late in the season as plants start to mature (Bernier *et al*., 1993) [20] *Alternaria brassicicola* and *A. brassicae* cause dark leaf spot of cultivated and wild crucifers (Smith *et al*., 1988) [21]. In cabbage leaf dark spots with concentric rings at centre observed in field and yellowing of surrounding tissue also observed in the infected plant. A foliage and pod blight of pea caused by *Alternaria alternate* described by Susuri L., *et al* 1982 [22]. The infected pod of pea developed black sunken lesions. Also in fied grown pea plant, the diseased plant showed lesion on leaves with concentric rings. *Alternaria linicola* Groves & Skolko and *Alternaria lini* there are two species known to produce *Alternaria* blight of linseed. The symptoms of *Alternaria* blight made its first appearance on the bottom leaves of linseed as a black spot that grew in size to become round, oval, or irregular in shape. Leaf blighting from the leaf edges is also frequent. Spots merge and cover a considerable region of the leaves with severe infestations. The diseased leaves eventually dry out and curl. Often the blighted leaf turns black colour due to spore production. *Alternaria* leaf spot of linseed does not have target board-like patches. *Alternaria brassicae* (Berk.) Sacc. causes the *Alternaria* blight disease of mustard has been documented from every continent in the globe and is one of the most serious diseases of rapeseed mustard (P.D. Meena., *et al* 2010) [9]. The disease in field is characterized by the presence of spots on the leaves, stems, and siliquae. Later these spots Coalescence and the leaf become necrotic.

Evaluation of morphological characters

All *Alternaria* spp. were microscopically observed for their morphological characters and compared with available literature. Under morphological character, length, width, beak length of conidia were observed for all *Alternaria* spp. Number of septa were also recorded. Differences in length width and beak length were observed among all *Alternaria* spp. isolated from different naturally infected host. The measurement of nine isolates of *Alternaria* spp. isolated from nine different host *viz*., onion, brinjal, beans, cabbage, linseed, mustard, pea, potato, tomato are presented in Table no 1. The overall measurement of length width beak of conidia varies 56.93-15.5 µm, 12.48-3.5 µm and 14.89-2.4 µm respectively. The horizontal and vertical septa no varies from 2-11,0-3 respectively. The measurement of nine different isolates revealed that length of conidia was highest in potato (Yadav *et al*., 2011) [17], followed by mustard (Bhatiya *et al*., 2016) [27], brinjal (Marak *et al*., 2014) [28] onion (B. Chethana *et al*., 2018) [4], tomato (Nikam *et al*., 2015) [23], pea (Nabahat Bessadat *et al*., 2014) [3] cabbage (S. Manika *et al.*, 2013) [8] beans (Nabahat Bessadat *et al*., 2014) [3], and linseed (Verma *et al.*, 2017) [24] respectively.

The width of conidia was highest in mustard followed by onion pea tomato potato brinjal cabbage beans respectively and lowest in linseed. The beak length measured highest in onion with average length 14.89 µm (B. Chethana *et al*., 2018) [4] followed by potato, mustard, tomato, brinjal, pea, beans, cabbage, linseed respectively. The no. of horizontal septa was found highest in potato and no. of vertical septa was highest in pea.

Cultural characteristics of *Alternaria* spp.

All nine *Alternaria* spp., isolated from nine different naturally infected host crop were grown on PDA media and colony colour, growth type was observed. The details of all nine isolates were presented in Table no. 2. There were difference in the colony colour, colony texture among all spp. observed. The colony colour of onion were olive green with smooth texture and regular margin (B. Chethana *et al.*, 2018) [4] and the colony colour of brinjal (Marak *et al*., 2014) [28], linseed (Verma *et al*., 2017) [24], mustard (B. Bhatiya *et al.*, 2016) [27], potatao (Ayad *et al.*, 2017) [29] were greyish white, the colony colour of beans, pea (Nabahat Bessadat *et al*., 2014) [3], tomato (Marak *et al.*, 2014), were olive grey, in cabbage (Sharma *et al*., 2013) [25] the colony colour was dark olivaceous green.

The study of colony growth of all nine *Alternaria* spp. reveals the difference in colony growth among all spp. All the nine *Alternaria spp.* were grown on PDA media and after 15 days the observation was recorded. All the nine isolates showed difference in their cultural characteristics.

Table 1: Conidial characters of different isolates of *Alternaria* spp.

Sr. No.	Host Crop	Conidia (µm)			No. of Septa	
		Width	Length	Beak	Trans	Longi
1.	Onion	12.29	44.14	14.89	2-6	0-1
2.	Linseed	3.5	15.5	2.4	5	0-2
3.	Cabbage	9.36	32.76	5.98	3-6	0-1
4.	Potato	10.16	56.93	11.81	4-11	0-2
5.	Tomato	11.58	39.23	9.69	3-6	0-2
6.	Beans	8.98	30.49	7.09	3-5	0-2
7.	Brinjal	9.927	46.32	9.21	4-7	0-2
8.	Mustard	12.48	52.78	9.88	3-8	0-2
9.	Pea	11.7	34.58	7.28	2-6	0-3

Table 2: Cultural characteristics of isolates of *Alternaria* spp.

Sr. No.	Host Crop	Colony		
		Colour	Texture	Margin
1.	Onion	Olive green	Smooth	regular
2.	Linseed	Greyish white	Fluffy	regular
3.	Cabbage	Dark olivaceous green	Velvety	irregualar
4.	Potato	Greyish white	Fluffy	irregular
5.	Tomato	Olive grey	Smooth	regular
6.	Beans	Olive grey	Cottony	regular
7.	Brinjal	Greyish white	Cottony	regular
8.	Mustard	Greyish white	Fluffy	regular
9.	Pea	Olive grey	Fluffy	Regular

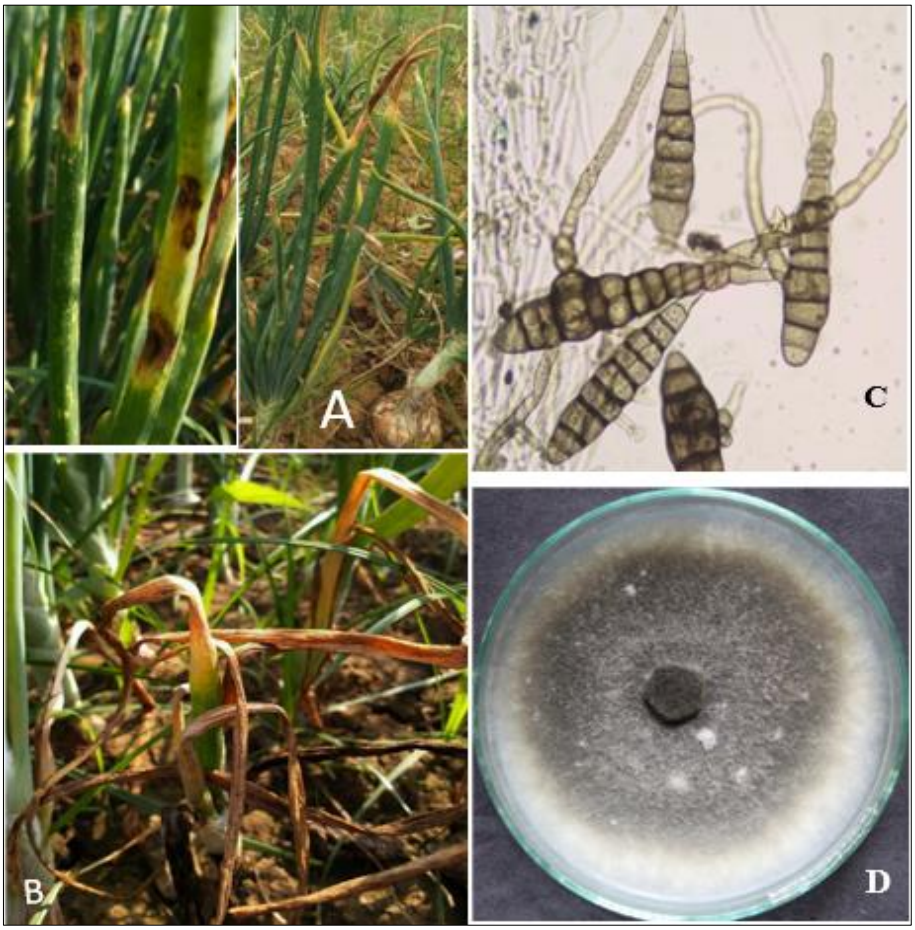
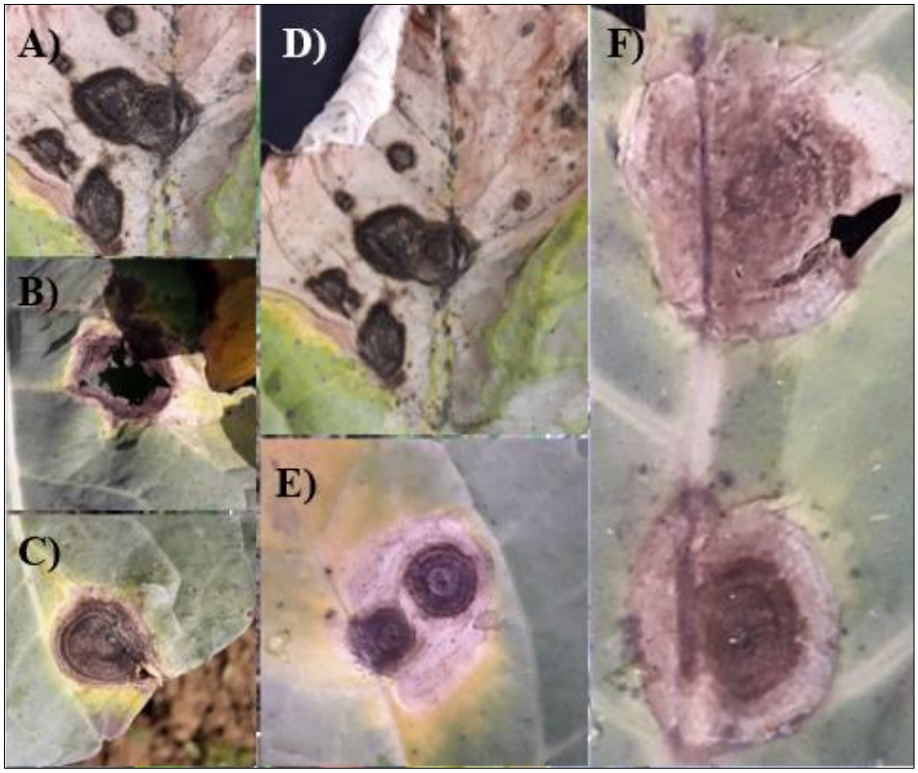


Fig 1 a: A B Naturally infected onion plant with purple blotch symptom; C) conidial morphology of *Alternaria* sp. spore derived from purple blotch infected onion plant; D) Pure culture derived from purple blotch infected onion plant

Fig 1 B: A B, C & D Naturally infected linseed plant with *Alternaria* leaf blight symptom; C) conidial morphology of *Alternaria* sp. Spore derived from infected linseed plant; D) Pure culture derived from *Alternaria* leaf blight infected linseed plant



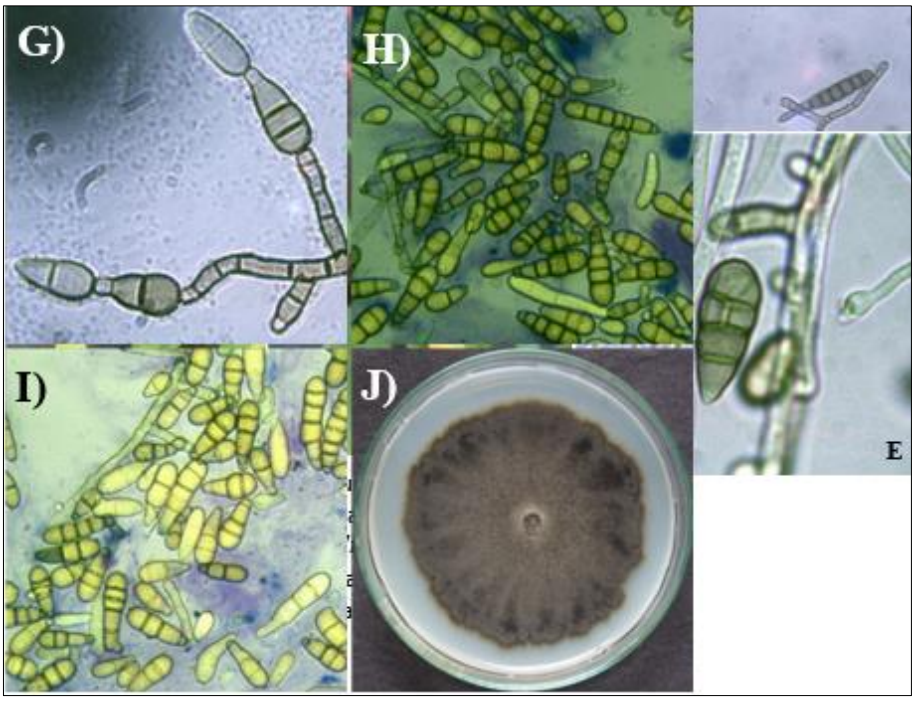
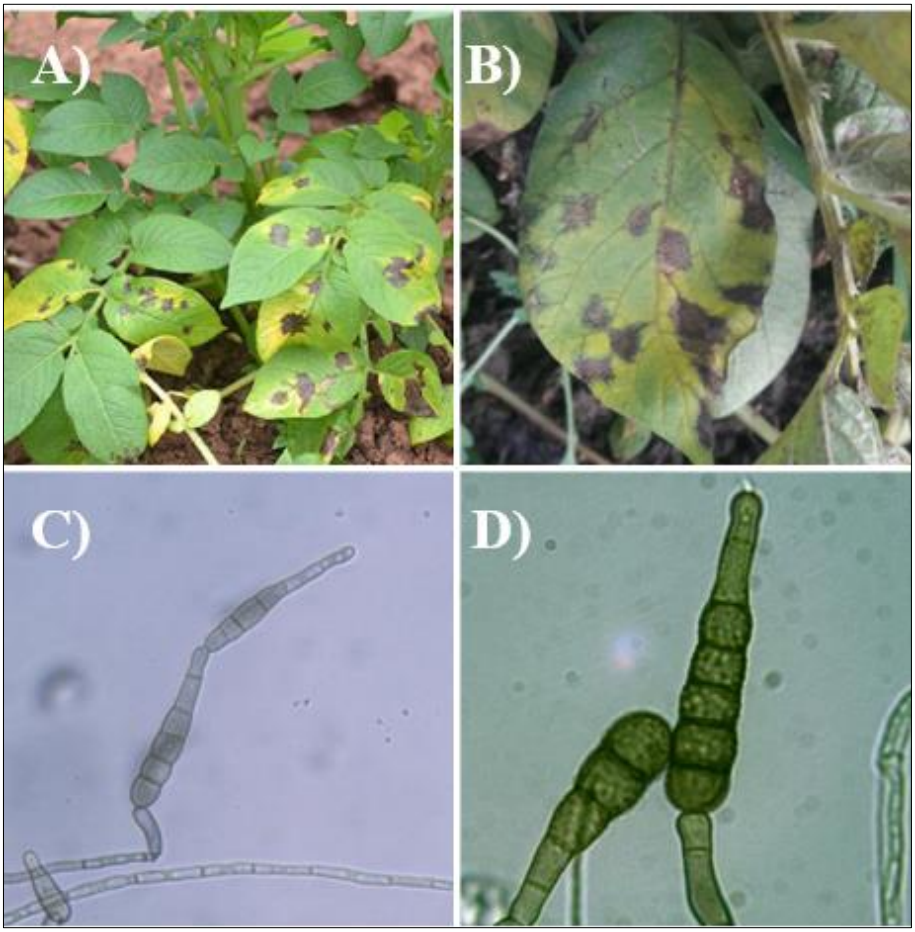


Fig 1 C: A to F naturally infected cabbage plant with leaf blight symptom; D, G to I) conidial morphology of *Alternaria* sp. spore derived from infected cabbage plant; J) Pure culture derived from *Alternaria* leaf blight infected cabbage plant



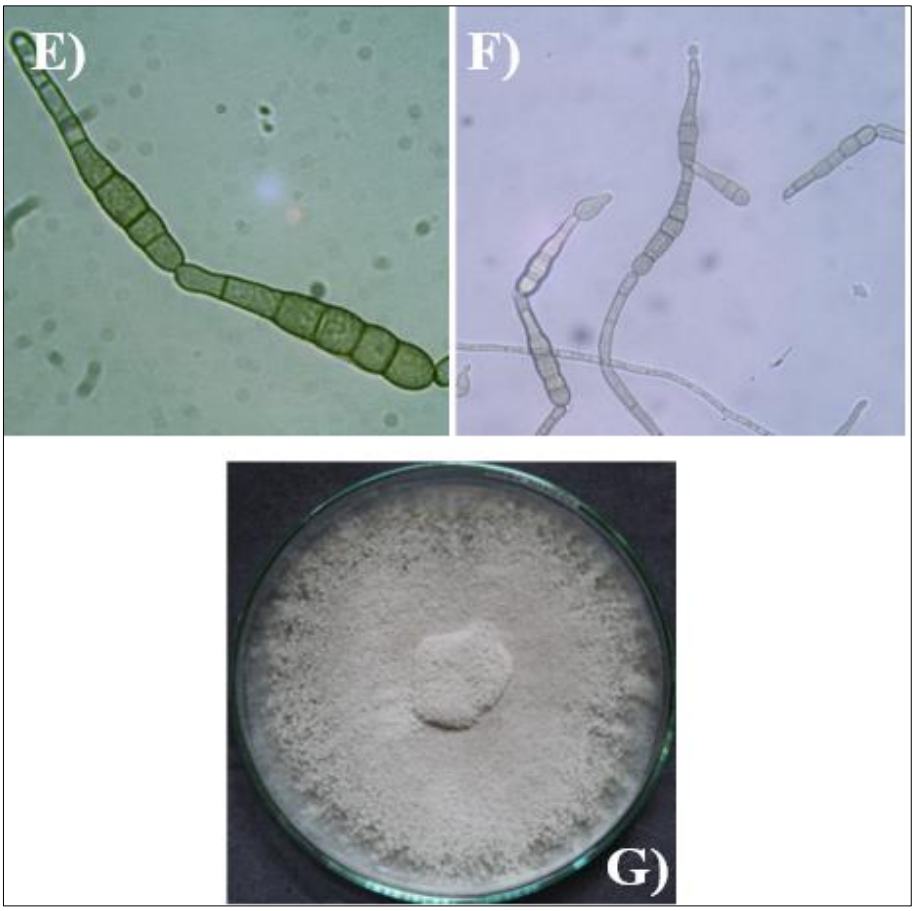


Fig 1 D: A& B Naturally infected potato plant with Early leaf blight symptom; C, D, E & F) conidial morphology of *Alternaria* sp. spore derived from infected potato plant; G) Pure culture derived from Early leaf blight infected potato plant

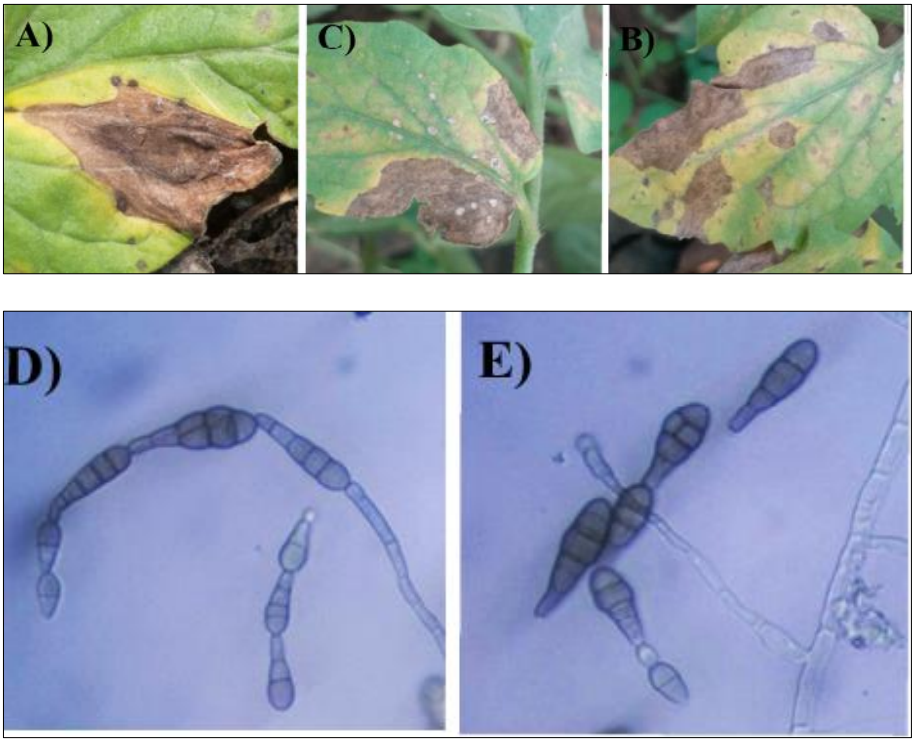
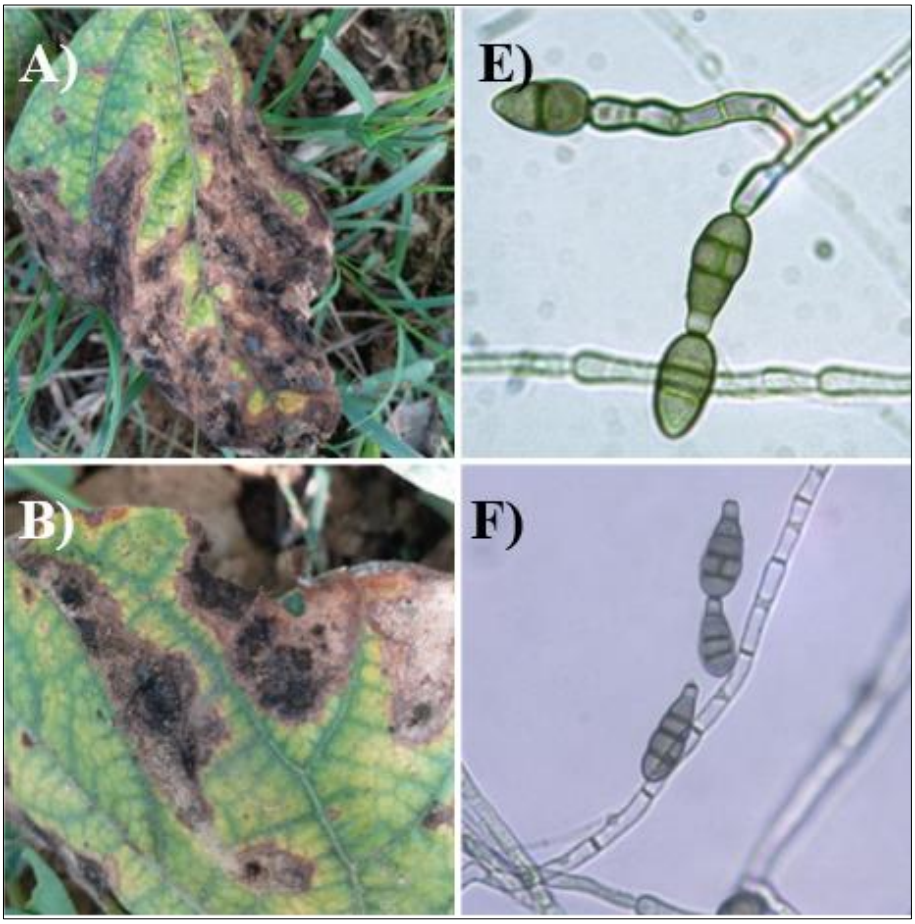


Fig 1E: A, B & C Naturally infected tomato plant with Early leaf blight symptom; D to G) conidial morphology of *Alternaria* sp. spore derived from infected plant tomato plant; H) Pure culture derived from Early leaf blight infected tomato



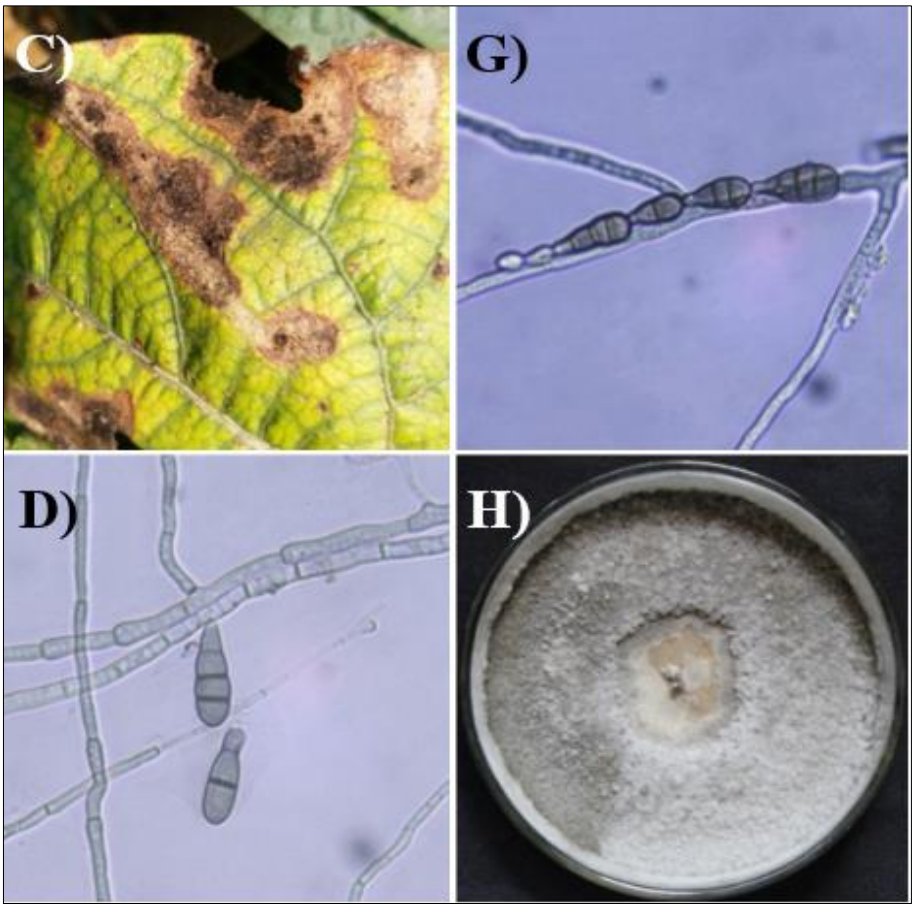
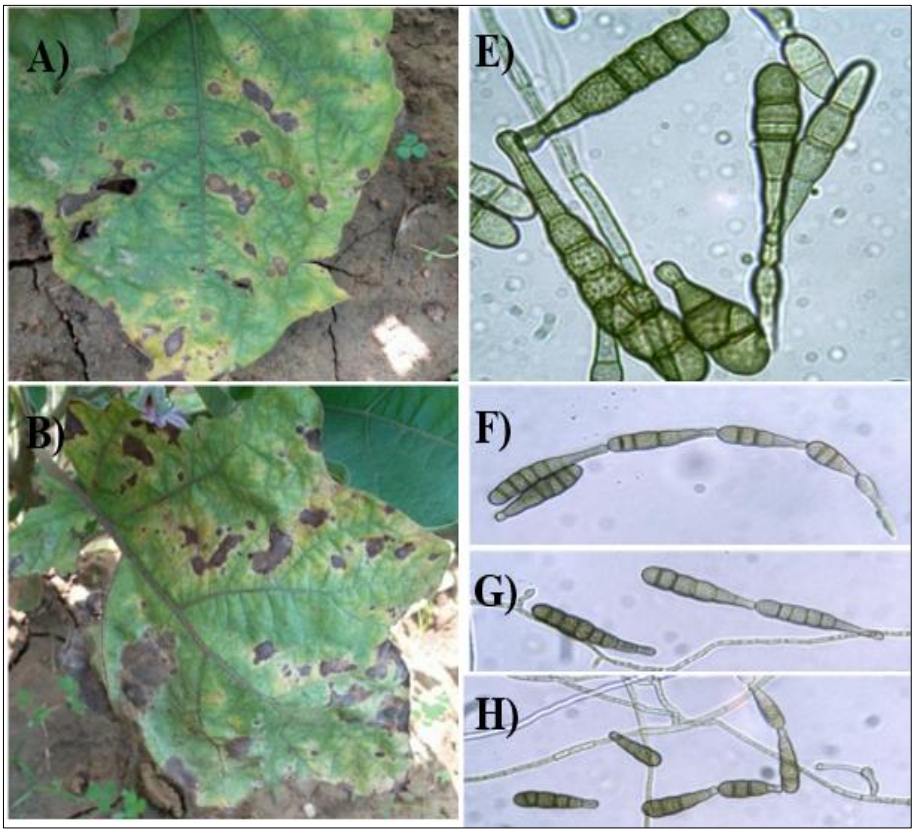


Fig 1F: (A, B, C) Naturally infected bean plant with *Alternaria* sp. expressing leaf blight symptom; (D to G) conidial morphology of *Alternaria* spore derived from naturally infected bean plant; (H) Pure culture derived from naturally infected bean plant with *Alternaria* sp.



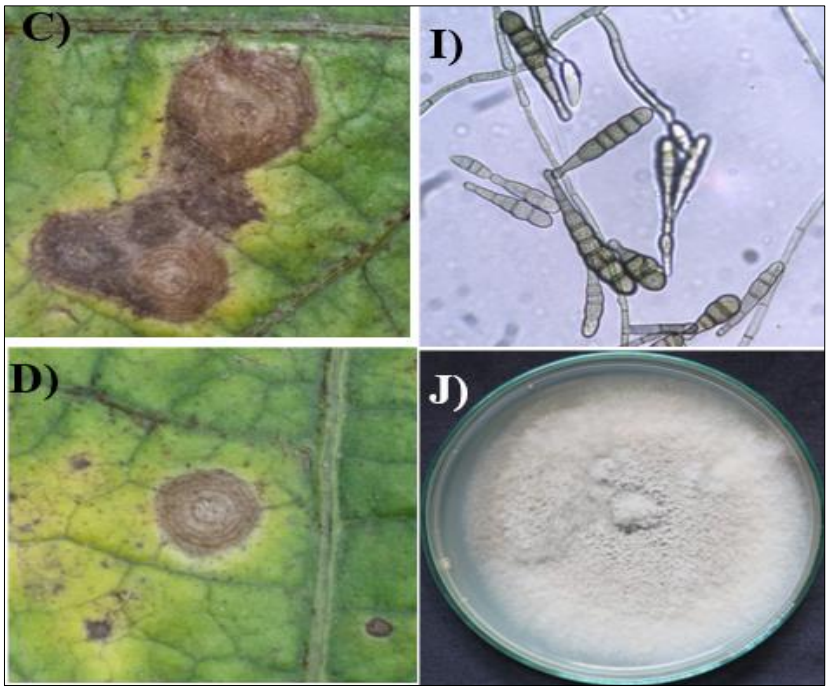


Fig 1 G: (A Naturally infected brinjal plant with *Alternaria* sp. expressing leaf blight symptom; (B to I) conidial morphology of *Alternaria* spore derived from naturally infected brinjal plant; J) Pure culture derived from naturally infected brinjal plant with *Alternaria* sp.

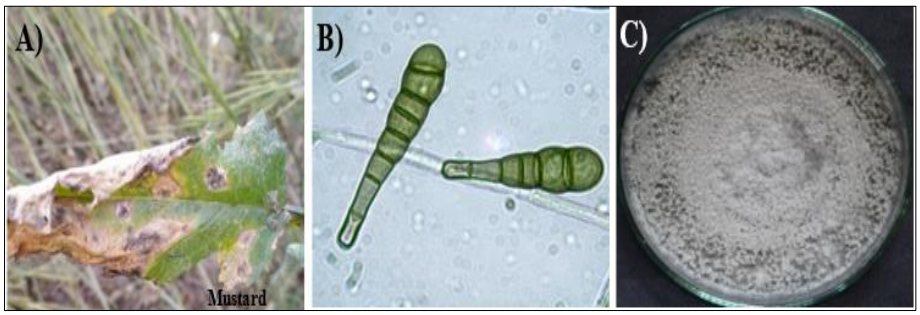
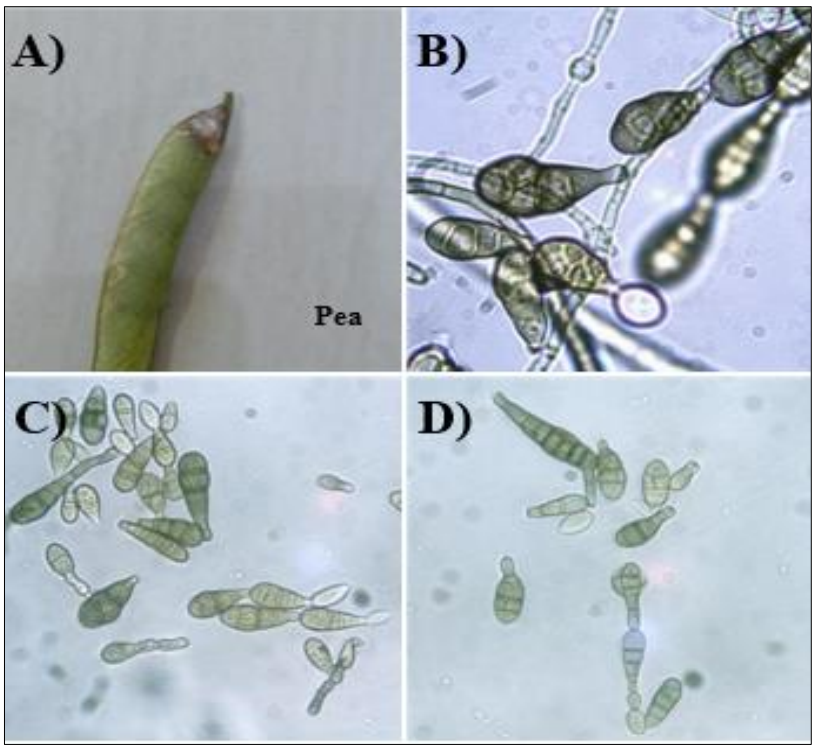


Fig 1H: (A) Naturally infected mustard plant with *Alternaria* sp.; (B) conidial morphology of *Alternaria* spore derived from naturally infected mustard plant; (C) Pure culture derived from naturally infected mustard plant with *Alternaria* sp



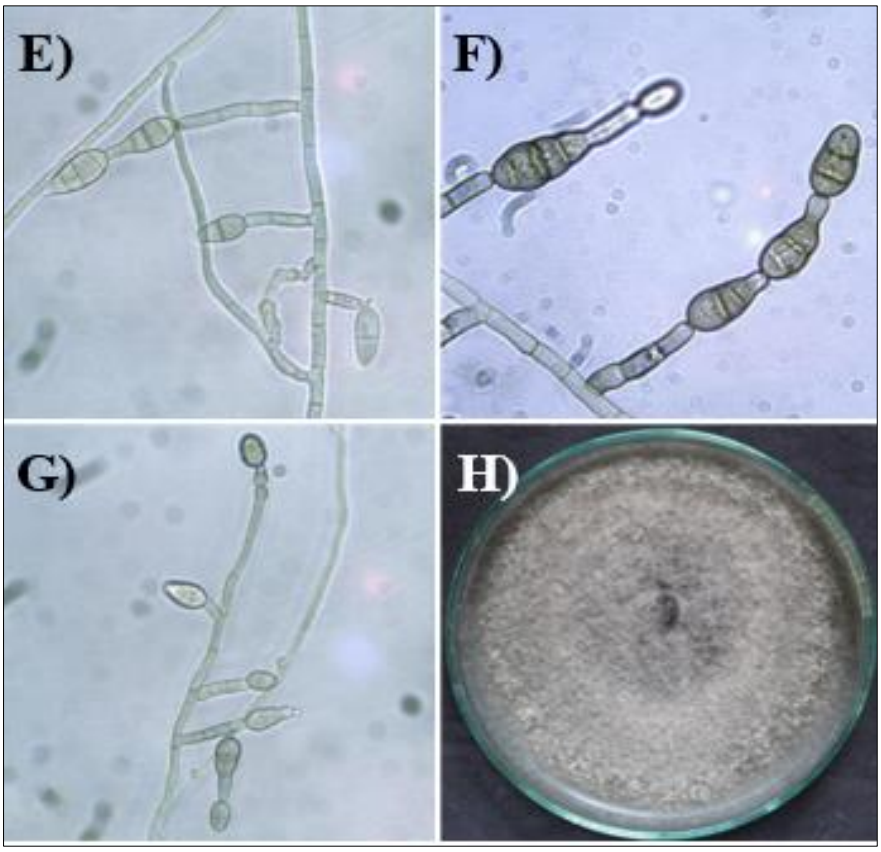


Fig 1I: (A) Naturally infected pea pod with *Alternaria* sp.; (B to G) conidial morphology of *Alternaria* spore derived from naturally infected pea pod; (H) Pure culture derived from naturally infected pea pod with *Alternaria* sp.

Conclusion

Variability in the multi-celled phaeodictyospore and cultural characteristics was observed in *Alternaria* spp. derived from nine different crops. Naturally infected plants of different crops i.e. Onion, Linseed, Cabbage, Potato, Tomato, Bean, Brinjal, Mustard, Pea *with Alternaria* spp. were identifies with their unique necrotic lesions, which sometimes have a target like appearance due to growth interruptions and black sooty growth on the necrotic lesions. Growth of the derived *Alternaria* spp. on PDA and microscopic preparations of conidia helped us to characterize the host specific *Alternaria* of the respective crops. Multi-celled phaeodictyospore from each naturally infected plant was isolated and were documented using Carl Zeis Primostar research microscope. Microscopic preparations of conidia revealed unique conidial morphology.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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