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Characterization of *Alternaria* species causing leaf diseases in some economically important vegetables of Assam, India

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Foliar diseases caused by *Alternaria* species in some economically important vegetable crops of Assam were investigated. Altogether eight different vegetable hosts were found to be infected by different species of *Alternaria*. The commonly occurring foliar diseases were leaf spot and blight. The symptoms of leaf spot was characterized by brown spots with concentric rings while blight symptoms was characterized by dark brown to black irregular shaped lesions on the leaf blades or petioles. The symptomology of the vegetable hosts and characteristics of the associated pathogens is being described. For the first time leaf spot disease of *Xanthosoma violaceum* caused by *Alternaria* sp. has been reported. Studies on reproductive structures of the pathogens indicated remarkable variation in the shape and size of the condia. Our present study is one of the first-hand information of diseases of vegetables caused by *Alternaria* in the state of Assam. This information might help in proper management and control of foliar diseases caused by *Alternaria* species.

Keywords: *Alternaria* species, foliar diseases, Vegetable crops, *Xanthosoma violaceum*

INTRODUCTION

Vegetables play an important role in human nutrition and disease prevention. It provides essential macro- and micro-nutrients, which are important for a balanced diet. India stands second in vegetables production in the world, next only to China with an annual production of 81 million tonnes from 5.1 million hectares of land (Karanth, 2002). During the last two decades, considerable emphasis has been laid to increase production of vegetable crops in India (Rao, 2004). However, many vegetable crops are attacked by fungal pathogens causing serious plant diseases. Amongst pathogenic fungi, diseases caused by the genus *Alternaria* are the major factors responsible for low production of crops like Cucurbitaceous, Brassicaceous and Solanaceous vegetables. These crops are often destroyed by blights causing serious disease rapidly in a few days.

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Therefore, the problem deserves immediate attention and effective control measures.Global climate change has increased the risk by altering pathogen evolution and host–pathogen interactions and facilitating the emergence of new pathogenic strains. This may also increase the pathogen's host range and spread of plant diseases in new areas. Studies have indicated that many fungal species are sensitive to climate change and some of them have the capacity to develop thermotolerance. Besides, a greater number of previously unharmful or underappreciated pathogenic fungal species may become infectious (Casadevall *et al.* 2019). The genus *Alternaria* is a widespread fungus found to be associated with several plant species. They are highly diverse and may exist in different form ranging from pathogenic, saprophytic to endophytic. Species of *Alternaria* are known as serious plant pathogens, causing major losses on a wide range of crops. (Woudenberg *et al.*2013). Over 250 species occurring world-wide have been described (Takaoka *et al.* 2014). Thus, it has become important to understand the

aggressiveness and host range of *Alternaria* species in the changing environment. This might help in proper management and control of vegetable diseases caused by the genus *Alternaria*. The aim of the present study was to investigate the different diseases cause by *Alternaria* species in some economically important vegetable crops and to characterize them in terms of their reproductive structures.

MATERIALS AND METHODS

Field survey and sample collection

Field visits were conducted to the vegetables cultivating areas of Kamrup (M) district of Assam, India. Diseased plant materials from infected parts (leaf) of the vegetable crops were collected from various fields and placed them separately in sterile polythene bags.Sampling was done during morning hours. The host plants were identified and assigned according to scientific nomenclature. Prior to the laboratory work, the specimens were photographed separately. Symptoms of the diseases were observed and studied in the field and laboratory conditions.

Microscopic observation and identification of the pathogen

A portion of the diseased plant part was placed on a clean glass slide with the help of a needle and forcep, cut in thin sections with the help of a blade or sometimes teased with a needle for the release of fungal spores and semi-permanent slides were prepared using lactophenol cotton blue stain. The prepared slides were observed under a compound microscope at 10X, 40X and 100X magnifications. The fungal pathogen associated with the diseased plant materials were identified based on the morphological characters of spores and spores bearing structure by using direct microscopy. Identification of fungi was also based on the structure and colour of mycelia and microscopic examinations of vegetative and reproductive structures.Pathogens were identified up to genus and species level taxa using manuals of Barnett and Hunter (1998) and referring manual of soil fungi by Gilman (1957). For presenting sizes, length and width, 20 structures were measured and given in the descriptions. Drawings of the conidial mass were made free hand using rotring pen and tracing papers.

RESULTS AND DISCUSSION

In the present study a total of 8 different host vegetable crops were found to cause diseased by *Alternaria* spp. (Table1). The host vegetable crops were identified as *Xanthosoma violaceum*, *Solanum lycopersicum*, *Brassica oleracea, Basella alba, Solanum melongena, Brassica juncea, Raphanus raphanistrum, and Raphanus sativus.* The common diseased symptoms were identified as fungal foliar leaf spots and blights diseases. The description of the diseased symptoms of each host along with morphology and microscopic characters of the causal organisms are described below:

Leaf spot of Xanthosoma violaceum

The afflicted leaf exhibited distinct characteristics, marked by the emergence of zonate necrotic lesions. These lesions were encircled by chlorotic regions featuring a subdued greyish center, which commonly gives rise to sooty-black blemishes. These blemishes adopt a target-like configuration reminiscent of the appearance displayed by *X. violaceum*, as depicted in Fig. 1 (A & B). Thus, based on the aforementioned description, the identified symptom corresponds to a leaf spot attributed to *X. violaceum*.The morphological attributes of the causal agent encompass the following features: branched and septate mycelium, along with conidia that are obclavate, possessing a smooth surface. These conidia exhibit a slight elongation in their apical cell and contain 6"12 transverse septa, coupled with 0"2 longitudinal divisions. These structures vary in size, spanning dimensions of 38"67 µm in length and 7"14 µm in width. Consequently, these distinctive traits lead to the identification of the causal agent as *Alternaria* sp., as illustrated in (Fig.1 C). Moreover, Fig.1 (D) portrays an accurate microscopic representation of the observed conidia.Top of Form

Leaf blight of Solanum lycopersicum

The affected leaf exhibited distinct features, characterized by the emergence of leaf blights

Table1: Vegetable host crops with their disease symptoms and causal organisms

Vegetable host	Disease symptom	Causal organism
Xanthosoma violaceum	Leaf spot	*Alternaria* sp.
Solanum lycopersicum	Leaf blight	*Alternaria tomatophila*
Brassica oleracea	Leaf blight	*Alternaria brassicicola*
Basella alba	Leaf spot	*Alternaria* sp.
Solanum melongena	Leaf blight	*Alternaria alternata*
Brassica juncea	Leaf spot	*Alternaria brassicicola*
Raphanus raphanistrum	Leaf spot	*Alternaria brassicae*
Raphanus sativus	Leaf spot	*Alternaria raphani*

in proximity to the lower part, which subsequently merge to give rise to substantial irregular lesions with a sooty tan-brown hue on *S. lycopersicum*, as depicted in Fig. 2 (A & B). Consequently, based on the aforementioned depiction, the identified symptom corresponds to the early blight ailment affecting *S. lycopersicum.*The morphological attributes of the causal agent encompass the following characteristics: branched and septate mycelium, accompanied by conidia that exhibit an obclavate shape and possess a smooth surface. These conidia demonstrate a slight constriction at the septa and can be found either singly or in pairs, linked together in chains of two. The conidia themselves assume a dark brown coloration, featuring 4-9 transverse septa, along with 0"3 longitudinal divisions. These structures exhibit dimensions that range from 55"79 µm in length and 5.5"9.5 µm in width. Consequently, these distinctive traits culminate in the identification of the causal agent as *Alternaria tomatophila*, as illustrated in Fig. 2 (C). Furthermore, Fig.2 (D) portrays an accurate microscopic representation of the observed conidia.

Leaf blight of Brassica oleracea

The ailing leaf exhibited distinct characteristics, marked by the manifestation of yellowing primarily on one side of the plant's lower leaves. Subsequently, these leaves undergo a transition into a brownish hue, with the appearance of spots that ultimately merge to form sizable necrotic regions. These areas further evolve into dark-brown patches on *B. oleracea*, as illustrated in Fig.(3 A & B)**.** Consequently, based on the detailed description provided, the identified symptom corresponds to leaf blight affecting *B. oleracea*.The morphological traits of the causal agent encompass the following features: mycelium that is branched, septate, and displays a brownish coloration. The conidia, on the other hand, assume a short conical shape without a beak, characterized by a brown color, and feature a smooth wall. These conidia possess a cylindrical to obclavate morphology and are produced in chains comprising 7-10 spores. Within each spore, 4"7 transverse septa are present, accompanied by 1"2 longitudinal divisions. These structures exhibit dimensions ranging from 57.5–66.5 µm in length and 13.5–17 µm in width. As a result of these distinctive characteristics, the causal agent responsible for the disease is identified as *Alternaria brassicicola*, as depicted in Fig.3 (C). Furthermore, Fig 3 (D) provides a precise microscopic illustration of the observed conidia.

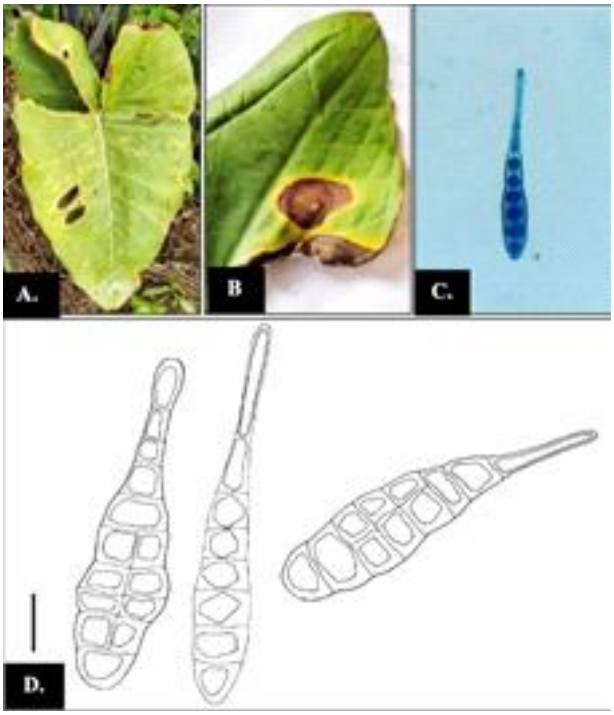

Fig. 1: Leaf spot disease of *Xanthosoma violaceum* and morphology of the causal pathogen. (A & B) Disease symptom of the host, (C.) Conidia of *Alternaria* sp. as seen in microscope, (D.) Microscopic drawing of the conidia of *Alternaria* sp. (scale bars = 10µm).

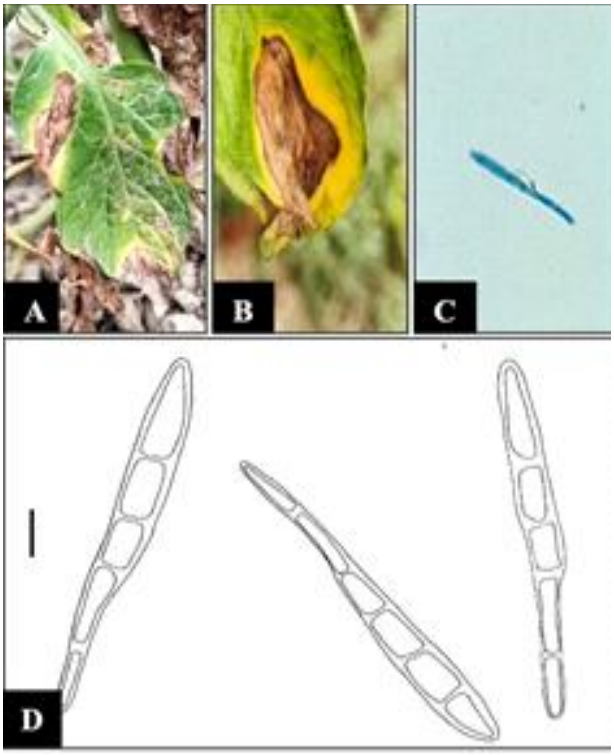

Fig.2: Leaf blight disease of *Solanum lycopersicum*and morphology of the causal pathogen. (A & B) Disease symptom of the host; (C.) Conidia of *Alternaria tomatophila* as seen in microscope (scale bar = 20 µm), (D.) Microscopic drawing of the conidia of *A. tomatophila* (scale bar = 10 µm).

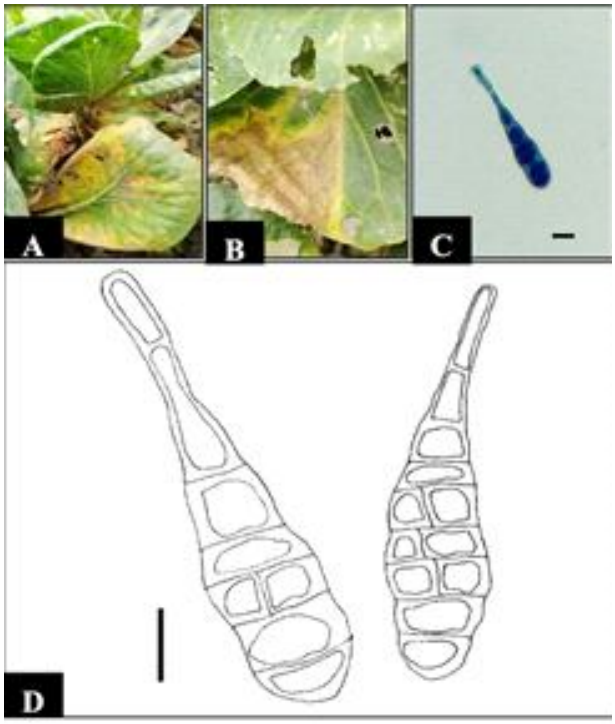

Fig. 3: Leaf blight disease of *Brassica oleracea* and morphology of the causal pathogen. (A & B) Disease symptom of the host, (C.) Conidia of *Alternaria brassicicola*as seen in microscope, (D.) Microscopic drawing of the conidia of *A. brassicicola* (scale bars = 10 µm).

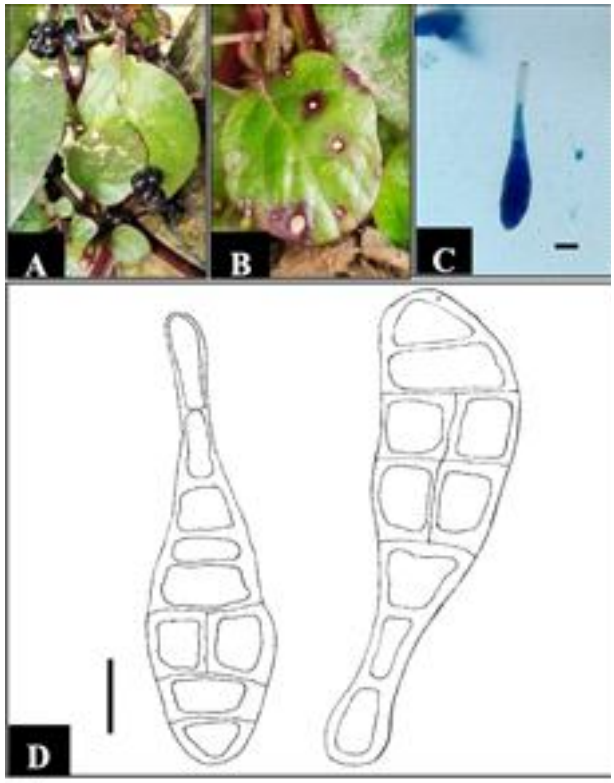

Fig. 4: Leaf spot disease of *Basella alba* and morphology of the causal pathogen. (A & B) Disease symptom of the host, (C.) Conidia of *Alternaria* sp. as seen in microscope, (D.) Microscopic drawing of the conidia of *Alternaria* sp. (scale bars = 10 µm).

Leaf spot of Basella alba

The afflicted leaf displayed distinct features, characterized by the emergence of circular to oval-shaped pinhead spots in a vivid purple hue. These spots contained a necrotic grey center, encircled by a border transitioning from purple to brown on *B. alba*, as depicted in Fig. 4 (A & B). As a consequence of the detailed description provided, the identified symptom corresponds to a leaf spot condition affecting *B. alba*.The morphological attributes of the causal agent encompass the following traits: branched and septate mycelium. The conidia, on the other hand, exhibit an obclavate shape, featuring a smooth surface. These conidia also possess a slightly elongated apical cell and are characterized by 4"9 transverse septa and 0"4 longitudinal divisions. Their dimensions range from 42.5"66.5 µm in length and 7.5"15.5 µm in width. Therefore, based on these distinct features, the causal organism responsible for the disease is identified as *Alternaria* sp., as depicted in Fig.4 (C). Furthermore, Fig.4 (D) provides a detailed microscopic representation of the observed conidia.

Leaf blight of Solanum melongena

The affected leaf exhibited discernible features, marked by the occurrence of chlorotic lesions. These lesions, which ranged from angular to irregular in shape, later merged together, giving rise to expansive patches that covered the leaf's surface. Over time, these patches assumed a greyish-brown hue on *S. melongena*, as illustrated in Fig.5 (A & B). As a result of the comprehensive description provided, the symptom at hand was recognized as leaf blight affecting *S. melongena*.The morphological attributes of the causative agent encompassed the following traits: mycelium that displayed branching and septation. The conidia, in contrast, took on an obclavate shape and featured a smooth surface. These conidia exhibited a slightly elongated apical cell and were distinguished by 6"11 transverse septa, alongside 0"3 longitudinal divisions. In terms of size, these structures ranged from 45"65 µm in length and 9"15 µm in width. Consequently, based on these distinct characteristics, the causal agent responsible for the disease was identified as *Alternaria alternata*, as depicted in Fig. 5 (C). Additionally, Fig. 5 (D**)** presented an accurate microscopic rendition of the observed conidia.

Leaf spot of Brassica juncea

The ailing leaf displayed distinctive features, characterized by the emergence of circular brown spots, enveloped by chlorotic zones. Over time, these zones underwent transformation, culminating in the formation of tan-brown concentric rings on *B. juncea*, as depicted in Fig. 6 (A & B). Consequently, based on the comprehensive description provided, the symptom in question was identified as leaf blight affecting *B. juncea*.The morphological traits of the causal agent encompassed the following attributes: mycelium that exhibited branching, septation, and a brownish coloration. The conidia, on the contrary, adopted a short conical shape with a rounded beak, displaying a dark brown hue and featuring a smooth surface. These conidia assumed a cylindrical to obclavate form and were generated in chains comprising 8"10 spores. Within each spore, 5"7 transverse septa were observed, alongside 1"2 longitudinal divisions. These structures exhibited dimensions ranging from 60–65 µm in length and 12.5–15.5 µm in width. Thus, based on these distinct characteristics, the causal agent responsible for the disease was determined to be *Alternaria brassicicola*, as illustrated in Fig. 6 (C). Additionally, Fig.6 (D) provided an accurate microscopic depiction of the observed conidia.

Leaf spot of Raphanus raphanistrum

The affected leaf exhibited distinct features, characterized by the emergence of small, dark or yellow leaf spots that eventually transitioned into dark brown patches. These patches contained a greyish center and underwent further development, giving rise to concentric rings adorned with black borders on *R. raphanistrum*, as depicted Fig. 7 (A & B). As a result of the comprehensive description provided, the symptom at hand was identified as leaf blight affecting *R. raphanistrum*.The morphological attributes of the causal organism encompassed the following traits: mycelium characterized by

septation and branching. The conidia, in contrast, exhibited an obclavate shape, featuring a prominent beak and a smooth surface. These conidia were adorned with 11"19 transverse septa, and 0"5 longitudinal divisions, displaying a slight constriction at the septa. When produced on their plant hosts, they were usually solitary, occasionally forming chains. The dimensions of these structures spanned from 74"126 µm in length and 9.5"16.5 µm in width. Therefore, based on these distinctive characteristics, the causal agent responsible for the disease was identified as *Alternaria brassicae*, as illustrated in Fig.7 (C). Additionally, Fig.7 (D) provided an accurate microscopic representation of the observed conidia.

Leaf blight of Raphanus sativus

The affected leaf exhibited distinct traits, characterized by the emergence of dark brown spots that gradually merged, giving rise to greyish-brown lesions. These lesions were adorned with irregular spots that resembled targets on *R. sativus*, as illustrated in Fig. 8 (A & B). Consequently, based on the detailed description provided, the symptom was identified as leaf blight affecting *R. sativus*.The morphological features of the causal agent comprised the following attributes: mycelium exhibiting septation and branching. The conidia, on the other hand, assumed a cylindrical to obclavate shape, featuring a bluntly rounded apical cell. This apical cell could develop through an abrupt transition into a broader form. The conidia were distinguished by 7"10 transverse septa and 1"2 longitudinal divisions. Their dimensions ranged from 88"126 µm in length and 14"20 µm in width. Hence, based on these specific characteristics, the causal organism responsible for the disease was determined to be *Alternaria raphani*, as depicted in Fig. 8 (C). Additionally, Fig. 8 (D) presented an accurate microscopic rendering of the observed conidia.

Many pathogenic fungi are known to cause diseases in vegetable crops. Among various diseases, systemic foliar pathogens are major causes for commercial crop losses and diminished production in tropical country like India (Thind *et al.* 2011). Therefore, rapid identification of fungal diseases by timely recognition of their symptoms is an effective management practice and may help control and prevent their spread and progress. In the present investigation foliar leaf diseases of some economically important vegetable crops was studied. The common fungal foliar disease symptoms were found to be leaf spots and blights. In many instances these diseases have been reported to cause by various species of pathogenic fungi declining both quality and productivity in many commercial crops including vegetables (Singh and Misra, 2017). In our study we found eight different species of *Alternaria* causing foliar diseases in different vegetable hosts.

The genus *Alternaria* is an emerging and noteworthy foliar fungal pathogen that has gained significant attention in regions where winter-summer vegetable crops are cultivated. This pathogen displays an exceptionally wide array of host susceptibilities, underscoring its virulent character (Macioszek *et al.* 2020). Most of the diseases under investigation were reported earlier but there is no information of occurrence of *Alternaria* in *Xanthosoma violaceum*. In fact, this is the first-hand report of leaf spot disease of *X. violaceum* cause by *Alternaria* sp. This indicates that in the changing environmental conditions this species might have adopted to boarder host range and speciation.There are several indications that plant pathogens may undergo rapid environmental speciation via changes in the host itself.It has also become important to study fungal leaf diseases to known well about environmental speciation. The key morphological feature of the genus *Alternaria* is the production of large, multicellular, brown to dark-brown colored conidia with longitudinal as well as transverse septa. These conidia are broader near the base and gradually taper to an elongated beak, providing an obclavate to club-like appearance. They are produced in single or branched chains on short, erect conidiophores. In our present study great variation in the shape and size of the conidia in the different species of *Alternaria* was observed. Such variation might have arisen due to the variability of its morphological characters, which are not only affected by intrinsic factors but also by environmental conditions. Furthermore, the examination of the conidial structure of *Alternaria*

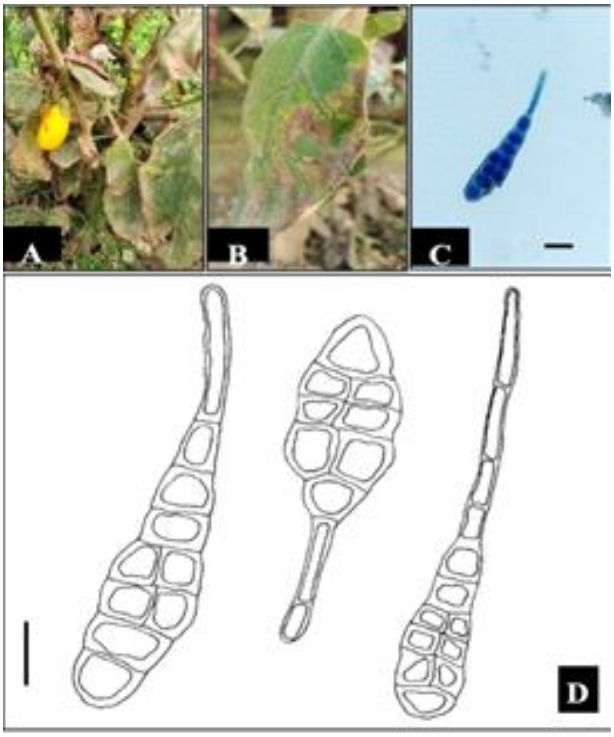

Fig. 5: Leaf blight disease of *Solanum melongena* and morphology of the causal pathogen. (A & B) Disease symptom of the host, (C.) Conidia of *Alternaria alternata* as seen in microscope, (D.) Microscopic drawing of the conidia of *A. alternata*(scale bars = 10 µm).

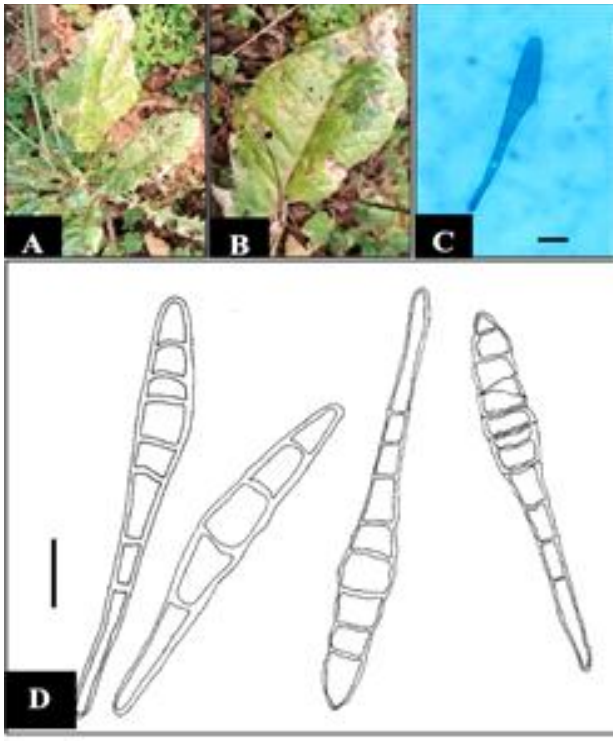

Fig.7: Leaf spot disease of *Raphanus raphanistrum* and morphology of the causal pathogen. (A & B) Disease symptom of the host, (C.) Conidia of *Alternaria brassicae* as seen in microscope (scale bar = 20 µm); (D.) Microscopic drawing of the conidia of *A. brassicae* (scale bar = 10 µm).

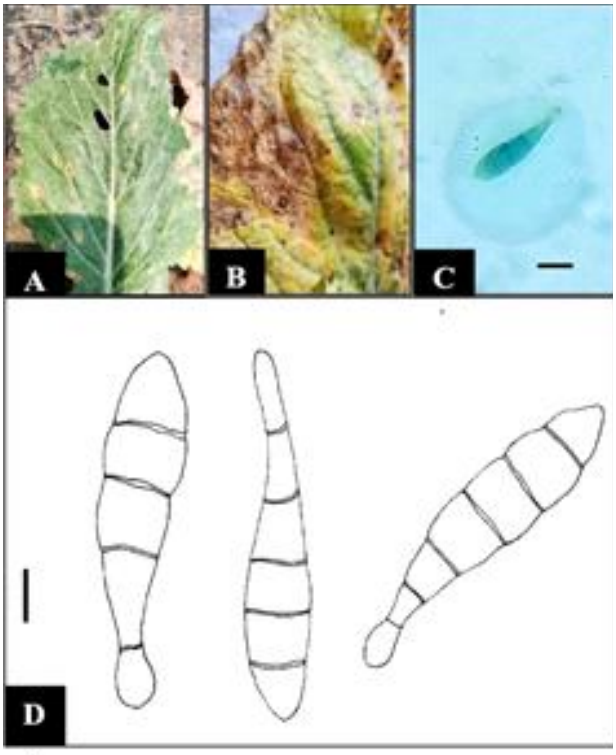

Fig. 6: Leaf spot disease of *Brassica juncea* and morphology of the causal pathogen. (A & B) Disease symptom of the host, (C.) Conidia of *Alternaria brassicicola* as seen in microscope (scale bar = 20 µm); (D.) Microscopic drawing of the conidia of *A. brassicicola* (scale bar = 10 µm).

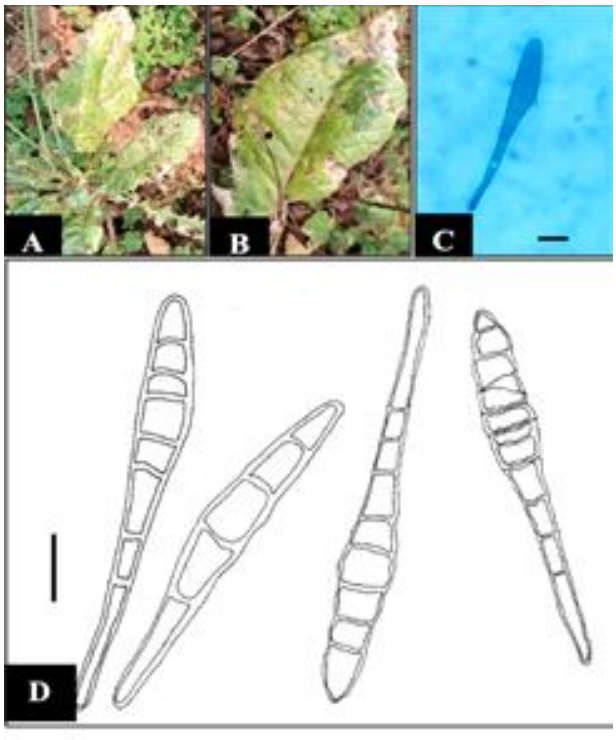

Fig.8: Leaf spot disease of *Raphanus sativus* and morphology of the causal pathogen. (A & B) Disease symptom of the host, (C.) Conidia of *Alternaria raphani* as seen in microscope (scale bar = 20 µm); (D.) Microscopic drawing of the conidia of *A. raphani* (scale bar = 10 µm).

pathogen assumes paramount importance, as shifts in environmental conditions exerts a profound impact on the conidial morphology and virulence of *Alternaria*. As elements of the environment, such as temperature, humidity, and nutrient availability, undergo fluctuations, *Alternaria* displays a dynamic adaptability, leading to modifications in the form and dimensions of its conidia. This responsive alteration plays a role in influencing mechanisms of dispersal, spore viability, and the pathogen's capacity to infiltrate host tissues. This complex interplay among the environment, conidial attributes, and virulence holds the capacity to initiate speciation occurrences and facilitate the evolutionary progression of the *Alternaria* genus across temporal spans. Additionally, variations in environmental conditions can prompt changes in the expression of genes associated with pathogenic traits, thereby influencing the pathogen's virulence characteristics. The diverse alterations in pathogenic traits, shaping the pathogen's virulence attributes, can extend the range of hosts susceptible to infection, broadening the spectrum of potential host organisms. Notably, escalated temperatures could potentially stimulate the synthesis of enzymes and toxins that amplify the pathogen's proficiency in colonizing and impairing host plants. Grasping the intricacies of these interdependent dynamics encompassing changing environmental conditions, conidial attributes, and virulence within *Alternaria* holds pivotal significance, enabling the anticipation of disease eruptions, refining disease management approaches, and ultimately preserving agricultural productivity in the midst of an ever-evolving pathogenic threat.Thus, information on fungal leaf diseases caused by *Alternaria* species is important to know about the environmental speciation of this organism. Further, there is a need to identify the factors influencing the emergence and the increasingincidences of these diseases. Such attributes might help in proper management and control of foliar vegetables diseases caused by *Alternaria* species.

CONCLUSION

Investigation of fungal foliar diseases is of paramount importance due to their pervasive impact on plant health and agricultural systems. These diseases, caused by various fungal pathogens, can have far-reaching consequences, leading to reduced crop yields, compromised quality, and economic losses for farmers. Understanding fungal foliar diseases also contributes to advancing our knowledge of plant-pathogen interactions, disease ecology, and the broader field of plant pathology. However, among the array of fungal foliar pathogens, the *Alternaria* pathogen stands out as a significant contributor to the challenges faced by agricultural crops. Its prominence lies in the extensive consequences it inflicts upon crop yield and quality. *Alternaria* pathogen is responsible for a broader spectrum of foliar diseases affecting diverse crops, spanning vegetables, fruits, and grains. The detrimental effects it brings about, including leaf damage, reduced photosynthesis, and compromised plant vigor, culminate in substantial economic losses for farmers and threaten global food production. Identifying emerging leaf diseases caused by *Alternaria* species may help in proper management and control of this pathogen.

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