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Fusarium wilt of bottle gourd (*Lagenaria siceraria*) caused by *Fusarium oxysporum* at high altitude region of Ladakh

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Abstract

Ladakh, a trans-Himalayan cold-arid region characterized by sub-zero temperatures and lower atmospheric pressure, limits the cultivation period of crops to the summer months. Bottle gourd, a nutrient-rich Cucurbitaceae vegetable, is grown from April to September in Ladakh. However, the favourable environmental conditions for fungal plant pathogens challenge bottle gourd cultivation during this period. During a field survey of the Muth region located at an elevation of 4221 m above sea level, wilting of bottle gourd leaves was observed in August, with a disease incidence of 14% and a severity index of 5%. Isolation and characterization of the pathogen on potato dextrose agar (PDA) medium identified it as *Fusarium* sp., with typical spores observed under the microscope. The pathogenicity test confirmed the pathogenic nature of the isolate. Amplification and sequencing of the ITS region showed high similarity with *Fusarium oxysporum* in the UNITE database and the sequence has been submitted to NCBI GenBank under the accession number OP453355. These findings provide valuable insights into the potential threats of fungal pathogens to bottle gourd cultivation in Ladakh and highlight the importance of disease management strategies in high-altitude regions.

Key words: high altitude, bottle gourd, wilt, *Fusarium oxysporum,* Ladakh

Introduction

Ladakh is a trans- Himalayan cold-arid region lying between 31°15’-36° N latitude and 75°15’-80°15’ E longitude. Due to high mountains and barren landscapes, the temperature fluctuates between -30° C in Leh to -70° C in Drass during the winter months of October to April yearly (Ballabh *et al.,* 2007). The temperature usually ranges from +25° C to -25° C in most regions suitable for human habitation. Ladakh also has distinctive climatic traits such as intense solar radiations, high wind velocity, decreased atmospheric pressure and low rainfall (Phour *et al.,* 2019). Moreover, the altitude of Ladakh ranges from 2700 to 6000 m above mean sea level. These cold-arid climatic conditions significantly affect crops’ cultivation period in different parts of Ladakh and limit the cultivation period (Ghai *et al.,* 2018). Vegetable production in Ladakh has increased over time with the introduction of new varieties of vegetables, and thus, there are diverse options, such as melons, capsicum, brinjal, potato, tomato, cauliflower, cabbage, squash, bottle gourd, etc., available for production and consumption now than it was a few decades back (Stobdan *et al.,* 2018). These diverse vegetables are necessary for fulfilling the nutritional requirements of the local population and cucurbits, such as bottle gourd laden with vitamins (A and B), minerals (potassium and calcium), flavonoids and saponins (Saha *et al.,* 2016). Bottle gourd (*Lagenaria siceraria*) is cultivated during April–September either in open fields or greenhouses, trenches and is available in the summer season for consumption. It contributes significantly in total cucurbits production in Ladakh. However, it is prone to various fungal diseases such as wilting, leaf blight, leaf spots, fruit rot, mildews and others (Avinash *et al.,* 2021). At the same time, the physiographical factors of Ladakh, however harsh they may appear, could not restrict the growth and adaptation of potential pathogens and pests (Singh and Dhiman, 2018; Phour *et al.,* 2018).

Different species of Fusarium such as *F. avenaceum, F. oxysporum, F. solani* and *F. eumartii,* cause Fusarium wilt. They are considered one of the most important plant pathogens in the world (Shah and Jiskani, 2014). *F. oxysporum* is a common soil pathogen primarily affecting economically important crops globally, and it survives in soil for a longer time, making its management much more difficult (Relevante and Cumagun, 2013). Fusarium wilt of bottle gourd also affects the yield and fruit quality. An incidence of 20-40% is usually seen, and more severe cases typically result in significant crop losses, limiting its production significantly (Li *et al.,* 2021). Consequently, it becomes vital to study its presence to prevent huge losses. It is of absolute importance in regions like Ladakh, where climatic conditions limit crop production, and locally grown vegetables are the primary source of nutrition for the inhabitants of this region.

This study aims to investigate the cause of wilt in bottle gourd in the high-altitude region of Ladakh, specifically examining the genetic and phenotypic characteristics of the pathogen responsible for this disease.

Materials and methods

Survey and sampling site: In August 2017, a random survey and sampling method were used to assess the prevalent fungal diseases of bottle gourd. Plants were observed for symptoms of any fungal infestation, such as mildew, rust, spots or wilt. The bottle gourd leaves showing visual symptoms of fungal diseases were collected from Muth (or Mood) village. Plants from four corners and centre of each field were assessed. Disease incidence (DI) was calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

Disease severity index (DSI) was calculated using a 0-4 scale where a score of 0 indicated no visible disease symptoms; 1 indicated less than 25% leaves showing symptoms; 2 indicated 25-50% of leaves with disease symptoms; 3 indicated 50-90% leaves with disease symptoms and 4 indicated completely wilted or dead plants. After scoring each plant, the Disease severity index was calculated using the following formula:

$$\text{Disease severity index (DSI)} = \frac{\sum(\text{Pi} \times \text{ni})}{\text{N} \times \text{P4}} \times 100$$

Pi is the score (0-4), ni is the number of plants in the corresponding DSI grade, N is the total number of plants assessed and P4 is the highest DSI grade (Li *et al.,* 2021).

Various physiographical parameters of the field location like temperature, relative humidity, altitude, latitude and longitude were noted. Infected leaves were collected and put in a portable refrigerator until it reached the lab for morphological and molecular identification of the causal organism.

Isolation of pathogen: The collected leaves were surface sterilized with 1% sodium hypochlorite and rinsed with distilled water thoroughly. These leaves were cut into small pieces, placed on Potato Dextrose Agar (PDA) media, and incubated at 25°C for 5-7 days. Pure culture was obtained by subsequent sub-culturing on the same media. The pure isolated cultures were transferred to PDA slants and kept at 4°C for further use.

Morphological characterization: Colony characteristics on PDA were observed for colour, texture, margins and other characteristics. The isolates were transferred to glass slides and stained with lactophenol cotton blue dye. Various descriptive structures of fungi, such as the shape and size of conidia, conidiophores, hyphae and septation were observed under the microscope. Based on these morphological characteristics, the fungus was primarily identified up to the genus level.

Pathogenicity test: Isolated cultures were then subjected to a pathogenicity test. For this, leaves from healthy plants were collected, surface sterilized with 1% sodium hypochlorite solution and rinsed thrice with sterile distilled water. These leaves were placed in a sterile petri plate containing sterile Whatman filter paper moistened with sterile distilled water and inoculated with fungal suspension. After 4-7 days of incubation at 25°C and 60-80% relative humidity, symptoms (if any) were observed.

DNA extraction and PCR amplification: Isolated cultures were grown in potato dextrose broth and harvested mycelia was used to extract genomic DNA using the method adapted from Samarrai and Schmid, 2000. Gene amplification of the extracted DNA was carried out using universal primer pair ITS-1/4 in C1000 Touch^{TM} Thermal cycler (Bio-Rad) and the amplified product was purified using QIAquick PCR Purification Kit (Qiagen).

Sequence analysis: Sanger sequencing of purified amplified DNA was done and the obtained sequences were compared with reference sequences using BLAST search in UNITE database.

Results and discussion

Survey: Muth village is located at an altitude of 4221 m above mean sea level in the Eastern part of Ladakh and at latitude of 33°12009 N and longitude of 78°41730 E. Maximum and minimum temperature observed of the village during the survey was 26° C and 5° C respectively with relative humidity of 41%. Plants showed onset of symptoms of wilting on the leaves. The disease incidence recorded was 14%, and the disease severity index was calculated to be 5%.

Morphological characterization: Colony characteristics on PDA media revealed white-coloured colonies with light pink centre, smooth margins and cottony texture. Microscopic examination showed oval to kidney-shaped micro conidia and thin-walled, sickle-shaped or spindle-shaped macroconidia with septation (Fig. 1). These characteristics helped in the primary identification of the isolate as *Fusarium sp.*

Pathogenicity test: The leaves started showing symptoms after four days of inoculation. The leaves showed discolouration, chlorosis and eventually turned brown after seven days (Fig. 2). The symptoms confirmed that the isolate is pathogenic to the bottle gourd leaves.

PCR amplification and sequence analysis: The amplified PCR product of ITS1/4 primers of ~550 bp length was run on 1% agarose gel. BLAST of the obtained ITS1 sequence showed 100% similarities with *Fusarium oxysporum* in UNITE database. The isolate was submitted in NCBI GenBank under accession number OP453355.

F. oxysporum is a notorious fungus affecting many crops worldwide, primarily causing wilting. In the Shaoxing and

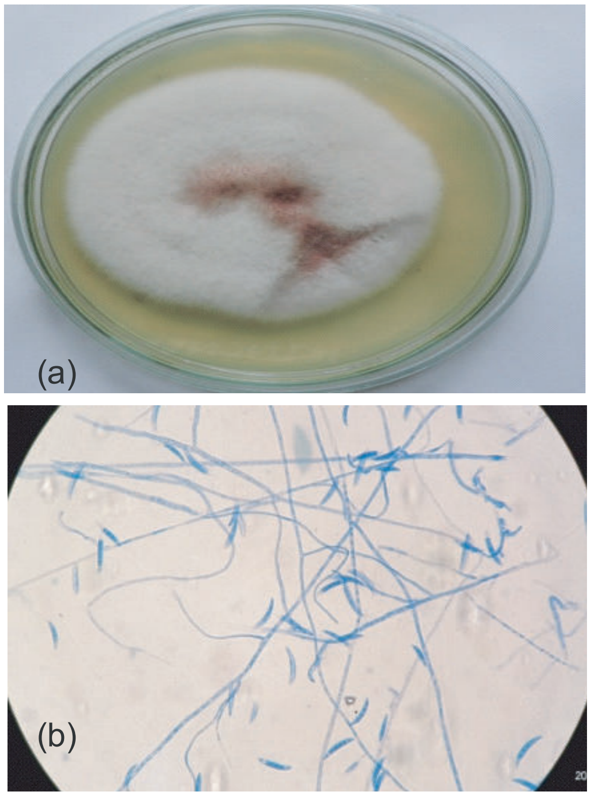

Fig. 1 (a) Colony morphology of isolate on PDA media, (b) conidial and hyphae structures at 40x

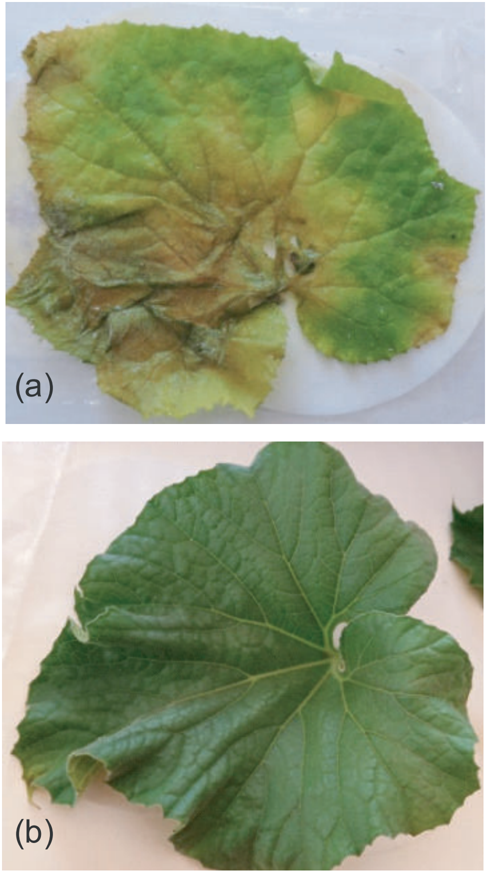

Fig. 2 (a) Innoculated leaf, (b) Control

Haining region of China, *F. oxysporum* was isolated. A total of 89 bottle gourd accessions were used to study their resistance against Fusarium wilt in a glasshouse in 2019 and 2020. All isolates' mean disease incidence was 46% in 2019 and 55% in 2020 (Li *et al.,* 2021). In another study of disease incidence and severity of Fusarium wilt, 12 isolates of *F. oxysporum* was obtained from bottle gourd stem and the soil. The major symptoms observed were wilting and vein clearing (Cumagun *et al.,* 2010). In the Batangas and Bulacan region of the Phillipines, *F. oxysporum* was isolated from bitter gourd and bottle gourd, respectively (Cumagun *et al.,* 2008). Bottle gourd seeds infected naturally with *F. oxysporum* showed a 1.25- 26.75% disease incidence in Rajasthan (Avinash *et al.,* 2021). Another study of cucurbits seeds showed 29.2% incidence of *F. oxysporum* on sponge gourd and a 22.2 % incidence of *F. verticillioides* on bottle gourd. The infection ratio was higher in seeds collected from farmer fields and wilt disease was the major culprit in decreasing the yield rate of cucurbits. *F. oxysporum* also showed incidence in cucumber and ridge gourd (Avinash *et al.,* 2013); fruit rot and wilt of tomato (Ignjatov *et al.,* 2012); foliar wilting, stunted growth and decreased fruit production of strawberry (Koike *et al.,* 2009); wilt of banana in Australia, Southeast Asia and Southwestern Pacific (Ploetz, 2015).

Currently, limited studies report Fusarium diseases of crops at high altitude regions. A survey of Fusarium wilt of chickpeas grown in Ethiopia with altitude ranging from 2148 to 2879 m above sea level (asl) showed 100% disease prevalence in all surveyed locations. Wilt incidence was higher at lower altitudes (≤2300m) as compared to higher altitude (≥2300m) (Ali and Terefe, 2021). A similar trend was seen in a survey of banana wilt disease incidence in regions located at 700-2012 m asl. The highest Fusarium wilt incidence was recorded at altitudes between 1000-1600 m asl in comparison to higher altitudes (>1600m asl) (Karangwa *et al.,* 2016). The effect of soil temperature and inoculum density of *F. oxysporum* f. sp. *ciceris* on disease development in chickpea indicated that asymptomatic or less to moderate disease development was observed when one of the two factors were sub-optimal. It was concluded that a deficient factor which can limit the process of disease development is compensated by another factor which is optimum for the growth of pathogen (Jiménez-Díaz *et al.,* 2015). Some *Fusarium* sp. causing wilt and dry rot in tomatoes has been isolated from Eastern and Western Ladakh at an altitude range of 9000- 14000ft asl (Phour *et al.,*2018). *Fusarium sp.* has also been reported to cause wilting in tomatoes and capsicum grown under protected cultivation in July-September in Ladakh (Phour *et al.,* 2019). The prevalence of pathogens such as *Fusarium sp.* at such high altitudes can result from various factors such as seed and water quality, temperature and atmospheric humidity during the cultivation period. Some indigenous plants, such as chikori, rumax, langthang, *etc*., might also serve as hosts for pathogens as these plants are cold tolerant, survive in sub-zero temperatures during winters and their seeds germinate without human intervention during the summer season (Phour *et al.,* 2018). High wind velocity can contribute to the transmission of fungal spores from one region to another along with farmers' inter and intra village exchange of seeds (Ghai *et al.,* 2018). It is well established that a single occurrence of disease in the field makes it highly likely to continuously survive in the soil for an indefinite period without any host and thus, affect crop yields significantly (Li *et al.,* 2021). Therefore, a non-intentional introduction of *Fusarium oxysporum* in the field will lead to its constant prevalence in the soil until appropriate remedial actions are taken.

At 4221 metres in Eastern Ladakh's Muth region, *Fusarium oxysporum* causes bottle gourd to wilt. The first Fusarium wilt in bottle gourd in high altitude Ladakh has occurred in this pathogen-, pest-, and insect-free region. Concerningly, the disease prevalence and severity suggest imminent crop losses, requiring immediate pathogen control. Most worrying is *F. oxysporum* ability to survive in soil after entry. Sustainable solutions are needed to control Fusarium wilt in bottle gourd.

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