

New Report on *Fusarium equiseti* Causing Yellow Leaf Disease of *Brassica juncea* (L.) Czern from Karandighi, Uttar Dinajpur, West Bengal, India

Shambhu Swarnakar, Papan Chowhan, Zerald Tiru, Parimal Mandal, Arka Pratim Chakraborty*

Department of Botany, Raiganj University, Raiganj, Uttar Dinajpur- 733134, West Bengal, India.

Abstract

Yellow leaf disease in B-9 variety of yellow mustard [*Brassica juncea* (L.) Czern] was noticed in the area of Khowaspur, Karandighi, Uttar Dinajpur District, West Bengal. Irregularly shaped dull yellow regions along the leaf margin and even in the midrib of leaf were observed. *Fusarium equiseti* (Corda) Sacc. (ON783721.1) was isolated and identified as causal potent fungal isolate causing yellows in leaves of *Brassica juncea*.

Keywords: *Fusarium equiseti*, yellow leaf disease, *Brassica juncea*

DOI: <https://doi.org/10.55734/nbujps.2022.v14i01.003>



Article info

Received **12 October 2022**

Revised **08 April 2023**

Accepted **03 May 2023**

Introduction

Yellows symptoms in leaves of *Brassica juncea* was noticed during January and February 2022 from the area of Khowaspur (Lat 25.772671^o; Long 88.032848^o), Karandighi, Uttar Dinajpur District, West Bengal (Figs. 1 & 2). In January, the temperature varied between 13.3-24.7 °C and 16.0-28.7 °C in February. In January, the average humidity was 53%, while in February humidity was recorded as 45%. The initial signs of the disease were irregularly shaped dull yellow regions along the leaf margins. These areas then spread to the leaf midrib and turned tan to brown (Fig. 1C). Lesions could be sparse and dispersed throughout the leaves and densely packed over vast areas of leaves. After surface sterilizing the diseased leaf sample with 0.1% HgCl₂ for 2 min, followed by ethanol for 2 min, the leaf sample (1–1.5 cm) was chopped into small pieces and placed on PDA (Potato Dextrose Agar) medium. An antibiotic Monocef-O 100 (each 5 ml of the reconstituted suspension contains cefpodoxime proxetil IP equivalent to cefpodoxime-100 mg) was added in PDA medium to prevent any

bacterial interference. On potato dextrose agar, a cefpodoxime proxetil IP equivalent to cefpodoxime-100 mg) was added in PDA medium to prevent any bacterial interference. On potato dextrose agar, a fungus was isolated from the diseased leaf. On the basis of morphology of the fungal mycelia, various shapes and sizes of conidia by compound and scanning electron microscopy, the fungus was supposed to be species of *Fusarium* (Fig. 3). For molecular identification, first step was DNA isolation followed by fragment of 18S rRNA gene that was amplified by ITS1 and ITS4 primers- ITS1- TCC GTA GGT GAA CCT GC GG and ITS4- TCC TCC GCT TAT TGA TAT GC. A single discrete PCR amplicon band of 1500 bp was observed when carried out with ITS1 and ITS4 primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 18S rRNA gene was generated from forward and reverse sequence data using aligner software and finally the 18S rRNA gene sequence was used to carry out BLAST with the “nr” database of NCBI GenBank database. *Fusarium equiseti* (ON783721) was identified and submitted to NCBI GenBank (Fig. 4).

* Correspondence -arka.botanyrgu@gmail.com



Fig. 1: Sample collection site (A & B); Disease symptoms (C) in leaf of B9 variety of yellow mustard (*Brassica juncea*)



Fig. 2: Earth map and GPS location of the sample collection site from where diseased leaves of *Brassica juncea* were collected (A-C)

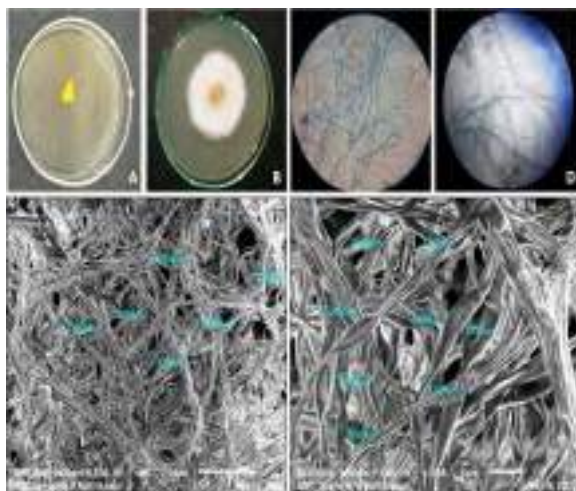


Fig. 3: Isolation on PDA medium (A); 7 days old growing isolate (B); Microscopic view of mycelia of



Fig. 4: Molecular identification of the isolate with GenBank accession number- ON783721

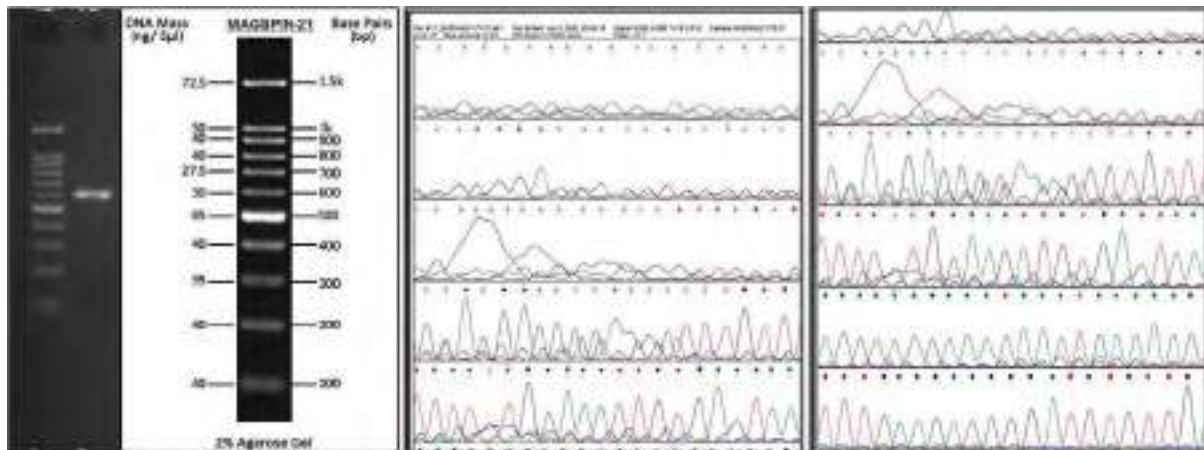


Fig. 5: Chromatogram of sequence of the fungus

resolved on agarose gel and forward and reverse DNA sequencing reaction of PCR amplicon was The Neighbour Joining (NJ) method [Saitou and Nei (1987), Tamura et al. (2004, 2021)] was used to do phylogenetic analysis of *Fusarium equiseti* (ON783721.1) with other ex types of *Fusarium*. *Fusarium equiseti* (ON783721.1) was found to be closely related with *Fusarium equiseti* isolate MLS029 (OM203485.1) (Figs. 5 & 6). Healthy mustard plant [*B. juncea* (L.) Czern] was re-inoculated with foliar spray of spore suspension of *F. equiseti* (ON783721.1) and similar leaf symptoms were appeared. To confirm the attachment of fungal mycelia to the leaf surface, scanning electron microscopy of the leaf surface was performed and the existence of fungal mycelia was confirmed. After Koch's Postulate, the isolated fungal morphology



Fig. 6: Phylogenetic analysis of *Fusarium equiseti* (ON783721) was performed with other ex types of *Fusarium* by Neighbour Joining (NJ) method

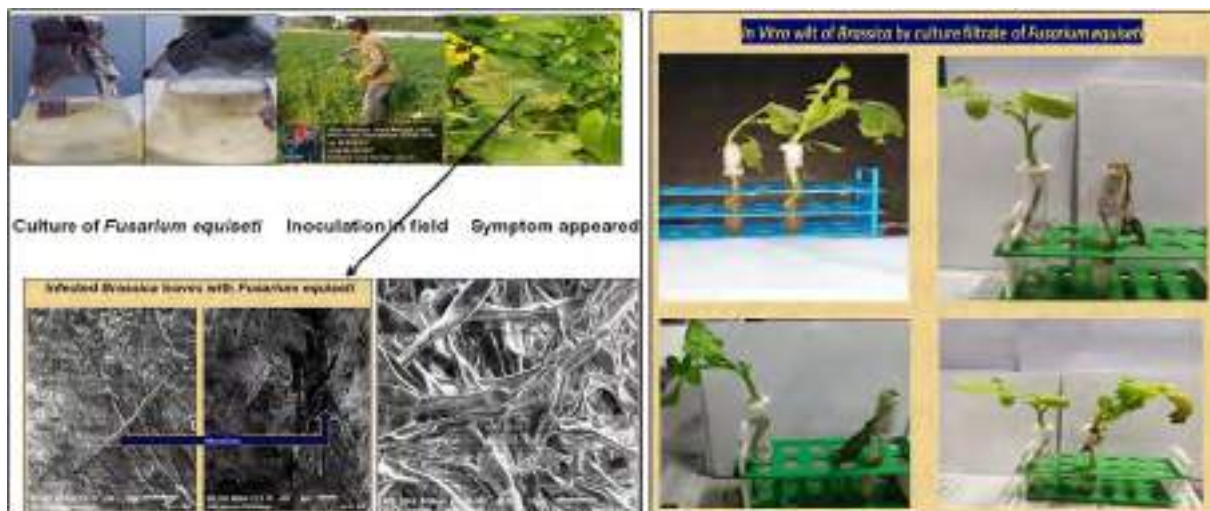


Fig. 7: Koch's postulate in field condition; establishment of leaf disease by *Fusarium equiseti* (ON783721) and *in vitro* wilt disease by *F. equiseti*.

was rechecked through scanning electron microscopy. *F. equiseti* (ON783721.1) was able to cause wilt of the plant when the plant was dipped into the spore suspension of the fungus *in vitro* condition (Fig. 7). This case report is regarded as new one on *Fusarium equiseti* (ON783721) causing yellow leaf disease of *Brassica juncea* from Karandighi, Uttar Dinajpur, West Bengal. Similar findings were found on first report of *Fusarium equiseti* causing wilt and seedling death in other crop plants in support of our new finding (Mishra et al. 2021; Khan et al. 2021; Astudillo-Calderón et al. 2019; Rajput et al. 2020; Aldakil et al. 2019).

Conflict of Interests

The authors declare no conflict of interests among them.

Acknowledgements

Authors take this opportunity to express earnest gratefulness to M/s Edison Life Science, Kolkata for molecular identification of the isolate and the Director of USIC-Scanning Electron Microscopy Unit, University of North Bengal for taking the scanning electron microscopic photographs.

References

- Aldakil H, Jaradat ZW, Tadros M, Alboom MH (2019) First Report of *Fusarium equiseti* causing crown rot on cucumber in Jordan valley. Plant Dis <https://doi.org/10.1094/PDIS-11-18-2073-PDN>
- Astudillo-Calderón S, Tello ML, Alonso de Robador JM, Pintos B (2019) First Report of *Fusarium equiseti* causing vascular wilt disease on *Vitis vinifera* in Spain. Plant Dis <https://doi.org/10.1094/PDIS-01-19-0067-PDN>
- Khan MF, Liu Y, Bhuyian MZR, Lashman D, Liu Z, Zhong S (2021) First Report of *Fusarium equiseti* causing seedling death on sugar beet in Minnesota, USA. Plant Dis doi: 10.1094/PDIS-10-20-2102-PDN
- Mishra RK, Mishra M, Bohra A, Naik S, Pr S. Kumar K, Patil P, Srivastava DK, Singh NP (2021) First report of *Fusarium equiseti* (Corda) Sacc. causing wilt of *Cajanus scarabaeoides*, a wild relative of pigeon pea in India. Plant Dis doi: 10.1094/PDIS-12-20-2723-PDN
- Rajput N, Zaman B, Huo C, JiFen C, Muhammad A, Abdul ML, Rehana NS, Babar K, Owais I, Zhijian Z (2020) First report of *Fusarium equiseti* causing stem rot disease of grape (*Vitis vinifera* L.) in Afghanistan. J Plant Pathol 102: 1277. <https://doi.org/10.1007/s42161-020-00557-8>
- Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425.
- Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.
- Tamura K, Stecher G, Kumar S (2021) MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol <https://doi.org/10.1093/molbev/msab120>