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Isolation and pathogenicity of pathogen causing diseases on dodder (*Cuscuta gronovii*)

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Abstract
The disease symptoms on Cuscuta vine were observed in severe form in the survey for host range of Cuscuta in different locations of Raigad and Thane districts of Konkan region during 2015-16. The Cuscuta vine was found to be infected by different pathogens; symptoms appeared as discoloration and shrivelling of vine, blighted portion, necrotic lesions and tip necrosis on vine. The infected samples of Cuscuta were collected and brought to the laboratory for further studies. Under microscopic examination there are three mounts were placed. In that different morphological structure were observed. Different pathogens were isolated from diseased Cuscuta samples on PDA medium. After isolation of diseased samples there are three isolates were obtained. There colony and morphological characters are different from each other. Pathogenicity of the test fungi was proved by spraying with the spore suspension of individual fungus (10^5 spores/ml) on *C. gronovii* parasitizing on green gram plants (two weeks old) already grown in plastic pots. Symptoms appeared on the artificially inoculated *C. gronovii* vine within period of 7 to 14 days after inoculation. Three fungi *viz*., *Fusarium incarnatum* (Desm.) Sacc, *Alternaria dianthicola* Neerg and *Curvularia pallescens* Boedijn were isolated from diseased Cuscuta samples on poatato dextrose agar medium (PDA). Further these three isolated fungi were confirmed as by the chief Mycologist, Agharkar Research Institute, Pune.

Keywords: *Cuscuta gronovii*, Symptoms, Spore, Pathogenicity, Pathogen, *Fusarium incarnatum* (Desm.) Sacc, *Alternaria dianthicola* Neerg and *Curvularia pallescens* Boedijn

Introduction
The name Cuscuta originates from the Arabic word "kushkut," which loosely translates as "a tangled wisp of hair" (Austin 1980) [2]. When Cuscuta species begin to cover a plant, a mass of tangled yellow to orange threads of various diameters and no leaves are produced. Cuscuta spp. are also known by various common names, such as dodder, love vine, tangle gut, devil's gut and strangle weed. The most common name dodder, possibly originates from the old German word "dotter" which means yolk (Dawson *et al*., 1994) [6].

Pulses are good sources of proteins and commonly called the poor man's meat. India ranks first in the world in terms of pulse production (25 per cent total worlds production). Madhya Pradesh, Maharashtra, Uttar Pradesh, Andhra Pradesh, Karnataka and Rajasthan are the major states growing pulses in India. These six states contribute 80 per cent of total pulse production and area (Anonymous, 2014) [1].

In Konkan region of Maharashtra, the *Cuscuta gronovii* was found to be parasitic on crops of *Rabi* season. In Raigad and Thane districts, it is serious problem on pulses like beans, green gram, kidney bean, cow pea etc. (especially in rice-fallows) in *Rabi* season. It is also parasitic on the other dicotyledonous crops and weeds. Its parasitic effects reduce the plant vigour and yield. In severe infestation the infested plants may die. (Dalvi *et al.*, 2014) [5].

Cuscuta is a complete stem parasite and infected by various pathogens reported earlier by researcher and literature. A strain of *Colletotrichum destructivum* was identified from *Cuscuta* spp. parasitizing alfalfa in Oregon, USA by Leach (1958) [7] and it was proposed as a potential biological control agent against Cuscuta. Zhang (1985) [9] reported that the fungus, *Colletotrichum gloeosporioides* infected Cuscuta in China. Bewick *et al*. (1987) [3] isolated the *Fusarium tricinctum* and *Alternaria* sp. from a diseased *C. gronovii* plant growing in an uncultivated marsh in Wisconsin. The *Alternaria* sp. was isolated on PDA and the *F. tricinctum* on a hay agar. Shakir *et al*. (1999) [8] isolated four fungi *viz*., *Alternaria alternata* (Fr.) Keissler, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Curvularia lunata* (Wakker)

Boed. and *Fusarium pallidoroseum* (Cooke) Sacc. from stem pieces of *Cuscuta reflexa* and *Cuscuta campestris* in Pakistan. Cook *et al*. (2009) [4] isolated *Alternaria* spp. from randomly chosen *Cuscuta pentagona* tissue samples. Considering this aspect as a new window of Biological control of *Cuscuta* for research, this research plan was conducted and carried out to isolate the pathogen associated with infected or diseased *Cuscuta* vine to find out their effectiveness against *Cuscuta*.

Materials and Methods

A) Isolation of pathogen causing diseases on *C. gronovii*

Collection of diseased samples of Cuscuta:

The Cuscuta vine showing typical symptoms of disease were collected in the paper bags from different locations of the Raigad and Thane districts of Konkan region during the survey of host range in 2015-16. These samples were brought to the laboratory for further studies.

Visual observations

Visual observations of disease symptoms on Cuscuta were recorded during the survey and host range of Cuscuta to know the development of the disease under natural conditions.

Microscopic examination

Fresh diseased samples of dodder showing typical symptoms of the disease were collected and brought to the laboratory. These samples were then washed under tap water to remove extraneous material. Temporary mounts were prepared from the diseased specimens and examined under compound microscope for presence of microorganism if any.

Isolation of causal organism causing diseases on *C. gronovii*

Fresh samples of diseased Cuscuta vine, showing the typical symptoms like browning or discoloured (blighted/necrotic) and shrivelling of vine samples were brought to the laboratory from different locations of Raigad and Thane districts of Konkan region of Maharashtra in 2015-16. These samples were washed with running tap water to remove extraneous material. Small pieces of the desired size were cut by taking care that each piece contained half infected and half healthy portion. Such pieces were then disinfected with 0.1 per cent mercuric chloride ($HgCl_2$) for 1 minute followed by three washings in distilled sterile water to remove the traces of mercuric chloride. These pieces were then placed on sterilized blotters for drying. Properly dried pieces were transferred aseptically in sterilized Petri plates containing sterilized, solidified PDA medium. The plates were incubated in BOD incubator at 25 ± 2°C till the fungal mycelium fully covered the surface of the medium. This fungal growth was transferred to PDA slants and maintained as stock culture for further studies.

Pathogenicity of the isolated organisms

Inoculation

Seeds of *C. gronovii* and its host like green gram were sown together in the 9 inch plastic pots containing desired potting mixture. Potting mixture comprising FYM and soil (1:2) was autoclaved for three successive days in order to kill the micro flora present if any. Few healthy growing pulse plants parasitized by dodder per pot were maintained and watered regularly. The host-parasite system was allowed to develop for two weeks after parasitism had occurred. At this time, plants were inoculated with spores from test pathogen. Spore suspension of the test pathogens were prepared by pouring the distilled sterile water in 7-8 days old culture plates. The resultant spore suspension was filtered through muslin cloth and filtrate obtained was suitably diluted with distilled sterile water to get inoculum concentration of (10^5) spores/ml determined by using a haemocytometer. Thirty days old plants of green gram parasitized by Cuscuta already grown in 9 inch plastic pots were artificially inoculated by spraying the spore suspension (10^5 spores/ml) of the test pathogens with an atomizer. Plants of green gram parasitized by Cuscuta grown in plastic pots and sprayed with sterile water (without inoculum) were served as control.

Development of symptoms

Pots both inoculated and uninoculated were incubated in glasshouse. Proper humidity (85-90%) and temperature (32^0C) were maintained in glasshouse. Plants of green gram parasitized by Cuscuta were watered as and when required till the development of typical disease symptoms.

Re-isolation

The causal organisms were re-isolated from the artificially inoculated Cuscuta vine showing typical disease symptoms. The fungal growth obtained on PDA medium on re-isolation was compared with the original culture obtained from naturally infected Cuscuta vine samples under natural conditions.

Identification of the causal organism

The re-isolated pure fungal culture was identified by comparing its morphological and colony characters with the information available in the reviewed literature as well as on the standard websites for fungal identification. The cultures were tentatively identified at Department of Plant Pathology, College of Agriculture, Dapoli and then sent to Chief Mycologist, Agharkar Research Institute, Pune for further confirmation of the fungus up to species level.

Results

Visual observations

The disease symptoms on Cuscuta vine were observed in severe form during the survey for host range of Cuscuta in different locations of Raigad and Thane districts of Konkan region during 2015-16. The disease symptoms appeared as discoloration and shriveling of vine, blighted portion, necrotic lesions and tip necrosis on Cuscuta vine. These necrotic lesions were elongated into gray to black colour. (PLATE I).

Plate I: Symptoms observed on Cuscuta vine during survey

Microscopic examination

In order to know the association of the pathogen with the disease, fresh diseased samples of Cuscuta plants showing typical symptoms were collected during the survey for host range of Cuscuta and brought to the laboratory. Temporary mounts were prepared from the diseased samples and examined under compound microscope. Microscopic examination revealed the presence of fungal structures such as mycelium and conidia. The different conidial structure were seen as follows

Mount I : Macro conidia were 2-5 septa and fusiform to sickle shaped; Micro conidia were 1-2 septa, smaller than macro conidia, pyriform, fusiform to ovoid and straight or curved.

Mount II : Conidia were narrow-ovoid, obclavate or muriform. The conidia had 6 - 10 transverse septa slightly darker and constricting and 0 to few longitudinal septa.

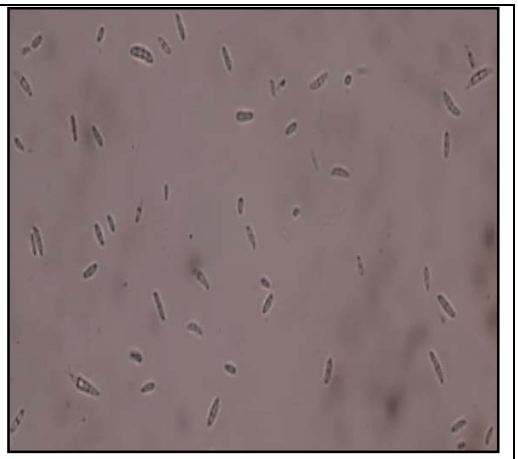
Mount III : Conidia were borne acropleurngenously, pale olivaceous to black, mostly straight nearly cylindrical with acute apical tip, 3 septate.

Isolation of causal organisms causing diseases on *C. gronovii*

Different fungi were isolated from diseased Cuscuta samples on PDA medium individually (PLATE II). Characteristics of colonies of isolated fungi are given in Table 1.

Table 1: Colony (mycelium) characters of isolates obtained from *C. gronovii*.

Sr. No.	Isolated fungi	Colonies (mycelium)
11	Isolate I	Growth of the fungus on PDA was initially creamy white turning later with brown cream to brown colour pigmentation on reverse of the colony.
22	Isolate II	Colonies were fast growing, black to olivaceous black or greyish and suede like to floccose.
33	Isolate III	Growth of the fungus on PDA was effuse, gray, becoming black on aging; mycelium mostly immersed, forming straight or flexuous and smooth conidiophores.



Fusarium incarnatum (Desm.) Sacc.

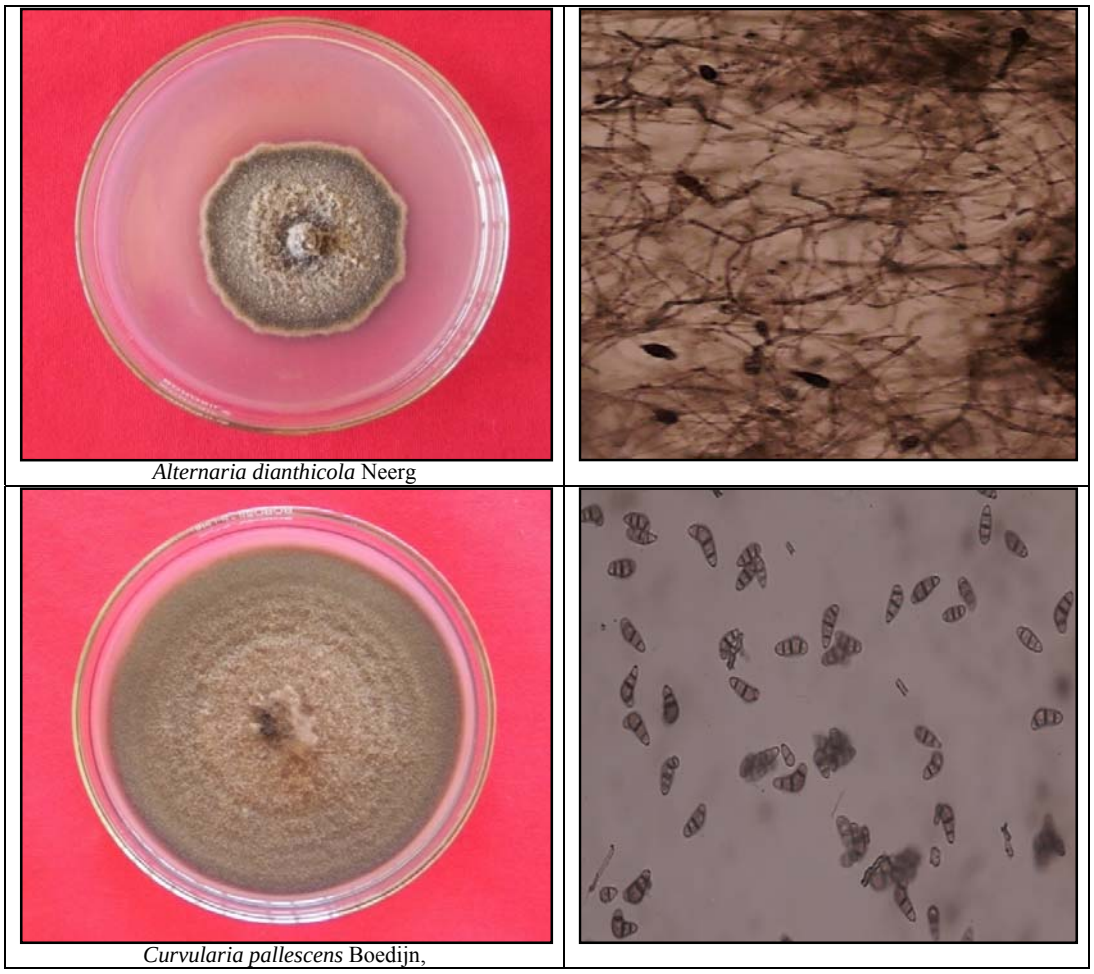


Plate II: Pure cultures and spores of isolated fungi

Sporulating culture of different fungi were mounted and observed under compound microscope at 45X. The conidial characteristics of different isolated fungi are given Table 2.

Table 2: Conidial characters of isolates obtained from *C. gronovii*.

Sr. No.	Isolated fungus	Conidial characteristics
1	Isolate I	Microconidia are 1-2 septate measuring 10-20 × 2.5-3.5 µm, macroconidia aerial, mycelius, 3-5 septate, straight to slightly curved, wedged shaped with pointed apex, measuring 24-40 × 3.8-4.2 µm.
2	Isolate II	Conidia were simple or sometimes branch, dilute yellowish, narrow-ovoid, obclavate or muriform. The conidia were measured 12.7 to 120 µm in length, 6.5 - 17.20 µm in width and had 6 - 10 transverse septa slightly darker and constricting and 0 – 4 longitudinal septa. Conidia had a short beak of length and the outer wall was smooth.
3	Isolate III	Conidia were borne acropleurngenously or acrogenourly, pale olivaceous to black, mostly straight nearly cylindrical with acute apical tip, 3 septate, measuring 22-28 × 6-11µm.

Pathogenicity of the isolated organisms

Inoculation

C. gronovii parasitizing on green gram plants (two weeks old) already grown in plastic pots was inoculated by spraying with the spore suspension (10^5 spores/ml) of the test fungi individually. The inoculated *C. gronovii* parasitizing on green gram plants were incubated in glasshouse. Proper humidity (85 to 90%) and temperature (32^0C) were maintained in glasshouse. Pots were watered as and when required till the development of typical disease symptoms.

Development of symptoms

Symptoms appeared on the artificially inoculated *C. gronovii* vine within period of 7 to 14 DAI. The symptoms produced on inoculated *C. gronovii* vine (parasitizing on green gram plants) were similar to those observed during survey for host range.

Re-isolation

The test fungi were re-isolated from artificially inoculated *C. gronovii* vine (parasitizing on green gram plants) on PDA medium individually and incubated at 25 ± 27 ^{0}C. One week after incubation, morphological and cultural characteristics of the test pathogens re-isolated from artificially diseased Cuscuta vine were compared with those of the original culture isolated from naturally infected Cuscuta samples. This proved that the pathogens responsible for causing diseases on *C. gronovii* were *Fusarium*, *Alternaria* and *Curvularia* spp.

Identification

On the basis of microscopic observations and cultural characters, the fungi were tentatively identified as *Fusarium*, *Alternaria* and *Curvularia* spp. The further identification at the species level was confirmed by the Chief Mycologist,

Agharkar Research Institute, Pune and the fungi were identified as

Isolate I : *Fusarium incarnatum* (Desm.) Sacc.
Isolate II : *Alternaria dianthicola* Neerg.
Isolate III : *Curvularia pallescens* Boedijn.

Discussion

The disease symptoms on Cuscuta vine were observed in severe form in the survey for host range of Cuscuta in different locations of Raigad and Thane districts of Konkan region during 2015-16. Disease symptoms appeared as discoloration and shriveling of vine, blighted portion, necrotic lesions and tip necrosis on Cuscuta vine. Similar symptoms were also reported by Bewick *et al*., (1987) [3] and Shakir *et al*., (1999) [8]. These findings are quite similar to those reported by Shakir *et al*. (1999) [8] who isolated four fungi viz., *Alternaria alternata* (Fr.) Keissler, *Curvularia lunata* (Wakker) Boed. and *Fusarium pallidoroseum* (Cooke) Sacc. from stem pieces of *Cuscuta reflexa* and *Cuscuta campestris*. The results of present study are in agreement with earlier findings of Bewick *et al*., (1987) [3] who isolated the *Fusarium tricinctum* and *Alternaria* sp. from a diseased *C. gronovii* plant growing in an uncultivated marsh in Wisconsin and Cook *et al*., (2009) [4].

The pathogenicity of the isolated fungi was confirmed by inoculating spore suspension (10^5) of the test pathogens by an atomizer on *C. gronovii* parasitizing on green gram plants (two weeks old) individually. Typical symptoms of disease such as discoloration and shriveling of vine, blighted portion, necrotic lesions and tip necrosis on Cuscuta vine appeared on the artificially inoculated *C. gronovii* plants within a period of 7 to 14 DAI were observed. In present study, symptoms developed on artificial inoculated *C. gronovii* plants were similar to those observed during survey. The test pathogens were re-isolated from artificially inoculated C. gronovii (parasitizing on green gram plants). These findings are quite similar to those reported by Shakir *et al*. (1999) [8] who proved the pathogenicity of four fungi viz., *Alternaria alternata* (Fr.) Keissler, *Colletotrichum gloeosporioides* (Penz.) Sacc, *Curvularia lunata* (Wakker) Boed and *Fusarium pallidoroseum* (Cooke) Sacc. Bewick *et al*. (1987) [3] also proved the pathogenicity of *Fusarium tricinctum* and *Alternaria* spp. The results of present study are also in conformity with the findings of Cook *et al*. (2009) [4].

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