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STUDY ON CULTURAL, MORPHOLOGICAL AND PATHOGENIC VARIABILITY AMONG THE ISOLATES OF *PYTHIUM APHANIDERMATUM*

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ABSTRACT

Damping-off is the most serious disease of tobacco in nursery conditions caused by *Pythium aphanidermatum*. Total fifteen isolates of *P. aphanidermatum* were recovered from infested soil samples of *bidi* tobacco growing areas of middle Gujarat which are designated as Pa 1 to Pa 15. The isolates showed incredible variability in cultural and morphological characters on oat meal agar media. The isolates show white to light white types of colony appearance whereas, he aerial, moderately aerial, flat, fluffy, fluffy and dense and fluffy aerial types of topography and margin were observed in the fifteen different isolates. Evident differences in size of mycelium, oospore and number of oospores were observed among the isolates even when same medium was used for the growth. The pathogenic variability among the different isolates revealed that maximum mortality due to damped-off was recorded in Pa 4 (98%), followed by Pa 14, Pa 8, Pa 3, Pa 7, Pa 11, Pa 12, Pa 2, Pa 13, Pa 9, Pa 15, Pa 10 Pa 1, Pa 6 and Pa 5 isolates. All the seedlings were healthy in uninoculated, which proved that all the fifteen isolates were pathogenic and isolates Pa 4 and Pa 14 were found highly virulent.

Key words : Damping-off, *Pythium*, Isolates, Variability.

Introduction

Tobacco (*Nicotiana tabacum* L.) is one of the most important non-food crops of the *Solanaceae* family in the world. Tobacco is an important cash crop in terms of revenue generation, export earnings and rural employment. *Bidi* tobacco is attacked by a varied range of pathogens including fungi, bacteria, viruses and nematodes from seedlings to transplanted crops. Damping-off, root-knot and frog-eye leaf spot diseases are major constraints for tobacco cultivation in the nursery stage. Among these, damping-off is the most serious disease in the nursery incited by *P. aphanidermatum* (Edson) Fitzpatrick, a soil-borne oomycete fungus. Pre-emergence damping-off kills young seedlings before they reach the soil surface. They may be killed even before the hypocotyls have broken through the seed coat (seed rot). When the radical and plumule emerge from the seed, they rot completely. The post-emergence damping-off is quite noticeable. This stage of the disease is distinguished by the toppling of infected seedlings at any point after they emerge from the soil until the stem has hardened sufficiently to resist invasion. Infection typically occurs at or below ground level and infected tissues appear water-soaked and soft. As the disease progresses, the stems at the base become constricted and the plants collapse. Seedlings that appear to be healthy one day may have collapsed on the next day. Understanding the disease epidemiology and host-pathogen interaction is greatly dependent on the knowledge of diversity of pathogen at field level as diverse population of a pathogen have different levels of interaction with the host under variable environment conditions (Singh, 1990).

Materials and Methods

Collection of samples

Infested soil samples showing typical symptoms of damping-off of tobacco were collected from respective

bidi tobacco growing areas of middle Gujarat during first week of July to second week of August 2021. Five soil cores of 5 cm diameter and up to 15 cm deep were collected from five sites of each tobacco nursery. A total 15 isolates of *Pythium* sp. were recovered from infested soil samples (Table 1) and placed in pots for further studies.

Isolation of Pathogen

The pathogen was isolated using standard method suggested by Saha *et al*. (2002) . In this technique, bottle gourd fruits were used as bait to stimulate pathogen growth. The fruits were treated for 12 hrs. in a solution containing carbendazim (500 ppm) and streptocycline (100 ppm) to prevent bacterial and fungal contamination. The treated portion of the fruits transversely cut into 2-4 small pieces and then placed at a depth of 5-8 cm below the soil surface in contaminated soil (taken from a different area). The soil was irrigated and kept moist. After 24 to 48 hrs, the fruit slices were removed from the soil with minimal disruption to the pathogen-infected fruit. Under aseptic conditions, each fruit slice was put into an air-filled plastic bag and maintained at room temperature for 24 hrs. The entire bottle guard fruit slice was covered with white fluffy mycelial growth within 24 hrs. The mycelial growth was observed under microscope and it was aseptically transferred to oat meal agar plate. The culture was purified and maintained for further investigation.

Purification and maintenance of the Pathogen

The fungus was purified by using hyphal tip method and pure culture was maintained by sub-culturing every eight days on plate of oat meal agar and preserved in refrigerator.

Determination of Cultural variability

The fifteen isolates of the pathogen were grown on

Table 1 : Cultural variability among the isolates of *P. aphanidermatum* on oat meal agar.

S. no.	Isolates	Place	Average radial growth (mm)			Cultural characteristics	Mycelial density
			18 hours	24 hours	48 hours		
1	Pa1	Vasana	20.75	42.50	90	Moderately aerial whitish mycelial growth, but initially slow	+
2	Pa2	Visnoli	53.75	69.75	90	Dense, thick, white fluffy aerial mycelial and fast growth	++++
3	Pa3	Dharmaj	50.00	62.50	90	Dense, thick, white fluffy aerial mycelial but fast growth	++++
4	Pa4	Jakhla	40.25	62.50	90	Moderately aerial whitish mycelial but slow growth	++
5	Pa5	Malsarpura	50.50	66.25	90	Moderately aerial whitish mycelial growth and fast growth	++
6	Pa6	Silod	51.75	68.75	90	Moderately aerial whitish mycelial growth and fast growth	++
7	Pa7	Varsola	49.25	67.50	90	Moderately aerial whitish mycelial growth and fast growth	++
8	Pa8	Naranpura lat	39.25	60.00	90	Flat and light white mycelial and slow growth	++
9	Pa9	Haripura lat	37.25	57.50	90	Moderately aerial whitish mycelial but slow growth	++
10	Pa10	Thasara	38.25	58.25	90	Moderately aerial whitish mycelial but slow growth	++
11	Pa11	Vinoti	54.25	72.50	90	Dense, thick, white fluffy aerial mycelial and fast growth	++++
12	Pa12	Rakhial	47.25	66.75	90	Moderately aerial whitish mycelial and fast growth	++
13	Pa13	Arera	53.00	71.25	90	Dense, thick, white fluffy aerial mycelial and fast growth	++++
14	Pa14	Vanoda	54.25	70.50	90	Moderately aerial whitish mycelial and fast growth	++
15	Pa15	Anand	32.50	56.25	90	Moderately aerial whitish mycelial but slow growth	++

+ = Poor, ++ = Moderate, +++ = Good, ++++ = Excellent.

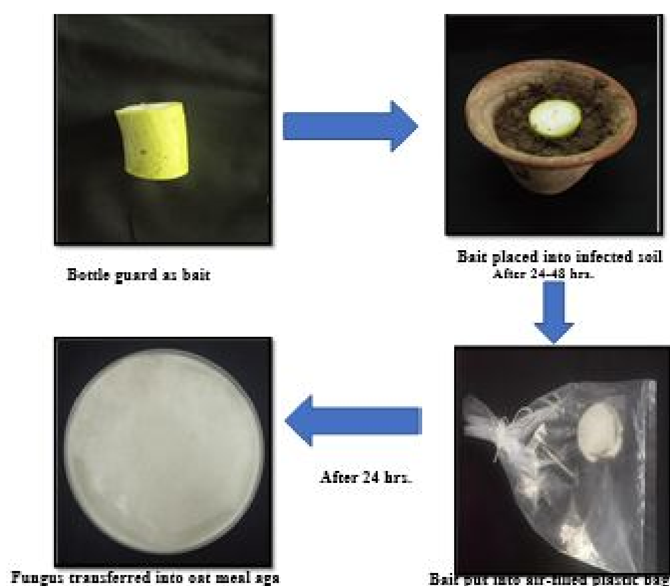

Plate 1 : Isolation of *Pythium aphanidermatum* using bottle guard as bait.

oat meal agar plates for cultural variability. The 5 mm disc of 3 days old pure culture of each isolate was inoculated at the center of the plates of oat meal agar with three repetitions and incubated at room temperature. The mycelial growth and colony characters were measured and visually observed, respectively after 18, 24 and 48 hrs of incubation till the complete growth of each isolate in Petri plates.

Determination of Morphological variability

The growth of each isolate was studied under a microscope and the morphological characteristics *viz.*, size of the mycelium, oospores and number of oospores were measured under 40x microscopic field.

Determination of Pathogenic variability

The pure cultures of isolates were used to prove pathogenic variability of *Pythium* sp. on *bidi* tobacco susceptible cultivar GT 7. The pathogenicity tests were carried out by soil inoculation method suggested by Ramesh (2004).

Soil inoculation

To prove the pathogenicity of each isolate *Pythium* sp. actively growing eight days old cultures were taken, mixed with sterile water and blended to prepare homogenized inoculums. The inoculum (100 ml/pots) was poured into earthen pots filled with sterilized soil. Approximately 100 seeds were seeded in each inoculated and un-inoculated pot after 10 days. As a control, seeds were sown in un-inoculated pots. The pots were labelled, watered gently and arranged in a glasshouse. The pot was irrigated frequently and regular observations related to symptomatology and the number of damped-off seedlings were recorded. The damped-off seedlings were subjected to re-isolation and comparison with the original culture to prove the pathogenicity of the fungus.

Results and Discussion

Identification of the pathogen

The identification of fungal pathogen was done by studying the cultural and morphological characters of fungus grown on oat meal agar medium

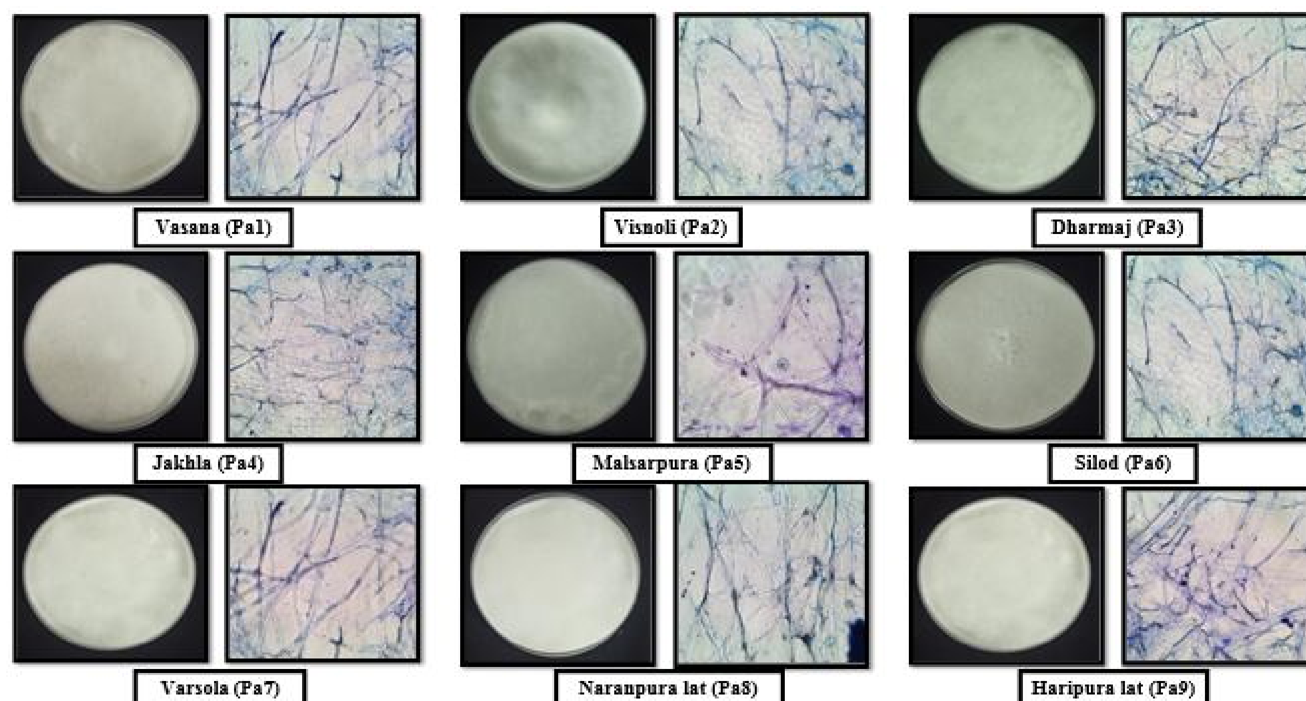

Plate 2 : Cultural and morphological variability among the isolates of *P. aphanidermatum.*

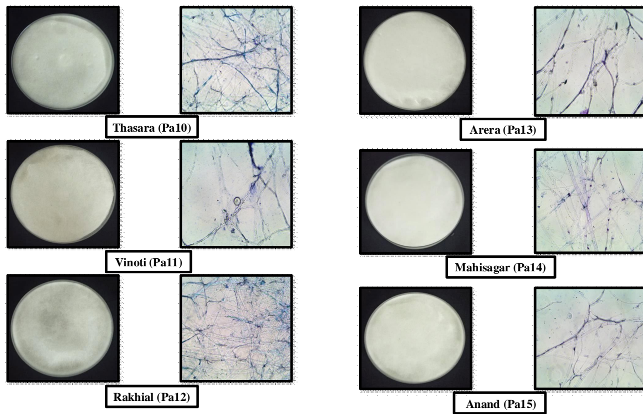

Plate 3 : Cultural and morphological variability among the isolates of *P. aphanidermatum*.

growth (Table 2).

Variability among the isolates regarding the radial growth of *P. aphanidermatum* was recorded. The isolates Visnoli (Pa 2), Dharmaj (Pa 3), Malsarpura (Pa 5), Silod (Pa 6), Varsola (Pa 7), Vinoti (Pa 11), Rakhial (Pa 12), Arera (Pa 13) and Vanoda (Pa 14) produced maximum colony diameter and fast growth. The least colony diameter and initially slow growth were found in isolate Vasana (Pa 1), Jakhla (Pa 4), Naranpura lat (Pa 8), Haripura lat (Pa 9), Thasara (Pa 10) and Anand (Pa 15) (Table 2).

Determination of Morphological variability

The result revealed that all the fifteen isolates produced hyaline and coenocytic mycelium ranging from 2.8 to 4.5 µm in diameter with an equal number of branching. Among all isolates, maximum mycelium size (4.5 µm) was observed in Vasana (Pa 1) isolate, while Rakhial (Pa 12) and Jakhla (Pa 4) isolate produced smallest mycelium size (2.8 ìm). The oospores produced after seven days in all the isolates ranged from 17.61-29.81 µm in diameter. Maximum oospore diameter (29 µm) was observed in Vasana (Pa 1) isolate. Vinoti (Pa 11) isolate showed maximum oospores 6 while in case of other isolates, 4-5 oospores were observed per microscopic field at 40X (Table 2).

However, differences in the mycelium and oospore size and number of oospores per microscopic field were noticed among the isolates even when the same medium

Table 2 : Morphological variability among the isolates of *P. aphanidermatum* on oat meal agar.

S. no.	Isolates	Mycelium		Oospore (After 7 days)	
		Average diameter (µm)	No. of branches	Average diameter (µm)	No. of oospore/microscopic field at 40X
1	Pa1	4.5	3	29.89	5
2	Pa2	4.0	3	17.61	4
3	Pa3	3.1	4	20.23	4
4	Pa4	2.8	3	20.97	5
5	Pa5	3.2	3	19.22	3
6	Pa6	2.9	4	14.86	4
7	Pa7	3.8	4	25.18	4
8	Pa8	3.4	3	21.82	2
9	Pa9	3.2	3	19.02	3
10	Pa10	3.3	3	22.76	4
11	Pa11	3.8	3	21.96	6
12	Pa12	2.8	4	17.61	4
13	Pa13	3.9	3	21.52	4
14	Pa14	3.4	3	21.07	3
15	Pa15	3.7	4	20.81	4

Determination of Cultural variability

Total fifteen isolates of *P. aphanidermatum* collected from *bidi* tobacco growing areas of middle Gujarat were cultured on oat meal agar media to study their cultural variability. The mycelial colour and type of growth were recorded and presented in Table 1. The majority of the isolates produced similar growth except at 18 and 24 hrs.

The isolates Visnoli (Pa2), Dharmaj (Pa3), Vinoti (Pa11) and Arera (Pa13) showed dense, thick, white fluffy aerial mycelial growth while moderately aerial whitish mycelium growth was observed in Vasana (Pa 1), Jakhla (Pa 4), Malsarpura (Pa 5), Silod (Pa 6), Varsola (Pa 7), Haripura lat (Pa 9), Thasara (Pa 10), Rakhial (Pa 12), Vanoda (Pa 14) and Anand (Pa 15) isolates. Naranpura lat (Pa 8) isolate showed flat and light white mycelial

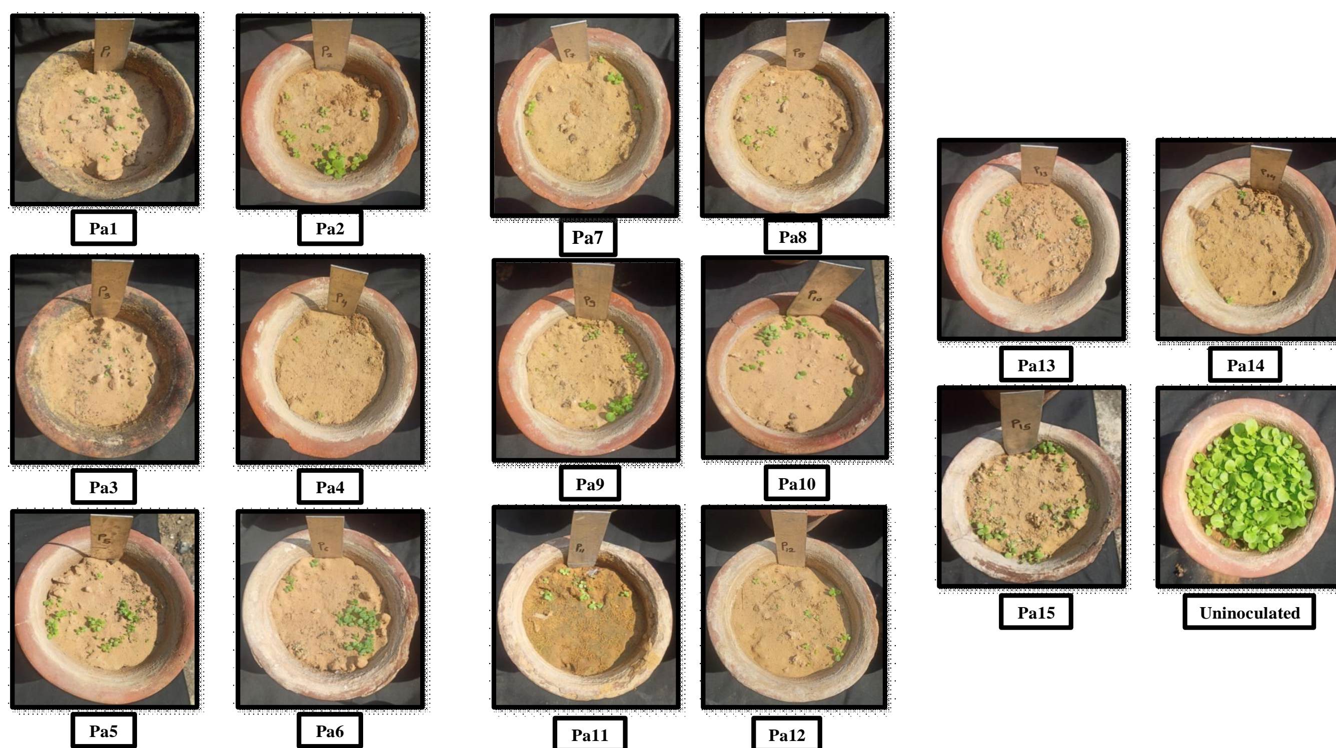

Plate 4 : Pathogenic variability of different isolates of the pathogen on *bidi* tobacco cv. GT 7.

Table 3 : Pathogenic variability of *Pythium* sp. on *bidi* tobacco cultivar GT 7 by soil inoculation method.

S. no.	Isolates	No. of seeds sown	No. of seeds germinated (14 DAS)	Mortality (%)	No. of seedlings survived (30 DAS)	Mortality (%)	Incubation period
1	Pa1	100	75	25	17	83	8-10
2	Pa2	100	78	22	15	85	4-6
3	Pa3	100	76	24	8	92	7-8
4	Pa4	100	70	30	2	98	12
5	Pa5	100	78	22	20	80	4-6
6	Pa6	100	76	24	18	82	4-6
7	Pa7	100	72	28	8	92	4-6
8	Pa8	100	70	30	7	93	4-6
9	Pa9	100	70	30	13	87	4-6
10	Pa10	100	76	24	16	84	4-6
11	Pa11	100	72	28	9	91	4-6
12	Pa12	100	76	24	9	91	7-8
13	Pa13	100	78	22	12	88	7-8
14	Pa14	100	72	28	3	97	8-10
15	Pa15	100	70	30	14	86	8-10
16	Uninoculated (Control)	100	94	6	91	9	-

DAS = Days after seeding.

was used for the growth of the isolates. It can be assumed that variation in the isolates may be inherent, since isolates were collected from *bidi* tobacco growing area in middle Gujarat. Hence, these variations indicated the existence of variability in this pathogen.

Variation in colony appearance and topography was similar as observed by earlier workers; Sagar (2006), Gaur and Chauhan (2007), Latha (2012), Yadav and Joshi (2012), Ashwathi *et al.* (2017). The morphological character of *P. aphanidermatum viz.*, mycelium size ranging from 2.8 to 4.0 ìm and oospores size ranging from 17-24 µm diameter were similar to the finding of Mehrotra and Agrawal (2004), Gherbawy *et al.* (2005) and Rangaswami and Mahadevan (2005).

Determination of Pathogenic variability

The pure cultures of the above isolates were used to prove pathogenic variability on *bidi* tobacco susceptible cultivar GT 7. The pathogenicity tests were carried out by soil inoculation method.

Soil inoculation

The result revealed that minimum germination was recorded in Pa 4, Pa 8, Pa 9, Pa15 followed by Pa 7, Pa 11, Pa 14, Pa 1, Pa 3, Pa 6, Pa 10, Pa 12, Pa 2, Pa 5 and Pa13, while in uninoculated (control) 94 per cent germination was observed. Thus, maximum pre-emergence mortality was recorded in Pa 4, Pa 8, Pa 9 and Pa 15 isolates of 14 days after seeding (Table 3).

Considering the post emergence damped-off (30 DAS) maximum mortality of 98 per cent was recorded in Pa 4 followed by Pa 14, Pa 8, Pa 3, Pa 7, Pa 11, Pa 12, Pa 2, Pa 13, Pa 9, Pa 15, Pa 10, Pa 1, Pa 6 and Pa 5 isolates. All the seedlings were healthy in uninoculated, which proved that all the fifteen isolates were pathogenic and isolate Pa 4 and Pa 14 were found highly virulent.

The variation in the *Pythium* sp. isolated from different locations of middle Gujarat by soil inoculation method has been observed in the same trend as earlier workers; Muthukumar *et al.* (2010), Jayalakshmi *et al.* (2021), Jukte *et al.* (2016) and Hassanisaadi *et al.* (2021).

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