Plant Archives Vol. 20, Supplement 1, 2020 pp. 3411-3416 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ISOLATION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF *PYTHIUM* SPECIES FROM BRINJAL GROWING TRACTS OF ERODE AND CUDDALORE DISTRICT

M. Subharathinam[1*], K. Sanjeevkumar[2], P.Balabaskar[3] and S.Kumar[4]

[1*,2,3]Depart. of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608002, (T.N.) India.
[4]Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608002 (T. N,) India.

Abstract

A roving survey conducted in districts of Tamil Nadu, *viz.*, Erode and Cuddalore district revealed endemic nature of the disease. Maximum mean severity of the disease was recorded at Vellithiruppur in Erode district registered the maximum incidence of 38.30 per cent followed by Matuvapattu (36.2%) in Cuddalore district. In general, the crop grown in red loam was more severely affected by damping-off. The isolates causing brinjal damping-off were identified as *P. aphanidermatum,* and *P. debarayanum.* Among the twenty one locations surveyed twelve isolates were identified as *P. aphanidermatum* was found to be more virulent and recording maximum disease incidence was isolate (P_1). Among the twenty one isolates of P_1, P_6, P_{11}, P_{15} and P_{20} the isolates was found to be more virulent. The fungus produced white colour cottony growth on PDA medium and recorded the maximum mycelial growth. Molecular identifications of the isolates were performed. *P. aphanidermatum* was identified based on the microscopic characteristics. TES buffer method was used for the isolation of DNA from *Pythium* isolates. Totally, 21isolates were examined for the amplification of Oomycete ITS region isolates showed amplified product with size range of 800 bp which showed these isolates were belongs to *Pythium* spp.

Key words : Isolates, *Pythium aphanidermatum*, molecular characterization, roving survey, *P. debarayanum*

Introduction

The brinjal (*Solanum melongena* L.) also known as the '*eggplant*' or '*aubergine*'/ '*Male insana*' and the '*Italian Melazana*', both of which translate to "*made apple*", is one of the most popular and principal vegetable crop grown in India and other parts of the world. The crop belongs the family Solanaceae and genus *Solanum.* The cultivated brinjal is presumed to be of Indian origin with China as secondary centre of origin. It has been cultivated for many centuries in India, China, Arabia, Bangladesh, Pakistan and Philippines and was probably introduced into Europe during the Moorish invasion of Spain in 16th century National horticultural board (www.ikisan.com, 2016-17). The crop is susceptible to diseases, such as leaf blight, leaf spot and rhizome rot. Among the various diseases, damping-off caused by *Pythium* spp. is a major problem in all brinjal growing areas of India. Damping-off resulted in yield loss of 20 to50% in Tamil Nadu (Kavitha *et al*., 2011).

The genus *Pythium* is one of the largest Oomycete genus and consists of more than 130 recognized species which are isolated from different regions of the world (Bala *et al*, 2010). Some species of *Pythium* are beneficial while most species are known to parasitize and cause infections in the roots of crop plants and ultimately damage them. Among the *Pythium* species, *P. aphanidermatum* is cosmopolitan in distribution and one of the most common plant parasitic pathogen of a number of different crop plants in warmer parts of the world. *P.aphanidermatum*is known to cause infection on a wide range of plant species, belonging to different families *viz*., Amaranthaceae, Araceae, Cruciferae, Cucurbitaceae, Euphorbiaceae, Gramineae, Leguminosae, Linaceae, Malvaceae, Moraceae, Solanaceae, Umbelliferae, Zingiberaceae (Waterhouse and Waterston, 1964). Different species of *Pythium* has been reported on brinjal. Historically, keys for identification of *Pythium* species are based on

***Author for correspondence :** E-mail : subhasakthi368@gmail.com

microscopic morphometrics and growth characteristics on specific media as well as biological characters (Mostowfizadeh-Ghalamfarsa and Banihashemi, 2005).

However, the overlapping of characters used for species classification frequently makes identification difficult and time intensive. Correct identification is necessary in order to adopt effective agricultural measures as soon as possible. Therefore alternative approaches must be developed to accurately identify and differentiate fungal species. Recently many molecular approaches including Polymerase Chain Reaction (PCR) have been tested to identify *Pythium* spp. (Klemsdal *et al.*, 2008) as well as other plant pathogenic fungi (Langrell *et al.*, 2011). Species-specific molecular primers are a powerful means for detecting *Pythium* in soil and plant samples. PCR method provides a rapid, simple and reliable alternative to conventional methods to identify common fungal isolates. Hence, the present study was conducted to assess the Isolation, identification and molecular characterization of *Pythium* species from brinjal growing tracts of Erode and Cuddalore district

Materials and Methods

Survey for occurrence of Damping-off caused by *P. aphanidermatum*

A field survey was conducted in 21 localities in Erode and Cuddalore District of Tamil Nadu to assess the extent of loss due to damping-off incidence. Disease incidence in brinjal crop was estimated, and the PDI was worked out by using the formula. The Percent Disease Incidence (PDI) was then calculated using the following formula (Mayee and Dator, 1986)

PDI (Pre-emergence) =

$$PDI\,(Post-emergence)=\frac{No.of\ plants\ affected}{Total\ No.of\ plants\ observed}\times 100$$

Isolation and identification of pathogen

The pathogen *Pythium* spp. was isolated from the diseased tissues of brinjal collected from different areas in Erode and Cuddalore districts during survey by tissue segment method (Rangaswami, 1958). The infected portions of diseased plants were cut into small pieces using sterilized scalpel and there were surface sterilized with 1 % sodium hypochlorite for one minute and washed in two changes of sterile distilled water and then placed on potato dextrose agar medium. These plates were incubated at 28 ± 2°C and the isolate was purified by single hyphal tip method. The culture was maintained on PDA slants and used for further studies.

Pathogenicity test

Paper cup of uniform size containing sterilized soil were used for proving pathogenicity. The inoculum of the pathogen multiplied in sand maize medium was mixed with soil @ 5:1 ratio at the time of sowing. About 5-10 brinjal (Var-CO_2) seeds were sown in each paper cup and after 15 days the plants showing the typical shrinkage at the collar region were pulled out and the pathogen was re-isolated on PDA slants. The culture thus obtained was compared with that of the original culture and the pathogenicity (Koch postulates) was proved. (Muthukumar *et al.*, 2010)

Morphological characters of *Pythium* spp. isolates

From the two to three days old culture plates 9mm culture disc of the pathogen was cut by using a sterilized cork borer and placed at the centre of the each sterile Petri dish containing 15 ml of previously sterilized solidified PDA medium and incubated for 2-5 days. The growth and morphological characters of the isolates *viz.*, colony morphology, mycelia growth rate, colony colour and septation were observed, measurements were taken under microscope after calibration with ocular and stage

Table 1: Survey on the incidence of damping-off of brinjal growing tracts in Erode and Cuddalore districts.

Sl. No.	Name of the village	Soil type	Damping-off incidence (%)
Erode District			
1	Vellithiruppur	Red loam	38.3 (38.23)
2	Bommanpatty	Red sandy soil	11.3 (19.64)
3	Anthiyur	Red sandy soil	13.5 (21.55)
4	Mylampadi	Literatic soil	19.1 (25.91)
5	Olagadam	Red loam	18.5 (25.47)
6	Bhavani	Red sandy soil	32.45(34.72)
7	Poonachi	Red loam	23.65 (29.09)
8	Ammapettai	Red sandy soil	21.6 (27.69)
9	Boothapadi	Red loam	15.4 (23.10)
10	Kaattur	Red sandy soil	23.89 (29.26)
Cuddalore District			
11	Annamalai nagar	Clay soil	28.45 (32.23)
12	Sivapuri	Clay loam	27.98 (31.93)
13	Vallampadugai	Clay soil	13.2 (21.30)
14	B.mutlur	Sandy soil	14.7 (22.54)
15	Jayakontapattinam	Sandy soil	26.4 (30.90)
16	Kurinjipadi	Sandy loam	12.9 (21.04)
17	Siruvathur	Clay soil	25.6 (30.39)
18	Angusettipalayam	Clay loam	25.3 (30.19)
19	Karumpur	Clay loam	20.6 (26.99)
20	Matuvapattu	Clay loam	36.2 (36.98)
21	Anuvampattu	Clay loam	16.2(23.67)

Data in parentheses indicate angular transformed values.

Table 2: Isolation and identification of *Pythium* Species from brinjal growing tracts of Erode and Cuddalore district.

Sl. No.	Name of the village	Isolate No.	District	Name of the species
1	Vellithiruppur	P_1	Erode	*P.aphanidermatum*
2	Bommanpatty	P_2	Erode	*P.aphanidermatum*
3	Anthiyur	P_3	Erode	*P.aphanidermatum*
4	Mylampadi	P_4	Erode	*P.aphanidermatum*
5	Olagadam	P_5	Erode	*P. debaryanum*
6	Bhavani	P_6	Erode	*P.aphanidermatum*
7	Poonachi	P_7	Erode	*P.debaryanum*
8	Ammapettai	P_8	Erode	*P.aphanidermatum*
9	Boothapadi	P_9	Erode	*P.debaryanum*
10	Kaattur	P_{10}	Erode	*P. debaryanum*
11	Annamalai nagar	P_{11}	Cuddalore	*P.aphanidermatum*
12	Sivapuri	P_{12}	Cuddalore	*P.debaryanum*
13	Vallampadugai	P_{13}	Cuddalore	*P.debaryanum*
14	B.mutlur	P_{14}	Cuddalore	*P.aphanidermatum*
15	Jayakontapattinam	P_{15}	Cuddalore	*P.aphanidermatum*
16	Kurinjipadi	P_{16}	Cuddalore	*P.debaryanum*
17	Siruvathur	P_{17}	Cuddalore	*P.debaryanum*
18	Angusettipalayam	P_{18}	Cuddalore	*P.aphanidermatum*
19	Karumpur	P_{19}	Cuddalore	*P.aphanidermatum*
20	Matuvapattu	P_{20}	Cuddalore	*P.aphanidermatum*
21	Anuvampattu	P_{21}	Cuddalore	*P.debaryanum*

Table 3: Cultural characteristics of various isolate of *Pythium* spp.

Sl. No.	Isolate No.	Mycelial growth (mm)	Colony character
1	P_1	90.00	White colour mycelium growth with fluffy colony
2	P_2	71.06	White colour mycelium growth with fluffy colony
3	P_3	73.12	White colour mycelium growth with fluffy colony
4	P_4	75.87	White colour mycelium growth with fluffy colony
5	P_5	76.95	White colour mycelium growth with fluffy colony
6	P_6	90.00	White colour mycelium growth with fluffy colony
7	P_7	81.66	White colour mycelium growth with fluffy colony
8	P_8	80.99	White colour mycelium growth with fluffy colony
9	P_9	74.43	White colour mycelium growth with fluffy colony
10	P_{10}	83.23	White colour mycelium growth with fluffy colony
11	P_{11}	90.00	White colour mycelium growth with fluffy colony
12	P_{12}	89.00	White colour mycelium growth with fluffy colony
13	P_{13}	67.90	White colour mycelium growth with fluffy colony
14	P_{14}	72.79	White colour mycelium growth with fluffy colony
15	P_{15}	90.00	White colour mycelium growth with fluffy colony
16	P_{16}	69.99	White colour mycelium growth with fluffy colony
17	P_{17}	87.76	White colour mycelium growth with fluffy colony
18	P_{18}	85.54	White colour mycelium growth with fluffy colony
19	P_{19}	78.00	White colour mycelium growth with fluffy colony
20	P_{20}	90.00	White colour mycelium growth with fluffy colony
21	P21	74.29	White colour mycelium growth with fluffy colony

micrometer.

Molecular characterization of *Pythium* spp. isolates

DNA extraction

DNA extraction, the fungal mycelial tissues were previously multiplied in liquid V8 medium (20% of V8 juice broth in distilled water) (King's Lynn Norfolk, USA) containing 2.5 g of $CaCO_3$. After 14 days of incubation under darkness at 25°C, the fungal tissues were harvested by separating the mycelium and the liquid medium. DNA was extracted from the harvested mycelia according to the procedure described by Mahuku, (2004). Mycelia were ground to a fine paste in a mortar containing TES extraction buffer (0.2 M TrisHCl [pH 8], 10 mM EDTA [pH 8], 0.5 M NaCl, 1% SDS) and sterilized acid-washed sea sand. Additional TES buffer containing proteinase K was added and the mixture incubated at 65ºC for 30 min. DNA was precipitated using ice-cold isopropanol and the pellet was washed twice with 70% ethanol, dried and dissolved in TE buffer (10 mM Tris-HCl [pH 8], 1 mM EDTA).(Nzungize1 *et al.,* 2011).

Polymerase chain reaction (PCR)

The internal transcribed spacer (ITS) region was amplified using universal primers ITS1 and ITS4. A reaction volume of 50 ìL containing 23.0µL nuclease free water, 25.0 ìL of Econo Taq PLUS GREEN 2X Master, 0.5 µl of each primer (10µM) [ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3')] and 1.0 ìL of DNA template (Lucigen Corporation 2505 Parmenter St, Middleton, WI 53562 USA) was used. Amplification conditions were achieved in a BIO RAD My Cycler thermal cycler programmed for initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 1 min. At the end of amplification reaction, a final extension step was accomplished at 72°C for 10 min. PCR products attained were run at 1% agarose gels dissolved in 1× TAE (Tris-Acetate EDTA buffer) concentration as the

Table 4: Effect of *Pythium* spp. isolates on the incidence of pre and post-emergence damping-off brinjal.

Sl. No.	Isolates	Pre-emergence damping-off (%)	Post–emergence damping-off (%)
1	P_1	37.81(37.94)	68.21(55.67)
2	P_2	11.21(19.56)	20.45(26.88)
3	P_3	18.17(25.23)	28.80(32.45)
4	P_4	20.12(26.65)	39.95(39.20)
5	P_5	19.87(26.47)	35.98(36.85)
6	P_6	33.25(35.21)	51.13(45.64)
7	P_7	22.11(28.04)	41.10(39.87)
8	P_8	21.11(27.35)	40.50(39.52)
9	P_9	19.49(26.19)	33.99(35.66)
10	P_{10}	23.58(29.05)	42.81(40.86)
11	P_{11}	25.67(30.44)	49.75(44.85)
12	P_{12}	25.53(30.34)	49.19(44.53)
13	P_{13}	13.55(21.59)	22.00(27.97)
14	P_{14}	21.11(27.35)	29.88(33.13)
15	P_{15}	24.32(29.54)	48.00(43.85)
16	P_{16}	16.85(24.23)	20.56(26.96)
17	P_{17}	24.81(29.87)	47.99(43.84)
18	P_{18}	24.33(29.55)	40.24(39.37)
19	P_{19}	20.59(26.98)	39.56(38.97)
20	P_{20}	33.92(35.62)	58.36(41.93)
21	P21	19.25(26.02)	31.82(34.33)
	SEdCD (p=0.05)	0.12 0.12	0.11 0.25

Data in parentheses indicate angular transformed values.

running solution followed with post staining of ethidium bromide (0.5 µg/ml). Electrophoretic migration was carried out for 1 h electrophoresed at 100 V. The amplified products were visualized and photographed under ultraviolet (UV) light. A 100 bp EZ Load molecular ruler (Bio-Rad Laboratories, Inc. CA, USA) was used to estimate the size of PCR products. (Binagwa *et al.*, 2016)

Results and Discussion

Survey on the incidence of damping-off in brinjal growing tracts of Erode and Cuddalore districts

Among the different locations of Erode and Cuddalore districts surveyed for damping-off incidence, Vellithiruppur in Erode district registered the maximum incidence of 38.30 per cent followed by Matuvapattu (36.2%) in Cuddalore district, Bhavani in Erode district (32.45%) and Annamalai Nagar in Cuddalore district (28.45%) in the decreasing order of merit. The other locations *viz*., Sivapuri (27.98 %), Jayakondapattinam (26.40 %), Siruvathur (25.60 %) and Anguchettipalayam (25.30 %) in Cuddalore district recorded moderate disease incidence while the minimum damping-off

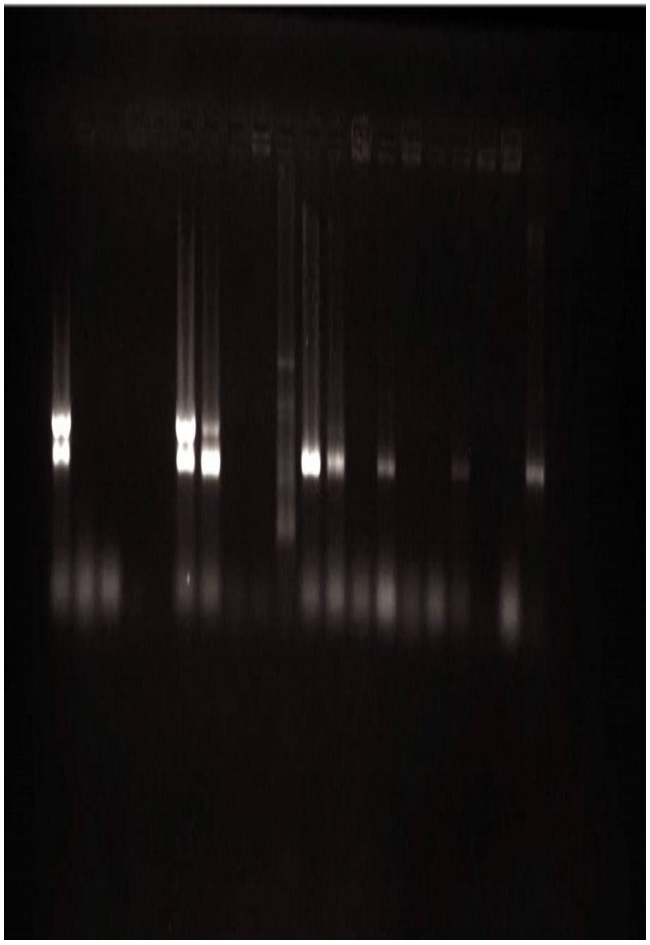

Fig. 1: PCR amplification of Oomycete ITS region of *Pythium* isolates.

incidence of 11.30 per cent recorded in Bommanpatty. The native strains of *Pythium* sp. were isolated from the surveyed locations and designated as P_1 to P_{21} (Table 1). The variation in the damping off incidence might be due to the interaction effect of the pathogen and environmental factors that prevailed in the respective locality (Rao and Krishnappa, 1996). Likewise, the earlier survey of Muthukumar (2008) in 21 locations of Tamil Nadu showed the occurrence of damping-off of chilli incidence to the extent of 38.9 per cent. In Cuddalore district in Tamil Nadu occurrence of damping-off incidence to the extent of 9.32 to 42.89 per cent in the brinjal crop (Rubini, 2013) has also been reported. These earlier reports corroborates with the present findings.

Isolation, identification and cultural characteristics of *Pythium* species

Among the species of *Pythium*, *P. aphanidermatum* (P_1) from Vellithiruppur area belonging to Erode district was found to be highly virulent in causing damping-off compared to other strains investigated in the present study. Among the twenty one locations surveyed twelve isolates (P_1, P_2, P_3, P_4, P_6, P_8, P_{11}, P_{14}, P_{15}, P_{18}, P_{19} and P_{20}) were

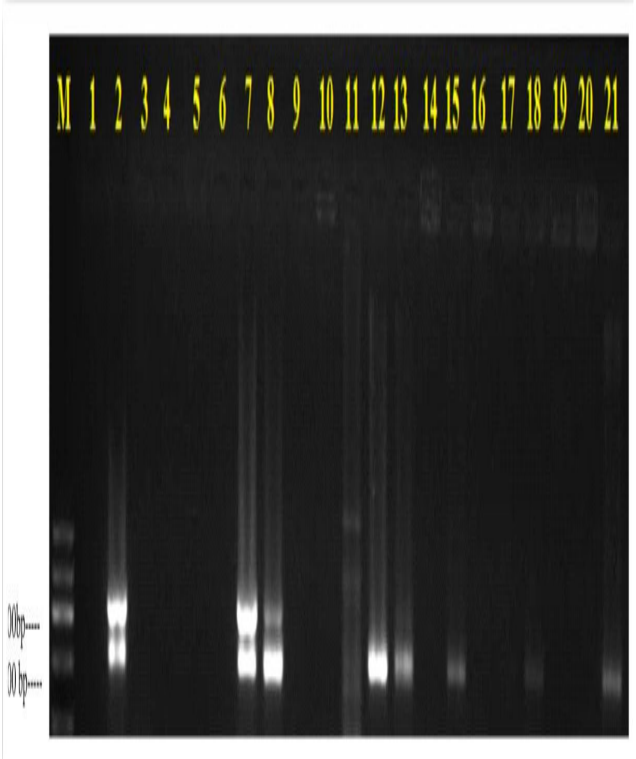

Fig. 2 : PCR amplification of *P. aphanidermatum* using species specific primer.

identified as *P. aphanidermatum* and the isolates from eight locations were identified as *P. debaryanum* (P_5, P_7, P_9, P_{10}, P_{12}, P_{13}, P_{16}, P_{17} and P_{21}). The various isolates of *Pythium*, 60% of the isolates belonged to *P. aphanidermatum* which explained that the isolate has adapted well to different ecological conditions and soil types. The next abundant species was *P. debaryanum* which was found to be associated up to 40% of the areas surveyed (Table 2). The 21 isolates of *Pythium* spp. exhibited variability with respect to mycelial growth, colony colour and colony character. Among the 21 isolates, the maximum mycelial growth was recorded by P_1, P_6, P_{11}, P_{15} and P_{20}. The minimum mycelial growth was recorded by P_{13}. Cultural characters like colony colour and colony pattern revealed that, all the isolates produced white colour cottony growth (Table 3).

The results depicted in table 4 showed significant difference in their virulence by all the 21 isolates of *Pythium* spp. in causing damping- off disease. However, the isolate of *P. aphanidermatum* collected from Vellithiruppur (P_1) was found highly virulent as it recorded the maximum pre and post-emergence damping-off (37.81 and 68.21 % respectively). This was followed by Matuvapattu (P_{20}) (33.92 and 58.36 % respectively). The minimum incidence of the disease was observed in Bommanpatty (P_2) (11.21 and 20.45 % pre and post-emergence damping off respectively). Studies on the different *Pythium* species in Tamil Nadu revealed the diversity of *Pythium* species such as *P. aphanidermatum* (Fitzpatrick, 1972), *P. debaryanum* (Water house, 1967) and *P. ultimum* (Trow, 1991). Emayavaramban, (1994) and Rafin and Tirily, (1995) have reported the variation in the virulence of the isolates of *P. aphanidermatum* in causing pre and post-emergence damping-off of tomato. Bhuvaneswari, (2008) reported that *P. aphanidermatum* isolate was highly virulent in causing damping-off in tomato. The results of the present study also corroborate with the findings of above researchers explaining that the isolate of *P. aphanidermatum* (P_1) is highly virulent in causing damping-off. Lai *et al*. (2015) tested pathogenicity at 25°C, and found that *P. ultimum* was the most pathogenic species, causing 97.0 per cent seed rot and 46.4 per cent damping-off and *P. aphanidermatum* was the second most pathogenic species, resulting in 88.5 per cent seed rot and 41.8 per cent damping-off in soybean.

Analysis of variability among the isolates of *Pythium* species from brinjal based on PCR-ITS

PCR analysis with twenty one isolate of *Pythium* sp indicated that molecular weight of DNA fragment ranged from 300-400 bp (Fig. 1& 2). PCR analysis has been used by several workers for identification of *Pythium* sp(Nzungize *et al*., 2011; Binagwa *et al*., 2016; Gichuru *et al*., 2016). The above reports lend support to the present findings. Further studies needed to confirm the interstain variation.

References

Bala, K.G., A.W.A.M. Robideau, Z.G. de Cock, A.M. Abad, S. Lodhi, A. Shahzad, Ghaffar, M.D. Coffey and C.A. Lévesque (2010). *Phytopythium gen. nov. Persoonia*, **24:** 136-137.

Bhuvaneshwari (2008). Biological control of damping-off disease of tomato caused by *Pythium aphanidermatum* (Edson) Fitz. *M.Sc. (Ag.) Thesis*, Annamalai University, India.

Binagwa, H., H. Bonsi and N. Msolla (2016). Morphological and molecular identification of *Pythium*spp. isolated from common beans (*Phaseolus vulgaris*) infected with root rot disease. *African Journal of Plant Science*, **10:**1-9.

Emayavaramban, S. (1994). Management of chilli (*Capsicum annuum* L.) incited by *Pythium aphanidermatum* (Edson) Fitz. With biocontrol agents, *M. Sc. (Ag.) Thesis*, TamilNadu Agricultural University, Coimbatore, India.

Fitzpatrick, H.M. (1972). Synonyms Nematosporangium. *Mycologia*, **24:** 683-687. *Fungal Biol*., **115:** 672-82.

Gichru, V., R. Buruchara and P. Okori (2016). Pathogenic and molecular characterization of *Pythium spp*. inducing root rot symptoms in other crops intercropped with beans in Southern western Uganda. *Journal of Applied*

Sciences,**104:** 9955-9964.

Kavitha, K., S. Nakkeeran and G. Chandrasekar (2011). Rhizobacterial mediated induction of defense enzymes to enhance the resistance of turmeric (*Curcuma longa* L) to *P. aphanidermatum* causing rhizome rot. *Arch. Phytopathol*., **45:** 199-219.

Klemsdal, S.S., M.L. Herrero, L.A. Wanner, G. Lund and A. Hermansen (2008). PCR based identification of *Pythium spp*. causing cavity spot in carrots and sensitive detection in soil samples. *Plant Pathol*., **57:** 877-886.

Lai, W., G.X. Allen, R.C. Elroy, B. Carolyn, Z. Jinxiu, Z. Shuzhen, L. Wenbin, W. Junjiang and L. Lijun (2015). Pathogenicity of *Pythium* species causing seed rot and damping-off in soybean under controlled condition. *Phyto. protection*, **91:** 3-10.

Langrell, S.R.H., O. Morel and C. Robin (2011). Touch down nested multiplex PCR detection of *Phytophthora cinnamomi* and *P. cambivora* from French and English chestnut grove soils. *Fungal Biol*., **115:** 672-82.

Mahuku, G. (2004). A simple extraction method suitable for PCR-based analysis of plant, fungal and bacterial DNA. *Plant Molecular Biology*, **22:**71-81.

Mayee, C.D. and V.V. Datar (1986). *Phytopathometry.* Technical bulletin, Marathwada Agricultural University, Parbhani, 125.

Mostowfizadeh-Ghalamfarsa, R. and Z. Banihashemi (2005). Identification of soil *Pythium* species in Fars province of Iran. *Iran. J. Sci. Technol*., **29:**79-87.

Muthukumar, A., A. Eswaran and G. Sangeeta (2010). Occurance, virulence and pathogenicity of species of *Pythium* inciting damping-off disease in chilli. *J. Mycol. Pl. Pathol*., **40:** 67-71.

Muthukumar, A. (2008). Management of chilli damping off caused by *Pythium aphanidermatum* (Edson) Fitz. with bacterial endophytes (*Pseudomonas fluorescens*) in glasshouse conditions. *Adv. Plant Sci*., **21:** 295-298.

Nzungize, J., P. Gepts and R. Buruchara (2011).Pathogenic and molecular characterization of *Pythium* species inducing root rot symptoms of common bean in Rwanda. *African Journal of Microbiology Research*, **5:** 1169-1181.

Rafin, C. and Y. Tirily (1995). Characteristics and pathogenisity of *Pythium* spp. associated with root rot of tomato in soilless culture in France. *Plant Pathology*, **44:** 779-785.

Rangaswami, G. (1958). An agar block technique for isolating soil microorganisms with special Pythiaceous fungi. *Sci.Cult*., **24:** 85.

Rao, V.K. and K. Krishnappa (1996). Ecology of soil borne plant pathogens with special references to *Meloidogyne* and *Fusarium* associated with chickpea wilt in Karnataka. *Indian J mycol. Pl. Pathol*., **26:** 64-68.

Rubini, R. (2013). Biological management of brinjal damping-off disease caused by *Pythiumaphanidermatum* (Eson) Fitz. *M.sc.(Ag.)Thesis*, Annamalai University, India.

Trow, A.H. (1991). Observation on the biology and cytology of *P. ultimum. Ann. Botany,***15:** 269-312.

Waterhouse, G.M. and J.M. Waterston (1964). *Pythium aphanidermatum*. CMI descriptions of pathogenic fungi and bacteria, No. 36. Commonwealth Mycological Institute: Kew, Surrey, UK. Waterhouse.

Weststeijn, W.A. (1990). Fluorescent Pseudomonads isolate E11-2 as biological agent for *Pythium* root rot in tulips.