INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 9 | ISSUE 1 | APRIL, 2016 | 358-361

● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

A CASE STUDY

DOI : 10.15740/HAS/IJPP/9.1/358-361

Symptomatology, isolation, identification and pathogenicity test of damping off disease in okra

■ S.R. JUKTE, S.L. BADGUJAR*, A.P. SURYAWANSHI, UTPAL DEY AND D.P. KULDHAR

Department of Plant Pathology, V.N. Marathwada Krishi Vidyapeeth, PARBHANI (M.S.) INDIA

ARITCLE INFO

Received : 12.09.2013
Accepted : 27.03.2016

KEY WORDS :
Damping off, Isolation, Okra, *Pythium* spp., Pathogenicity, Symptomatology

***Corresponding author:**
Email: sandeepbadgujar@rediffmail.com

ABSTRACT

Damping off diseases in okra is an economically most important and destructive disease of okra. The main characteristic symptom of the disease is pre- emergence damping off *i.e.* rotting of the seeds and seedlings before actual emergence from the soil and post-emergence damping off which is severe when the seedlings are in cotyledonous stage. The infected tissues become soft and water soaked resulting in toppling over of the entire plant on the soil surface. The test pathogen (*Pythium aphanidermatum*) was isolated successfully on the basal culture medium Potato dextrose agar, from the seedlings showing typical symptoms of damping off. The pathogen produced non-septate, well branched, colourless to white mycelium, lobed sporangia on indeterminate sporangiophores, and formation of resting spore (oospore) when observed under the microscope. Pathogenicity of *P. aphanidermatum* was proved by sick soil method in pot culture, sowing okra cv. PARBHANI KRANTI under screen house condition and by water agar method. The pathogen was reisolated on PDA from artificially diseased okra seedling, and compared its cultural and morphological characteristics with the original fungus isolated from the naturally damping off diseased okra plant.

How to view point the article : Jukte, S.R., Badgujar, S.L., Suryawanshi, A.P., Dey , Utpal and Kuldhar, D.P. (2016). Symptomatology, isolation, identification and pathogenicity test of damping off disease in okra. *Internat. J. Plant Protec.*, **9**(1) : 358-361.

INTRODUCTION

Okra [*Abelmoschus esculentus* L. (Moench)], is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. It belongs to the family Malvaceae originated from Ethiopia. Okra provides an important source of vitamins, calcium, potassium and other mineral matters which are often lacking in the diet in developing countries. It is called lady's finger in England, gumbo in the United States of America, guino-gombo in Spanish, guibeiro in Portuguese and okra in India.

Okra plant is affected by many diseases *viz.*, yellow vein mosaic (Yellow Vein Mosaic Virus), *Cercospora* leaf spot (*Cercospora abelmoschi*, *C. malayensis*, *C. hibisci*), *Fusarium* wilt (*Fusarium oxysporum* f. sp. vasinfectum), Powdery mildew (*Erysiphe*

cichoracearum), Damping off (*Pythium* spp., *Rhizoctonia* spp., *Phytopthora* spp.), Enation Leaf curl. Among these diseases damping off (*Pythium* spp., *Rhizoctonia* spp. *Phytophthora* spp.) cause heavy losses in initial plant stand in the field.

Pre and post emergence damping off disease caused by *Pythium* spp. in vegetable crops is economically very important worldwide (Ramamoorthy *et al.*, 2002). The genus *Pythium* consisting of over 120 species with life histories that range from saprotrophic, facultative parasites with wide host ranges known. In India species of *Pythium* recorded are *P. aphanidermatum, P. debaryanum, P. deliense, P. graminicola, P. ultimum* and *P. myriotylum* on various hosts (Muthukumar *et al.*, 2010). Of the different species of *Pythium, P. aphanidermatum* (Edson) Fitz. is reported from a large number of hosts (Mishra, 2010). *P. aphanidermatum* causes great loss in agriculture production. This fungus is an unspecialized parasite that has a wide host range, young tissues and plants are infected and affected much more severely by this pathogen (Jayaseelan *et al.*, 2012). Damping off incited by *Pythium aphanidermatum* causes more than 60 per cent losses in seedlings both in nursery and main field (Manoranjitham *et al.*, 2000). Considering the economic importance of the disease the present investigation was undertaken.

MATERIAL AND METHODS

The okra seedlings showing the symptoms of damping off were collected in the (from the Department of Horticulture) polythene bags, labeled and brought to the laboratory. These samples were processed after surface sterilization (0.15% $HgCl_2$) for isolation of *Pythium aphanidermatum*. The isolate of the test pathogen were purified, numbered and maintained on Potato dextrose agar slants and stored at 8 to 10°C in a refrigerator.

Pythium selective medium (PSM) :

The boiled and filtered extract of potatoes (200 g) is mixed with dextrose (20 g), agar-agar (20 g), streptocycline (250 ppm), carbendanzim (15 ppm) and mancozeb (50 ppm) dissolved by indirect heating (in boiling water 1000 ml), sterilized media in autoclave at 15psi pressure for 15 min.

Isolation :

Isolation of the test pathogen from local cultivar of okra seedlings showing discoloration and softening of collar region was collected. Isolation was carried under aseptic conditions on PDA medium and PSM medium. The previously collected rotten seedlings were washed and surfaces sterilized with 0.1 per cent $HgCl_2$ for 15 sec and then pass through three changes of sterile distilled water to remove traces of $HgCl_2$. After this, collar region stem bits comprise 0.5cm discolored and 0.5cm healthy portion were cut and placed on each of the sterilized PDA/PSM Petriplates. Three petriplates per replication were maintained. These Petriplates were labeled and incubated in an inverted position at 26 ± 2^0C. After 7-8 days of incubation, the well isolate colonies of fungus showing white mycelial growth were transferred onto PDA. The purified fungus culture was maintained on PDA/PSM slants in test tubes for further studies.

Pathogenicity test :

Pathogenicity of the test fungus was confirmed by sick soil method in pots under screen house conditions and by water agar method. Pure culture of test pathogen was multiplied on sand : maize meal medium (Sieved fine river sand 100g + maize meal 80g + distilled water 150 ml) for ten days and uniformly mixed (@ 100g / kg soil) with sterilized potting mixture of soil : sand : FYM (2:1:1).

In sick soil method, three pots were disinfected with 5 per cent Copper sulphate solution, pots were filled with potting mixture and inoculated with pure culture of the test fungus. One pot filled with sterilized potting mixture and without culture of *Pythium aphanidermatum* was maintained as uninoculated control. These pots were incubated for 15 days at room temperature, frequently stirred, watered regularly and allowed susceptible pathogen or fungus to colonize better in the pots. Then surface sterilized seeds of okra parbhani kranti variety were sown (@ 10 seeds/pot) and kept in screen house at room temperature and watered regularly. Observations on pre-emergence seed root and post emergence seedling mortality were recorded. The seedlings which showed dropping, toppling and discolorations with softening of collar region test were subjected to reisolation by passing through 0.1 per cent $HgCl_2$ and three changes of sterile water and then placed on sterile PDA Petri plates. Growth of the reisolated test fungus obtained was

transferred on PDA slants and compared with original pure culture of the test fungus obtained from naturally damping off diseased plants.

In water agar method, five test tubes were filled with the mixture of water agar and inoculum of the test pathogen where as other test tubes were filled with plain water without culture of *Pythium aphanidermatum* which were maintained as control. Then, seedlings of okra cv. PARBHANI KRANTI were placed in all test tubes. After 3-4 days observations on seedling mortality were recorded.

Identification of test pathogen :

Based on morphological and cultural characteristics *viz.*, nonseptate, well branched, colourless to white mycelium, lobed sporangia on indeterminate sporangiophore were observed under microscope and the pathogen was identified and confirmed as *Pythium aphanidermatum*.

Mass multiplication of *Pythium aphanidermatum:*

The mass multiplication of *Pythium aphanidermatum* pathogen was done by Potato Dextrose Broth and Sand:Maize medium for various studies.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Symptomatology :

The symptoms observed under glass house and field conditions were: pre- emergence damping off caused seed, any young seedlings to rot before they emerge from the growing medium while post-emergence damping off kill newly emerged seedlings. In post-emergence damping off the pathogen cause a water soaked soft brown lesion at the stem base near the soil line the pinches off the stem causing the seedling to topple over and die. The symptoms observed in present studies were similar to those described by Patil *et al.* (2005) and Zagade (2007).

Isolation :

Isolations were made from damping off diseased plant parts (Collar region rotten seed) of okra showing typical symptoms of brown discoloration and softening of collar region on Potato Dextrose Agar medium. Through sub culturing, the test pathogen was purified and pure culture of *P. aphanidermatum* obtained was maintained on PDA slant for further studies.

Pathogenicity test :

Pathogenicity of the test pathogen was proved *in vivo* by sick soil method in pot culture and by water agar method. In sick soil method, pathogenicity of the test pathogen was proved by sowing susceptible okra cultivar (Parbhani kranti) under screen house condition. The multiplication of the pathogen and it's infection to the seeds and seedlings followed by seedling mortality. Initially, infected seedlings exhibited discoloration, softening of collar region, toppling and finally diseased seedlings died. The symptoms observed in pathogenicity test on cv. Parbhani kranti are exhibited .

In water agar method, seedlings were placed in test tubes containing water agar with inoculums of *Pythium aphanidermatum* and another test tubes containing plain water which was kept for control. After 3-4 days seedling mortality was observed.

The fungus was re-isolated on PDA from artificially diseased seedlings of Parbhani kranti cultivar of okra. The cultural and morphological characteristics observed were found exactly identical to those of the original fungus isolated from naturally diseased okra plants. Thus pathogenicity of the test pathogen (*Pythium aphanidermatum*) was proved.

Identification :

Based on typical damping off symptoms *viz*. discoloration, seed rot softening of collar region of the seedling of the naturally and artificially diseased okra plants. Cultural and morphological characteristics, and pathogenicity test; the pathogen under investigation was identified and confirmed as *Pythium aphanidermatum.* Isolation, characterization and pathogenicity of the *P. aphanidermatum* identification of causing damping off of okra were successfully attempted and reported earlier by several workers (Bisht *et al.*, 1997; Ramamoorthy *et al*., 2002; Bhat and Shrivastava, 2003; Abdelzaher *et al.*, 2004; Arya, 2004; Bhora *et al.*, 2006 and Muthukumar *et al.*, 2010)

REFERENCES

Abdelzaher, M.A., Imam, M.M., Shoulkamy, M.A. and

Gherbowy, Y.M.A. (2004). Biological control of *Pythium* Damping off of Bush okra using Rhizosphere strains of *Pseudomonas fluorescens*. *Mycobiol.*, **32**(3): 139-147.

Arya, S. (2004). Evaluation of tomato seed treatment with biocontrol agents and soil amendment with neem cake against damping off. *Pl. Dis. Res.*, **20**(20) : 187-189.

Bhat, N.M. and Srivastava, L.S .(2003). Evaluation of some fungicides and neem formulation some fungicides and neem formulation against six soil borne pathogens and three *Trichoderma* spp. *in vitro*. *Pl. Dis. Res*., **18**(1): 56-59.

Bisht, G.S., Joshi, C., Bisht, D. and R.D. Khobe (1997). Distribution and pathogenicity of *Pythium* spp. from tomato. *Indian Phytopath*., **50**(1): 83-97.

Bohra, B., Vyas, B.B.N. and Mistry, K.B. (2006). Eco-friendly management of damping off in winter vegetables and tobacco using microbial agents and neem formulation. *J. mycol. Pl. Pathol.,* **36**(2): 178-181.

Jeyaseelan, E.C., Tharmila, S. and Niranjan, K. (2012). Antagonistic activity of Trichoderma spp. and Bacillus spp. against *Pythium aphanidermatum* isolated from damping off. *Archives Appl. Sci. Res*., **4**(4): 1623-1627.

Manoranjitham, S.K., Prakasam, V., Rajappan, K. and Amutha, G. (2000). Effect of two antagonists on damping off disease of tomato. *Indian Pyhtopath*., **53**(4): 441-443.

Mishra, V.K. (2010). *In vitro* antagonism of *Trichoderma* species against *Pythium aphanidermatum*. *J. Phytol.*, **2** (9): 28-35.

Muthukumar, A., Eswaran, A. and Sangeetha, G. (2010). Occurrence, virulence and pathogenicity of species of P*ythium* inciting damping off disease in chilli. *J. Mycol. Pl. Pathol.,* **40**(1): 67-71.

Patil, V.B., Deshpande, G.D., Suryawanshi, A.P., Khalikar, P.V., Mogle, T.R., Patil, M.A. and Zote, K.K. (2005). Isolation technique for *Pythium* spp. from soil with tomato seedling. *Indian Phytopath*, **58**(3): 359.

Ramamoorthy, V., Raguchander, T. and Samiyappan, R. (2002). Enhancing resistance of tomato and hot pepper to *Pythium* diseases by seed treatment with *fluorescent pseudomonads*. *J. Pl. Pathol*., **108**: 429-441.

Zagade, S.N. (2007). Investigation on damping off of chilli with reference to predisposition, varietal screening and control measure. M.Sc. (Ag.) Thesis, Marathwada Agricultural University, Parbhani, M.S. (INDIA).