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Isolation, identification, pathogenicity test and screening of brinjal cultivars against damping off in brinjal

■ V.M. GHOLVE*, V.R. TATIKUNDALWAR AND S.S. WAGH

Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, PARBHANI (M.S.) INDIA

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ABSTRACT

Brinjal or egg plant (*Solanum melongena* L.) is widely grown vegetable crop in India. Of the various diseases affecting brinjal, damping off caused by *Pythium ultimum* Trow. is one of the most destructive diseases causing several yield losses. The pathogen (*P.ultimum*) from naturally diseased brinjal plant showing typical symptoms of damping off was successfully isolated on the basal culture medium potato dextrose agar. The fungus *P.ultimum* produce nonseptate, well branched, colourless to whitish mycelium, sporangia on indeterminate sporangiophores when observed under the microscope. Pathogenicity of *Pythium ultimum* Trow. was proved by sick soil method in pot culture, sowing brinjal cv. HADGAON LOCAL under screen house condition. The pathogen was reisolated on PDA from artificially diseased brinjal seedling, and compared its cultural and morphological characteristics with the original fungus isolated from the naturally damping off diseased brinjal plant. Based on the typical symptoms of damping off, morphological and cultural characteristics, microscopic observations and pathogenicity test; the test pathogen was identified and confirmed *as P. ultimum.* Results revealed that of 13 brinjal cultivar lines, five lines *viz.*, EPM-564, Ajay, Kranti seed, Brinjal MG and Puneri Kateri were moderately susceptible with disease incidence in the range of 11.10 to 16.66 per cent and five lines *viz.*, Vishal, Arnav, Local Pingali, Local Kinwat, Local Hadgaon were susceptible with the disease incidence of about 22.21 to 44.44 per cent.

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

INTRODUCTION

The brinjal growers were facing the problem of pre-emergence and post emergence damping off diseases in brinjal at nursery stage every year. Among the several factors responsible for reduction in plant stand at nursery as well as in field, *Pythium ultimum* Trow. is one of the most important and destructive disease of brinjal. The pathogen has been reported to inflict losses upto 80 per

cent in germination in tomato (Patil *et al.*, 2005); chilli, brinjal, cabbage and cauliflower (Zagade, 2007). Bhora *et al.* (2006) reported 68.40 per cent damping off (*P. ultimum* Trow.) in brinjal.

The damping off in brinjal is caused by *Pythium* spp, including *P. aphanidermatum, P.irregulare and P.ultimum* Trow, which can cause Pre -emergence damping off results in seed rot before these emerge out of the soil . The post emergence damping off phase is characterized by infection of the young juvenile tissues of the collar at the ground level.

The infected tissue become soft and water soaked, the collar portion rots and ultimately seedlings collapse and die. The guaranteed supply of quality seedlings in required quantities is a major pre requisite for stabilized production of Brinjal. While raising seedlings in beds, the farmers face major problem of damping off incited by *Pythium* spp.

Keeping in view and importance of brinjal and losses incurred damping off (*Pythium ultimum* Trow.) disease, present investigations on the aspects *viz.*, isolation, pathogenicity and identification of the pathogen and screening were undertaken.

MATERIAL AND METHODS

Collection of disease samples :

The brinjal seedlings on nursery beds showing the symptoms of damping off were collected in the (at the Department of Horticulture) polythene bags, labeled and brought to the laboratory. These samples were processed after surface sterilization (0.1% $HgCl_2$) for isolation of *Pythium ultimum* Trow. The isolate of the test pathogen were purified, numbered and maintained on Potato dextrose agar slants and stored at 8 to 10^0C 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 fungus from local cultivar of Brinjal seed expressing pre-emergence rotting of the seeds were collected. Similarly, Brinjal toppled seedlings of local variety showing discolorations and softening of collar region was collected. Isolation was carried out under aseptic conditions on PDA medium and PSM medium the previously collected rotten seeds and seedlings were washed and surface 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 0.5cm healthy portion were cut and placed on each of the sterilized PDA / PSM petriplates. Likewise 3 to 4 rotten seeds were placed on sterilized PDA / PSM Petriplates. There petriplates replication were maintained. These petriplates were labeled and incubated in an inverted position at 26 $\pm$ 2^0C. After 7-8 days of incubation, the well isolated 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 of isolated fungal culture :

Pathogenicity of the test fungus was confirmed by sick soil method in plastic pots under screen house conditions pure culture of test pathogen was multiplied on sand : maize meal medium(Plate A) (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).

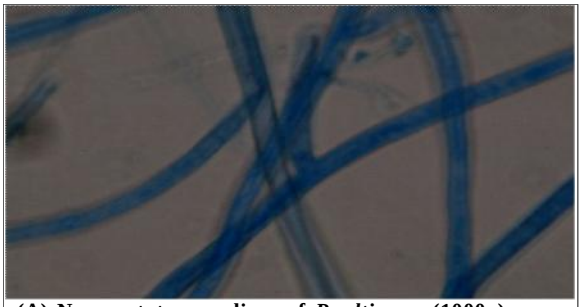
Plate A : Mass multiplication of *P. ultimum* on sand : maize medium

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

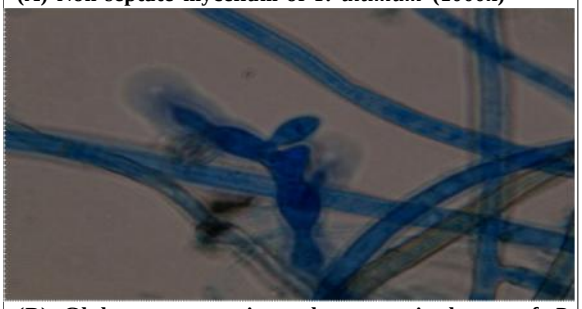
pot filled with sterilized potting mixture and without culture of *Pythium ultimum* Trow. was maintained as uninoculated control. These pots were incubated for 15 days at room temperature, frequently stirred, watered regularly and allowed susceptible pathogen or fungus to colonize better in the pots. Then surface sterilized seeds of brinjal susceptible Hadgaon local variety were sown (@6 seeds /pot) and kept in screen house at room temperature and watered regularly. Observations on pre – emergence seed rot 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.

Identification of test pathogen :

Based on morphological and cultural characteristics *viz*., nonseptate, well branched, colourless to white mycelium, sporangia on indeterminate sporangiophores were observed under microscope and the pathogen was identified and confirmed as *Pythium ultimum* Trow. (Plate B).



(A) Non-septate mycelium of *P. ultimum* (1000x)



(B) Globous sporangia and sporangiophores of *P. ultimum* (1000x)

Plate B : Microphotographs of *P. ultimum*

Varietal screening :

To identify the sources of damping off disease resistance in Brinjal varieties/ cultivars/ were screened against *P. ultimum* Trow., applying sick soil technique in pot culture under screen house conditions at the Department of Plant Pathology during *Kharif*, 2011. For these pathogen multiplied on sand: maize medium was mixed with autoclaved (30 lbs for 30 min) potting mixture soil : sand : FYM (2:1:1) (@ 25 gm / kg potting mixture) and filled in to pots, (disinfected with 5 % solution of copper sulphate) watered lightly and incubated at room temperature for two weeks in screen house. Within this period test pathogen multiplied in the pots.

Disinfected plastic pots were filled with sterilized soil and inoculcated with sand: maize base culture of *Pythium ultimum* Trow. was inoculated and water regularly to make sick soil. A total 13 Brinjal lines *viz.* Brinjal EPH-574, Ajay, Vishal, Kranti, Manjari, Safal (Brinjal-MGL) Puneri Kateri selection, Kalpataru, Panch Ganga, Arnav, Local Pingali, Local Hadgaon, Local Kinwat were sown (6 seeds/pot) in the pots containing sick soil and incubated in screen house.

Observations on number of plants per cultivar damped off cultivar were recorded at 7, 14, 21 28 and 35 days after at sowing and average of four replication was calculated. Finally the per cent incidence was calculated by the given formula devised by Mayee and Datar (1986).

$$\% \text{ disease incidence} = \frac{\text{Number of infected plant per units}}{\text{Total no. of plant observed (Healthy and infected)}} \times 100$$

Table A : Scale/Description of the symptom for damping off of brinjal

Sr. No.	Reaction	% disease incidence
1.	Immune	No symptoms on plants
2.	Resistant (R)	1% or less mortality
3.	Moderately resistance (MR)	1 - 10 % mortality
4.	Moderately susceptible (MS)	11-20% mortality
5.	Susceptible(S)	21-50% mortality
6.	Highly susceptible (HS)	51% or more mortality

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 (Plate 1). 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 brinjal showing typical symptoms of brown discoloration and softening of collar region on Potato Dextrose Agar medium. (Plate 2) Through sub culturing, the test pathogen was purified and pure culture of *P. ultimum* the culture obtained was

Plate 1 : Brinjal seedlings showing damping off (*P. ultimum)*

Plate 2 : Pure culture of *Pythium ultimum Trow.* On PDA medium

Table 1 : Pre and post–emergence mortality caused by *P. ultimum* in brinjal cv. HADGAON LOCAL

Treatments	Germination (%)	Mortality (%)		Total mortality (%)
		Pre emergence	Post emergence	
Sick soil (inoculated)	55.00	45.00	40.00	85.00
(Control)	100.00	0.00	12.00	12.00

* Average of three replication

Table 2 : Reactions of brinjal cultivars against *Pythium ultimum*

Tr. No.	Cultivars	Mean PDI*	Disease reactions
V_1	EPM-574	11.10	Moderately susceptible
V_2	Ajay	11.10	Moderately susceptible
V_3	Vishal	22.21	Susceptible
V_4	Kranti seed	16.66	Moderately susceptible
V_5	Manjari	05.55	Moderately resistant
V_6	Brinjal MG	16.66	Moderately susceptible
V_7	Puneri Kateri	16.66	Moderately susceptible
V_8	Kalpataru	05.55	Moderately resistant
V_9	Panchaganga	05.55	Moderately resistant
V_{10}	Local Pingali	27.77	Susceptible
V_{11}	Local Kinwat	27.77	Susceptible
V_{12}	Arnav	22.21	Susceptible
V_{13}	Local Hadgaon	44.44	Susceptible

*Mean of four replications

maintained on PDA slant for further studies.

Pathogenicity test :

Pathogenicity of the test pathogen was proved *in vitro* by sick soil method in plastic pot culture (Plate 3), sowing susceptible local brinjal cultivar (Hadgaon local) under screen house condition. The detailed observations on seed germination, pre-emergence and post-emergence mortality were recorded (Table 1). Isolation, characterization and pathogenicity of the *P.ultimum* identification of causing damping off of brinjal were successfully attempted and reported earlier by several workers (Bisht *et al.,* 1997; Bhat and Shrivastava 2003; Arya, 2004; Bhora *et al.,* 2006; Usharani and Satheesh, 2007; Rani and Kumar, 2007 and Muthukumar *et al.*, 2010).

Plate 3 : Pathogenicity on brinjal cv. HADGAON LOCAL

The result (Table 1) on pathogenicity test revealed that the test pathogen (*P. ultimum*) caused considerably reduced seed germination (55%), maximum pre-emergence (45%) and post–emergence seedling mortality (40%) as compared to uninoculated control germination. 100 per cent pre-emergence seed rot over post emergence mortality (12%).

Initially, infected seedlings exhibited discoloration, softening of collar region, toppling and finally diseased seedlings died. The fungus was re-isolated on PDA from artificially diseased seedlings of local Hadgaon cultivar of brinjal. The cultural and morphological characteristics observed were found exactly identical to those of the original fungus isolated from naturally diseased brinjal plants. Thus pathogenicity of the test pathogen (*Pythium ultimum*) was proved.

Varietal screening :

A total of 13 brinjal germplasm lines, varieties/ cultivars were screened using sick soil in pot culture under screen house conditions (Plate 4). On the basis of per cent damping off incidence, the test brinjal lines were categorized as: resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible.

Plate 4 : *In vitro* screening of Brinjal cultivars/ varieties against *P. ultimum*

The results (Table 2) indicated that, among 13 varieties/ cultivars of brinjal evaluated, none was found resistant to tolerant to the disease (Plate 4). The mean damping off disease incidence was ranged from 05.55 to 44.44 per cent.

Of the 13 varieties/ cultivars of brinjal, three lines *viz.,* Manjari Kalpataru and Panch ganga were moderately resistant with disease incidence of 05.55 per cent; five lines *viz.*, EPM-564, Ajay, Kranti seed, Brinjal MG and Puneri Kateri were moderately susceptible with disease incidence in the range of 11.10 to 16.66 per cent and five lines *viz.*, Vishal, Arnav, Local Pingali, Local Kinwat, Local Hadgaon were susceptible with the disease incidence of about 22.21 to 44.44 per cent.

Thus, majority of the brinjal cultivars/varieties, genotypes screened were found, moderately resistant, moderately susceptible and susceptible to damping off disease and none was resistant. Results of the present study on varied reactions of brinjal entries against damping off disease (*P.ultimum*) are on the same line are reported earlier by Sharma, 2003; Bhora *et al*, 2006.

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