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STUDIES ON ISOLATION PURIFICATION AND PATHOGENICITY TEST OF DIFFERENT FUNGAL ISOLATES OF *MACROPHOMINA PHASEOLINA* CAUSING ROOT ROT DISEASE TO *RAUWOLFIA SERPENTINA*

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ABSTRACT:

Rauwolfia serpentina is an important medicinal herb used in Ayurveda and Alleopathy. Reserpine is an indole alkaloid present in *Rauwolfia serpentina* viz. reported to possess anti-hypertensive and tranquilizer property. Reserpine is present in all plant parts, but more in roots. Various factors are responsible for growth of plants and active constituent present in it (Dey, Abhijit and De, J. N. (2010). *Macrophomina phaseolina* (Tassi) Goid is a soil borne fungus causes root rot diseases to Sarpagandha (*Rauwolfia serpentina*) that affects active constituent of root. The fungus infects the root and lower stem of over 500 plant species and is widely distributed in the United States (Wyllie, 1988). **The healthy and infected roots were collected from medicinal plant garden, M. A. University, Parbhani and Medicinal plant garden,** M. P. K. V., Rahuri. **The healthy and infected roots were brought to laboratory, labelled properly and preserved for further study.**
Key words:- *Macropham phaseolina , Sarpagandha, roots, etc.*

INTRODUCTION:

The fungal pathogen *Macrophomina phaseolina* (Tassi) Goid was isolated from the *Rauwolfia serpentina* Benth ex. kurz roots collected from medicinal plant garden, M. A. University, Parbhani and Medicinal plant garden, M. P. K. V., Rahuri showing typical root rot symptoms i.e. black conductive tissue. The infected roots were sterilized with 0.5% sodium hypochlorite solution. The sterilized root were used for isolation of fungal pathogen i.e. ***Macrophomina phaseolina.***

MATERIALS AND METHODS:

Isolation of the pathogen:

The diseased roots of Sarpagandha were collected from medicinal plant garden M.A. University, Parbhani, Medicinal plant garden M. P. K. V., Rahuri. The diseased roots were designated as IRS-1and IRS-2 respectively. The healthy and diseased roots were brought to laboratory and preserved for further study. These infected roots were used for isolation of fungal pathogen.

The fungal pathogen was isolated from the Sarpagandha roots collected from different gardens showing typical root rot symptoms. The diseased roots were sterilized with 0.1% Sodium hypochloride solution. The isolation of pathogen was carried out by taking small portion from four different diseased roots and inoculated aseptically on Potato Dextrose Agar medium (PDA). These plates were incubated for seven days at room temperature,

Purification:

The Purification was carried out by using hyphal tip technique as given by Reddy, et. al. (2006). The two isolated fungal mycelia hyphal tips were transferred subsequently for three times on fresh PDA media and incubated for 7 days at room temperature to obtain pure culture. Thus pure culture of two isolates of fungal pathogens was obtained. These cultures were maintained on PDA for further studies.

Identification:

The fungal pathogens i.e. *Macrophomina phaseolina* was identified on the basis of culture characters, growth, vegetative and reproductive

structures. The mycelium and reproductive structures are as described by Barnett. (1970), Alexopolous, et. al. (1996). Mukadm et. al., (2006) and Nagmani et. al. (2006).The pure culture of *Macrophomina phaseolina* was sent to Agharkar Research Institute, Pune for identification. They also confirmed that the pure culture is of *Macrophomina phaseolina*

Pathogenicity test:

The Pathogenicity test was carried out by inoculating with homogenized fungal mycelial suspension of *Macrophomina phaseolina* (Tassi) Goid Mp-1 on dried roots of *Rauwolfia serpentina.* Ten days after inoculation the symptoms appeared on inoculated roots as black colored. Reisolated and purified culture from these artificially inoculated roots was similar to that of original culture and symptoms were also similar to that of infected root. The roots which were not inoculated with the homogenized mycelial suspension did not show any symptoms of this disease. Thus, pathogenicity test is proved as Kareppa, (1992), Kareppa, (1999) and Wakle and Kareppa, (2000).

Experimental results and discussion:

Isolation of fungal pathogen:

The isolation of pathogen was made by taking small portion of the infected root and inoculated aseptically on Potato Dextrose Agar medium (PDA). The plates were incubated for 7 days at room temperature. After 7 days fungal growth of *Macrophomina phaseolina* was observed on PDA media plates as shown in Plate-I and II. Two different isolates were isolated i.e. Mp-1 from IRS1 root sample and Mp-2 from IRS2 root sample of *Rauwolfia serpentina.*

Purification:

Purification of *Macrophomina phaseolina* was carried out by hyphal tip technique as given by Reddy et.al. (2006). Isolated fungal mycelium was transferred for three times on fresh PDA media to obtain pure culture (Plate-III& IV).

Identification:

The *Macrophomina phaseolina* was identified on the basis of growth, vegetative and reproductive characters. The mycelium and reproductive structures are described as Alexopolous and Mims (1979). The pure culture of *Macrophomina phaseolina* was also sent to Agharkar Research Institute, Pune for identification. They also confirmed that the pure culture was *Macrophomina phaseolina.*

Morphological characters of the fungus:

The *Macrophomina phaseolina* fungal mycelium is septate and black colored. The conidiophores were formed in groups, straight and olivaceous brown in color. The conidia were solitary straight, oblong, ellipsoidal tapering to beak, olivaceous brown colored, length 145-290 µm and 14-18 µm thick in the broadest part with 8 to 10 transverse and up to 4 longitudinal septa. The beaks were flexuous, pale and branched. Thus, the pathogen causing root rot of *Rauwolfia serpentina* has been identified as *Macrophomina phaseolina* (Tassi) Goid.

The microsclerotia of *Macrophomina phaseolina* was formed in seven days old isolates and it is highly variable in size and shape in different media. The microsclerotia are formed by joining of hyphal tip cells. The 40 to 200 hyphal tip cells joined with each other by melanin material. Each cell of hyphal tip produced individual microsclorotia.

Maintenance of pure culture:

The pure fungal culture of *Macrophomina phaseolina* isolates Mp-1and Mp-2 was maintained on PDA slants for further studies. (Plate-V)

Pathogenicity test:

For the study of pathogenicity test Mp-1 isolate was selected. The Pathogenicity test was carried out by inoculating homogenized fungal mycelial suspension of Mp-1 isolate on dried

roots of Sarpagandha. Ten days after inoculation the symptoms appeared on inoculated roots as black spots. The fungal pathogen was artificially inoculated on roots shows similar characters to that of original culture and symptoms were also shows similar to that of naturally infected roots. The roots which were not inoculated with the fungal mycelial suspension did not show any symptoms of the disease (Plate-VI).

It was also observed that the fungal infection shows black conducting tissues of Sarpagandha roots. The reisolated fungal pathogen from a+rtificially inoculated roots showed similar fungal growth on potato dextrose agar (PDA) media.

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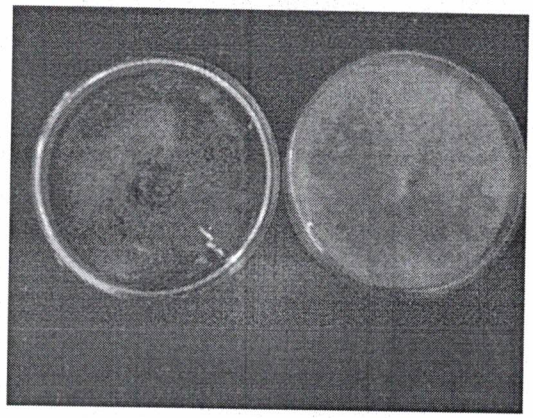
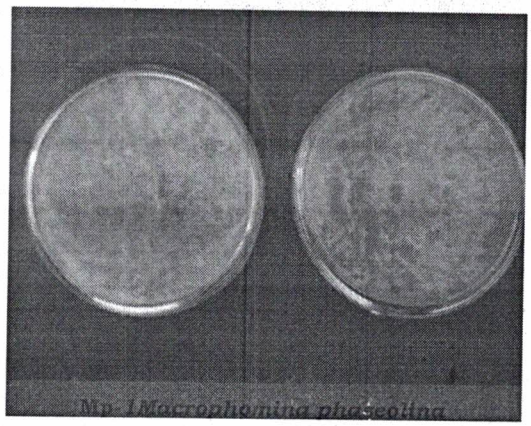


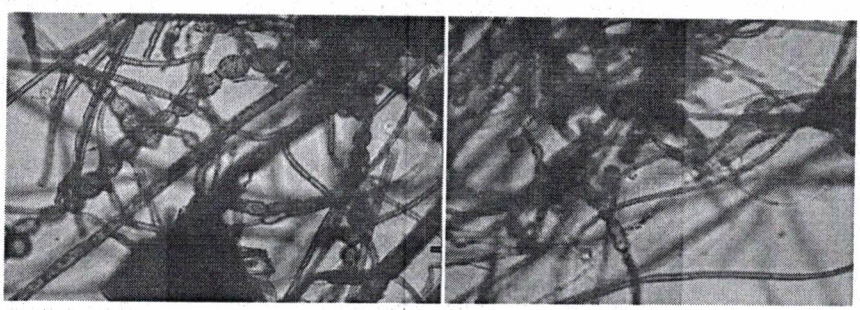
Mp-*1Macrophomina phaseolina*

Plate-I: Isolated *Macrophomina phaseolina* (Mp-1*)* from diseased roots collected from M. A. University, Parbhani.



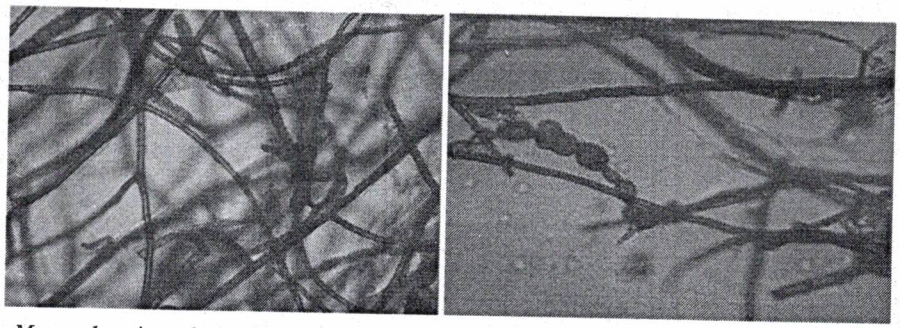
Mp-*2Macrophomina phaseolina*

Plate-II: Isolated *Macrophomina phaseolina* (Mp-2*)* from diseased roots collected from M. P. K.V., Rahuri.



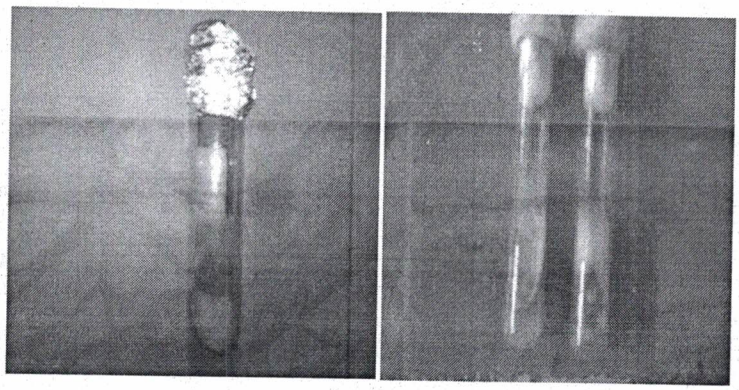
Macrophomina phaseolina (Mp-1) *Macrophomina phaseolina* (Mp-1)

Plate- III: Mycelium, conidia and sclerotia of Mp-1 isolates of *Macrophomina phaseolina* and its identification.



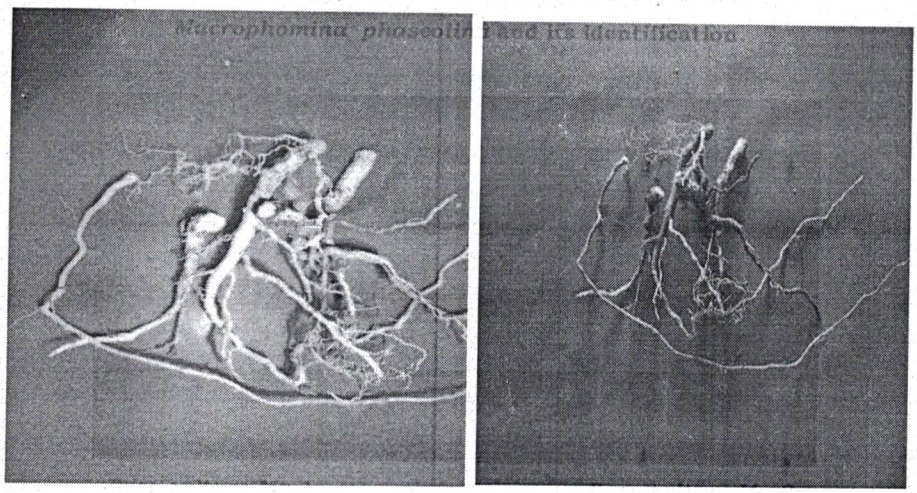
Macrophomina phaseolina (Mp-2) *Macrophomina phaseolina* (Mp-2)

Plate- IV: Mycelium, conidia and sclerotia of Mp-2 isolates of *Macrophomina phaseolina* and its identification.



Pure culture Mp-1 **Pure culture Mp-2**

Plate-V: Pure culture of *Macrophomina phaseolina*.



Healthy root **Inoculated root**

Plate- VI: pathogenicity test of *Macrophomina phaseolina*.

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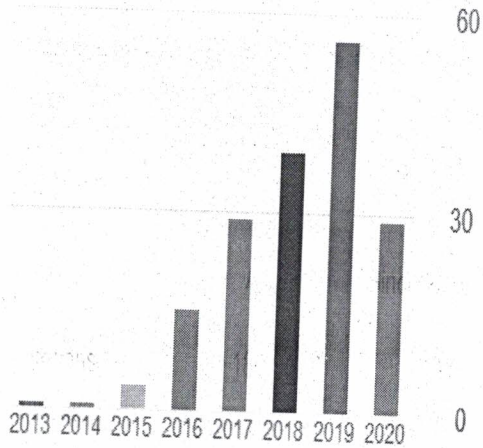

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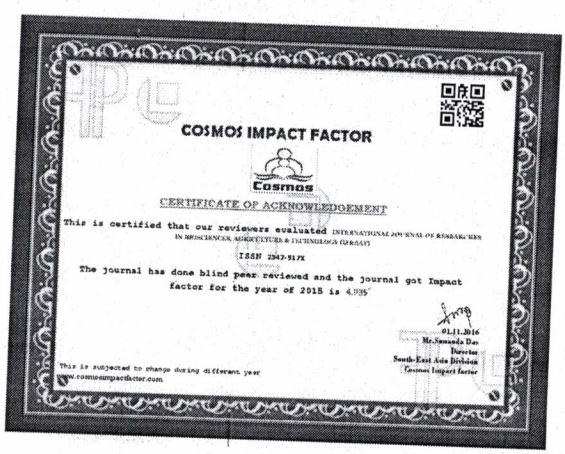

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