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# Inhibitory effect of bacterial antagonists on the growth of *Macrophomina phaseolina* (Tassi.) Goid. Causing charcoal rot of sunflower *(Helianthus annus L.) invitro*

**A. Karmel Reetha*[1] and S. Mohan[2]**

[1]Department of plant pathology, Vanavarayar Institute of Agriculture, Pollachi - 642103 Tamil Nadu, INDIA
[2]Department of plant Pathology, Agricultural Collège and Research Institute, Madurai - 625104 Tamil Nadu, INDIA
*Corresponding author. E-mail: reelee.pat@gmail.com

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**Abstract:** Charcoal rot caused by *Macrophominaphaseolina*is a major disease causing in sunflower plant. The pathogen invasion occurs from the seedling to maturity stage. To overcome this problem *in vitro*, sensitivity of *M. phaseolina* was determined through inhibition zone technique to various isolates of antagonistic bacteria like seven isolates of *Pseudomonasfluorescens*($EPf_2$, $EDPf_3$, $APf_4$, $CPf_5$, $MPf_6$, $KPf_7$and$Pf_1$)andseven isolates *Bacillus subtilis* ($EBs_1$, $EDBs_2$, $ABs_3$, $CBs_4$, $MBs_5$, $KBs_6$and$Bs_{10}$) amended into PDA medium. The results showed that the entire bacterial antagonist were effective against the fungus *M. phaseolina* and exhibited appreciable amount of inhibition. Among these bacterial antagonistsignificantly compared to the control *P.fluorescens (*Pf1) proved to be the most effective (71.49 %) with an inhibition zone of 5.00 mm reducing the colony growth of *M. Phaseolina*followed *B. subtilis* (65.92 %)inhibition zone of 17.80 mm respectively over control. However, from these studies it is concluded that an isolate of various antagonist can vary in its sclerotia producing ability on root.

**Keywords:** Bacterial biocontrol, *In-vitro*, *Macrophomina phaseolina,* Sunflower

## INTRODUCTION

Sunflower (*Helianthus annus, L.*) is an important oil seed crop in India popularly known as "Surajmukhi." The name "*Helianthus*" is derived from 'Helios' meaning 'sun' and 'anthos' meaning 'flower'. It is known as sunflower as it follows the sun by day, always turning towards its direct rays. It is one of the fastest growing plants which belong to family Asteraceae (Compositae) (Rodriguez *et al.*,2002). *M. phaseolina* the causal agent of charcoal rot is a serious threat for sunflower crop especially in the arid regions of the world (Hoes, 1985).

It has been estimated that diseases can cause an average annual loss of 12 per cent in yield from nearly 12 million hectares of the world (Zimmer and Hoes, 1978; Kolte, 1985). The fungus has a host specific behaviour and a high degree of variation in its morphological, cultural and pathological properties, even when it is isolated from different parts of the same plant (Khan, 2007). Bhutta*et al*. (1995) studied the transmission process of *M. phaseolina*from root to upward growth of the sunflower and development of fungus establishment in the seedlings within 48 hours of entering in the host tissue. Shekhar *et al*. (2006) on the basis of colony colour, divided seven isolates of *M. phaseolina* into four groups namely greyish white, blackish grey, dark black and cottony white colonies Muhammad *et al.* (2010) observed that in dual culture assays, all antagonists inhibited the growth of *M. phaseolina*. *Rhizobium melilot*iand *Bacillus subtilis*showed maximum inhibition in the growth of *M. phaseolina*in sunflower. Cook and Baker (1983) reported that the use of biological agent for the control of plant diseases is an alternative method of chemical control. Several disease management strategies are available *viz.*, cultural, biological, resistant cultivars, crop rotation and chemical control (Kamal, 2006). The objective of the study was toisolate and identifydifferent isolates of pathogens isolates of antagonists from the rhizosphere region of sunflower root and;*In vitro* screening of different isolates of antagonists against *M. phaseolina*.

## MATERIALS AND METHODS

**Isolation of pathogen (*M. phaseolina*):** The pathogen inciting root rot caused by*M. phaseolina*was isolated from the diseased stems and roots of sunflower collected from different places of Tamil Nadu. The surface sterilized tissues were plated on potato dextrose agar (PDA)in sterile Petri plates and incubated at room temperature (28±2˚C) for seven days.slants and sand maize media for further studies(Rangaswami, 1993).

**Sand maize medium:**

Broken maize or Maize Powder - 100g
Sieved white sand - 1900g

Seven isolates of *M. phaseolina*collected from various locations were multiplied on sand maize medium (sand and ground maize grains mixed in the ratio of 19:1, moistened and autoclaved in saline bottles at 20lb for two hours) and incubated at 28±2°C for 21 days.

**Morphological characters of *M. phaseolina*isolates:** From the seven days old culture plates, nine mm 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 and solidified PDA medium. The plates were incubated at room temperature (28±2$^0$C) for five days. The growth and morphological characters of the isolates *viz.*, colony morphology, mycelia growth rate, colony colour and shape of sclerotia were noted. The pycnidia were observed under microscope (magnification 45X) after calibration with ocular and stage micrometer (Fig 1).

**Isolation of antagonists from the Rhizosphere region:** Antagonistic fungi and bacteria were isolated from the rhizosphere soil collected from different sunflower growing areas of Tamil Nadu (Table 2). The plants were pulled out gently with intact roots and the excess soil adhering on roots was removed gently. Ten gram of rhizosphere soil was transferred to 250 ml Erlen Meyer flask containing 100 ml of sterile distilled water. After thorough shaking, the antagonist in the suspension was isolated by serial dilution plate method. From the final dilutions of $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$, one ml of each aliquot was pipetted out, poured in sterilized Petri dish containing King's B medium (King *etal.*, 1954) and nutrient agar medium separately and they were gently rotated clockwise and anti-clockwise for uniform distribution and incubated at room temperature (28±2$^0$C) for 24 hours. Colonies with characteristics of *Bacillus* spp., *Pseudomonas* spp. were isolated individually and purified by streak plate method (Rangaswami,

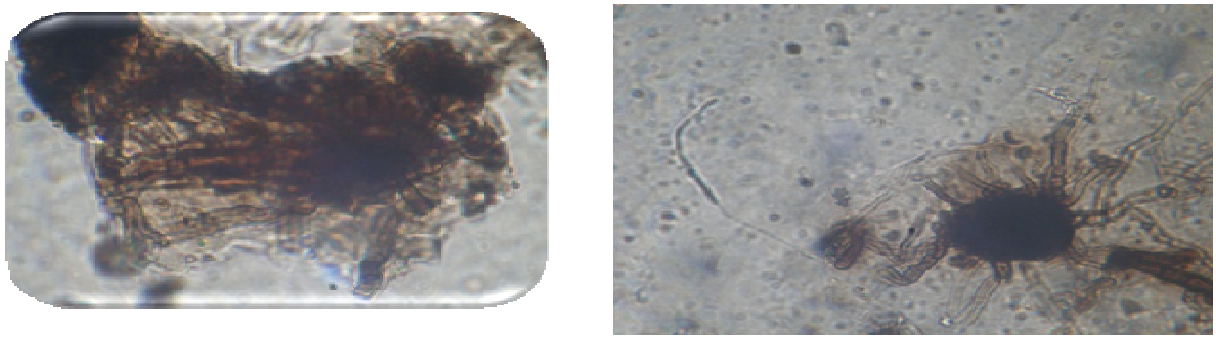

**Fig. 1.** *Morphological character of Sunflower root rot***.** (a) *Pycnidia* (b) *Micro sclerotia.*

**Table 1.** Isolation of Biocontrol agents screened against *M. Phaseolina.*

| S. N. | Location | Crop | Source | Isolates code |
|---|---|---|---|---|
| **a)** | **Biocontrol agents isolated from different locations** | | | |
| **I.** | ***Bacillus* spp.** | | | |
| 1. | Erur | Sunflower | Rhizosphere | $EBs_1$ |
| 2. | Edaiyar | Sunflower | Rhizosphere | $EDBs_2$ |
| 3. | Aruppukottai | Sunflower | Rhizosphere | $ABs_3$ |
| 4. | TNAU | Sunflower | Rhizosphere | $CBs_4$ |
| 5. | Madurai | Sunflower | Rhizosphere | $MBs_5$ |
| 6. | Thoothukudi | Sunflower | Rhizosphere | $KBs_6$ |
| **II.** | ***P. fluorescent*** | | | |
| 1. | Erur | Sunflower | Rhizosphere | $EPf_2$ |
| 2. | Edaiyar | Sunflower | Rhizosphere | $EDPf_3$ |
| 3. | Aruppukottai | Sunflower | Rhizosphere | $APf_4$ |
| 4. | TNAU | Sunflower | Rhizosphere | $CPf_5$ |
| 5. | Madurai | Sunflower | Rhizosphere | $MPf_6$ |
| 6. | Thoothukudi | Sunflower | Rhizosphere | $KPf_7$ |
| **b)** | **Biocontrol agents obtained from other source** | | | |
| **I.** | ***P.fluorescens*** | | | |
| i. | $Pf_1$ | | Department of Plant Pathology, TNAU, Coimbatore | |
| **II** | ***B.subtilis*** | | | |
| i. | $Bs_{10}$ | | Department of Plant Pathology, TNAU, Coimbatore | |

**Fig 2.** *Symptoms of M. phaseolina in sunflower root.*

1993) on nutrient agar medium and King's B medium. The pure cultures were maintained on the respective agar slants at 4°C.

**Screening of biocontrol agents *in vitro*:** The antagonistic effect of the biocontrol agent's*viz*., seven isolates in each of *fluorescenspseudomonas* and *Bacillus*spp were tested against *M. phaseolina*by dual culture technique (Dennis and Webster, 1971). Five-mm-diamycelial disc of *M. phaseolina*was placed at one end of the Petri plate containing PDA and the bacterial antagonist were streaked at the opposite end each of the Petri plates. Inoculation of *M. phaseolina* without antagonists served as control and each treatment was replicated three times. When the fungus attained full growth in the control plate, growth of the pathogen and inhibition zone were measured and per cent reduction in growth over control was calculated.

After four days of incubation, mycelia growth of the pathogen and inhibition zone was measured in treated as well as control plates. Per cent inhibition (PI) of mycelia growth was calculated using the formula suggested by Pandey*et al*. (2000).

$$PI = \frac{De - Dt}{De} \times 100$$

De - average diameter of fungal growth (cm) in control
Dt - average diameter of fungal growth (cm) in treatment

**Statistical analysis:** The experiments were conducted by Completely Randomized Design (CRD). The percentage values were transformed into "Arcsine" and "Square -root". The statistical analysis of the experiment was done by following the methods suggested by Gomez and Gomez (1984). Per cent values were transformed by arcsine or square root transformation.

## RESULTS AND DISCUSSION

**Symptoms of sunflower charcoal rot (*M. phaseoliina*):** The symptoms of sunflower root rot incidence observed in different location were examined. In general the disease occurred during flowering to maturity stage. At this stage, roots were turn into dark brown external, inner tissues appeared greyish because of the large number of sclerotia embedded in them. The stem had silvery grey discoloration extending up from the base, and in many cases, the epidermis was split, the roots were black and mostly decomposed (Fig. 2). *M. phaseolina*soil borne species which infect root, stem and collar region of plant host and caused cortical and vascular discoloration, was prevalent in arid regions,

**Table 2.** Effect of bacterial antagonists on the growth of *M. Phaseolina.*

| S. N. | Isolates | Mycelial growth (cm)* 7 DAI* | Inhibition over Control(%) | Inhibition zone (mm) |
|---|---|---|---|---|
| 1. | $Pf_1$ | 2.56 | 71.49 | 5.00 |
| 2. | $EPf_2$ | 4.70 | 47.66 | 2.00 |
| 3. | $EDPf_3$ | 5.26 | 41.42 | 1.00 |
| 4. | $APf_4$ | 4.30 | 52.11 | 4.20 |
| 5. | $CPf_5$ | 3.20 | 64.36 | 1.00 |
| 6. | $MPf_6$ | 6.13 | 31.73 | 2.10 |
| 7. | $KPf_7$ | 6.36 | 29.17 | 2.20 |
| 8. | $Bs_{10}$ | 3.06 | 65.92 | 17.80 |
| 9. | $EBs_1$ | 6.33 | 29.51 | 5.20 |
| 10. | $EDBs_2$ | 6.10 | 32.07 | 4.10 |
| 11. | $ABs_3$ | 5.86 | 34.74 | 1.50 |
| 12. | $CBs_4$ | 3.30 | 63.25 | 2.30 |
| 13. | $MBs_5$ | 4.56 | 49.22 | 13.10 |
| 14. | $KBs_6$ | 5.70 | 36.52 | 2.10 |
| 15. | Control | 8.98 | - | - |
| **CD (P=0.05)** | | | **0.60** | |

but can be found in moderate climates when high temperature and dry conditions occur in sunflower by Sonja *et al.* (2012).

**Inhibitory effect of bacterial antagonists on the growth of *M. phaseolina*:** Preliminary screening to identify best antagonist among the fourteen bacterial antagonists was conducted *in vitro.* The result showed that the entire bacterial antagonist were effective against the fungus *M. phaseolina* and exhibited appreciable amount of inhibition.

Among the seven isolates of *P. fluorescens* Pf1 allowed minimum mycelial growth of *M. phaseolina* 2.56 cm with 71.49 per cent growth reduction and with an inhibition zone of 5.00 mm followed by $CPf_5$ minimum mycelial growth of 3.20 cm with 34.36 per cent growth reduction and with an inhibition zone of 1.00 mm (Table. 2). *Pseudomonas* spp. showed antifungal activity against the alfalfa pathogen *M. phaseolina* inthe *in-vitro* as well as in the *in-vivo* assays (Guinazu*et al.*, 2012).

Among the seven *Bacillus* isolates $Bs_{10}$ allowed minimum mycelial growth of 3.06 cm with 65.92 per cent growth reduction and inhibition zone of 17.80 mm (Table. 2) followed by $CBs_4$ 3.30 cm with 63.25 per cent growth reduction and inhibition zone of 2.30 mm. The control Petri dish received maximum mycelial growth of 8.98 cm within seven days after inoculation.Priyadharshni (2012) reported that the dual culture in blackgram antagonist *B. subtilis*($MB_3$) inhibited the growth of *M. phaseolina* which recorded the mycelia diameter of 4.8 cm and it leads to 42.06 per cent of inhibition over control.

## Conclusion

The present study was successful in selecting effective isolates of bio control agent like *P.fluorescens* and *B.subtilis.* The results on *in vitro* antagonistic effect against *M. phaseolina* was maximum with *Pf1*, followed by $Bs_{10}$comparison of the control Petri dish received maximum mycelial growth of 8.98 cm within seven days after inoculation.Even though today numerous different strategies have been employed to prevent plant diseases, evidence has shown that harnessing indigenous or introduced soil microbial inoculants influence plant health and productivity.

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