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

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Abstract: Charcoal rot caused by Macrophomina phaseolina 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 (EP1, EDPI, API, CP1, MP1, KP1, andfP1) andseven isolates Bacillus subtilis (EB1, EDBS, AB1, CBS1, MB1, KB1, andBS1) 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 antagonists significantly compared to the control P. fluorescens (P11) 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 melilotian and Bacillus subtilishowed 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 to isolate and identify different 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 each sterile Petri dish containing 15 ml of previously sterilized and solidified PDA medium. The plates were incubated at room temperature (28±2°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, 10⁻⁵, 10⁻⁷ and 10⁻⁹, one ml of each aliquot was pipetted out, poured in sterilized Petri dish containing King’s B medium (King et al., 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°C) for 24 hours. Colonies with characteristics of *Bacillus* spp., *Pseudomonas* spp. were isolated individually and purified by streak plate method (Rangaswami,

![Image of pycnidia and micro sclerotia](image_url)

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

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<th>S. N.</th>
<th>Location</th>
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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. phaseolina):**

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,
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, 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* in the *in-vitro* as well as in the *in-vivo* assays (Guinazu et al., 2012). Among the seven *Bacillus* isolates Bs 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, 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. Priyadharshini (2012) reported that the dual culture in blackgram antagonist *B. subtilis* (MB1) inhibited the growth of *M. phaseolina* which recorded the mycelial 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, 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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**REFERENCES**


