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# Screening of Soil Bacteria for Efficacy in Bio-control of *Parthenium hysterophorus* L.

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**Abstract**

Agricultural crops serve a source for number of industrial products, besides their major use for human food. The average per hectare yield of these crops in India is less as compared to other advanced countries due to many factors like lack of irrigation, availability of fertilizers and other ecological factors. Out of which the problem of weeds is the major barrier in the loss of production. Weeds are the unwanted plants that grow in agricultural fields and compete with the main crops for nutritive and other ecological needs and thus reduces the crop yield. The weeds are of human health concerns as well as they are responsible for causing allergy, nausea and other health disorders. Weeds are of great concern for farmers as they reduce the crop yields very significantly. Hence there is need to control weed populations and their possible infestation by methods that will not cause any harm to the environment. There are several physical and chemical methods used for weed management in agriculture sector. Chemical control of weeds is dominating in the fields by farmers, however use of chemicals results in environmental pollution. The use of biological methods is found to be more economical than other methods and is found to play a significant role in sustainable weed management. The biological methods employs use of various biological agents including microorganisms. The present research work was undertaken to isolate bacteria from the soil and to test to inhibit the germination and growth of Parthenium hysterophorus and crop plants in laboratory conditions. Two prominent bacteria belonging to Pseudomonas species were found to be promising with respect to inhibition of weed growth.

**Keywords:** Weeds; Biological control; Rhizospheric bacteria; Hydrogen cyanide

## Introduction

Weeds are the plants, which grow where they are not wanted. Weeds differ from other plants in being more adaptive and having peculiar characteristics that make them more competitive (Dangwal et al., 2010). They are non-indigenous plants that can invade or negatively alternative plant communities (Muhammad et al., 2009). Weed flora and its composition in a crop is influenced by the type of cultivation, time or season of cultivation, soil type, soil PH, climatic conditions, cultivation practices like irrigation, tillage systems, application of fertilizer and weed management.

Weeds represent one of the greatest limiting factors to efficient crop production. They Weeds cause greater economic losses on agricultural lands than all other pests combined (Kremer and Kennedy, 1996). These weeds effectively compete with the crop for nutrients, water, space and reduce the yield ranging from 12 to 51 % (Mukherjee and Singh, 2005; Halder and Patra, 2007). Weeds also serve as reservoir for plant pathogens that may cause significant loss in crop production. The decrease in crop quality and yield due to weeds has promoted the use of herbicides. However, in recent years, some weed populations have developed multiple herbicide resistances to these products leading to higher rates of application and to more complicated soil and water contamination problems. In addition, herbicides have an impact on human health (Bankar etal., 2023; Dheer et al., 2023; Kumawat, 2023; Singh et al., 2023; Culliney, 2005).

The conventional methods of weed control are not suitable for complete eradication of weeds. A large number of chemicals have been tried for Parthenium control. But the timing of chemical control is critical. In addition, herbicides have negative impact on human health (Culliney, 2005). The use of microorganisms as bio-herbicides

has several advantages. Deleterious rhizobacteria (DRB) are overlooked as biological control agents of weeds (Flores-Vargas and O´Hara, 2006; Kremer and Kennedy, 1996). Many DRB are plant specific and their potential as a biological control agent has only recently been investigated. DRBs are ubiquitous and likely common to all plant root systems. Selection for bacteria that are specifically detrimental to weed seedling growth could benefit agriculture. Thus the objective of this research was to isolate, identify and characterize potential DRB from soil.

## Materials and Methods

### Collection of soil and bacteria isolation

Soil samples were collected in sterile containers by using standard methodology. Standard microbiological methods was used for isolation of bacteria(Araujo et al.,1996). Plates were incubated at RT for 3 days. Representative colonies were selected and subcultured by streaking growth onto King's B agar. Purified cultures were preserved at 4°C.

### Agar plate bioassay for inhibitory effects of bacteria on weed seedlings and crop plants

Seeds of *Parthenium hysterophorus* and crop plants were surface sterilized and blotted on sterilized filter paper. Bacterial Cultures grown for 2 days at RT in glucose minimum salt medium (Brown and Dilworth, 1975) were centrifuged and 2 ml of supernatant was added to the surface of water agar plates. The supernatant was allowed to absorb into the agar for 3 hours. Five surface sterilized seeds of each weed species and crop plants separately were then placed on each plate and incubated in the dark at RT for 5 days. Controls were inoculated with 2 ml of sterile medium. The study was carried out in triplicates. After 5 days, germination percentage was recorded. Seedlings were then removed and shoot, root lengths measured.

### Screening for production of secondary metabolites

The determination of hydrogen cyanide production was carried out by Lorck method (Lorck, 1948) modified by Alstrom (Alstrom and Burns, 1989).

### Characterization and identification of strains

Strains that inhibited the target weed plants in laboratory conditions were identified using standard literature (Claus and Berkeley, 1986).

## Results and Discussion

A total of 16 distinct bacterial strains were obtained from the soil. These isolates were screened on agar plates under laboratory conditions to investigate inhibitory effects on the seedlings of weed and crop plants. Two bacterial isolates significantly reduced the root and shoot lengths of weed seedlings compared to the crop plants. The results are represented as per Table 1 and 2. In a laboratory screening, bacterial isolate 1(B1) inhibited root length of selected crop plants- *Arachis hypogea* and *Glycine max* by 19 and 21% respectively whereas it inhibited 36% root growth of Parthenium weed. Bacterial isolate 2 (B2) inhibited root length of selected crop plants- *Arachis hypogea* and *Glycine max* by 12.40 and 19.12% respectively whereas it inhibited 33% root growth of Parthenium weed. The bacterial isolates also inhibited shoot length of *Arachis hypogea* and *Glycine max* by 18 and 22% respectively and shoot length of Parthenium weed by 38%. Bacterial isolate *2 (*B2) inhibited root length of selected crop plants- *Arachis hypogea* and *Glycine max* by 8.31 and 11% respectively whereas it inhibited 32% root growth of Parthenium weed.

**Table 1**. Laboratory screening of bacteria and metabolites on growth of weed seedlings

| Weed | Bacterial treatment | Studied characters | | | | |
|---|---|---|---|---|---|---|
| | | Germination at day 5(%) | Root length(cm) | % reduction | Shoot length(cm) | % reduction |
| *Parthenium hysterophorus* | Control* | 100 | 2.33±0.16 | | 3.83±0.42 | |
| | B1 | 40 | 1.48±0.08 | 36 | 2.39±0.06 | 38 |
| | B2 | 40 | 1.54±0.05 | 33 | 2.60±0.14 | 32 |

* Sterile medium without bacteria is used in control.

% reduction= [(Control root or shoot length - DRB strain root or shoot length)/Control root or shoot length]× 100

Bacterial isolates 1 and 2 on the basis of morphological, cultural and biochemical characteristics were identified as *P. aeruginosa* and *P. fluorescens* respectively by referring to Bergey's Manual of Determinative Bacteriology.

*P. aeruginosa* and *P. fluorescens* have been reported to be producing HCN as a secondary metabolite and are known to be associated with growth inhibition (Albert and Anderson, 1987; Kremer and Kennedy, 1996). Further, the role of these type of bacteria in inhibiting the growth of several weeds including *P. hysterophorus* from various crops is reported by Kremer and Kennedy, 1996; Adom and Zdor, 2001; Dheer et al., 2023). This indicates that soil bacteria have potential to interfere with weed seedling growth and reducing weed infestation.

**Table 2**. Laboratory screening of bacteria on growth of crop seedlings

| Crop seedlings | Bacterial treatment | Studied characters | | | | |
|---|---|---|---|---|---|---|
| | | Germination at day 5(%) | Root length | % reduction | Shoot length | % reduction |
| *Arachis hypogaea* | Control* | 100 | 7.58±0.41 | | 3.49±0.26 | |
| | B1 | 80 | 6.10±0.69 | 19 | 2.87± 0.35 | 18 |
| | B2 | 80 | 6.64±0.87 | 12.40 | 3.20±0.13 | 8.31 |
| *Glycine max* | Control* | 100 | 6.38±0.62 | | 10.36±0.92 | |
| | B1 | 40 | 5.06± 0.99 | 21 | 8.06± 1.08 | 22 |
| | B2 | 80 | 5.16 ±0.59 | 19.12 | 9.22±1.16 | 11 |

* Sterile medium without bacteria is used in control.
% reduction= [(Control root or shoot length - DRB strain root or shoot length)/Control root or shoot length]× 100
% reduction is in comparison with its respective control plate.

**Conclusion**

In the present study, the two promising bacterial isolates namely *P. aeruginosa* and *P. fluorescens* were found to reduce the growth of weed seedlings significantly than the selected crop plants. The results reported indicate that these HCN producing *Pseudomonas sp* have a great potential reduce weed infestation in crops. Our investigations indicate the potential for possible inhibition of the weed seedlings through introduction to their specific DRB with identified detrimental activity. However, further study is necessary to understand inoculum responses in different cropping and soil systems, and to determine environment effects on responses to inoculum for the development of these DRBs as a weed control against *P. hysterophorus* in agriculture fields.

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PVS and PSV conceived the concept, wrote and approved the manuscript.

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