J. Environ. Res. Develop.
Journal of Environmental Research And Development **Vol. 8 No. 3, January-March 2014**

ISOALTION, CHARACTERIZATION AND IDENTIFICATION OF RHIZOSPHERIC BACTERIA WITH THE POTENTIAL FOR BIOLOGICAL CONTROL OF *Sida acuta*

Vishwas Shankar Patil

Department of Microbiology, Lal Bahadur Shastri College, Satara, Maharashtra (INDIA)

Received October 15, 2013 *Accepted January 16, 2014*

ABSTRACT

Sida acuta is a weed in agriculture crops. It reduces crop production. Weed control is important in crops management to maintain its vigor and productivity. The increasing threat of herbicide resistance and negative impact of herbicides on environment provides an opportunity for the development of biological agent for weed management. The current work was undertaken to isolate, identify and characterize potential deleterious rhizospheric bacteria from weed species. Rhizospheric bacterial isolates that inhibit weeds, but not crops, will have a potential to develop an effective bacteria based weed management control method for ecologically based weed management systems.

Key Words : *Sida acu1sta*, Weed control, Herbicide resistance, Biological agent, Rhizospheric bacteria

INTRODUCTION

Sida acuta Burm. F., commonly referred to as tea weed or iron weed, is a weed from malvaceae family. The weed is native to Mexico and Central America but has spread throughout the world. It is commonly encountered with the crops in Indian agricultural sector. It is an erect annual and/or perennial shrub that can grow to a height of three feet. One weed plant can produce hundreds of seeds throughout the growing season. Each seed is wedge-shaped, brown to black, with two stiff spikes at the tip. Ironweed is considered a weed in agriculture. The weed reduces crop production. It also dominates in improved pastures, waste and disturbed places.[1]

The conventional methods of weed control like physical methods, mechanical, burning and pasture management are not feasible for complete eradication of weed. Chemical control of weed in India is gaining popularity. A large number of chemicals have been tried for Ironweed. But the timing of chemical control is critical. In addition, herbicides cause soil and water contamination so there is need to turn towards safer control methods such as biological control using living organisms to manage weeds.[2,3]

Use of soil microorganisms to control weeds is an alternative method to the currently used conventional methods.[4] Deleterious rhizobacteria (DRB) are non-parasitic bacteria that are associated with plant roots and inhibit the plant growth.[5] DRB usually cause reduced seed germination, growth inhibition and reduced root elongation by producing phytotoxins, phytohormones or cyanides but they can also reduce plant growth directly by competing with the plant for nutrients or indirectly by reducing the colonisation of beneficial rhizobia or mycorrhiza. Deleterious rhizospheric bacteria are likely common to all plant root systems. Selection of those rhizospheric bacterial isolates that specifically colonize and inhibit growth of weeds but not that of crop plants can be considered in the development of biological control technologies[6-9] and this could benefit agriculture by contributing to increased crop yields, by reducing weed competition and by reducing the use of chemical herbicides.

AIMS AND OBJECTIVES

The objective of this research was to isolate, identify and characterize the potential DRB from weed species.

MATERIAL AND METHODS

Collection of weed and bacteria isolation

Seedling and mature *Sida acuta* plants were collected and stored in sterile plastic bags at 4°C until processing in the laboratory. Standard microbiological methods were used to isolate bacteria from the rhizosphere using King's B agar medium. Purified cultures were stored at 4°C.

Laboratory screening for inhibitory effects of bacteria and their metabolites on germination and growth of weed seedlings and crop plants

Bacterial cultures grown for 2 days at 28°C in glucose minimum salt medium were centrifuged and 2 ml of supernatant was allowed to absorb into 0.9% water agar plates for 3 hours. Five surface- sterilized seeds of weed and crop plants separately were then placed on each plate and incubated in the dark at 20°C for 5 days. Controls were inoculated with 2 ml of sterile medium. This study was carried out in triplicates. After 5 days, germination percentage was recorded. Then seedlings were removed and shoot, root lengths measured.

Glasshouse screening of bacteria for inhibitory effects on weed seedlings and crop plants

Surface sterilized seeds of *Sida acuta* and crop plants were germinated for 2 days on 0.9% water agar and three seedlings each were planted into a pot containing a pasteurized soil. The three seedlings in each pot were inoculated with a 0.5 ml of bacterial isolate suspension (OD at 600 nm =0.9) of an individual strains. A thin layer of sterile sand was placed over the soil. Plants were grown at 25°C and watered every second day with plant nutrient solution. The study was carried out in triplicates. After 4 weeks the plants were harvested, shoot and root lengths measured. Roots and shoots were dried at 60°C for 24 hours and dry weights of shoots and roots were recorded.

Screening for production of secondary metabolites

Qualitative cyanide determination was performed by sub culturing the isolates on Nutrient Agar medium containing glycine (4.4 g/l). The production of cyanide was detected using picrate/Na_2CO_3 paper fixed to the underside of the Petri-dish lids , sealed with parafilm before incubation at 28° C. A change from yellow to orange, red or reddish brown was recorded at 4,24 and 48 hours as an indication of weak, moderate or strongly cyanogenic potential, respectively. Reactions from inoculated plates were visually compared with corresponding control plates containing no culture.

Characterization and identification of strains

Bacterial isolates that inhibited the target weed plants under glasshouse conditions were characterized and identified based on their morphological, cultural, physiological and biochemical characteristics.[10-13]

RESULTS AND DISCUSSION

A total of 15 bacterial strains were obtained from the rhizosphere of *Sida acuta*. All the strains were relatively fast growing with most producing single colonies after overnight incubation at 28°C on Kings B media. Agar plate bioassay was carried out to investigate inhibitory effects of rhizobacterial isolates on the seedlings of weed and crop plants under laboratory conditions. Five bacterial isolates significantly reduced the root and shoot lengths of weed seedlings compared to the crop plants. In a laboratory screening, bacterial isolate 1(later identified as *Xanthomonas* sp.) inhibited root and shoot length of crop plants in a range 25-36% and 8-34% respectively whereas it caused 46% and 39% inhibition of root and shoot length of target weed respectively. Bacterial isolate 2 (later identified as *B.cereus*) inhibited root and shoot length of crop plants in a range 23-29 % and 12-31 % respectively whereas it caused 34% and 17% inhibition of root and shoot length of target weed. Bacterial isolate 3 *(*later identified as *B. subtilis*) inhibited root and shoot length of crop plants in a range 18-25 % (with the exception of 29% reduction of Jowar plant) and 14-34% respectively whereas it caused 30% and 13% inhibition of root and shoot length of target weed. Bacterial isolate 4 *(*later identified as *P. aeruginosa*) inhibited root and shoot length of crop plants in a range 15-35 % and 12-26% (with the exception of 66%

reduction of Maize plant) respectively whereas it caused 38% and 23% inhibition of root and shoot length of target weed. Bacterial isolate 5 *(*later identified as *P. fluorescens*) inhibited root and shoot length of crop plants in a range 5-21 % and 6-13% respectively whereas it caused 27% and 30% inhibition of root and shoot length of target weed.

Five rhizobacterial isolates caused the germination inhibition of *Sida acuta* by 75%, 50%, 25%, 50% and 50% respectively **(Fig.1)**. These five rhizobacterial isolates were further screened to investigate inhibitory effects on the weed and crop plants under glasshouse conditions. All five rhizobacterial isolates exhibited the variety of effects on root and shoot length reduction of *Sida acuta* weed **(Fig.2)** and also on selected crop varieties. Bacterial isolate 1 caused root and shoot length reduction of crop plants in a range 39-47% and 10-16% respectively whereas it caused 50% and 18% reduction of root and shoot length of *Sida acuta.* It caused 32-54% and 12-19 % reduction of root and shoots dry weight of crop plants respectively. It also caused 46% and 23% reduction of root and shoot dry weight of *Sida acuta* respectively. Bacterial isolate 2 caused root and shoot length reduction of crop plants in a range 17-36% and 6-17% respectively whereas it caused 20% and 10% reduction of root and shoot length of *Sida acuta.* It caused 26-34% and 9-19 % reduction of root and shoots dry weight of crop plants respectively. It also caused 21% and 14% reduction of root and shoot dry weight of *Sida acuta* respectively. Bacterial isolate 3 caused root and shoot length reduction of crop plants in a range 19-34% and 7-15% respectively whereas it caused 26% and 10% reduction of root and shoot length of *Sida acuta.* It caused 24-31% and 8-14 % reduction of root and shoots dry weight of crop plants respectively. It also caused 20% and 17% reduction of root and shoot dry weight of *Sida acuta* respectively. Bacterial isolate 4 caused root and shoot length reduction of crop plants in a range 33-40% and 7-11% respectively whereas it caused 44% and 10% reduction of root and shoot length of *Sida acuta.*

Similarly, significant reduction in dry weight of roots of target weed and crops was observed inoculated with the DRB strains in comparison to the noninoculated control **(Fig. 3)**. It caused 26-41% and 10-14 % reduction of root and shoots dry weight of crop plants respectively. It also caused 39% and 14% reduction of root and shoot dry weight of *Sida acuta* respectively. Bacterial isolate 5 caused root and shoot length reduction of crop plants in a range 23-40% (with 44% in Jowar plant) and 5-15% respectively whereas it caused 41% and 12% reduction of root and shoot length of *Sida acuta.* It caused 20-33% and 10-14 % reduction of root and shoots dry weight of crop plants respectively. It also caused 41% and 17% reduction of root and shoot dry weight of *Sida acuta* respectively.

Bacterial isolate-1,4 and 5 were found to be producing HCN, a gaseous metabolite. Bacterial isolate-1 showed strong HCN production ability whereas isolate-4 and 5 exhibited weak potential of HCN production whereas HCN production ability was absent in isolate 2 and 3 **(Table 1)**. Bacterial isolates 1, 2, 3, 4 and 5 were further characterized and identified as *Xanthomonas* sp., *Bacillus cereus, Bacillus subtilis, P. aeruginosa* and *P. fluorescens* respectively based on their morphological, cultural, physiological and biochemical characteristics **(Table 2)**, as described in Bergey's Manual of Systematic Bacteriology.

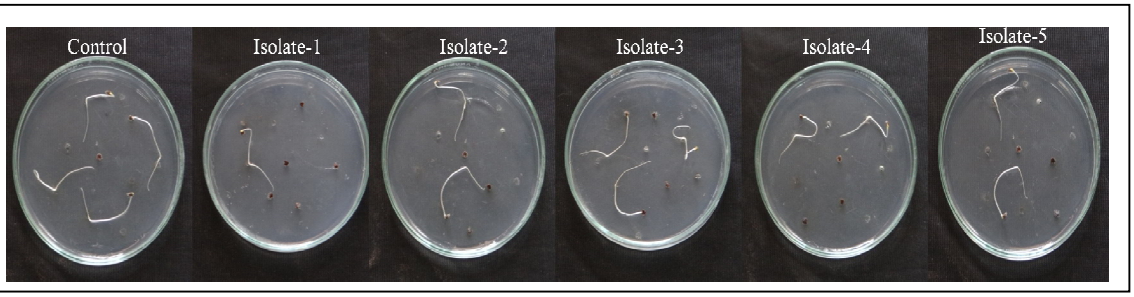

Fig.1 : Agar plate bioassay of rhizobacterial isolates on *Sida acuta* under laboratory conditions

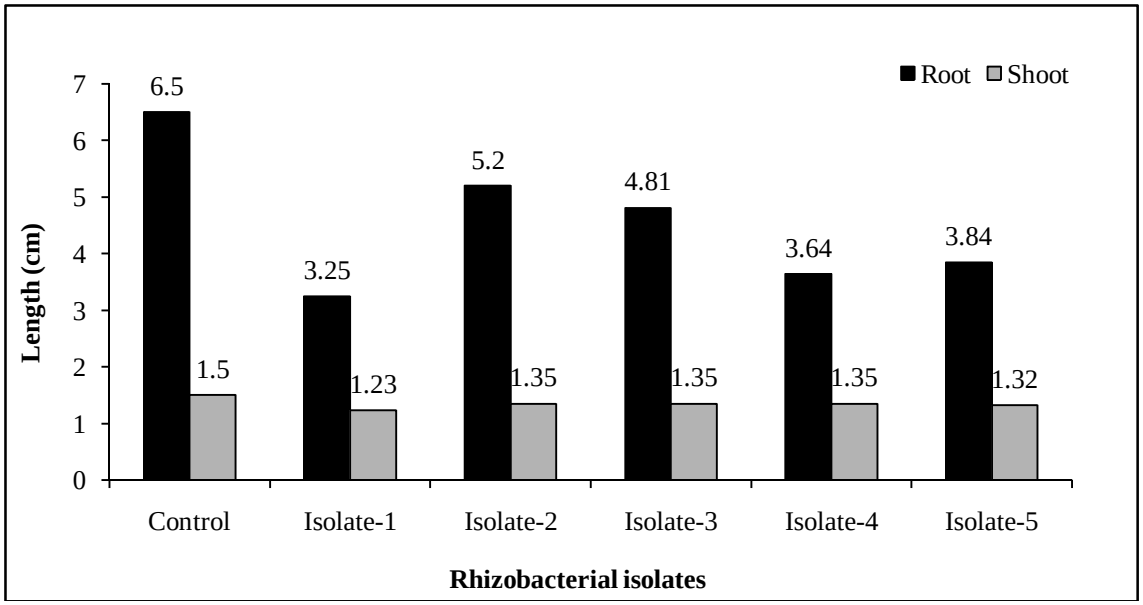

Fig. 2 : Effect of rhizobacterial isolates on root and shoot length reduction of *Sida acuta*

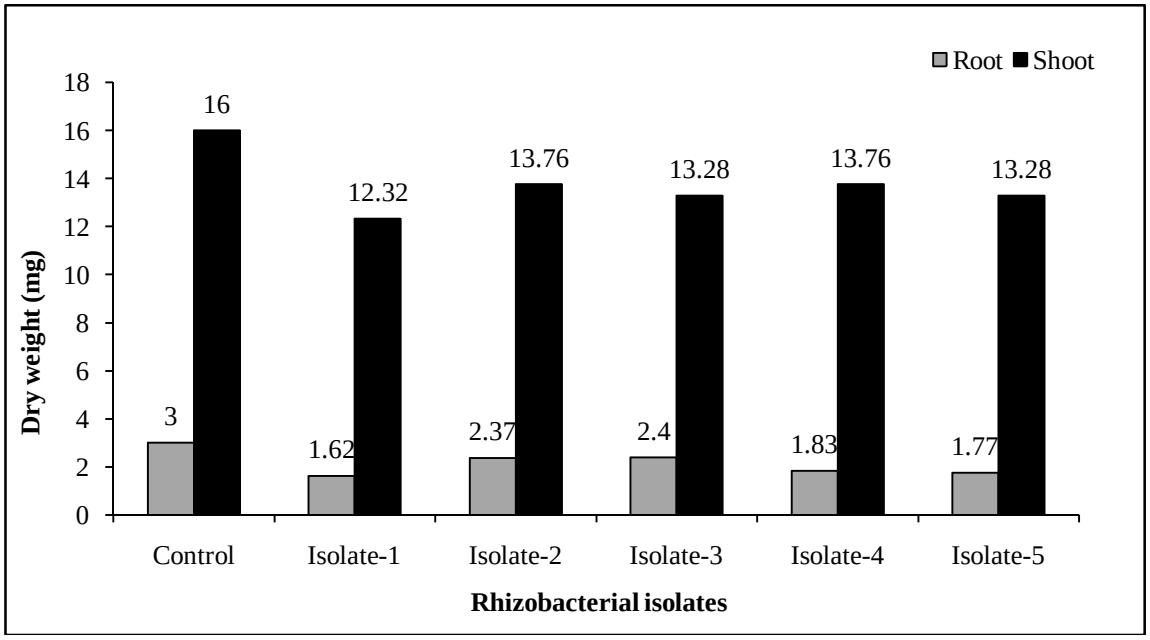

Fig. 3 : Effect of rhizobacterial isolates on root and shoot dry weight reduction of *Sida acuta*

Table 1 : Qualitative detection of HCN

Isolate code number	HCN production[a]
Isolate-1	+++[b]
Isolate-2	-
Isolate-3	-
Isolate-4	+
Isolate-5	+

a=Intensity of HCN reaction with picrate/Na_2CO_3 indicator:
none,-; weak, +; moderate, ++; strong, +++.
b=Reaction detectable at 4 hours after initiation of HCN assay.

Table 2 : Characterization and identification of rhizobacterial isolates

Test	Observation				
	Bacterial isolate 1	**Bacterial isolate 2**	**Bacterial isolate 3**	**Bacterial isolate 4**	**Bacterial isolate 5**
Colony characters on Kings B agar (incubation at 30^{o}C /24hrs)					
Size(in mm)	3mm	3mm	5mm	3mm	2mm
Shape	Circular	Circular	Circular	Circular	Circular
Margine	Regular	Regular	Irregular	Irregular	Irregular
Elevation	Convex	Flat	Flat	Flat	Raised
Consistancy	Smooth	Smooth	Smooth	Smooth	Smooth
Opacity	Opaque	Opaque	Opaque	Transparent	Transparent
Colour	Yellow	White	White	Bluish-green	Bluish-green
Morphological characteristics					
Gram nature	Gram negative (-)	Gram positive (+)	Gram positive (+)	Gram negative (-)	Gram negative (-)
Morphology	Thin,slender,short rods	Thick long rods	Thick long rods	Thin, slender short rods	Thin, slender, short rods
Motility	motile	motile	motile	motile	motile
Endospore	Absent	Present	Present	Absent	Absent
Physiological and biochemical characteristics					
Opt pH		7.0	6-9	7	7
Opt temp(^{0} C)	25-30	35(15-45)	35-40	37	37
O_2 reqt	aerobic	aerobic	aerobic	aerobic	aerobic
D-Glucose	+(Aonly)	+	+	-	-
D-Galactose	+(Aonly)	-	-	+	+
Oxidase	-(or weak positive)	-	+	+	+
Catalase	+	+	+	+	+
Gelatinase	+(weakly)	+	+	+	+
Urease	-	-	+	-	-
H_2S production	H_2S production	-	-	-	-
HCN production	+(strong)	-	-	+(weak)	+(weak)
Pigment production	Yellow, Xanthomonadin	-	-	Fluorescein and pyocyanin	Fluorescein
Some other specific tests	Mucoid and yellow colony growth on Nutrient agar with 5% Glucose; Do not grow on aspargine medium	Utilizes Fructose Sucrose and Maltose	Amylase, caseinase, Nitrate reduction tests positive, Utilizes Fructose, Sucrose and Maltose	Grow both at 4^{0} C and 41^{0}C; Nitrate reduction test, Arginine utilisation test positive; Chitinase and Lipase	Grow only at 4^{0} C; Nitrate reduction test, Arginine utilisation test positive;

				synthesis.	Chitinase and Pectinase synthesis.
Identification	*Xanthomonas sp.*	*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Pseudomonas fluorescens*

CONCLUSION

The results reported confirm with earlier studies and indicate that the rhizospheric bacteria have a great potential to interfere with the growth of weed seedlings. Five rhizobacterial isolates exhibitted the variety of effects on *Sida acuta* weed and also on selected crop varieties. Rhizobacterial isolates reduced very significantly the germination of seeds of target weed species. They inhibited the growth of crops to a great extent by 17-47 % and growth of *Sida acuta* by 32-53 %. They reduced very significantly the root length of target weed in glasshouse screening, maximum inhibition (50%) was found to be caused by *Xanthomonas* sp and least inhibition (20%) was caused by *B. subtilis* strains. No significant change was found in shoot length reduction of target weed. Similarly, significant reduction in dry weight of roots was observed inoculated with the five weed inhibitory strains in comparison to the noninoculated control. By contrast there was effect on shoot dry weight reduction of weed species. Our results indicated that *Xanthomonas* spp, *P.aeruginosa* and *P. fluorescens* has the ability to produce HCN as a secondary metabolite. It is reported that cyanogenic rhizobacteria are involved in the reduction of plant development. Cyanide is proved to be a potential inhibitor of enzymes involved in plant respiration, carbohydrate metabolism, CO_2 and nitrate assimilation. HCN production ability was lacking in *B.cereus* and *B.subtilis* isolates. Reports indicate that the inhibitory effect of *Bacillus subtilis* IJ-31 on the growth of plant seedlings could be due to the production of indole 3- propionic acid and indole 3-acetic acid. Sodium vanillate and 2-aminobenzoic acid are the substances reported to be produced by *Bacillus cereus* EJ-121 that have inhibitory activity on lettuce seedling. A majority of rhizobacteria screened in the glasshouse conditions reduced weed and crop seedling growth equally. Therefore, further studies are necessary for developing these rhizobacteria as a potential biological agent for weed control.

ACKNOWLEDGEMENT

Author is thankful to the U.G.C., New Delhi, India for providing financial assistance.

REFERENCES

1. Mann A., Gbate M. and Umar A.N., *Sida acuta* subspecies *acuta*., Medicinal and economic plant of Nupeland, *Jube Evans Books and Publi.*, 241, **(2003).**
2. Alavanja M.C., Hoppin J.A. and Kamel F., Health effects of chronic pesticide exposure : Cancer and neurotoxicity, *Annu. Rev. Pub. Heal.*, **25**(1), 155-197, **(2004).**
3. Shepard J.P., Creighton J. and Duzan H., Forestry herbicides in the United States : An overview, *Wild. Soc. Bull.*, **32**(2), 1020-1027, **(2004).**
4. Flores-Vargas R.D. and O´Hara G.W., Isolation and characterization of rhizosphere bacteria with potential for biological control of weeds in vineyards, *J. Appl. Microbiol.*, **100**(2), 946-954, **(2006).**
5. Kremer R.J. and Kennedy A.C., Rhizobacteria as biocontrol agents of weeds, *Weed Technol.*, **10**(2), 601-609, **(1996).**
6. Kremer R.J., Bioherbicides: potential successful strategies for weed control, In: Koul, O., Dhaliwal, G. **(Eds.)**, *Microbial Biopesticides*, Taylor & Francis, London, **33**(2),307–323, **(2002).**
7. Claus D. and Berkeley R.C.W., *Bergey's Manual of Systematic Bacteriology*,

Williams & Wilkins, Baltimore, 244-254, **(1986).**

8. Adam O. and Zdor R., Effect of cyanogenic rhizobacteria on the growth of velvetleaf (*Abutilon theophrasii*) and corn (*Zea mays*) in autoclaved soil and the influence of Supplemented glycine, *Soil Biol. Biochem.*, **33**(1), 801–809, **(2001)**.
9. Choksi Nikita and Hemangi Desai, Isolation, Identification and characterization of lactic acid bacteria from dairy sludge sample, *J. Envion. Res. Develop.*, **7**(1A), 234-244, **(2012)**.
10. Li J. and Kremer R.J., Growth response of weed and crop seedlings to deleteious rhizobacteria, *Biol. Control,* **39**(2), 58-65, **(2006).**
11. Lee H.J., Kim W.C., Jeon S.Y., Kim J.W., Joo G.J., Rhee I.K. and Song K.S., Growth inhibitors of soybean seedling from *B. cereus* IJ-31, *Agric. Chem. Biotech*, **46**(3), 100–104, **(2003).**
12. Mukati A., Vyas A. and Vyas H., A study of Natural population of *Neurospora* and isolation of novel morphological mutants, *J. Environ. Res. Develop.*, **7**(2A), 923-936, **(2012)**.
13. Hoang L., Joo G.J., Kim W.C., Jeon S.Y., Choi S.H., Kim J.W., Rhee I.K., Hur J.M. and Song K.S., Growth inhibitors of lettuce seedlings from *Bacillus cereus* EJ-121, *Plant Growth Regulation*, **47**(1),149–154, **(2005).**

We have modified our environment so radically that we must now modify ourselves to exist in this new environment.

Norbert Wiener