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Development of Herbicide Tolerant *Rhizobium* Species From Different Leguminous Plants

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ABSTRACT

The present study was planned to determine the herbicide tolerance Rhizobium species were selected from three different leguminous plants namely Arachis hypogaea L, Vigna mungo L., and Vigna radiate L. Herbicides such as Atrazine, Paraquat Dichloride and Pendimethalin were used for this purpose in the concentrations such as .0.1, 0.2, 0.3%. Rhizobium species are designated R1, R2 and R3 viz, Groundnut, Black gram and Green gram. R1 isolates from groundnut leguminous plants were showed that better performance on Pendimethalin than compared to Atrazine and Paraquat Dichloride. Pendimethalinwas showed zone of inhibition at 0.1% level. The efficient improved Rhizobium isolates from groundnut are subjected to again herbicide tolerance, and the results are same like that, when the herbicides concentrations was increased maximum zone of inhibition was noticed. Overall results are highlighted that R1 (Arachis hypgaea L.,) isolates had a adverse effect on the Pendimethalin at 0.3 % level was safer to Rhizobia.. If Rhizobial strains show sensitivity to herbicides, herbicide-resistant strains need to be developed. Further study was extended to test effect herbicides under field conditions on the growth of Rhizobia.

Keywords: *Rhizobium, Herbicide, Leguminous plants and strain improvement.*

INTRODUCTION

Herbicides are strong chemical products that not only action their target weeds but may also display significant toxicity to other organisms. Herbicide usage has been a common agricultural practice since the middle of the 20th Century, and over the years has led to the emergence of multiple resistance events in weeds (218 species tolerant to 148 different herbicides as of October 2013 are reported in http://www.weedscience.org). To address this problem, seed companies have resorted to producing new recombinant crops tolerant to broader-range non-selective herbicides such as glyphosate. However, owing to the continued use of these products, new weed tolerance events are increasingly taking place as well.

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Herbicides may also influence the growth of Rhizobia, herbicides may have negative effects on the growth of Rhizobia[1].Herbicides may influence nodulation and biological nitrogen fixation in legumes either by affecting Rhizobia, the plant or both. There is a need to study and thus separate the possibility of the direct effects of herbicion the growth of Rhizobia. Herbicides may influence nodulation and biological nitrogen fixation in legumes either by affecting Rhizobia, the plant or both. There is a need tostudy and thus separate the effects of herbicides on nodulation and the possibility of the direct effects of herbicides on Rhizobia. The effects of herbicides on nodulation and nitrogen fixation by examination of the effects of these chemicals on plant growth have been reported previously[2].

Atrazine: Atrazine is a herbicide of the triazine class. Atrazine is used to prevent pre and post emergence broadleaf weeds in crops such as maize (corn) and sugarcane and on turf, such as golf courses and residential lawns. It is one of the most widely used herbicides in US[3].

Paraquat Dichloride : Although first synthesized in 1882,Paraquat's herbicidal properties were not recognized until 1995. Paraquat was first manufactured and sold by ICI in early 1962 and is today among the most commonly used herbicides.It kills a wide range of annual grasses and broad leaved weeds and the tops of established perennial weeds. Problems with herbicide resistant weeds may be addressed by applying herbicides with different modes of action, along with cultural methods such as crop rotation, in integrated weed management systems. Paraquat, with its distinctive mode of action is one of few chemical options that can be used to prevent and mitigate problems with weedsthat have become resistant to the very widely used non selective herbicide glyphosate[4].

Pendimethalin: Pendimethalin is strongly adsorbed by soil organic matter and clay and said do not readily leach through the soil. The agency contents that 'Pendimethalin' should not cause a water contamination problem'' and has therefore waived ground water data requirements[5].

Rhizobium: *Rhizobium* - *legume* symbiosis are the primary source of fixed nitrogen in land based systems and can provide well over half of the biological source of fixed nitrogen. Atmospheric nitrogen fixed symbiotically by the association between *Rhizobium* species and legumes represents a renewable source of nitrogen for agriculture .Rhizobia forms symbiotic association with legumes. The organisms belonging to genus *Rhizobium* are Gram negative, short rods 0.5 -0.9 µ wide and 1.2 -3.0 µ long, motile by polar or sub-polar flagella, non-spore forming, and aerobic with opt. temp. 25-30ºC. Most strains produce gum (extracellular Polysaccharide slime) of varying composition. Members of the genus *Rhizobium,* upon infection of the appropriate legume, can cause the formation of nodules and participate in the symbiotic acquisition of nitrogen. The legume Rhizobia symbiosis has a unique importance in agriculture. The symbiosis results in huge quantities of nitrogen fixation throughout the world and any adverse effect on Rhizobia results in reduced rates of biological nitrogen fixation. Many factors influence the growth of nitrogen-fixing Rhizobia. The effects of temperature, light, moisture, soil pH and nutrition on Rhizobia are well documented[6]. *Rhizobium* - *legume* symbiosis are the primary source of fixed nitrogen in land based systems and can provide well over half of the biological source of fixed nitrogen. Atmospheric nitrogen fixed symbiotically by the association between *Rhizobium* species and legumes represents a renewable source of nitrogen for agriculture[7]. *Arachis hypogaea* L. is the most legume oilseed, protein rich crop India. Even though India ranks first in area, its production is low compared to other groundnut growing countries. Among various reasons, Nutrient supply and uptake by the plant decides the yield of groundnut is cash crop such screening of groundnut cultivars to acetic acid can be beneficial for obtaining the higher yields. *Vigna mungo* L. is highly prized pulse very rich in phosphoric acid,it is grown all over India,annually a little less than one million tonnes of grains are produced from an area of about 2.5 milion hectares. Black gram is considered to have been

domesticated in Indian from its wild ancestral formGreen gram (*Vigna radiata* Linn) is spread all over the country with a production of about 0.8 million tones. The *Vigna radiata* 24% Crude protein, 1.3% fat, 56.5% carbohydrate[8]. Green gram is highly rich in fiber content, it belongs to absorb high level of bad cholesterol in the body.

MATERIALS AND METHODS

Sample collection

Three different leguminous plants such as groundnut (*Arachis hypogaea* L.) Black gram (*Vigna mungo* L.) and Green gram (*Vigna radiata* L.)

Isolation of *Rhizobium*

Healthy pink nodules from legume roots were selected and subjected to surface sterilization with 95% ethanol and H_2O_2 followed by successive washes with sterile distilled water. Suspension was made by crushing single nodule in sterile Petri plates containing few drops of sterile normal saline. A loopful of suspension was streaked onto Congo Red Yeast Extract Mannitol Agar (CRYEMA) by four quadrant method & incubated at 25º C for 3 days. A well isolated colourless colony was re-streaked onto same medium and incubated at 25º C for 3 days for purification. Isolated colonies were transferred onto slant of sterile Yeast Extract Mannitol agar and incubated at 25ºC for 3 days. Then they were placed in refrigerator at 4º C for preservation. After every two months, transfer on fresh slant was given. Four confirmatory tests were performed viz. Congo Red Dye Absorption Test, Growth on Glucose Peptone Agar to confirm isolates as Rhizobium not the *Agrobacterium* or other bacteria, which frequently come as contaminant.

Identification of *Rhizobium*

The bacterium was identified by the following tests.

Gram stainingwas also done by the standard method[9] Carbohydrate utilization test was also done byDanish[10].

Congo red test

An aliquot of 2.5 ml of 1 per cent solution of the dye in water was added to a liter of YEMA. The isolated bacterial culture was inoculated on to the plated YEMA and observed.

RESULT AND DISCUSSION

Confirmative test and sugar fermentation properties are also tested (Table 1 & 2)Effect of herbicides on survival of Rhizobia and nodulation of peas, groundnuts and Lucerne. Our findings stated that Pendimethalin herbicide was effective for R1 isolates from *A.hypogaea* than Atrazine. Thirteen herbicides were tested for toxicity against strains of *Rhizobium* used in South African legume inoculants for lucerne, clover, soybeans, groundnuts and lupines, respectively. The slow growing strains of *Rhizobium japonicum, Rhizobium lupini* and *Rhizobium* sp. (groundnuts) were less affected by at least two of the herbicides tested than strains of the fast growing *R. meliloti* and *R. trifolii.* Toxicity of a herbicide to Rhizobia in vitro did not necessarily correlate with its effect on nodulation and some are considered suitable for field application[11].

Disc diffusion method-Effect of herbicide on the growth of *Rhizobium* strains

This experiment was conducted for the purpose of the effect of herbicide on the growth of *Rhizobium* strain. The *Rhizobium*

strains (R1 (R-*Rhizobium*) from *Arachis hypogaea*,L., R2 from *Vigna mungo*, L. and R3 from *Vigna radiata* L. were gave following results respectively, maximum zone of inhibition observed in 0.3 ml/ 100 ml of all the three herbicides like

Pendimethalin, Paraquat Dichloride 24% SL and Atrazine 50% WP. The maximum zone of inhibition was observed in Paraquat Dichloride (0.3 ml/ 100 ml) 35 mm 33 mm and 34 mm from (R1,R2,R3). The moderate zone of inhibition was observed in Atrazine (0.3 ml/ 100 ml) 20 mm, 21 mm and 20 mm and the minimum zone of inhibition was observed in Pendimethalin (0.3ml/100 ml) 13 mm, 12 mm and 11 mm in diameter. The minimum zone of inhibition was observed at 0.1% level in Pendimethalin (0.1 ml/100 ml and 0.2 ml/100 ml), Atrazine (0.1 ml/100 ml). The *Rhizobium* strain was highly inhibited by Paraquat Dichloride and Atrazine respectively.in R2 isolates from *Vigna mungo*L. was showed better zone of inhibition on Pendimethalin (12mm) and Paraquat

dichloride (33mm). in R3- isolates from *Vigna radiata*,L., were subjected to zone of inhibition on Pendimethalin (11mm),followed by Atrazine (20mm) and Paraquat dichloride (34mm) (Table-3).

Strain Development

Rhizobium strain had the maximum N_2 fixing efficiency growth and yield as well as suitable to field application, so it was selected for the strain tolerant experiment. The *Rhizobium* isolate was improved in its resistance capacity towards chemical herbicides Pendimethalin, Paraquat Dichloride 24% SL and Atrazine 50% WP. R1 isolates from A.hypogaea were showed better performance on the tested herbicides. So it was selected for strain improvement.The isolate was found sensitive at 0.4 ml/100 ml in 4th generation 0.5 ml/100 ml in 5th generation of herbicides Paraquat Dichloride, Pendimethalin and Atrazine respectively. After the strain improvement treatment, the isolates attained tolerances to herbicide were again subjected to disc diffusion method.

Table: 1: Carbohydrate utilization by Rhizobial isolates

Sugars	Rhizobial isolate code		
	R-1	R-2	R-3
Arabinose	+	+	+
Xylose	+	+	+
Rhamnose	+	+	+
Glucose	+	+	+
Galactose	+	+	+
Fructose	+	+	+
Sucrose	+	+	+
Maltose	+	+	+
Raffinose	+	+	+

Table 2: Confirmation tests for isolates

Confirmatory test	Rhizobial isolate number		
	R-1	R-2	R-3
Congo red dye absorpition	-	-	-
Growth on glucose peptone	-	-	-
Nile blue reduction	-	-	-
Ketolactose	-	-	-

Table: 3 Disc diffusion methods, Effect of herbicide on the growth of strain improvement *Rhizobium* strain (Zone of inhibition in mm)

Treatments	Concentrations (ml/100 ml)	R1 *Rhizobium* (Zone of inhibition in mm)	R2 *Rhizobium* (Zone of inhibition in mm)	R3 *Rhizobium* (Zone of inhibition in mm)
Pendimethalin	0.1	6	5	5
	0.2	6	6	5
	0.3	7	7	6
Paraquat Dichloride	0.1	24	23	21
	0.2	23	22	22
	0.3	21	20	21
Atrazine	0.1	6	6	5
	0.2	15	14	13
	0.3	20	18	19

SUMMARY AND CONCLUSION

Overall results are highlighted that R1 (*Arachis hypgaea*) isolates had a adverse effect on the Pendimethalin at 0.3 % level was safer to Rhizobia. However, there is a need to test more Rhizobial strains against various herbicides commonly used for the control of weeds in all major legumes for their possible adverse effect on Rhizobia. If Rhizobial strains show sensitivity to herbicides, herbicide-resistant strains need to be developed. Further study was extended to test effect on the growth of Rhizobia, but it will adversely affect the nodulation process and nitrogenase activity or not.

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