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EFFECT OF PESTICIDES ON THE GROWTH OF *RHIZOBIUM NEPOTUM* ISOLATED FROM "STEM (COLLER) NODULES OF *AESCHYNOMENE INDICA*"

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ABSTRACT

Pesticides are used for effective protection of plants from pest. Extensive use of pesticide result into disturbance in natural biological system. Pesticides used to control the pest can be harmful for nitrogen fixing bacteria. The objective of present study is to assess the effect of 2,4- dichlorophenoxy acetic acid, Atrazine, Glyphosate on the growth of Rhizobium nepotum (LT56076) isolated from Aeschynomene indica in vitro. The Rhizobium nepotum (LT56076) morphologically characterized on CRYMA medium and authenbticated by using 16s rRNA sequence. Rhizobium nepotum (LT56076) was treated with 10,20,30 µl/ml Atrazine and Glyphosate inhibit the growth of Rhizobium nepotum (LT56076) and compared with control.

KEYWORDS: *Pesticide, Rhizobium nepotum, Aeschynomene indica.*

INTRODUCTION

Rhizobium legume symbiosis is a primary source of fixed nitrogen, any adverse effect on *Rhizobia* result into reduced rate of nitrogen fixation. Many factors that contribute the effect viz. temperature, pH, light, moisture, nutrition to *Rhizobia* Gibson (1977). The amount of applied pesticides reaching to target organism is about 0.1% w bulk remain in soil and contaminate the soil environment Carrigen et.al., 2006. Extensive use of pesticide in contemporary agriculture, the issue of impact of these chemical on soil microorganisms received move attention (Pimentel 1995) and (Andrea et.al., 2000)

Symbiotic nitrogen fixing bacteria *Rhizobium* and free nitrogen fixer *Azotobacter* can stimulate plant growth and control soil-borne plant pathogens (Shahzad et.al., 2012) and has been used as biofertilizer to increase the crop yield (Kloepper et.al., 1989). Besides this pesticide are used to control fungi, herbs, insects and pests in cultivated crops.

Pesticide in soil undergo different processes viz. Degradative, transport, adsorption or desorption depending upon chemical nature (Ayansina et.al., 2006). Pesticide interact with soil microorganisms and alter metabolic activity and bring the changes in physiological and biochemical behaviour of soil microbes (Weber J.B. et.al., 2004).

Pesticides used are harmful for *Rhizobium* which inhibit the nitrogen fixation processes also reduce bacterium respiration rate. (Tate ,1995, San-Tos and Flores, 1995). Pesticide also affects efficiency and activity of nitrogenise enzyme Sough and Wright (1994). The objective of present study was to study the effect of pesticide on *Rhizobium nepotum* (nitrogen fixing bacteria) isolated from wild type *Aeschynomene indica.*

MATERIAL AND METHODS:

Collection of Sample: The stem nodules (coller) from *Aeschynomene indica* were collected from Ratnagiri Konkan area (MS) India.

Pesticide Tested: The pesticide 2,4- Dichlorophenol acetic acid, Atrazine, Glyphosate.

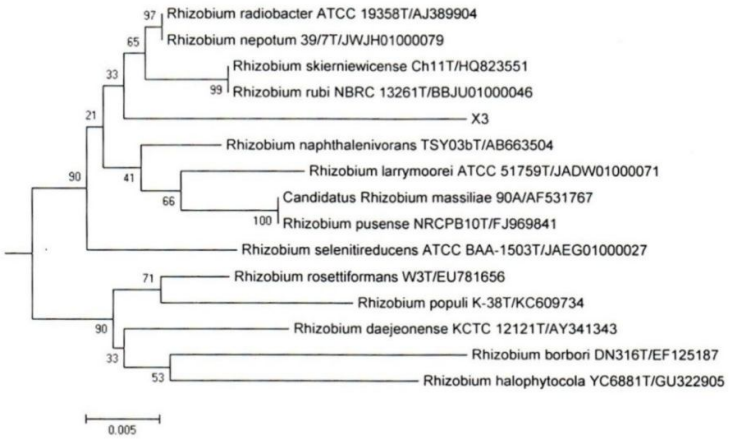
Isolation and Identification of *Rhizobium nepotum*:

Root nodules from *Aeschynomene indica* were collected. Healthy stem nodules were washed under tap water and surface sterilization was carried out with H_2O_2 for 1-4 minutes, followed by washing with distilled water. Healthy stem nodule crushed in a test tube containing sterilised distilled water and suspension was prepared. A loopful of suspension was streaked on Congo Red Yeast Extract Mannitol Agar plate (CRYMA). Plates were incubated at 28± 2^0C for 3 days. Well isolated typical single colony was restreaked on freshly prepared Congo Red Yeast Extract Mannitol Agar plates in order to obtain pure culture. Series of biochemical tests were carried out and finally identification carried by 16s rRNA gene sequencing method by using Sigma's Gene Elute Bacterial Genomic DNA kit (ARI,Pune)

>X2

GCCAATGCCGCGTGAGTGATGAATGCCTTAGGGTTGTAAAGCTCTTTCACCGGAGAAGATAATGACGGTATCCGG
AGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGAATTACTG
GGCGTAAAGCGCACGTAGGCGGATATTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGAT
ACTGGGTATCTTGAGTATGGAAGAGGTAAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTACATATTCGGAGGA
ACACCAGTGGCGAAGGCGGCTTACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTAGCCGTCGGGCAGTATACTGTTCGGTGGCGCAGCTAA
CGCATTAAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
GGTGGAGCATGTGGTTTAATTCGAAGCACCGCGGCAGAACCTTACCAGCTCTTGACATTCGGGGTATGGGCATTN
GAGACGATGTCCTTCAGTTAGGCTGGCCCCAGAACAGGTGCCTGCATGGCTGTCCTCAGCTCGTGTCCTGGAAAT
GTTGGGATTAAGTCCCGCAACGAGCGCAACCCCTCCCCCCTTAATTTGCCAGCATTNATTTGGGCCCTCTAAGGGG
ACTGCCGGT

Strain Designation	Closest phylogenetic affiliation	Max ident
X2	*Rhizobium nepotum* 39/7 (T) 16S ribosomal RNA gene partial sequence (JWJH01000079)	97.59%



Effect of pesticides by Agar Well Diffusion Method:

The agar diffusion methods (cylindrical-plate or cup-plate, well-method) are most wildly used for estimation of potency and bioactivity of antimicrobial compound. The Agar well diffusion method based on diffusion of antimicrobial compounds from well in solidified agar layer in petri dish.

In present study the Congo Red Yeast Extract Mannitol Agar plate was taken and the culture of *Rhizobium nepotum* was spread on the plate with the help of sterile cotton bud. Three wells having diameter 6mm were prepared with the help of cork borer in which 10µl of 2,4- Dihydrophenoxy acetic acid, Atrazine, Glyphosate each pesticide were added in to three different wells. The plates were incubated at 28± 2^0C for 3 days. After incubation the zone of inhibition was observed around each well of pesticide (Boner et.al., 2008).

RESULT AND DISCUSSION:

Rhizobium nepotum isolated from stem nodules of *Aeschynomene indica* were tested for effect of pesticide on growth by using Agar Well Diffusion method.

It was found that nitrogen fixing bacteria *Rhizobium nepotum* were inhibited by pesticides tested. Out of three pesticides 2,4-dichlorophenoxy acetic acid inhibit *Rhizobium nepotum* at 30µg/ml concentration, Atrazine and Glyphosate at 50µg/ml concentration.
The results are shown in the table,

Effect of pesticides on the growth of *Rhizobium nepotum*

	Concentration mg/ml				
Name of pesticide	10	20	30	40	50
2,4 –Dichlorophenoxy acetic acid	+	+	-		
Atrazine	+	+	+	+	-
Glyphosate	+	+	+	+	-

+ = sensitive, - = insensitive

Khudhur et.al., (2013) reported that *Azotobacter* from soil and *Azotobacter vinelandii* isolated from soil was inhibited by two different pesticides. Thus pesticide had negative effect on growth of nitrogen fixing bacteria from phyllospheric soil.

Sudhakar et.al., (2000) reported that there was variable effect of pesticide on the growth of nitrogen fixing bacteria from root nodule of soybean and pea plant *Rhizobium*. Both the organism *Rhizobium and Azotobacter* was tested against pesticide inhibited the growth of both nitrogen fixing bacteria.

Digrak et.al., (2001), Nasima et.al., (2005) in endosulfan pesticide singnificant reduction in soil microorganisms occurred at higher concentration. Birhan ayalemert et.al., (2017) assessed the effect of glyphosate and mancozeb on the growth of *Viciafaba Rhizobia* isolates in vitro on nitrogen fixation performance and found to be almost all isolates were affected. In this connection our result confirmed with legume *Rhizobia* also showed similar effect of glyphosate and Atrazine.

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