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EFFECT OF ANTIBIOTICS ON THE GROWTH OF *RHIZOBIUM NEPOTUM* ISOLATED FROM "STEM NODULE OF *AESCHYNOMENE INDICA*"

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ABSTRACT

Different antibiotics were used for efficacy viz. chloramphenicol, Cloxacillin, erythromycin, ampicillin, kanamycin against Rhizobium nepotum was studied in vitro at 10, 15, 30 µg and 10 unit. Chloramphenicol, ledermycin, gentamycin, cephalosporin,streptomycin, tetracycline and kanamycin inhibit the growth of Rhizobium nepotum (LT56076) at all concentrations while cloxacillin, erythromycin, ampicillin, amoxycillin and penicillin were ineffective.

KEYWORDS: *Rhizobium nepotum*, antibiotics, *Aeschynomene indica*, *HPLC, MIC, MBC, MPC.*

INTRODUCTION

Plants require nitrogen, they acquire either by soil or atmosphere by symbiotic nitrogen fixation (Vance, 1990) *Rhizobium* capable of fixing atmospheric nitrogen in symbiotic association with improving soil nitrogen content (Sanginsg et.al., 1988). Crop productivity will be better when crop inoculated with *Rhizobium* strain (Bhide, 1956)

Chemotherapeutic agents like antibiotics are used to kill or inhibit the growth of microorganisms. They destroy the pathogenic microorganisms which causes diseases by inhibiting their growth at low concentration avoiding undesirable damage to host (Dafale et.al., 2012, Denyer et.al.,2004).

Different antibiotics were tested for their efficacy to control different diseases of stem nodulating legumes (Bowler and Atherton, 1972)

Antibiotics play role in fighting against diseases but misuse of antibiotics foster the spread and increase of antibiotic resistance. The effectiveness of antibiotic depends on many factors such as location of infection, concentration of antibiotic, presence of interfering substance, nature of pathogen (Prescott et.al., 2008).

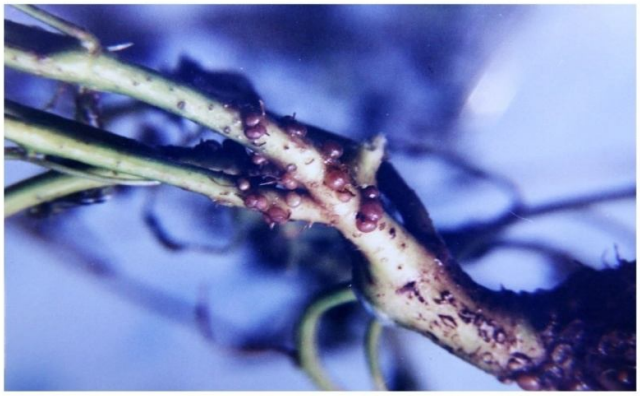
Potency of antibiotic can be determined by many methods like microbiological assays. HPLC, Immunological assay, Radio immune assay (Pofaller et.al., 1984). Commercially produced antibiotics require determining potency by microbiological assay (Greco, 1997)

Microbiological assays precisely determine both potency and by bioactivity of antibiotic and does not require specialised equipment (Pinto et.al., 2007)

Microbiological assay has several parameters such as Minimal inhibitory concertation (MIC), Minimal bactericidal concentration (MBC), Mutation prevention concentration (MPC) and critical concentration (Ccr) were used to describe the potency of antibiotic.

Microbiological assays are simple, sensitive cost effective, precise which gives reproducible result similar to HPLC. HPLC quantify the potency of antibiotics but not the bioactivity where as microbial assay estimate both potency and bioactivity of antibiotics. Hence Microbiological assay is most convenient method to determine potency of antibiotics (Cazedey and Salgado, 2011)

Fig: Stem nodules of *Aeschynomene indica*



In present study, *Rhizobium nepotum* (LT56076) isolated from stem (coller) nodule of *Aeschynomene indica* and experiments were conducted to find out effect of different antibiotics.

MATERIAL METHODS:

Antimicrobial susceptibility test performed three ways viz. broth inoculation, agar dilution and disc diffusion method. In broth dilution method multiple concentrations of antibiotics added in broth tubes and subsequently inoculated with test organism. Inhibition of bacterial growth related to concentration of antibiotics.

In agar dilution method the dilution is made in agar and bacteria to be tested are inoculated on to surface of multiple agar plates containing different concentration of antibiotics. For present study Disc diffusion method of Kirby Bauer et.al.,1966 were used. Testes were carried out with *Rhizobium nepotum* (LT 56076) isolated from stem (coller) nodules of *Aeschynomene indica*, with 12 different antibiotics.

Filter paper discs were obtained from SPAN Diagnostic India. Susceptibility testes were performed with CRYEMA. Zones of inhibition were measured after 48hrs of incubation at $28^0 \pm 2^0$ C. The minimum concentration (MIC) was recorded as the lowest concentrations resulting inhibition of growth.

The comparative stability of discs (12) was determined by storing the discs in open containers and closed storage rack with desiccant. Both containers were stored at room temperature (28^0C) and refrigerator (18^0C) for 7 days. The discs were removed and tested against *Rhizobium nepotum* (LT 56076) by using agar overlay modification of Kirby and Bauer (Barry et.al., 1970) the diameter of inhibitory zones was recorded after 48hrs of incubation at $28^0 \pm 2^0$ C.

The zone diameters around the antibiotics (12) discs varied not more than 1mm during 7 days study regardless the storage condition thus confirming reproducibility of the method.

RESULT AND DISCUSSION:

Amongst 12 antibiotics assayed Chloramphenicol, ledermycin, gentamycin, cephalosporin, streptomycin, tetracycline, kanamycin inhibits the growth of *Rhizobium nepotum* (LT 56076) at all concentrations while cloxacillin, erythromycin, ampicillin, amoxycillin and penicillin does not inhibit the growth of *Rhizobium nepotum* (LT 56076).

The effect of antibiotics on the growth is shown in the table

Rhizobium strain	Ch	Cl	Le	Er	Gn	Ap	Am	Ce	St	Tr	Kn	Pn
Rhizobium nepotum (LT 56076)	S	R	S	R	S	R	R	S	S	S	S	R

S= sensitive R=Resistant

Tirussel et.al., 1943 studied the effect of antibiotic substance upon Rhizobial culture and it was found that Actinomycin, Erythrocin, Eyrocidine hydrochloride had antirhizobial effect. Keczhe's and Manninger (1962) observed the antagonistic effect of Penicillin, streptomycin, chloramphenicol and terramycin towards growth of different strains of Rhizobia.

Graham (1963) reported that streptomycin, auromycin, terramycin and ledermycin are known to exhibit antimicrobial effect.

In this connection our results confirmed with legume *Rhizobia*, *Rhizobium nepotum* (LT 56076) showed similar effect under investigation with different antibiotics.

REFERENCES:

Barry A. L., Garcia F. And Thrupp L. D. (1970) An improved single disc method for testing the antibiotic susceptibility of rapidly growing pathogen Amer. J. Clin. Pathol. 53:149-158

Bauer A. W., Kirby W. M. M., Sherris J. C. and Tourck M. (1966) Antibiotic susceptibility testing by a standardised single disc method. Amer. J. Cln. Pathol. 45(4):493-496

Bhide V. P. (1956) Cross inoculation studies with some Rhizobia of cowpea group.

Bowler J. W. And Atherton J. G. (1972) Effect of tetracycline antibiotics on plants affected by legume little leaf disease. Aust J. Biol. 25:43-51.

Cazedey E. C. L., Salgado H. R. N. (2011) Development and validation of microbial bioassay for determination of orbifloxacin in pharmaceutical preparations Pharmaceutics 3:572-581

Dafale N. A, Semwal P. K., Agarwal (2012) Quantification of ceftriaxone sodium in pharmaceutical preparation by new validated microbiological bioassay. Anl. Method 41:2490-2498

Denyer S. P., Hodges N. A., Gorman S. P. (2004) Hugo and Russell's Pharmaceutical Microbiology seventh ed. Backwell Publishing company U. K.

Graham P. H. (1963) Antibiotic sensitivity of root nodule bacteria Aust. J. Biol. Sci. 16:557-559

Greco G. M. (1997) Microbiological Assay Systems for analysis of antibiotics in pharmaceutical formulations (PhD dissertation) the state University of New Jersey U. S. A.

Keczkes H. and Manninger E. (1962) Effect of antibiotics on growth of Rhizobia. Can:J. Microbiol 8:157-159

Pfaller M. A., Krogstad D. J., Granich C. G. (1984) Laboratory evaluation of five assay methods for Vancomycin bioassay high pressure liquid chromatography, fluorescence polarisation immune assay, radioimmune assay and fluorescence immune assay. J. Microbiol 20:311-316.

Pinto TJ. A., Lourenco F. R., Kaneko T. M. (2007) Microbiological assay of gentamycin employing an alternative experimental design, AOAC - Annual meeting and Expostion Anais. Anahein - California 157

Prescott L. M. Harley J. P., Klein D. A. (2008) Microbiology seventh ed. Mcgraw - Hill Newyork 825-858.

Sanginga N. Mulongoy K. and Ayanoba A. (1988) Response of leucaena /Rhizobium symbiosis to mineral nutrients in South Western Nigeria plant and soil. 112:121-127

Trussel P. C. and Sarles W. B. (1943) Effect of antibiotic substances upon Rhizobia J. Bacterio. 45:29-32

Vance C. P. (1990) Symbiotic Nitrogen fixation recent genetic advance in the biochemistry of plants: Intermediary nitrogen metabolism Mifin B. J. Lea P. J. eds. 16:48-88 Academic press, San Diego.