A Study on Effect of 2, 4 D herbicide on Growth of Nitrogen Fixing Bacteria

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Abstract

Soils contain microorganisms viz. bacteria, fungi, yeasts, photosynthetic organisms including algae and microorganisms such as protozoa, nematodes, mites, springtails, spiders, insects and earthworms. A study was carried out to determine the effect of 2, 4 D on growth of . **selected nitrogen fixing bacteria** All the Selected six species shown equal resistance to 2, 4 D.

INTRODUCTION

The assessment and monitoring of soil life and soil health can be used to develop more sustainable and productive farming systems (K.K.Barman and Jay G Varshney 2008). herbicides may also greatly influence soil biota populations indirectly by their effects on vegetation which provide habitat and food for many of them. The soil organisms may respond differentially due to the changes in vegetation rather than to direct herbicide effect (Grossbard and Davies 1976, Haugland 1994). Herbicidal impact on legumes are conflicting, several studies have conclusively shown that some of these chemicals are incompatible with *Rhizobium* (Singh and Wright, 2002; Aamil et al., 2004)

The use of herbicides is an important component of the production process of most crops, mainly those capable of fixing atmospheric nitrogen. The fixation of the dinitrogen through soybean-rhizobium symbiosis helps with over 70% in the requirement of total N of the culture (Marenco et al., 1993; Thurlow & Hiltbold, 1985). Effects of some pre emergence and post emergence herbicides individually and in combinations on soil microflora have been studied earlier. These studies showed that the herbicides in general cause detrimental effect on soil microflora, although the effect was not stable (Bandopadhyay and Mandal,1991). *Rhizobium* strains isolated from pea nut nodules may be harmed by Trifluralin (Algawadi and Reddy,1986) *Rhizobium Japonicum* has been reported more resistant to MIC of some pesticides (Nair et al ., 1989). At low concentrations, 2, 4-D might stimulate growth of the organism by cell division and elongation (Armita Nahi et .al 2016)

MATERIAL AND METHODS

Cultures of *Bradirhizobium japonicum,Rhizobium meliloti,Rhizobium leguminosarum bivor trifoli ,Rhizobium leguminosarum bivor viceae, Rhizobium leguminosarum bivor phaseoli and Bradirhizobium* species were made available by the Department of Microbiology, University of Pune from United states Department of Agriculture.

The cultures were revived in Yeast extract mannitol broth at 30 oC on shaker (200 rpm) for a week . A loopful of culture was streaked on yeast extract mannitol agar plates and incubated at 30 oC for 48 hrs. Suspensions were made in sterile yeast extract mannitol broth

The herbicide used is 2,4 D (Monsanto chemical company Mumbai) Tenfold serial dilutions of this herbicide were made in sterile tap water. In each dilution equal volume of melted double strength yeast extract mannitol agar (YEMA) was added to get YEMA media with 10^{1} 10^{2} 10^{3} ……10^{10} 2,4 D dilutions. Plates of each dilution agar were poured and three plates were used for every species of *Rhizobium*

0.1 ml fresh culture (1x 10^{6} cells) of each Rhizobium species was spread on 2,4 D dilution agar in triplicate and incubated at 30 0C for 48 hrs . Plain yeast extract mannitol agar (without herbicide) were spread with 0.1 ml of each *Rhizobium* species in triplicate as control. The MIC was calculated On the basis of concentration of active ingredient of the 2,4 D.

RESULTS

Table one show the minimum inhibitory dilution of 2,4 D. At higher concentrations the growth was totally inhibited. It has been observed that minimum inhibitory dilution (MID) was 10^{3} for all *Rhizobium* species . Microbial analysis indicate a significant increase in the populations of*Azospirillum*sp. following the application of 2,4-D under flooded conditions, while the populations of*Azotobacter*and anaerobic nitrogen fixers were rather inhibited. (G.K.Patnaik s.al.1994) Herbicides may physiologically influence the nodulating potential of certain strains of rhizobium (Pl.Eberach and L.A. Douglas 1989)

The MIC for of 2, 4 D for six selected species of *Rhizobium* was 800 ppm. From the results obtained it can be concluded that six species of *Rhizobium* selected for study showed equal resistance to 2,4 D.

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Table 1: Effect of 2 4 D on growth of *Rhizobium species*

Sr.No	Dilution →	10^{1} *	10^{2}	10^{3}	10^{4}	10^{5}	10^{6}	10^{7}	10^{8}	10^{9}	10^{10}	C
01	*Bradyrhizobium japonicum*	_	_	_	+	+	+	+	+	+	+	+
02	*Rrhizobium meliloti*	_	_	_	_	+	+	+	+	+	+	+
03	*R.leguminosarum bivor trifoli*	_	_	_	+	+	+	+	+	+	+	+
04	*R.leguminosarumbivor viceae*	_	_	_	+	+	+	+	+	+	+	+
05	*R.leguminosarum bivor phaseoli*	_	_	_	+	+	+	+	+	+	+	+
06	*Bradirhizobium species*	_	_	_	+	+	+	+	+	+	+	+

*80 000 ppm

No growth → - Growth → +

Control → C → YEMA without Herbicide

Table 2 : Minimum Inhibitory Concentration in ppm

Sr. No	Name of the species	2,4 D
01	*Bradyrhizobium japonicum*	800
02	*Rrhizobium meliloti*	800
03	*R.leguminosarum bivor trifoli*	800
04	*R. leguminosarum bivor viceae*	800
05	*R. leguminosarum bivor phaseoli*	800
06	*Bradirhizobium species*	800

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