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Effect of Humic Acid on Nitrogen Fixation by *Azospirillumlipoferum*

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Abstract: *The present investigation shows the effect of humic acid or nitrogen fixation Azospirillumlipoferum Inoculated soil.There was an increase in nitrogenase activity of soil amended with humic acid(in the form of sodium humate) and Azospirillum alone or in combination of each other. The maximum activity was recorded on 10th day of incubation .Humic acid alone was having a stimulatory effect or nitrogenase activity of soil only up to 6th day after that there was declinein the activity .The stimulatory effect of humate on population of Azotobacter and biological nitrogen fixation has been reported by Gaur and Co-workers. (Gaur and Mathur: 1966;Gaur,Mathur and Bhardwaj, 1968; Bhardwaj and Gaur; 1970)..*

Keywords: Azospirillumlipoferum

I. INTRODUCTION

Soil health is one of the key important factors in determining crop performance. Use of organic materials in agriculture is to reduce pollution causing bythe use of chemical fertiliser and is a positive step to achieve sustainable agriculture and fertiliser efficiency. The effect of fulvic and humic acid application on yield and nutrient uptake in Sunflower (Helianthus annus) was seen by Aydin and Turan(1999). The effect of foliar application of humic acid on growth and yield of durum wheat was seen by Deifineet.al. in 2005. The fruit yield and quality of watermelon affected by hybrids and humic acid application was seen by Salman et.al. in 2005. The effect of lignite humic acid and fertiliser on yield of onion and nutrient availability was seen by Sangeetha .M in 2006.

The effect of different factors was examined on nitrogen fixation by *Azospirillum* inoculation using acetylene -ethylene assay method. Rice and Paul (1972) found that nitrogenase activity in a waterlogged soil straw system was related to the number of nitrogen fixing clostridia and to some extent to the microbial counts. The nitrogen is activity indicated a change in the microbial activity at about the 8th day of incubation this was attributed to the sequential use of the different organic components with a short lag in activity between the periods of maximum hemicellulose and cellulose utilization. Bhowmick and Magu(1982) reported that application of MCPB had inhibitory effect on oxygen consumption in soil particularly at low concentrations. The application of disystone showed beneficial effect on microbial activity by increased Co 2 evolution and were further stimulated by application of FYM.

II. MATERIALS AND METHODS

Method of extraction of humic acid: 500 g air dried compost sieved through 2 mm mesh wastaken in a 1 liter well stoppered conical flasks. 2.5 litre (1:5 compost extractant ratio) of freshly prepared sodium pyrophosphate- sodium hydroxide mixture was added. The contents were shaken for 1 hr and then allowed to stand for 18 hrs. The solution was filtered. The residue is the humin insoluble fraction while the filtrate contains humic and fulvic acids.

Precipitation of humic acids: Humic acids start precipitating when the pH of the filtrate isadjusted to about l preferably with conc. HCl. Allow it to stand for 12 hrs to ensure completion of the precipitation and then filter. Gel of humic acid remains on the filter paper while fulvic acid is collected as the soluble fraction. Humic acid is washed thoroughly in running water to make it chloride free. Humic acid obtained above was redissolved in l N-NaOH (soium hydroxide) and solution is known as sodium humate. After oven drying it was found that 10 ml solution contained 0.26 g humic acid. 15 ml humate at the rate of 0.4% wad added in 100 g soil.

The treatments were as given below: -

Soil (Alone)

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Soil + *A. lipoferum*
Soil +Humic Acid (HA)
Soil +HA+*A. lipoferum*
Samples were drawn at 6th, 10th, 15th, and 20th day of incubation for determination ofnitrogenase activity.

III. RESULTS

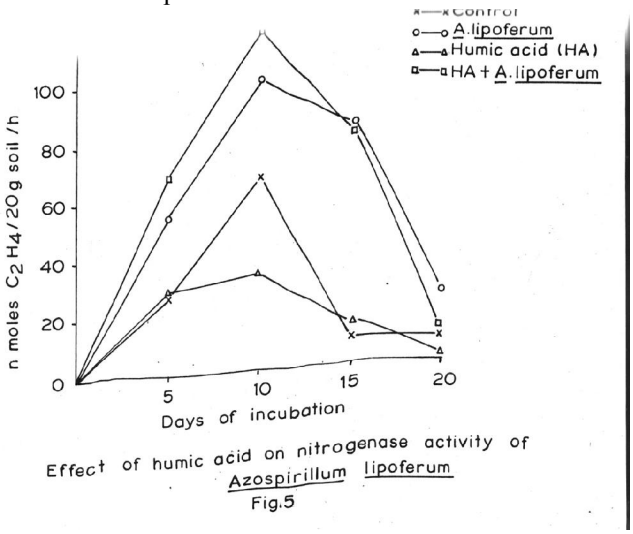
The data on the effect of humic acid on nitrogenase activity of *Azospirillumlipoferum* are presented in Table. The nitrogenase activity in control soil, studied at different intervals, varied from 66.48 to 8.10 n moles C_2H_4/20 g soil/hr. The mean value worked out to be 27.23 n moles C_2H_4. The nitrogenase activity of the soil due to *Azospirillum* inoculation alone or in presence of humic acid was significantly higher over control. Soil amended with humic acid also stimulated the nitrogenase activity on 6th and 15th day of inculation but was significantly suppressed on 10th and 20th day of incubation.

Table: Effect of humic acid on nitrogenase activity n moles C2 H4/20 g soil/hr Of *A.lipoferum*

Treatments	**Incubation**	**Period**	**Days**		**Mean**
	6	**10**	**15**	**20**	
Control(soil)	**25.62 (656.22)**	**66.48 (4419.59)**	**8.73(76.21)**	**8.10(65.61)**	**27.23**
A.lipoferum	**54.45 (2965.16)**	**99.56 (9912.19)**	**82.29(6771.64)**	**24.83 (616.53)**	**65.28**
Humic acid(HA)	**28.74 (825.80)**	**33.55 (1125.60)**	**14.43(208.22)**	**3.94 (15.52)**	**20.16**
HA+*A.lipoferum*	**68.76 (4727.94)**	**117.47 (13799.20)**	**80.95 (6552.90)**	**8.46 (71.57)**	**68.91**

	44.39	79.27	46.60	11.33
	Treatments	stages	interaction	
S. em+	0.5914	0.5914	1.18	
C.D.5%	1.70	1.7 0	3.41	

Actual values are given in the parenthesis.
values obtained after / Transformation are outside the parenthesis



Effect of humic acid on nitrogenase activity of Azospirillum lipoferum
Fig.5

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The maximum nitrogenase activity was found on 10th day followed by a decrease on 15th and 20th days. On 6th day of incubation where *A. lipoferum*, humic acid (0.5%) alone or in combination of each other were applied to soil, stimulated the nitrogenase activity significantly as compared to control. The maximum activity was found in humic acid plus *A. lipoferum* treatment (68.76 n moles C_2H_4) followed by
A. lipoferum (54.45 nn moles C_2H_4) alone.
On 10th day of incubation *A.lipoferum* alone or in presence of humic acid had significantly higher value than control while humic acid had lower value than control. On 15th day of incubation each treatment had significantly higher value than control. On 20th day of incubation with *A. lipoferum* resulted in significantly higher value than uninoculated soil while humic acid plus *A. lipoferum* were as par with control and humic acid was showing significantly lower value.

IV. DISCUSSION

There was an increase in nitrogenase activity of soil amended with humic acid (in the form of sodium humate) and *Azospirillum* alone or in combination of each other . The maximum activity was recorded on 10^{th} of incubation. Humic acid alone was having a stimulatory effect on nitrogenase activity of soil only up to 6th day after that there was decline in the activity. However inoculation of *A.lipoferum* in presence of humate was having higher values than *A . lipoferum* alone up to 10 days ofincubation but after that there was a slight decrease. The stimulatory effect of humate on population of *Azotobacter*and biological nitrogen fixation has been reported by Gaur &co-workers(Gaur and Mathur :1966; Gaur, Mathur& Bharadwaj1968; Bharadwaj &Gaur ,1970)
Recently the use of variety of organic acids to improve the quality and quantity in the garden crops is increased. Very small quantities of organic acid significantly effects in improving the physical and chemical properties of soil and also improves the quality of agricultural products . The test (Aydin(1999) showed that adding humus to the soil material in barley, sugar beet and potatoes cultivation gave a significant better performance .
Since sodium humate was not dialysed probably after 10 days ,excessive sodium ions and alkalinity might have suppressed the microbial population and their activity,

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