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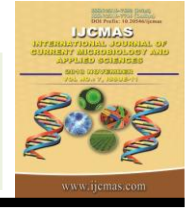

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Effect of Humic Acid on Soil Microbial Population and Enzymatic Activity

P. Chaitra* and K.K. Math

Department of Soil Science and Agricultural Chemistry, College of Agriculture, Dharwad University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India

**Corresponding author*

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A B S T R A C T

A pot culture experiment was conducted to study the effect of humic acid on soil microbial population and enzymatic activity in groundnut in an Alfisol at Main Agricultural Research Station, Dharwad. The experiment was laid out in Completely Randomized Design (CRD) with thirteen treatments and three replications. Maximum population of free living N2 fixing bacteria (15.33 cfu × 107 g-1 soil) and phosphorus solubilizing bacteria (14.08 cfu × 104 g-1 soil) was recorded in the treatment which received soil application of humic acid extracted from vermicompost @ 20 kg ha-1 in combination with the foliar spray of 0.2 per cent at 30 DAS. Similar trend was observed in soil enzymatic activity. Significantly highest dehydrogenase (24.20 µg TPF g-1 soil-1 day-1), urease (5.01 µg NH4-N g-1 soil day-1), and phosphatase (15.12 µg PNP g-1 soil hr-1) activity was also recorded in the treatment which received soil application of humic acid from vermicompost @ 20 kg ha-1 along with foliar spray of 0.2 per cent humic acid at 30 DAS.

Introduction

Agriculture is highly dependent on the use of chemical fertilizers, but the excess use of high analysis fertilizers leads to depletion of macro and micronutrients in soil.

Imbalanced nutrients application causes micronutrient deficiencies in soil viz., zinc, boron and iron which are limiting the crop productivity. Organic manures contain all the nutrients though in lower concentration and hence maintain optimum soil, physical, chemical and biological environment.

Plant growth promoting substances like humic acids are often novel and potential tools to provide substantial benefits to agriculture. Efficient use of organic wastes can be useful for the extraction of humic substances.

Materials and Methods

A pot culture experiment was conducted in an Alfisol at MARS, Dharwad. The experiment was laid out in Completely Randomized Design (CRD) with thirteen treatments and three replications. Recommended fertilizers FYM: 7.5 t/ha, 25:50:25 N: P_2O_5: K_2O kg/hain the form of urea, DAP and MOP, respectively, $ZnSO_4$: 25 kg/ha, $FeSO_4$: 25 kg/ha were applied as basal. Application of 500 kg gypsum ha^{-1}(at flowering stage) and seed treatment with *Rhizobium*@ 500 g ha^{-1} was common to all the treatments. Soil samples were collected at peak flowering stage (35 DAS) and analysed for total microbial population and enzymatic activity. Pour plate and serial dilution method was used for estimating the population of free living N_2 fixing bacteria and phosphorus solubilizing bacteria in soil. The test soil samples were serially diluted and aliquots of suitable dilutions were plated with appropriate culture media. The culture media used were Norris N-free medium for free living N_2fixing bacteria and Pikovskaya's medium for phosphorus solubilizing bacteria. Dehydrogenase activity in the soil samples was determined by following the procedure as described by Casida *et al.*, (1964). Phosphatase activity of soil samples was determined by following the procedure of Evazi and Tabatabai (1979). Urease activity of soil samples was determined by following the procedure of Tabatabai and Bremner (1972).

Results and Discussion

The results on the effect of humic acid application on population of free living N_2 fixing bacteria and phosphorus solubilizing bacteria (PSB) are given at table 1. Significantly highest population of free living N_2 fixing bacteria (15.33 cfu × 10^7 g^{-1}) and PSB (14.08 cfu× 10^4 g^{-1} soil) were observed in the treatment which received which received soil application of humic acid extracted from vermicompost @ 20 kg ha^{-1} in combination with the foliar spray of 0.2 per cent humic acid at 30 DAS (T_9), which was on par with the soil application of humic acid extracted from vermicompost @ 20 kg ha^{-1} in combination with the foliar spray of 0.1 per cent humic acid at 30 DAS (T_7). The lowest population of free living N_2 fixing bacteria (8.33x 10^7cfu g^{-1} soil) and PSB (8.08x 10^4cfu g^{-1} soil) was observed with the application of RPP alone (T_1).

The increased population of beneficial microorganisms might be due to the application of humic acid which produce the beneficial influence on microbes in several ways. They act as a source of food and energy for the growth and development of many microorganisms.

The addition of organic materials in the form of humic acid resulted in increased soil microbial biomass than that of inorganic fertilizers which is mainly due to higher organic carbon content. Perez-Piqueres *et al.*, (2005) reported that application of organic matter to soil increased the population of beneficial microorganisms in soil by serving as a source of nutrients. The findings are also in accordance with Shahein *et al.*, (2014) who reported that soil and foliar application of humic acid along with 50 per cent N, P and K increased soil microbial population when compared to control with no humic acid.

The results on the effect of humic acid application on soil dehydrogenase, urease and phosphatase activity are given at table 2. The results showed significantly highest dehydrogenase (24.20µg TPF g^{-1} $soil^{-1}$ day^{-1}), urease (5.01µg NH_4-N formed g $soil^{-1}$ day^{-1}) and phosphatase (15.12µgPNP g^{-1} soil hr^{-1}) activity with the soil application of humic acid from vermicompost @ 20 kg ha^{-1} along with foliar spray of 0.2 per cent humic acid at 30 DAS (T_9).

Table.1 Effect of soil and foliar application of humic acid to groundnut on the population of free living N_2-fixing bacteria and PSB at 35 DAS

Treatments	Free living N_2-fixing bacteria (CFU × 10^7 g^{-1} soil)	PSB (CFU × 10^4 g^{-1} soil)
T_1: RPP (control)	8.33	8.08
T_2: SA of HA @ 10 kg ha^{-1}	12.67	12.15
T_3: SA of HA @ 20 kg ha^{-1}	13.67	13.42
T_4: FA of HA @ 0.1 % @ 30 DAS	9.33	9.42
T_5: FA of HA @ 0.2 % @ 30 DAS	10.33	9.58
T_6: SA of HA @ 10 kg ha^{-1} + FA of HA @ 0.1 %@ 30 DAS	12.33	12.23
T_7: SA of HA @ 20 kg ha^{-1} + FA of HA @ 0.1 %@ 30 DAS	14.48	13.23
T_8: SA of HA @ 10 kg ha^{-1} + FA of HA @ 0.2 %@ 30 DAS	13.33	12.23
T_9: SA of HA @ 20 kg ha^{-1} + FA of HA @ 0.2 %@ 30 DAS	15.33	14.08
T_{10}: FA of commercial HA @ 0.1 % @ 30 DAS	9.33	9.08
T_{11}: FA of commercial HA @ 0.2 % @ 30 DAS	9.67	9.23
T_{12}: SA of commercial HA @ 10 kg ha^{-1}	12.00	11.40
T_{13}: SA of commercial HA @ 20 kg ha^{-1}	13.33	12.23
S.Em. ±	0.32	0.46
C.D. @ 0.05	0.93	1.35
CV (%)	4.71	4.52

RPP – Recommended package of practice; SA – Soil application; FA – Foliar application; HA – Humic acid
RPP is common for all the treatments; DAS – Days after sowing

Table.2 Effect of soil and foliar application of humic acid to groundnut on enzyme activity in soil at 35 DAS

Treatments	Dehydrogenase (µg TPF g^{-1} soil day^{-1})	Urease (µgNH_4-N g^{-1} soil day^{-1})	Phosphatase (µg PNP g^{-1} soil hr^{-1})
T_1: RPP (control)	16.50	3.13	13.20
T_2: SA of HA @ 10 kg ha^{-1}	20.48	4.03	14.27
T_3: SA of HA @ 20 kg ha^{-1}	22.54	4.34	14.60
T_4: FA of HA @ 0.1 % @ 30 DAS	18.58	3.43	13.52
T_5: FA of HA @ 0.2 % @ 30 DAS	19.30	3.55	13.97
T_6: SA of HA @ 10 kg ha^{-1} + FA of HA @ 0.1 %@ 30 DAS	21.90	4.56	14.72
T_7: SA of HA @ 20 kg ha^{-1} + FA of HA @ 0.1 %@ 30 DAS	23.80	4.91	14.96
T_8: SA of HA @ 10 kg ha^{-1} + FA of HA @ 0.2 %@ 30 DAS	22.20	4.79	14.80
T_9: SA of HA @ 20 kg ha^{-1} + FA of HA @ 0.2 %@ 30 DAS	24.20	5.01	15.12
T_{10}: FA of commercial HA @ 0.1 % @ 30 DAS	18.30	3.28	13.40
T_{11}: FA of commercial HA @ 0.2 % @ 30 DAS	19.00	3.35	13.80
T_{12}: SA of commercial HA @ 10 kg ha^{-1}	19.80	3.71	14.23
T_{13}: SA of commercial HA @ 20 kg ha^{-1}	20.50	4.15	14.42
S.Em. ±	0.46	0.07	0.17
C.D. @ 0.05	1.34	0.22	0.50
CV (%)	3.89	3.33	2.11

RPP – Recommended package of practice   SA – Soil application   FA – Foliar application   HA – Humic acid
RPP is common for all the treatments   DAS – Days after sowing

The highest enzymes activity in the T_9 treatment was on par with T_7 treatment which received combined soil and foliar application of humic acid from vermicompost, respectively @ 20 kg ha^{-1} and 0.1 per cent concentration.

Lowest dehydrogenase (16.50µgTPF g^{-1} soil day^{-1}), urease (3.13 µg NH_4-N g^{-1} $soil^{-1}$ day^{-1}) and phosphatase (13.20 µg PNP g^{-1} soil hr^{-1}) activity was recorded in T_1 (RPP alone) treatment. Soil enzymatic activity showed positive correlation with microbial population.

Higher enzymes activity in soil due to the application of humic acid might resulted in higher biomass production and extensive root exudates in the rhizosphere and this might have promoted the build-up of microbial population in soil. Application of humic acids induce better proliferation of roots and is responsible for the higher soil enzymes activity.

Spier and Ross, (1976) reported that enzymes activity in soil increased due to the addition of organic matter to soil. The application of organic matter in the form of humic acid to soil and foliage resulted in the release of higher quantity of root exudates and hence induced the enzymes activity in soil. Thenmozhi (2001) reported increased enzymes activity in the treatment receiving humic acid application which created favourable condition for microbial activity and the humic acid also acts as a carbon source for the growth of microorganisms.

Total population of free living N_2 fixing bacteria and phosphorous solubilizing bacteria were accelerated upon application of humic acid. The increased microbial population in turn contributed for an improvement in the enzymatic activities at peak flowering stage. Highest microbial population and enzymatic activity was observed in the treatment which received soil application of humic acid extracted from vermicompost @ 20 kg ha^{-1} along with foliar spray of 0.2 per cent humic acid at 30 DAS.

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