A Stimulating Effect of Humic Compounds on The Growth of Legumes, Nodulation and Biological Nitrogen Fixation

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ABSTRACT

Humic substances have been reported to increase plant growth. A stimulating effect on the growth of legumes may improve biological nitrogen fixation in soils. The present investigation studied dry matter production, nodulation and N content in nodules of Pigeon peas [*Cajanus cajan* (L.) Millsp. Variety Prabhat], Guar *[Cyamopsis tetragonoloba* (L.) Taub *variety Santa* cruz]. and Mungbean *{Vigna radiata* (L.) Wilczek variety VC2419], grown in sand cultures containing a fertilizer mixture and 0 to 750 mg Kg^{-1} of fulvic acid (FA) or humic acid (HA). The results indicated that FA and HA were capable of stimulating dry matter production in the plants. Dry weights of roots and nodules showed a tendency to increase over the control at 75 to 350 mg Kg^{-1} of FA or HA. The increases in total dry matter production were statistically significant at 750 mg Kg^{-1} of FA or HA, and a positive correlation was noticed between increases in dry weights of shoots, roots, and nodules and FA or HA concentrations. Fewer nodules were produced by plants receiving FA and HA treatments than the control, but the nodule mass was greater with FA and HA treatments. The N content of nodules was little affected by the treatments; although a tendency can be noticed that 75 mg Kg^{-1} of FA had decreased the N content in the nodules of both pigeon peas and Guar plants.

Key Words: Nitrogen fixation, legumes, fulvic acid, humus, soil organic matter.

INTRODUCTION

Humic substances have been reported to influence plant growth directly or indirectly. The indirect effects of humic compounds have been attributed to the improvement of soil physical, chemical, and biological conditions. directly, these compounds appeared to be capable of affecting plant growth through the acceleration of respiratory processes (Khristeva, 1968; Smidova, 1960; Averett *et al.,* 1989; Xing and Chen, 1999), by increasing cell permeabilities (Gawronski, 1969; Ayuso *et al.*, 1997; Malcolm, 1990), by hormonal growth responses (Vaughan, 1974; Azo and Yamaguchi, 1971; Poapst and Schnitzer, 1971; Cameron and, 1992; Davies *et al.,* 1997), or by a combination of these processes. However most of these investigations were limited to study seed germination, shoot growth of young seedlings, and elongation of excised roots in vitro. Recently, evidence has been presented that humic compounds could also affect nutrient uptake (Dormaar, 1975, Levesque, 1970; Flaig and Sochtig, 1962; Concheri *et al.,* 1996; Cook and Langford, 1998) and dry matter production of plants (Guminski *et al.*, 1977; Lee and Bartlett, 1976; Cook *et a/.*, 1996 Nardi *et al.*, 1996 & 2000).

Soil organic compounds, such as humus, may be beneficial to crop production. If the humic fraction is capable of increasing the growth of pigeon peas , guar, other legumes and improving biological N fixation, the latter may aid in the economical production of plant protein. Information on the role of humic compounds in the growth of legume crops is very limited.

The use of dilute sodium humate solutions has been reported to increase soybean yields(Varshney and Gaur, 1974; Muscolo *et al.*, 1998). The use of various concentrations of humic compounds may shed some light as to the optimum levels required to induce beneficial growth effects (Stevenson, 1994; Hu *et al.*, 2000). Therefore, the objectives of current study was to study dry matter production, nodulation· and N content of nodules in pigeon peas, guar and mungbean plants grown in sand cultures containing a fertilizer mixture and different levels of soil fulvic acid (FA) and humic acid (HA).

MATERIALS AND METHODS

Experiment Layout:

Fulvic and humic acids were extracted with 0.1 *N* NaoH, from clay surface soil samples (0- 15 cm) according to procedures as described previously (Tan, 1977, 1975; Page *et al.*, 1982). The humic fractions obtained were freeze-dried and ground for further analysis. The purified humic fractions had an ash content of < 1.0%, and elemental analysis revealed to very small concentrations of macronutrient and micronutrient elements (Tan, 1977; Page *et al.*, 1982). Two grams of freeze dried FA were dissolved in 75 ml of distilled water. After the P^H was adjusted to 7.0, the volume was made up to 100 ml with distilled water. This procedure yielded a FA solution containing 20000mg L^{-1} of FA and was used as the stock solution in the greenhouse experiments I for the preparation of 0 to 750 mg L^{-1} of FA.

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Sand purchased locally was treated with concentrated Hcl and washed thoroughly with deionized water until CI free. It was then heated in an over for 48 h at 148°C to eliminate contamination with organisms. Block plastic pots (4L capacity) were sterilized with Clorox and washed with sterile water, after they were filled with 3L (4.0 kg) of sand and mixed with 3g of a fertilizer mixture (0% N-10% P_2O_5 – 20% k_2o) and 2g of calcites limestone. These amounts of fertilizer and lime are equivalent to 706 and 471 kg/Fed, respectively . A 0, 75, 350 or 750 mg $.L^{-1}$ of FA were added to the mixture by pipetting the appropriate FA solutions per pot and mixing it with the sand. Based on 4kg of sand per pot, 75 ppm of FA required the addition of 15 ml of FA stock solution, whereas 350 and 750 ppm of FA required 70 and 150 ml FA solutions, respectively.

Five Pigeon peas [*Cajanus cajan* (L.) Millsp Var. Prabhat), five Guar [*Cyamopsis tetragonoJoba* (L.) Taub Var. Santa cruz) or ten Mungbean *[Vigna radlata* (L.) Wilczek var VC 2419) were seeded in the pots in May 2011.Each treatment was triplicated prior to planting, the seeds were inoculated with Okadin, a commercial inoculants, at the rate suggested by the manufacturer. The experimental design was a completely randomized design (Snedecor and Cochran, 1981; SAS, 1985) in the greenhouse and rotated at 5 days intervals. Watering was done every day as needed with deionizer water to approximately field capacity. Field capacity was approximately by observing the amount of water held by the sand medium against gravity, after which the pots were watered to a predetermined weight. With mungbean, the plant population per pot was thinned after germination to 5 plants. The plants were allowed to grow for 40 days, after they were harvested and separated into shoots (leaf and stem), roots, and nodules. The number of nodules was counted. Because the nodules and roots of the mungbean plants were so small, no nodule counts were made, and the mungbean roots and nodules were combined together for further analysis. The various plants parts were rinsed thoroughly with deionized water and dried overnight at 75°C in a forced - draft oven for dry matter weight determination. The nodules of Pigeon peas and guar plants were then ground and analyzed for total N content by the micro -Kjeldahl method (Jackson, 1960; Page *et al.*, 1982).

The greenhouse experiments were repeated in May 2012 with HA. The HA was prepared and added to sand in similar fashion, as was FA in the summer before.

Statistical analysis:

The data obtained were analyzed for significance by analysis of variance. And the least significant difference ($LSD_{o\cdot o1}$) was calculated at the 90% confidence level. In addition• linear regression analysis were performed (Snedecor and Cochran. 1981; SAS, 1985).

RESULTS and Discussion

Dry Matter Production:

The weight of dry matter roots and nodules of 40 days old pigeon peas plants showed a tendency to increase over that of the control by treatments with 75 to 350 mg kg^{-1} of FA (Table 1). Dry weights of roots and nodules of pigeon peas plants were significantly increased at 75mg.kg^{-1} FA concentration. But, dry weights of shoots were significantly decreased (Table 1). These results were coincided with the results of Davies *et al.*, (1997); Nardi *et al.*, (1996 & 2000). The differences in results between 0 to 750 mg kg^{-1} of FA were not significant by analysis of variance for dry weight of shoots but were significant at 750 mg kg^{-1} of FA for dry weights of roots and nodules. The experiments with HA yielded supporting evidence for the observations with FA. Statistically the differences were not significant for shoots but were significant at 350 and 750 mg kg^{-1} of HA for roots and nodules (Averett *et al.*, 1989; Stevenson, 1994; Ayuso *et al.*, 1997; Hu *et al*, 2000).

Regression analysis revealed a linear regression between shoot, root, and nodule dry weights and FA and HA treatments (Fig.1). The data showed a good fit, and the values of the correlation coefficients(r) were indicative of significance at the 99 to 95% level of probability.

The dry matter in shoots and roots of guar plants (Table2) exhibited a similar increasing trend as that discussed for pigeon peas. The effect of increasing concentrations of FA to increase dry matter content over the control was still demonstrated, although individual comparisons of data at 0, 75, and 350 mg kg^{-1} of FA revealed differences that were statistically non significant. At 750 mg kg^{-1} of FA, the increase in dry weight of shoots and roots was significant at the 90% confidence level. Dry weight of guar nodules was not affected by FA. The results with HA were less obvious, but the tendency of an increase in dry weight of roots was still noticed (Cameron and Sohn, 1992; Muscolo *et al.,* 1998).

The effect of FA and HA on dry matter production of mungbean plants are presented in Table 3. The data from the FA experiments showed that dry weight of shoots, roots, and nodules increased progressively as the FA treatments increased from 0 to 750 mg kg^{-1}. These increases were statistically significant at 750 mg kg^{-1} of FA or HA (Cook and Langford, 1998; Concheri *et al.*, 1996).

Table 1. Dry matter weights of shoots, roots, and nodules (gm per pot per 5 plants) of pigeon peas plants (40 days) as affected by various FA and HA concentrations*

Treatment (mg kg-^{1})	Shoots	Roots	Nodules	Total
0 FA	2.841	0.803	0.112	3.756
75 FA	2.655	0.880	0.118	3.653
350 FA	3.126	0.971	0.129	4.226
750 FA	3.188	1.064	0.146	4.398
$LSD_{o.o1}$	0.485	0.190	0.024	----
Regression Coefficient ,r**				
Shoots	y = 2.77 + 0.0006 x		r = 0.849*	
Roots	y = 0.829 + 0.0004 x		r = 0.980**	
Nodules	y = 0.113 + 0.00005 x		r= 0.999**	
Total	y = 3.712 + 0.00105 x		r = 0.936**	
0 HA	2.836	0.807	0.112	3.755
75 HA	2.987	0.897	0.136	4.020
350 HA	3.021	1.108	0.151	4.280
750 HA	3.131	0.984	0.141	4.256
$LSD_{o.o1}$	0.696	0.133	0.023	----
Regression Coefficient,r**				
Shoots	y = 2.90 + 0.0004 x		r = 0.915**	
Roots	y = 0.877 + 0.0003 x		r = 0.626	
Nodules	y = 0.126 + 0.00004 x		r= 6.34	
Total	y = 3.903 + 0.00074 x		r = 0.825*	

*,** Significant at the 0.05 and 0.01 levels, respectively.

+ $LSD_{0.01}$ = Least significant difference at the 90% confidence level.

++ Accordin9 to Snedecor and Cochran (1981) and SAS (1985), r stands for correlation coefficient, whereas theregression coefficient refers to the slope of the regression curve or the *b* units in *Y= a+bx. R*eview process insisted that use regression coefficient for r.

Table 2. Dry matter weights of shoots, roots, and nodules (gm per pot per 5 plants) of guar plants (40 days) as affected by various FA and HA concentrations*

Treatment (mg kg-^{1})	Shoots	Roots	Nodules	Total
0 FA	3.329	1.997	0.081	5.407
75 FA	3.370	2.087	0.061	5.518
350 FA	3.460	2.054	0.074	5.588
750 FA	4.079	2.827	0.077	6.983
$LSD_{o.o1}$	0.562	0.336	0.028	---
Regression Coefficient ,r** .				
Shoots	y = 3.27 + 0.0010 x		r = 0.945**	
Roots	y = 1.93 + 0.0011 x		r = 0.895*	
Nodules	y = 0.072 + 0.00006 x		r= 0.221	
Total	y = 5.272 + 0.00216 x		r= 0.921**	
0 HA	3.822	2.010	0.080	5.912
75 HA	3.925	2.101	0.057	6.083
350 HA	4.3032	2.196	0.079	6.5782
750 HA	4.025	2.348	0.088	6.461
$LSD_{o.o1}$	0.842	0.320	0.019	---
Regression Coefficient ,r** .				
Shoots	y = 3.87 + 0.0003 x		r = 0.835*	
Roots	y = 2.04 + 0.0005 x		r= 0.990**	
Nodules	y = 0.068 + 0.00003x		r= 0.622	
Total	y = 5.978 + 0.00083 x		r = 0.808*	

*,** Significant at the 0.05 and 0.01 levels, respectively.

+ $LSD_{0.01}$ = Least significant difference at the 90% confidence level.

++ According to Snedecor and Cochran (1981) and SAS (1985), *r* stands for correlation coefficient, whereas the regression coefficient refers to the slope of the regression curve or the *b* units in Y= *a+bx* . Review process insisted that use regression coefficient for *r.*

Table 3. Dry matter weights of shoots, roots, and nodules (gm per pot per 5 plants) of mungbean plants (40 days) as affected by various FA and HA concentrations* .

Treatment (mg kg-[1])	Shoots	Roots+ Nodules	Total
0 FA	1.210	0.440	1.650
75 FA	1.439	0.527	1.966
350 FA	1.604	0.593	2.197
750 FA	1.634	0.864	2.498
$LSD_{0.01}$	0.351	0.182	---
	Regression Coefficient, r**		
Shoots	Y = 1.33 + 0.0006 x	r = 0.857*	
Roots+Nodules	Y = 0.444 + 0.0006 x	r = 0.980**	
Total	Y = 1.774 + 0.0012 x	r= 0.964**	
0 HA	1.207	0.434	1.641
75 HA	1.443	0.458	1.901
350 HA	1.558	0.454	2.012
750 HA	1.619	0.515	2.134
$LSD_{0.01}$	0.341	0.051	----
	Regression Coefficient,r ** .		
Shoots	Y = 1.32 + 0.0005 x	r = 0.863*	
Roots+Nodules	Y = 0.436 + 0.00010 x	r =0.923**	
Total	Y = 1.756 + 0.0006 x	r= 0.899*	

*, ** significant at the 0.05 and 0.01 levels, respectively.

+ $LSD_{0.01}$ =Least significant difference at the 90% confidence level.

++ According to Snedecor and Cochran (1981) and SAS (1985), *r* stands for correlation coefficient, whereas the regression coefficient refers to the slope of the regression curve or the *b* units in Y= *a+bx*. Review process insisted that use regression coefficient for *r*.

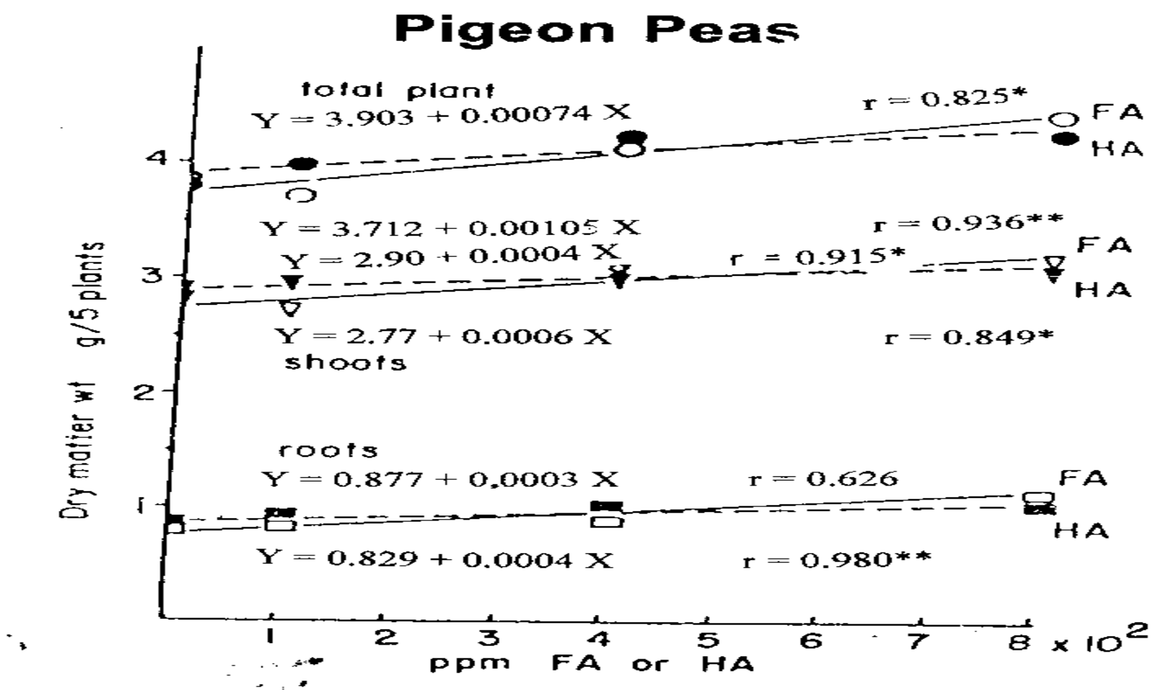

Fig. 1. Relationship between the increase in dry matter production of pigeon peas plant and FA and HA treatments, Regression coefficients (r) show correlations significant at the 95% (*), and 99%(**) levels of probability.

Nodulation and Percent of Nitrogen in Nodules:

Fewer nodules were formed as a results of treatments with FA and HA. (Tables 4 and 5). The decrease in nodulation was noticeable at 750 mg kg^{-1} of FA and HA. However, when nodule dry weights were studied , it appeared that nodule weight increased in Pigeon peas plants (Table 4) as the applied concentrations of FA and HA increased from 0 to 750 mg kg^{-1} . The latter result was supported by results with guar plants at 350 and 750 mg kg^{-1} of FA or HA (Table 5).

The N content of the nodules, as affected by FA or HA treatments, was inconsistent (Xing and Chen, 1999 and EL-Sayed 2002). Although a tendency to decrease in N content was noted in Pigeon peas nodules as a result of FA treatments, the data at 350 and 750 mg kg^{-1} of HA were nonsignificantly different from the control (Table 4). Lack of definite trend in N content of guar nodules was also observed as a result of treatment with FA (Table 5). However, application of HA seemed to increase somewhat the N content of guar nodules, with 75 mg kg^{-1} of HA yielding the highest levels (Cook *et al.*, 1996; Malcolm. 1990; EL-Khtatib *et al* ., 2013).

CONCLUSIONS

It can be concluded that the soil humic fraction contribute to a better growth of legume plants by stimulating total dry matter production. Both FA and HA were capable of increasing the total amount of dry weight in pigeon peas, guar, and mungbean plants. In general, shoot, root, and nodule dry weights showed a tendency to increase by treatment of the plants with 75 to 350 mg kg^{-1} of FA and HA.

Fewer nodules were produced from pigeon peas and guar plants receiving FA and HA than for the control plants, but the nodules had greater mass. Although some variations were noted in N content of these nodules. A small percent of N in nodules in case of FA and HA treated compared to control.

Table 4. Effect of FA and HA concentrations on number, weights, and N content of nodules of pigeon peas plants *

Treatment (mg kg^{-1})	No. of nodules/5 Plants	Weight/5 Nodules, (mg)	% of N
0 FA	109	11.7	5.51
75 FA	84	15.2	4.42
350 FA	88	15.8	4.96
750 FA	66	23.4	5.16
$LSD_{0.01}$	9.8	2.01	0.79
Regression Coefficient ,r**			
Number of nodules	Y = 99.6 - 0.043 x	r = - 0.861*	
Nodule weight	Y = 12.3 + 0.014 x	r = 0.957**	
% of N	-------------------	r = 0.077	
0 HA	111	11.2	5.16
75 HA	84	17.4	4.96
350 HA	87	18.5	5.09
750 HA	79	19.1	5.10
$LSD_{0.01}$	15.3	2.7	0.46
Regression Coefficient ,r**			
Number of nodules	Y = 98.5 - 0.004 x	r = - 0.713	
Nodule weight	Y = 14.1 + 0.008 x	r = 0.742	
% of N	---------------------	r= 0.124	

*, ** Significant at the 0.05 and 0.01 levels, respectively.

+$LSD_{0.01}$ = Least significant difference at the 90% confidence level.

++ According to Snedecor and Cochran (1981) and SAS (1985), *r* stands for correlation coefficient, whereas the regression coefficient refers to the slope of the regression curve or the *b* units in *Y= a+bx* . Review process insisted that use regression coefficient for *r*.

Table 5. Effect of FA and HA concentrations on number, weights, and N content of nodules of guar plants* .

Treatment mg kg-[1]	No. of nodules/5 Plants	Weight/5 Nodules, (mg)	% of N
0 FA	70	12.7	5.51
75 FA	50	12.7	4.42
350 FA	40	19.8	5.12
750 FA	22	37.4	5.64
$LSD_{o.o1}$	9.1	2.2	0.43
Regression Coefficient, r** .			
Number of nodules	y = 62.8 - 0.06 x	r = - 0.953**	
Nodule weight	y = 10.23 + 0.033 x	r = 0.976**	
% of N	----------------------	r= 0.471	
0 HA	82	10.8	4.17
75 HA	88	7.5	5.76
350 HA	70	12.4	5.11
750 HA	58	16.4	5.64
$LSD_{o.o1}$	7.8	1.97	0.55
Regression Coefficient, r** .			
Number of nodules	y = 86.0 - 0.05 x	r = - 0.952**	
Nodule weight	Y =8.73 + 0.010 x	r = 0.885*	
% of N	-----------------------	r= 0.527	

*, ** Significant at the 0.05 and 0.01 levels, respectively.

+ $LSD_{o.o1}$ = Least significant difference at the 90% confidence level.

++ According to Snedecor and Cochran (1981) and SAS (1985), *r* stands for correlation coefficient, whereas the regression coefficient refers to the slope of the regression curve or the *b* units in Y= *a+bx* . Review process insisted that use regression coefficient for *r* .

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الملخص العربي

التأثير المنشط للمركبات الدبالية على النمو وتكوين العقد البكتيرية والتثبيت الحيوى للنيتروجين فى البقوليات

سعيد عباس مُحَمَّد السيد

تعمل المواد الدباليه على زيادة نمو النباتات؛ وذات تأثير منشط لنمو البقوليات حيث تعمل على تحسين التثبيت الحيوى للنيتروجين بالتربه.

تم دراسة الوزن الجاف وتكوين العقد البكتيرية والمحتوى النيتروجينى بالعقد فى كل من بسلة الطيور والجوار وفول المانج حيث تم نموهم فى بيئة رملية تحتوى على خليط من اسمدة حمض الهيوميك (HA) وحمض الفلفيك (FA) واشارت النتائج الى ان كل من حمض الهيوميك (HA) وحمض الفلفيك (FA) ادت الى زيادة انتاج المادة الجافة فى النباتات وكذلك الوزن الجاف لكل من الجذور والعقد البكتيرية حيث اظهرت زيادة عن الكنترول عند تركيز (75و350) جزء فى المليون لكل من حمض الفلفيك (FA)؛ وحمض الهيوميك (HA) على الترتيب.

الزيادة الكلية فى المادة الجافة الناتجة كانت معنوية عند تركيز (750) جزء فى المليون لكل من حمض الفلفيك (FA)؛ وحمض الهيوميك(HA) حيث لوحظ وجود تلازم موجب بين الزيادة فى الوزن الجاف لكل من السيقان والجذور والعقد البكتيرية وتركيز كل من حمض الفلفيك (FA)؛ وحمض الهيوميك (HA).

لوحظ وجود قليل من العقد البكتيرية بالنباتات المضاف اليها حمض الفلفيك (FA)؛ وحمض الهيوميك(HA) وان حجم هذه العقد البكتيرية كان كبيرا بالمقارنه بالكنترول.

محتوى النيتروجين فى العقد البكتيرية كان قليلا عند تركيز (75 \) جزء فى المليون عند المعاملة بحمض الفلفيك(FA) فى كل من نباتات بسلة الطيور والجوار.