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ENRICHMENT OF HUMIC ACID AND AZOLLA WITH IRON OR ZNIC AND THEIR IMPACTS ON FABA BEAN PLANTS GROWN ON A CALCAREOUS SOIL

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ABSTRACT

A pot experiment using surface calcareous composite soil (0-20cm) as growth medium for faba bean plants (*Vicia faba,* C.V. Giza 2) was carried out to study the effect of both humic acid and Azolla enriched with Fe and/or Zn individually or impregnated in comparison with those of Fe-EDTA (5% Fe), FeSO4 (56% Fe), Zn-EDTA (14.2% Zn) and ZnSO4. 7H2O (22.6%Zn) on the growth of faba bean plants. Status of N, P, K,Fe and Zn, number, dry weight of root nodules, nitrogenase (N2-ase) activity, CO2 evolved and total counts of bacteria, fungi and actinomycetes were observed. Results obtained could be summarized as follows:

- All treatments caused increases in dry weight of faba bean plants compared with the control treatment.
- The dry matter yield and concentrations of N, P, Fe and Zn of faba bean. Plants were positively affected by humic acid and Azolla enriched with Fe and/or Zn. The increases were higher in case of humic acid or Azolla impregnated with Fe or Zn as compared with humic acid or Azolla combined with Fe or Zn.
- The combinations between any of the humic acid or Azolla and Fe or Zn were more effective for increasing the number and dry weight of nodules, N2-ase and CO2 evolution. However, the increases seemed to be dependent not only on the applied but also on type of the manure.
- Humic acid or Azolla enriched with iron and/or zinc could be considered as equivalent and effective source of iron or zinc to those of the chelated forms. Iron and zinc enriched humic or Azolla was equivalent to the highly expensive iron or zinc chelate like Fe- EDTA and Zn-EDTA.
- Results indicated that the soil supplemented with humic acid or Azolla impregnated or combined with Fe or Zn showed higher counts of bacteria, fungi and actinomycets than that recorded with the control treatment.

Key words: Humic acid – Azolla – Fe – Zn – bean plants – Enrichment - Calcareous

INTRODUCTION

The availability of trace elements to plants is governed by a variety of reactions that include complication with organic and inorganic ligands, ion exchange and adsorption, precipitation and dissolution of solids and acid-base equilibria. (Mattigod *et al.*, 1981).

Chen *et al.* (1982) found that peat enriched with iron (3.7% Fe) reduced symptoms of chlorosis and increased yield of peanuts. El-Hedek (2007) found that addition of poultry manure, compost and sulfur to a high calcareous soil caused relative increase in DTPA-extractable Fe of about 8.3%, 22.8% and 11.0% compared to control.

Shams El-Den (1993) noticed that Fe concentration in shoot and seeds of wheat plants significantly increased with increasing Fe application rate in a mineral or chelated form.

Basyouny (1996) showed that iron application as Fe-EDTA was superior for increasing the Fe content in wheat plants grown on non calcareous soil, whereas Fe-EDDHA was more effective in the calcareous soil and $FeSO_4$ was inferior as compared with the other two Fe chelated sources. Zn-EDTA gave higher dry matter production and Zn concentration than $ZnSO_4$ in wheat plants grown on Giza, Ismailia and Nubaria soils.

Bayoumi (2000) noticed that the addition of either $ZnSO_4$ or Zn-EDTA to the sandy or calcareous soils resulted in increases dry weight of shoots and grains of wheat plants as well as Zn uptake.

El-Hedek (2000) found that application of Fe-EDTA increased iron concentration in both straw and grains of barely plants grown on calcareous and non calcareous soils compared to those treated with $FeSO_4$.

Some reports emphasized that Azolla improves the soil physical properties by increasing the porosity and decreasing the specific gravity of soils which decreased the bulk density of the soil. These changes in soil properties are important, since they reduce the amount of energy required for soil tillage and improve water infeltration, aeration and soil temperature (Venture and Watanabe, 1993 and Mandal *et al.* 1999). Consequently, the beneficial effect of Azolla is not only the result of its potential for nitrogen fixation but also related to its improving effect the physiccal and chemical properties of soil (Badawy *et al.*, 1996).

Tanagaraju and Kannaiyan (1993) reported that Azolla application improve soil fertility by increasing total nitrogen, organic carbon, available P and the soil structure. The transformation and availability of Fe, Mn, Zn and Cu in the flooded rice soils were positively affected with addition of organic matter in combination with Azolla as green manure (Mandal *et al.*, 1997).

A range of elemental composition of *Azolla pinnata* was given by Singh (1979) and Liu (1979) as follows (percent on dry weight basis): Ash, 10.5; Nitrogen, 4-5; Phosphorus, 0.5-0.9; Potassium, 2-4.5; Iron, 0.06-0.26.

Heller (1989) showed that dried Azolla enriched with 2% Fe (on dry weight basis) applied to peanuts plants grown in calcareous soil resulted in leaf chlorophyll content equal or higher than those in plants treated with Fe-EDDHA.

EL-Badawy (2003) indicated that Azolla enriched with iron and/or zinc significantly increased the dry matter yield of maize plants as compared with the control treatment whereas Fe EDTA, Zn EDTA, $FeSO_4$ and $ZnSO_4$ treatments were insignificant.

Organic matter particularly the humic and fulvic acids plays an important role in the chemical behaviour of several materials in soils through their functional groups which have the ability to retain the metal in complex or chelate form (Stevenson, 1981).

Basyouny (2001) showed that application of organic manures or $FeSO_4$ alone or in combination to calcareous and non calcareous soils had a marked stimulatory effect on the content of Fe and Zn in wheat plants at different stages of growth. The highest values of Fe and Zn contents were obtained at the highest rates of organic manures mixed with the highest rates of $FeSO_4$ or $ZnSO_4$.

El-Ghanam and EL-Ghozoli (2006) found that application of humic acid increased the dry matter yield, N concentration and uptake, N_2-ase activity as well as the number and dry weight of nodules of bean plants grown on a calcareous soil.

The objective of this investigation were to (1) evaluate the performance of faba bean plants under humic acid and Azolla enriched with Fe and Zn in comparison with Fe and Zn sources (mineral and chelate sources). (2) compare the effect of humic acid or Azolla impregnated or combined with Fe and/or Zn on growth and concentrations of N, P, K, Fe and Zn in faba bean plants.

MATERIALS AND METHODS

A surface calcareous composite soil sample (0-20 cm) was taken from soil a field in EL-Hammam village, which located in North West of Egypt. The soil sample was air dried, crushed with a wooden roller to pass through a 2mm screen. The collected soil sample was prepared to determine particle size destruction, soluble salts, $CaCO_3$ content, organic matter and pH using the standard methods outlined by Piper (1955) and Jackson (1967) and the results obtained are presented in Table (1a).

Table (1 a): Some characteristics of the investigated soil sample

Particle size distribution (%):		**CO_3^{2-}**	0.0
Sand	41.6	**HCO_3^-**	1.14
Silt	27.5	**Cl_2^-**	22.3
Clay	30.9	**SO_4^{2-}**	18.0
Textural class	Clay loam	**Soil moisture**	
Organic matter (%)	0.45	**Constant %**	
Total $CaCO_3$ (%)	27.1	**Field capacity**	18.5
Active $CaCO_3$ (%)	21.4	**Wilting point**	8.4
pH (1:2.5 soil-water suspension)	7.97	**Available water**	10.1
EC(dSm^{-1})	4.1	**Available Fe**	2.88
Soluble Ions ($mmol_cL^{-1}$)		**Available Zn ($mgKg^{-1}$)**	1.23
Ca^{2+}	17.7		
Mg^{2+}	1.73		
Na^+	19.7		
K^+	2.31		

Humic acid:

Humic acid (HA) substances were extracted by 0.1 N NaOH and then purified according to Page *et al.* (1982) and were finely ground in porcelain mortar and thoroughly mixed with the soil.

Elementary analysis of humic acid:

Elementary analysis of purified humic acid (extracted from biogas manure) was carried out in order to determine the percentage of total C, N, H, O and S (Table, 1b). Total organic carbon of humic acid was determine using potassium dichromate method and O-phenanthroline as an indicator (Jackson, 1967). Total nitrogen was determined using microkjeldahl method (Sposito *et al.,* 1976). Hydrogen was determined using the dry combustion, it was oxidized to water, which was absorbed by calcium chloride and weighted (Karrer, 1950). Sulfur was determined using barium chloranilate method (Beaton *et al.,* 1968). Oxygen was calculated by difference (Goh and Stevenson, 1971).

Table (1b): Elementary analysis of humic acid extracted from biogas manure

C %	N %	C/N ratio	H %	O %	S %	C/H ratio	C/O ratio	O/H ratio	N/H ratio
45.2	1.73	26.1	7.84	39.8	5.43	5.76	1.13	5.07	0.22

Azolla strain (AZO.):

Azolla pinnata strain kindly provided by the Agricultural Microbiology Research Department, Soils, Water and Environment Research Institute, ARC, Giza, Egypt. Azolla plants were propagated in cement nurseries for 3 weeks in the greenhouse to obtain the Azolla material required for organic treatments. Azolla and humic acid were enriched with $FeSO_4$ iron and $ZnSO_4$ according to Plessener *et al.* (1998).

Inocula preparation:

To prepare Rhizobium inoculum, yeast minnitol broth medium (Vincent, 1970) was inoculated with *Rh. leguminsarium* and incubated at 32°C for 7 days. Faba bean seeds were successively washed with water and air dried, then soaked in cell suspension of Rhizobium sp. (8.3 X 10^7 viable cell/ml) for 30 min. gum Arabic (16%) was added as an adhesive agent prior to inoculation, then air dried for one hour before sowing.

Biological experiment

A greenhouse pot experiment was conducted at Training Center for Recycling of Agricultural Residues, Moshtohor, Qalyoubia Governorate, Soils, Water and Environment Research Institute, Agricultural Research Center (ARC). Five kilograms of soil were placed in plastic pots with the dimensions of 16cm height and 21cm diameter. Humic acid, Azolla, Fe or Zn were added individually or in combination mixture at a rate of 25mgkg^{-1} soil and thoroughly mixed with the soil. Eight seeds of faba bean (*Vicia faba*, C. v. Giza 2) were broadcasted in each pot. The experimental design was randomized complete block with three replicates. The experiment included the following treatments:

1. Control (without any additions).
2. Humic acid (HA)
3. Azolla (Azo.)
4. Fe-EDTA
5. ($FeSO_4$ alone)
6. Zn-EDTA
7. $ZnSO_4$
8. HA impregnated with $FeSO_4$
9. HA impregnated with $ZnSO_4$
10. Azo. impregnated with $FeSO_4$
11. Azo impregnated with $ZnSO_4$
12. HA combined with $FeSO_4$
13. HA combined with $ZnSO_4$
14. Azo. combined with $FeSO_4$
15. Azo. combined with $ZnSO_4$
16. HA impregnated with $FeSO_4$ and $ZnSO_4$
17. Azo. impregnated with $FeSO_4$ and $ZnSO_4$
18. HA combined with $FeSO_4$ and $ZnSO_4$
19. Azo. Combined with $FeSO_4$ and $ZnSO_4$

After two weeks of germination, the seedlings were thinned to three plants and the moisture content was kept at field capacity by means of daily compensation of water loss with distilled water. After 90 days from germination, the plants were harvested, dried at 70°C and the dry matter yield was recorded. Plant samples were digested using H_2SO_4 and $HClO_4$ for chemical analyses.

Sampling and analysis:

Data of nodules number and nodules weight/plant were recorded. N_2-ase activity of nodules was estimated according to Hardy *et al.* (1973). After forty days from germination of faba bean plants, the soil samples were taken from rhizosphere area to determined CO_2 evolution according to Page *et al.* (1982). The viable microbial counts were estimated by plate count technique using soil extract agar medium (Allen, 1959). Total nitrogen, phosphorus, potassium and micronutrients (Fe and Zn) were determined using microkjeldahl (A.O.A.C, 1980), Murphy and Riley (1962) as modified by John (1970). Dewis and Freitas (1970) and Atomic Absorption Spectrophotometer, respectively.

Analysis of variance for the results of the experimental treatments was conducted using Minitab program according to Ryan and Joiner (1994).

RESULTS AND DISCUSSION

Dry matter yield of faba bean plants:

Data presented in Table (2) showed that the dry matter yield of faba bean plants was positively affected as compared with control treatment. The high increase in dry weight of faba bean plants (72.9%) was achieved at treatment of HA impregnated with Fe, while low increase percentage (14.1%) was obtained at the treatment of $FeSO_4$. The dry matter yield of faba bean plants increased when humic acid or Azolla impregnanted with Fe or Zn than that those individually added. Almost similar results were achieved by El-Badawy (2003). Chen *et al.* (1982) reported that application of peat enriched with Fe to peanuts grown on a soil containing 42%

$CaCO_3$ resulted in yields amounted 130% and 110% of those of control and Fe-EDDHA treatments, respectively.

Enriching humic acid with Fe or Zn caused a pronounced effect on the dry matter yield of faba bean plants. It may be concluded that the inducing effect of humic acid on the productivity of soil could be attributed to humus formed in the soil, which improve the physical and chemical properties of soil and partly to extra supply of nutrients. In addition, attributed the beneficial effect of humic acid on plant growth to its acting as plant growth hormones (Nardi *et al.*, 1999).

Humic acid or Azolla impregnated with Fe or Zn increased the dry matter yield to be higher than that individual application of each them. The regression equation described this relation as follows:

D.M. yield (g pot^{-1})= 10.2 + 0.117 treat. ($r=0.327$)**

N, P and K concentrations of faba bean plants:

Data presented in Table (2) show the effect of humic acid and Azolla whether applied solely, impregnated or combined with Fe and/or Zn on N, P and K concentrations in faba bean plants.

The obtained results revealed that N concentration of faba bean plants was positively affected with all treatments compared with control treatment. The highest value was achieved due to the treatment of HA impregnated with Fe and Zn, while the lowest one was obtained due to the treatment of $ZnSO_4$. it is obvious that N concentration of faba bean plants increased slowly with humic acid and Azolla addition or combined with Fe and/or Zn. The beneficial effect of humic acid and Azolla as a nitrogen sources might pre-serve and support the soil fertility to several crops. These results agree well with those obtained by El-Badawy (2003). This relation could be described by the following equation;

N-concentration % = 2.62 + 0.0235 ($r=0.209$)*

Table (2): Effect of humic acid or Azolla enriched with Fe and/or Zn on dry weight and concentrations of N, P, K, Fe and Zn of faba bean plants grown on the calcareous soil.

Treatments	Dry weight (g pot^{-1})	N %	P %	K %	Fe $mgkg^{-1}$	Zn $mgkg^{-1}$
Control	8.5	1.33	0.261	1.10	445.2	61.7
HA	10.6	2.90	0.28	1.74	620	107.5
Azolla	10.8	3.46	0.267	2.27	616.6	90.7
Fe-EDTA	10.0	2.99	0.275	3.06	717.5	70.7
$FeSO_4$	9.7	2.57	0.259	1.59	969.6	153.3
Zn-EDTA	9.8	2.34	0.219	1.87	632.5	424.1
$ZnSO_4$	9.9	2.13	0.214	1.46	633.3	401.3
HA impregnated with $FeSO_4$	14.7	3.51	0.317	2.74	788.3	216.7
HA impregnated with $ZnSO_4$	12.4	3.40	0.32	3.92	670.8	441.2
Azolla impregnated with $FeSO_4$	10.4	2.96	0.409	2.31	746.6	213.3
Azolla impregnated with $ZnSO_4$	14.6	2.89	0.381	1.78	691.6	344.5
HA combined with $FeSO_4$	13.8	3.28	0.305	1.63	681.6	141.3
HA combined with $ZnSO_4$	10.6	3.14	0.265	2.24	669.2	353.0
Azolla combined with $FeSO_4$	11.3	2.76	0.267	1.77	672.5	135.3
Azolla combined with $ZnSO_4$	13.8	2.21	0.247	1.57	652.5	347
HA impregnated with $FeSO_4$ and $ZnSO_4$	10.7	3.10	0.307	1.51	670	247
Azolla impregnated with $FeSO_4$ and $ZnSO_4$	9.1	2.59	0.29	1.44	645.0	100
HA combined with $FeSO_4$ and $ZnSO_4$	11.1	3.53	0.313	2.15	630.8	294.3
Azolla combined with $FeSO_4$ and $ZnSO_4$	12.9	2.45	0.36	1.81	625.0	339.2
L.S.D. 0.01	0.449	0.589	0.025	0.264	5.52	9.69

With respect to P concentration in faba bean plants, data in Table (2) revealed that P-concentration in faba bean plants increased as the humic acid and Azolla were applied either in presence or absence of Fe or Zn compared with control treatment. This increase could be attributed to the beneficial effect of Azolla due to its potentially for nitrogen fixation beside its improving effect on the physical and chemical properties of soil (Badawy *et al.*, 1996).

The regression equation and correlation coefficient relating P concentration with different treatments is as follows:
P-concentration % = 0.262 + 0.00395 treat. $(r=0.317)^{**}$

Regarding to K-concentration in faba bean plants, it was increased due to all the treatments as compared with control treatment, however, increasing of K concentration was variant with the different treatments. The highest value of K-concentration was performed with the treatment of H.A. impregnanted with Zn while the lowest one was obtained with the treatment of $ZnSO_4$.

The relationships between K- concentration and other treatments were governed by the following equation:
K-concentration % = 1.94 + 0.0071 treat. $(r=0.254)^{*}$

It can be deduced from the above-mentioned results that humic acid or Azolla impregnated with Fe and/or Zn was the most efficient for increasing concentrations of N, P and K. The effectiveness of Fe-organo complexes is usually attributed to their similarity to soil organic matter and in particular to the humic substances contained therein.

Fe-concentration of faba bean plants:

Data presented in Table (2) indicated significant and positive effects of the different treatments on Fe-concentration of faba bean plants as compared with control treatment. Similar results were reported by El-Badawy (2003). The increase in Fe concentration could be due to production of acids and chelating compounds as a result of soil organic matter decomposition which play a vital role in the availability of essential plant nutrient elements. This conclusion stand in well agreement with that of El-Ghozoli (1998). The highest Fe-concentration (788.3 $mgkg^{-1}$) was obtained due to the treatment of HA impregnated with Fe, which was higher than those attained with Azolla impregnated with Fe and other treatments. Data showed also that humic acid and Azolla when impregnated with Fe could be considered as being equivalent to or higher than those that treated with Fe-EDTA or $FeSO_4$. These results agree with those obtained by Heller (1989) ad Plessner (1998).

As for Fe applied as impregnated or combined with Fe on faba bean plants, results indicated that Fe impregnated with humic or Azolla induced positive effect more than that recorded with individual application. With respect to HA impregnated with Fe or Zn applied to faba bean plants, the obtained results revealed an decrease of Fe concentration more than that recorded when HA impregnated with Fe was applied. This could be attributed to competitive binding between metal ions for reactive sites on humic acid.

The effect of different treatments could be presented according to the following equation:
Fe-concentration % = 635+2.58 treat. $(r=0.212)^{*}$

Zn-concentration of faba bean plants:

Results presented in Table (2) revealed that all tested treatments increased Zn-concentration of faba bean plants over the control treatment. Such increases in Zn-concentration of faba bean plants could be attributed to the high content of Zn in humic and Azolla or to the effect of acidic compounds or CO_2 evolved from the biological activities of soil microorganisms that may reduce the soil pH value. The highest Zn concentration value (441.2 $mgkg^{-1}$) was achieved with HA impregnated with Zn.

These results are accordance with those obtained by El-Badawy (2003). Results showed that plants received HA or Azolla impregnated with $ZnSO_4$ recorded high concentration of Zn as compared with individual addition HA, Azolla or $ZnSO_4$. The above mentioned results indicated that the Zn associated with humic acid and Azolla caused increase in concentration of Zn of the plants grown on the investigated soil, however, the magnitude of increase seemed to be dependent on type of organic manure.

Finally, results obtained indicated that humic acid or Azolla enriched with Fe and/or Zn could be considered as equivalent and effective source of iron or zinc to those of chelated sources which highly expensive i.e., Fe-EDTA and Zn–EDTA.

The effect of different treatments could be presented according to the following equation:

Zn-concentration % = 163 + 7.72 treat. $(r=0.325)^{**}$

Number and dry weight of nodules and Nitrogenase activity (N_2-ase).

Results in Table (3) show the effect of humic and Azolla enriched with or without Fe and Zn on number and dry weight of nodules and N2-ase. The number and dry weight of nodules and N_2-ase of faba bean plants were positively affected with humic acid and Azolla enriched with Fe or Zn addition. These results agree with those obtained by El-Ghanam and El-Ghozoli (2006) who found that the number and dry weight of nodules of faba bean plants increased with increasing humic acid application. The maximum increase in number of nodules, dry weight and its N_2-ase were achieved by HA impregnated with Fe.

The percentage increase in number of nodules and N_2-ase of treatment of HA impregnated with Fe were 380 and 332.2%, respectively. While this increase were 220 and 186.7.7% in case of Azolla impregnated with Fe, respectively.

Table (3): Effect of humic acid and Azolla enriched with Fe and/or Zn on nodulation and nitrogenase activity of faba bean plants grown on calcareous soil.

Treatments	No. of nodules/ plant	Dry weight of nodules (mg/plant)	N_2-ase (n.moles C_2H_4/hr/g dry nodules)
Control	10	61.2	12.1
HA	17	213.5	17.2
Azolla	15	218.4	16.8
Fe-EDTA	12	70.7	14.0
$FeSO_4$	12	77.5	14.4
Zn-EDTA	13	103.3	15.2
$ZnSO_4$	11	73.1	13.0
HA impregnated with $FeSO_4$	48	378.6	52.3
HA impregnated with $ZnSO_4$	39	270	42.8
Azolla impregnated with $FeSO_4$	32	265.2	34.7
Azolla impregnated with $ZnSO_4$	23	180.0	22.9
HA combined with $FeSO_4$	19	143.9	20.5
HA combined with $ZnSO_4$	18	136.3	19.7
Azolla combined with $FeSO_4$	33	237.9	35.4
Azolla combined with $ZnSO_4$	46	279.4	50.1
HA impregnated with $FeSO_4$ and $ZnSO_4$	36	272.8	40.2
Azolla impregnated with $FeSO_4$ and $ZnSO_4$	23	184.9	23.6
HA combined with $FeSO_4$ and $ZnSO_4$	27	114.2	29.5
Azolla combined with $FeSO_4$ and $ZnSO_4$	25	172.9	26.3
L.S.D. 0.01	2.45	34.5	0.228

Data in Table (3) showed also that plants received HA or Azolla impregnated with Fe and/or Zn recorded higher number and dry weight of root nodules and N_2-ase as compared due to the individual additions.

The regression equations representing these relations are:

No. of nodules= 15.3 + 0.958treat. $(r= 0.397)^{**}$

D.W. of nodules= 177+1.49 treat. $(r=0.183)^{*}$

N_2-ase= 16.5 + 1.10treat. $(r=0.484)^{***}$

CO_2 evolution:

Data presented in Table (4) show the effect of HA or Azolla enriched with Fe and/or Zn as impregnated or individual form on CO_2 evolved from the rhizosphere. Results indicated that CO_2 evolved from the soil increased markedly with HA or Azolla added impregnated or in combination with Fe and/or Zn as compared with the control treatment. These results are coincide with those of Eh-Ghanam and El-Ghozoli (2006). The relative increase of CO_2 evolved corresponding to HA, Azolla, H.A. impregnated with Fe, H.A. impregnated with Zn, Azolla impregnated with Fe and Azolla impregnated with Zn were 139.1, 80.0, 265.1, 390.1, 205.1 and 320.1%, respectively.

Table (4): CO_2 evolved, pH and Total count of bacteria, fungi and actinomycetes as affected by humic acid and Azolla enriched with Fe and/or Zn addition

Treatments	CO_2 (µg/g dry soil/ hr.)	Total bacteria counts X 10^7	Total fungi counts X 10^3	Total count of actinomycetes X 10^5
Control	59.1	1.35	1.5	10
HA	141.3	7.0	3.5	105
Azolla	106.4	7.5	2.5	68
Fe-EDTA	90.1	2.1	2.0	14
$FeSO_4$	92.5	1.75	2.1	23.5
Zn-EDTA	59.1	2.0	1.8	15.5
$ZnSO_4$	82.8	1.8	1.6	11.5
HA impregnated with $FeSO_4$	215.8	29	10.0	275
HA impregnated with $ZnSO_4$	289.7	26	8.0	205
Azolla impregnated with $FeSO_4$	141.9	20	7.0	190
Azolla impregnated with $ZnSO_4$	248.3	19.5	6.0	155
HA combined with $FeSO_4$	148.3	20	4.5	175
HA combined with $ZnSO_4$	159.7	13	5.5	105
Azolla combined with $FeSO_4$	180.3	19	2.0	85
Azolla combined with $ZnSO_4$	130.1	11	1.0	69
HA impregnated with $FeSO_4$ and $ZnSO_4$	195.1	25.5	7.0	47.5
Azolla impregnated with $FeSO_4$ and $ZnSO_4$	133.0	16.0	9.5	115
HA combined with $FeSO_4$ and $ZnSO_4$	177.4	13	5.0	65
Azolla combined with $FeSO_4$ and $ZnSO_4$	127.1	27	9.0	95
L.S.D. 0.01	5.31	0.361	0.318	0.801

The relative increase of CO_2 evolved from the rhizosphere in case of HA combined with $FeSO_4$, HA combined with $ZnSO_4$, Azolla combined with $FeSO_4$ and Azolla combined with $ZnSO_4$, were 150.9, 170.2, 140.1 and 120.1%, respectively.

Concerning the effect of HA and Azolla individually added or impregnated with Fe and Zn on CO_2 evolved from rhizosphere, data in Table (4) revealed that the values of CO_2 evolved from soil as affected by HA or Azolla impregnated with Fe and Zn recorded higher values than that individually added. However, these values were low than that of HA or Azolla impregnated with Fe or Zn.

The effect of treatments could be summarized by the following equation:
$CO_2 = 108 + 4.29$ treat. $(r=0.388)^{**}$

It was worthy to indicate that the plants grown on soil treated with organic manure enriched with mineral fertilizers showed higher Fe and Zn than the plants treated with either organic manure only or mineral fertilizers alone. Meanwhile, the HA impregnated with Fe or Zn showed stimulation effect on both Fe and Zn concentrations in the plants grown on the studied soil.

Total counts of bacteria, fungi and actionmycetes

The bacterial, fungi and actinomycetes plate counts in the soil treated with humic acid or Azolla individually or in combination with Fe and Zn are presented in Table (4). Results showed that the soil supplemented with HA or Azolla individually or impregnated with Fe and Zn showed higher counts than that recorded with the control treatment. Humic acid impregnated with Fe or Zn possessed the highest activity among all treatments. The increases of microbial counts might be due to the activities of a large number of living microorganisms in the presence of HA or Azolla and impregnated with Fe or Zn.

These relations could be summarized by the regression equations as follow:

Total bacteria counts = 4.31 + 1.06 treat. $(r= 0.612)^{***}$

Total fungi counts = 2.41 + 0.263 treat. $(r= 0.463)^{***}$

Total counts of actinomycetes = 69.7 + 2.86 treat. $(r= 0.214)^{*}$

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اثراء حامض الهيومك والآزولا بالحديد أو الزنك وتأثيرهما على نباتات الفول البلدي النامية فى ارض جيرية

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أقيمت تجربة أصص باستخدام تربة جيرية من قرية الحمام بشمال غرب مصر كبيئة نمو لنباتات الفول صنف جيزة ٢ وذلك لدراسة تأثير إضافة حامض الهيوميك والأزولا منفرداً أو محملا مع الحديد أو الزنك بالمقارنة بكل من Fe-EDTA (٥% حديد) وكبريتات الحديد (٥٦% حديد) وZn-EDTA (١٤,٢% زنك) وكبريتات الزنك (٢٢,٦% زنك) علي نمو نباتات الفول وحالة كل من النيتروجين والفوسفور والبوتاسيوم وعدد والوزن الجاف للعقد البكتيرية ونشاط إنزيم النيتروجينيز وثاني أكسيد الكربون المنطلق من منطقة الريزوسفير وكذلك العدد الكلي للبكتريا والفطريات والأكتينوميسيتات وأوضحت النتائج ما يلي:-

- ازداد إنتاج المادة الجافة لنباتات الفول بإضافة كل المعاملات مقارنة بالكنترول.
- تأثرت كل من المادة الجافة لنباتات الفول وتركيز كل من النيتـروجين والفوسـفور والبوتاسـيوم والحديـد والزنك إيجابيا بإضافة كل من الهيوميك والأزولا المثراة بالحديد أو الزنك أو كلاهما وكانت هـذه الزيـادة هي الأعلي في حالة التحميل عنه في الإضافة المنفردة .
- كانت عملية خلط أى من حامض الهيوميك أو الأزولا مع الحديد أو الزنك أكثر كفاءة في زيادة كـل مـن العدد والوزن الجاف للعقد البكتيرية ونشاط إنزيم النيتروجينيز وكذلك ثاني أكسيد الكربون المنطلق وكانـت أعلي القيم لا تعتمد فقط علي الإضافة بل علي نوع المخلف العضوي .
- أشارت النتائج أن حامض الهيوميك والأزولا المثراه بالحديد أو الزنك يمكـن أن تكـون فعالـة ومـؤثرة كمصدر للحديد أو الزنك بنفس فاعلية المصادر المخلبية وقد وجد أن الهيوميك أو الأزولا المثراة بالحديـد أو الزنك تكافئ وتزيد في تأثيراها أعلي المركبات المخلبية مثل Fe-EDTA أو Zn-EDTA.
أشارت النتائج أن التربة المضاف إليها حامض الهيوميك أو الأزولا بصورة منفردة أو مخلوطاً مع الحديـد أو الزنك أعطت أعلي الأعداد للبكتريا والفطريات والاكتينوميسيتات عنه بالمقارنة بمعاملة الكنترول.