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IMPROVED GROWTH AND TOLERANCE OF COWPEA TO IRRIGATION WITH WASTE EFFLUENTS FROM FERTILIZER'S FACTORIES USING MYCORRHIZAL FUNGUS *(Glomus Fasiculatum)*

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ABSTRACT

Effect of preinoculation of mycorrhizal fungus *(Glomus fasiculatum)* on growth and tolerance of cowpea plants irrigated with different concentrations of waste effluents from industrial fertilizers was studied. The results indicated that, the relative growth rate, dry weight, nodule formation, total pigment content as well as tolerance indices of mycorrhizal cowpea plants were significantly reduced by irrigation with different levels of polluted water. Mycorrhizal association was found not only to improve all above parameters but also aid in overcoming the detrimental growth effect of irrigated water pollution. The results also revealed that, macro- -and micro-element contents, (P, N, Na, Mg, Cu, Co, Zn and Fe) of cowpea plants grown on polluted soil were much higher than that of unpolluted one. Results emphasized that VA mycorrhizal symbiosis can increase metal tolerance of cowpea plants. The VA mycorrhizal symbiosis could accumulate the heavy metals Co, Zn, Fe in mycorrhizal plant root than that of mycorrhizal plant shoot. The percentage of VA mycorrhizal infection showed marked reduction with raising the pollution level. Evidently from the results proved that mycorrhizal dependencies for plant dry mass decreased at 10 & 20% pollution level, but, it increased at higher pollution level (40%).

INTRODUCTION

At present, water is becoming an increasingly scarce commodity in many countries, and accordingly planners are searching for new additional sources. In fact, the Egyptian government efforts to increase water resources has taken three main directions, development of upper Nile projects, increase of current water distribution and efficient use of all available water resources inside the country as well as re-use of waste water.

Organic and inorganic pollutants are routinely liberated by a variety of industrial processes as well as domestic sewage disposal and frequently contaminate water effluents which can lead in turn to pollution of fresh water environments. These pollutants result in serious degradation of water quality[(1)].

Polluted water used for irrigation of farm soils can lead in turn to accumulation of some elements like heavy metals in growing plants. These elements well be strongly toxic to crops, inhibit the formation of mycorrhizae, and consequently affect the growth yield of the host plant[(2)]. Organic nitrogen, ammonia, copper, zinc and iron, as well as other pollutants are important contaminants of industrial effluents liberated from industrial fertilizer's factories in Egypt.

Vesicular arbuscular VA mycorrhizae are natural means of assuring plant health and production, but their presence is too often ignored in both physiological and agricultural contexts [(3-5)].

The role of mycorrhizal symbiosis in plant growth and metal tolerance was proved by the work of many investigators[(6-9)]. They found that VA mycorrhizal infection to plants may increase dry weight, pigment content, uptake of macro-and micro-elements as well as metal tolerance. Previous studies found that there is a great deal of variability on the effect of different types of mycorrhizae on host metal tolerance. In some cases, VA mycorrhizae are able to provide a measure of protection against metal toxicity[(10)], but in other cases, they increase the uptake of metals, whereby reducing growth,[(11)]. Under metal-contaminated field conditions, VA mycorrhizae may either fail to form, or the mycobiont involved many changes [(12)].. However, root colonization and spore numbers of VA mycorrhizal fungi in the metal polluted field were relatively high compared with unpolluted field [(13)]. On the other hand it was showing that, high nitrogen levels arrest and prevent mycorrhizal formation and sometimes terminate symbiosis[(14)].

The aim of the present work is to study the effects of preinoculation of mycorrhizal fungus (*Glomus fasiculatum)* on the growth and tolerance of *Vigna sinensis* plant irrigated with waste water effluents liberated from Talkha fertilizer factory Dakhlia province, Egypt.

MATERIALS AND METHODS

Experimental Design:

Two mycorrhizal inoculation treatments were examined using four levels of polluted waste water (0%, 10%, 20% and 40% diluted with tap water) provided from industrial fertilizers factory of Talkha region, Dakhlia. The specific qualification of polluted waste water used in these experiment are listed in (Table 1). These treatments were replicated, several times for a total of 96 pots.

The Soil :

The soil was collected from Dakhlia province, Egypt (Clay : Sand 2:1 v/v, available phosphorus 21 ug.g^{-1}, total phosphorus, 150 μg.g^{-1}; total nitrogen 0.16%, pH 7.2).

It was then sieved through a 4 mm sieve to remove large vegetative material and then steamed for 2h at 100°C in an often to eliminate any indogenous VA mycorrhizal fungi. The soil was subsequently dried and potted into plastic-pots (18 cm diameter).

Table (1): Specification of polluted waste water from fertilizers factory of Talkha region Dakhlia province.

Constituents	Quantities
Carbonate $\mu g.g^{-1}$	54.0
Bicarbonate $\mu g.g^{-1}$	30.5
Calcium hardness $\mu g.g^{-1}$	15.0
Magnesium hardness $\mu g.g^{-1}$	30.0
Ammonia $\mu g.g^{-1}$	282.9
Nitrate $\mu g.g^{-1}$	34.9
Nitrite $\mu g.g^{-1}$	6.0
Organic nitrogen $\mu g.g^{-1}$	197.0
Phosphorus $\mu g.g^{-1}$	96.0
Iron $\mu g.g^{-1}$	1.2
Zinc $\mu g.g^{-1}$	0.19
Copper $\mu g.g^{-1}$	1.5
Total hardness	25.0
Chloride $\mu g.g^{-1}$	102.0
Total suspended compounds $\mu g.g^{-1}$	83.0
Conductivity μ mol.cm^{-1}	1451
pH	9.8

Mycorrhizal Treatments :

Two mycorrhizal inoculation treatments (non-inoculated and inoculated with *Glomus fasiculatum* (*Thaxter sensu Gerdemann*). The inocula consisted of our mycorrhizal onion root fragments (mycorrhizal infection = 72%) from stock pot cultures of this fungus.

The inoculum was placed 3 cm below the surface of the soil before sowing date to produce mycorrhizal-infection plants and non-inoculated plants were supplied with filtered washing of the inoculum to supply the same microflora other than mycorrhizal fungus.

Growth conditions :

Seeds of cowpea (*Vigna sinensis L.*) were surface sterilized with 7% calcium hypochlorite for 20 min. and subsequently washed with distilled water and germinated for 4 days in moist sterilized vermiculate in plastic dishes. Uniform seedlings were transplanted (One plant/pot) into 18 cm diameter plastic pots each containing 1200 g of the sterilized soil. All plants received a standard inoculum of cowpea *Rhizobium*. Pots were randomly distributed in a glasshouse with a normal condition of light and temperature (22 ± 2°C). Plants for each treatment were carefully watered with equal amount of the suggested (as mentioned in experimented design) concentrations of the polluted waste to maintain soil moisture near field capacity. Four replicates of the plants from each treatment were harvested at 5,7 and 9 weeks after planting from sowing date.

Analytical methods :

a-Growth parameters :

At each harvest, each plant was separated into roots and shoots. Fresh and dry weights of shoots and roots were recorded. Leaf area and nodulation rate were also determined. Shoots and roots were ground after drying at 80°C for 48 h and three subsamples were chemically analyzed. Total phosphorous was determined by the vanadono-molybdophosphoric colorimetric method[15], Total nitrogen was determined by kjeldahl method [16], K and Na were assayed by using flame spectrophotometer, while other elements including Mg, Ca, Zn, Cu, Co and Fe were determined by atomic absorption technique [17].

Photosynthetic pigments (Chlorophyll a,b & carotenoids) of plant leaves were extracted and determined by the method of Harborne et. al [18].

Mycorrhizal dependency (MD) and relative field mycorrhizal dependency (R.F.M.D.) of infected plants were calculating according Gerdemann[19] at each pollution level after 5,6, and 9 weeks respectively as follow :

M.D = dw M plant/dw NM plant x 100

R.F.M.D = dw M plant - dw NM plant/dw M plant

where, dw (dry weight), M (mycorrhizal), NM (non-mycorrhizal).

Tolerance indices (Ti) of mycorrhizal and non-mycorrhizal cowpea plants to polluted water were determined according to Shetty et al[20] at each harvest stage as follow:-

Ti=dw plant at pollution level/dw plant at control x 100

b-Estimation of VA mycorrhizal infection :

Random root samples were cleared and stained according to [21]. The percentage of mycorrhizal infection rate was determined by the method of Trouvelot and Gianinazi-Pe-arson[22].

c-Statistical analysis :

The effect of both diluted waste water and the VA mycorrhizal infection on the measured parameters was analysed using two factorial analysis of variance (ANOVA)[23].

Correlation matrix was used as a measure of the strength of the relationship between VA mycorrhizal infection and the measured parameters at different levels of pollution by the method of Crow et al[24].

The data were fed into a computer *(Apple L.C$_2$)* and we have used **"State View Set + Graphics"** program.

RESULTS

1- Growth Changes

The ecological ramifications of V.A. mycorrhiza increased cowpea (*Vigna sinensis*) plant tolerance to irrigation with polluted water are best assessed by evaluating the effects on plant growth (Table 2). The exposed cowpea plant to irrigation with different concentrations of polluted water showed variable changes in relative growth rate and dry matter accumulation. Comparing the use of 40% polluted water to the other dilutions in irrigation of cowpea plant, it was found that the relative growth rate and dry weight of either mycorrhizal and non-mycorrhizal treated plants were reduced to about 50% of the equivalent control (0.0% polluted water) except after first harvest stage (5 weeks planting).

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Table 2 : Effect of different dilutions of waste water on growth measurement (relative growth rate, dry weight, number of nodules, leaf area and tolerance indices) of mycorrhizal (VAM +ve) and non-mycorrhizal (VAM-ve) cowpea plant.

Weeks after Planting	Waste Water (%)	V.A.M State	Relative % Growth Rate	Dry Weight (g/plant)	Number of Nodules (No/Plant)	Leaf area cm/leaf	Tolerance Indices
5	0.0 % Control	-	0.037	0.193	20.5	37.4	
		+	0.044	0.414	37.5	46.4	
	10%	-	0.017	0.198	17.3	39.6	1.03
		+	0.035	0.324	34.7	43.3	1.25
	20%	-	0.007	0.24	12.5	36.5	1.25
		+	0.032	0.292	26.3	36.7	1.51
	40%	-	0.01	0.197	9.2	35.7	1.18
		+	0.27	0.228	21.7	37.8	1.18
7	0.0 % Control	-	0.048	0.369	23.8	53.6	
		+	0.057	0.54	42.4	55.8	
	10%	-	0.038	0.269	19.6	41.6	0.73
		+	0.048	0.494	37.2	50.4	1.34
	20%	-	0.038	0.302	11.0	39.1	0.23
		+	0.065	0.453	21.3	48.3	0.82
	40%	-	0.023	0.234	6.1	37.1	0.63
		+	0.032	0.395	19.3	43.4	1.07
9	0.0 % Control	-	0.072	0.777	25.1	39.1	
		+	0.094	1.082	52.1	50.1	
	10%	-	0.056	0.589	15.4	31.2	0.76
		+	0.062	0.764	28.6	36.6	0.98
	20%	-	0.041	0.508	8.0	30.1	0.55
		+	0.058	0.597	18.1	34.9	0.65
	40%	-	0.036	0.390	3.5	24.8	0.50
		+	0.04	0.593	17.5	33.2	0.76

N.P. : Control: Tap water used for irrigation. VAM : Vesicular arbuscular mycorrhizae.

However, generally mycorrhizal plant still exhibit higher growth rates and dry weights than that of non-mycorrhizal Nodulation of the roots of mycorrhizal and non-mycorrhizal plants is reduced with increasing the percentage of water pollution (Table 2).. However number of nodules produced by V.A mycorrhizal cowpea was about two times greater than that produced by non-mycorrhizal plant for each pollution level at different harvest stages.

The results presented in (Table 2) also indicated that leaf areas of *G. fasiculatum* infected plants was still higher than that of non-infected one when irrigated with polluted water all over the experimental periods. In general, leaf area of both infected and non-infected plants was reduced by increasing the level of water pollution.

The absolute values of tolerance indices of cowpea plants were rised by association with the mycorrhizal fungus (*Glomus fasiculatum*) as already presented in (Table 2). This was particularly marked at second harvest wherein, the tolerance index of the non-mycorrhizal plant in 10%, 20% and 40% being rised from about 0.73 to 1.34; .0.23 to 0.82 and 0.63 to 1.07 in mycorrhizal plants, respectively. Moreover, tolerance indices of mycorrhizal plants were already significantly higher than that equivalent non-mycorrhizal plant at the studied levels of pollution.

Such increasement were related to the extent of mycorrhizal infection.

2- Pigments Content :

The data given in table 3 show that total pigment content of mycorrhizal and non-mycorrhizal plants were generally reduced by using diluted polluted water at different harvesting stages.

The value obtained for the mycorrhizal plants remained greater than those given by non-mycorrhizal plants. The lowest value of total pigment contents observed at 20% polluted water after both first and second harvest stages, while in third harvest stage was observed at 10% pollution level.

At the end of the first and third harvesting stages, chlorophyll a & b were decreased with increasing water pollution. However, the carotenoid content behaved oppositly with increasing the levels of pollution.

The same picture of results was observed after the second harvesting stage with respect to chlorophyll a & b, while, carotenoid content was decreased with increasing level of water pollution (Table 3).

3- Elements Content :

Table 4, shows that the concentration of different elements detected in cowpea plants treated with different levels of polluted water were significantly higher than those of untreated plants.

Table 3: **Effect of different pollution levels of waste water on pigment contents of mycorrhizal (VAM +ve) and non-mycorrhizal (VAM-ve) cowpea plants.**

			Pigment contents (mg/g fresh wt)			
Weeks after Planting	Waste Water (%)	VAM State	Chlorophyll a	Chlorophyll b	Carotenoid Pigment	Total Pigment
5	0.0	-	14.21	8.05	1.07	23.33
	Control	+	17.78	11.71	1.83	31.32
	10%	-	10.57	8.76	1.45	20.78
		+	11.28	8.47	1.55	21.3
	20%	-	9.67	3.78	1.02	14.47
		+	10.76	5.10	1.08	16.94
	40%	-	8.04	5.24	9.8	23.08
		+	10.44	5.48	10.1	26.02
7	0.0	-	15.24	11.27	3.49	30.0
	Control	+	18.41	13.78	4.27	36.38
	10%	-	11.68	9.27	2.74	23.69
		+	12.72	9.23	2.78	24.73
	20%	-	8.95	7.08	2.46	18.49
		+	10.64	8.4	2.92	21.96
	40%	-	9.82	7.76	2.70	20.28
		+	10.66	8.42	2.92	22.0
9	0.0	-	5.13	4.42	4.27	10.8
	Control	+	5.40	4.75	1.25	11.4
	10%	-	3.84	3.74	1.14	8.72
		+	4.5	3.66	1.02	9.18
	20%	-	3.47	3.19	9.5	16.16
		+	3.54	3.25	9.7	16.49
	40%	-	2.81	1.76	7.20	11.77
		+	3.39	2.12	8.60	14.11

Furthermore, the concentration of such elements in mycorrhizal plants were higher than those of non-mycorrhizal one. The results of (Table 4) revealed also that phosphorus content (P) of mycorrhizal and non-mycorrhizal plants, decreases with increasing the level of pollution compared with control especially after 7 and 9 weeks plantation..

The content of both nitrogen (N) and (sodium) (Na) of the growing plants decreased with increasing the level of water pollution compared with control treatment after the first harvest stage in both mycorrhizal and non-mycorrhizal plants. However, N and Na contents of mycorrhizal and non--mycorrhizal plants shows higher values in second and third harvest stages with rising of water pollution.

Low content of potassium ions (K) was detected with increasing pollution levels at all harvest stages in both infected and non-infected plants. On the other hand, magnesium (Mg) and calcium (Ca) contents were not affected by increasing pollution of irrigated water all over the three harvest stages of both mycorrhizal and non-mycorrhizal cowpea plants (Table 4).

From the results recorded in table 4,.copper (Cu) and cobalt (Co) contents showed the highest values compared to controls by increasing pollution levels of irrigated water in both mycorrhizal and non-mycorrhizal plants at the three harvest stages. In addition, zinc concentration of mycorrhizal plant decreased, but in non-mycorrhizal plants, it increased compared to control with rising pollution of irrigated water after 5 weeks.

On the other hand, zinc content increases with increasing the pollution levels compared to control and the maximum concentration was observed at 20% pollution level after 7 & 9 weeks in mycorrhizal and non-mycorrhizal plants. However, ferrous (Fe) concentration increases with rising the pollution level in both mycorrhizal and non-mycorrhizal plant all over the experimental harvest stages; except in case of non-mycorrhizal plant at level 10% after 9 weeks.

The results of table 4 showed also that, the percentage of heavy metals (Cu, Co, Zn & Fe) content in roots to its content in the whole plant increases with the increase in the pollution level in the water for irrigation used. The percentage of Co and Zn content in mycorrhizal plant roots were generally higher than that of mycorrhizal plant shoots in polluted soil.

On the other hand the percentage of Cu content (>50%) in mycorrhizal plant roots were observed at higher pollution levels (20 & 40%) after 7 weeks planting.

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4- Nitrogen Phosphorus ratios, Mycorrhizal infection, Mycorrhizal dependence and relative field mycorrhizal dependence:

The relation between plant nitrogen-phosphorus ratios (N/P) and mycorrhizal infection (%M), mycorrhizal dependence (MD) and relative field mycorrhizal dependence (RFMD) of cowpea plants irrigated with polluted water are shown in (Table 5). The N/P ratios were increased with the aging of the plant at each pollution level. Nevertheless, these ratios in the sometime, did not related to level of pollution.

The rate of mycorrhizal root colonization (M%) decreases with rising the level of water pollution as already presented in (Table 5). Moreover, the mycorrhizal infection was increased with aging of the plant. In addition, the results shown in the table indicate that MD as well as RFMD decreased by rising pollution level up to 20%. There after, MD and RFMD were increased again.

5- Effect of mutual interaction of water pollution and mycorrhizal infection on plant:

From the results of two-factor analysis of variance presented in (Table 6) it was noticeable that, the levels of water pollution have shown highly significant effect ($P \leq 0.01$) on relative growth rate, nitrogen and copper contents of cowpea plants. Moreover, it also significantly affected ($P \leq 0.05$) dry weight, number of nodules, N/P ratio as well as zinc and cobalt content of grown plants. On the other hand, mycorrhizal infection showed a highly significant effect ($P \leq 0.01$) on relative growth rate and number of nodules of irrigated plants. However, mycorrhizal infection also induced significant effect ($P \leq 0.05$) on dry weight, total pigment content, phosphorus, nitrogen, magnesium, calcium and zinc contents of plants as well as N/P ratios. Furthermore, the combined action of water pollution levels and mycorrhizal infection showed significant effects on dry weight, number of nodules, magnesium, calcium and cobalt contents of cowpea plants. The remaining measurements listed in table 6 did not show any significant responses to water pollution and mycorrhizal infection or both together.

6- Strength relationship between VA mycorrhizal infection and plant measurements:

Correlating the mycorrhizal infection with plant measurements at various levels of pollution by using correlation coefficient "r" as already presented in (Table 7) revealed that, the relative growth rate, number of nodules and N/P ratio exhibited positive significant correlation with mycorrhizal infection. More over, zinc content of cowpea plant also significant showed inversely correlation with vesicular mycorrhizal infection at the three levels of polluted water. However, N/P ratio as well as nitrogen content of plants showed proportional significant correlation with mycorrhizal infection at the three levels of pollution, except at 10% level where nitrogen content was inversely correlated with mycorrhizal infection. On the other hand, mycorrhizal infection was significantly inversely correlated with phosphorus and cobalt contents of irrigated plants at 10% of water pollution, while at 20% of water pollution, calcium content of plants was positively correlated with mycorrhizal infection in a significant value. In addition, mycorrhizal, infection induced positive significant correlation with dry weight and negative significant with copper content of plants, at a pollution level of 40%. Furthermore, mycorrhizal infection was significantly correlated positively with relative growth rate, number of nodules, N/P ratio and potassium content of cowpea plants, while negatively correlated with phosphorus, sodium and zinc contents at normal conditions of irrigation with control (Table 7).

DISCUSSION

As previously reported, increasing the period of irrigation with polluted water markedly changed the physical and chemical properties of the exposed soil. These changes includes increase in the soil elements N, P, K, Zn, Cu, Mn, Co and Fe as well as other heavy metals [25]. Current evidence indicates that the level of pollution in irrigated water can be considered as an important factor controlling nutritive or morphological characters of the growing plants[26,27]. In this connection, our results revealed that the relative growth rate as well as dry weight of cowpea plants were significantly ($P \leq 0.05$) affected by irrigation with different levels of polluted water. Marked reduction in relative growth rates was observed by rising the level of water pollution reaching to about 50% of the control at a level of 40% polluted water.

It is evident from the present study that mycorrhizal infection improves relative growth rate, dry weight and tolerance indices of cowpea plant growing in soil exposed to different concentrations of polluted water, compared to non-mycorrhizal plant. These findings agrees with previous results [2,28-30] and suggest that management of VA mycorrhizal symbiosis could not only improve growth but also help in overcoming the detrimental growth effects of irrigated water pollution.

In this investigation, nodule formation by cowpea plants showed significant sensitive responses ($P \leq 0.05$) toward polluted water, where, its numbers decreases as pollution level increases. This may be attribute to high nitrogen content in polluted water and this correlation with proved previously [31].

However, the effect of water pollution on inhibition of nodule formation in mycorrhizal plants was greatly reduced compared with the non-mycorrhizal plants. Such reduction was related to the extent of mycorrhizal infection ($P \leq 0.01$).

Table 4 : Analysis of elements of mycorrhizal (VAM +ve) and non mycorrhizal (VAM -ve) cowpea plants treated with various levels of waste water.

Weeks after Planting	Waste Water (%)	VAM State	Mineral contents in whole plant: mg g⁻¹ Dwt		mmole g⁻¹ Dwt		mmole g⁻¹ Dwt	mg g⁻¹ Dwt	Heavy metal content in whole plant in mg g⁻¹ dry weight: Cd^{2+}		Cu^{2+}		Zn^{2+}		Pb^{2+}	
			P	N	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}	Total	% R	Total	R	Total	R	Total	R
5	Control 0.0	-	4.4	17.6	0.8	1.76	0.22	6.76	54	33	16.7	27	92.2	26	81.2	32
		+	5.6	23.6	0.9	1.59	0.27	7.9	61.7	41	33.3	29	157.1	31	146	21
	10%	-	2.52	15.6	0.5	1.65	0.24	6.7	52.5	33	40.9	31	122.4	24	125	20
		+	4.2	23.1	0.46	1.58	0.29	6.91	71.2	46	83.3	47	141.3	49	139.4	22
	20%	-	2.1	14.6	0.56	1.49	0.22	6.76	60	35	55.3	43	128.6	30	175	24
		+	3.9	24.8	0.46	1.19	0.27	6.83	74	42	90	51	139.3	56	169.8	36
	40%	-	1.8	16.6	0.59	1.53	0.31	6.63	73.1	29	63.3	36	116.4	46	91.8	36
		+	3.7	22.6	0.46	1.33	0.28	6.94	84.1	46	96.7	55	121.7	52	112.2	43
7	Control 0.0	-	1.13	9.37	0.4	1.69	0.29	6.1	38.6	43	33.3	28	67.2	30	74.8	25
		+	2.5	13.15	0.5	1.76	0.31	6.46	46.3	44	66.7	32	54.5	34	107	28
	10%	-	1.16	14.5	0.57	1.69	0.31	6.68	61.7	36	33.3	33	154	25	132.2	27
		+	2.8	25.8	0.56	1.7	0.33	6.29	69.3	49	70	57	168	47	166	35
	20%	-	1.4	16.6	0.82	1.52	0.31	6.79	58	41	56.9	32	175	28	128.6	23
		+	3.3	32.7	0.61	1.87	0.31	6.91	70.1	59	80	58	186	56	142	40
	40%	-	2.0	18.6	0.92	1.37	0.32	6.88	58.5	32	59.3	40	136.2	34	97.8	28
		+	4.1	33.6	0.65	1.44	0.31	6.96	76.1	50	83.3	59	153.6	52	121.6	45.5
9	Control 0.0	-	1.5	8.5	0.36	1.82	0.27	6.5	23.2	32	39.4	36	16.5	32	65.6	21
		+	2.7	11.1	0.41	1.96	0.28	6.89	23.2	38	70.3	36	23.5	31	86.7	30.7
	10%	-	1.6	15.4	0.58	1.34	0.28	6.82	38.6	39	53.6	43	103	40	53.6	31
		+	3.1	29.5	0.57	1.61	0.29	6.9	46.3	42	93.6	59.8	125	51	139.4	42
	20%	-	1.9	18	0.67	1.39	0.31	6.81	60.8	45	59.5	45	116	35	124	21
		+	3.7	26.3	0.59	1.59	0.30	6.89	55.2	46	96.7	60.5	134	54	149.6	45.5
	40%	-	2.1	19.2	0.79	1.53	0.28	6.83	45.3	34	74.6	41	66	40	79.2	28
		+	4.6	56.3	0.63	1.7	0.28	6.98	86.3	41	113.5	61	81	62	105.2	53.6

Dwt : dry weight.

$$\%R: \text{The Percentage of heavy metals in root} = \frac{\text{amount of heavy metals in root}}{\text{amount of heavy metals in whole plant}} \times 100$$

Table 5 : Nitrogen/Phosphorus ratio of plant tissues, (N/P), % of mycorrhizal infection (%M), mycorrhizal dependance (M.D) as well as relative field of mycorrhizal dependance (R.F.M.D) of infected cowpea plant by G. fasiculatum irrigated with various levels of polluted water.

Weaks after Planting	10% Polluted Water				20 % Polluted Water				40% Polluted Water			
	N/P	% M	M.D	R.F.M.D.	N/P	% M	M.D	R.F.M.D	N/P	% M	M.D	R.F.M.D.
5	5.5	18.5	1.6	0.39	6.2	12.5	1.2	0.2	6.1	8.3	1.2	0.14
7	9.2	36	1.8	0.46	9.9	29	1.5	0.33	8.2	18	1.7	0.4
9	9.5	53	1.3	0.23	7.1	35	1.2	0.15	14.2	22.7	1.5	0.34

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Table 6 : Two-factor analysis of varience (ANOVA) test for the effect of treatment of cowpea plants with polluted water and mycorrhizal infection on plant measurements

F.ratio thioritical / Two-factores / Measurements	Polluted Water	Mycorrhizal infection	Polluted water and mycorrhizal infection
F.ratio at 0.05	3.41	2.96	1.83
F.ratio at 0.01	7.35	5.26	3.16
Relative growth rate (%)	8.14**	6.93**	1.32
Dry weight g/plant	4.61*	4.32*	1.95*
Number of nodules No/plant	5.31*	10.21**	2.16*
Total pigments mg/g Fwt	2.89	3.63*	1.17
Phosphorus (P) content $mg.g^{-1}$ Dwt	0.63	3.51*	1.72
Nitrogen (N) content $mg.g^{-1}$ Dwt	9.13**	5.15*	0.73
N/P ratio (%)	3.65*	3.18*	1.11
Potassium content $m.mole.g^{-1}$ Dwt	2.19	1.2	1.68
Sodium content $m.mole.g^{-1}$ Dwt	2.31	2.16	1.79
Magnesium content $mg.g^{-1}$ Dwt	3.36*	3.1*	2.6*
Calcium content $m.mole.g^{-1}$ Dwt	3.21	2.98*	2.19*
Zinc content $Ug.g^{-1}$ Dwt	4.63*	3.42*	1.53
Iron content $Ug.g^{-1}$ Dwt	2.19	2.61	1.8
Copper content $Ug.g^{-1}$ Dwt	7.46**	1.17	1.56
Cobalt content $Ug.g^{-1}$ Dwt	4.21*	2.57	2.13*

* Significant → F : $P \leq 0.05$
** Highly significant → F : $P \leq 0.01$

Dwt : Dry weight
Fwt : Fresh weight

Table 7: The correlation between mycorrhizal infection and Plant measurements (growth parameters) at different levels of water pollution .

Correlation value / Measurements	Waste water (%) 0.0 %	10 %	20%	40%
Relative growth. rate	0.53*	0.52*	0.55*	0.67*
Dry Weight	0.51	0.48	0.51	0.59*
Number of nodules	0.83*	0.69*	0.72*	0.82*
Total pigment,	0.42	0.31	-0.1	0.28
Phosphorus (P) content	-0.76*	-0.52*	-0.31	-0.26
Nitrogen (N) Content	-0.51	-0.58*	0.69*	0.77*
N/P ratio	0.74*	0.67*	0.63*	0.72*
Potasseum content	0.56*	0.48	0.39	0.24
Sodium content	-0.57*	0.11	0.01	-0.15
Magnesium content	-0.43	0.14	0.4	0.31
Calcium content	0.17	0.44	0.58*	0.42
Zink content	-0.58*	-0.63*	-0.61*	-0.59*
Iron content	0.14	0.45	-0.12	-0.46
Copper content	-0.46	0.40	-0.51	-0.57*
Cobalt content	0.26	-0.63*	-0.1	-0.17

Critical value of tail = 0.52 $r \leq 0.52$ insignificant $r \geq 0.52$ significant *

The benefit role of VA mycorrhizal in nodule formation by pea plant was previously reported by some investigators [33-34].

The present study demonstrates that the reduction in chlorophyll a & b and total pigment contents as well as leaf area of cowpea plant was induced by rising level of irrigated water pollution. However, these measurements still higher in mycorrhizal plant than non-mycorrhizal one. In this connection, evidence from the previous study [27,35-37] indicates that polluted water may increase heavy metal accumulation in exposed plant. This accumulation may reduce the concentrations of chlorophyll a & b, cytochrome b and ferrodoxin. Based on aforementioned data we suggest that, VA mycorrhizal association may interfere with and reduce the toxic effects of heavy metal accumulation in exposed plant.

It appears from the present study that, macro- and micro-element contents, including P, N, Na, Mg, Cu, Co, Zn and Fe, of cowpea plants exposed to polluted water were much higher percentage than that of unexposed plant (Control). These findings confirm previous results findings[2,27]. They found that irrigation of farm soil with polluted water increased their macro- and micro element contents, which lead to more consequently to more accumulation of these elements in the growing plants. However, some of these elements may cause nutritive or morphological problems to these plants.

In the present investigation, it is of interest to note that, although the heavy metals; including Cu, Co, Zn and Fe, content of mycorrhizal plants are much higher than that of non-mycorrhizal one, the mycorrhizal plant showed better growth than that of non-mycorrhizal one. These results emphasize that VA mycorrhizal increase metal tolerance of the cowpea plants in the present. Similar observations have been made from the previous workers [19, 36-39].

Two main mechanisms for the effect of mycorrhiza on host metal tolerance have been reported. The first was proposed by [40], who suggested that metals may bind to the interfacial matrix found between the hyphae in ericoid mycorrhizas. This binding would then either, reduce further movement of the metal through the mycorrhizae to host tissue, or removed metabolically by the fungus and sequestered in harmless form within its hyphae. However, either of these possibilities, passive binding or metabolic detoxification by mycobiont, should lead to increased amounts of metal in the mycorrhizal root systems and decreased of these amounts in the above-ground parts of resistant plant. This negative correlation between shoot and root metal content was proved for zinc, iron, and cobalt in the present study. Similar observations were previously reported[37, 41-42]. Moreover, it was found that the mycobionts did not affect translocation of copper to the shoots of cowpea plants which was in agreement with other investigation [39]. The presence of metal-binding mechanism is conjecture, however, until information is obtained on metal localization in fungal and host tissues.

The second means by which infection by mycorrhizal fungi may affect metal tolerance is through improved phosphorus nutrition [10]. Evidence from the present study indicates that VA mycorrhizal infection significantly increase the concentration of phosphorus in cowpea plant tissues at various levels of water pollution, compared with non-infected plant. Our results support the previous works by [19,43,44]. They found that phosphorus uptake is usually enhanced in mycorrhizal plants than non-mycorrhizal one. According to aforementioned results, it is conceivable to suggest that, the two mechanisms; bind and improved phosphorus nutrition effects may play a role in increasing metal tolerance.

In the present investigation, the percentage VA mycorrhizal root infection showed marked reduction with rising the level of pollution. Moreover, there is a significant increases in nitrogen content of both exposed soil and growing plant under a long period of irrigation with polluted water. Similar observations have been made before [14,45,46,47]. They showed that high nitrogen levels arrest and prevent mycorrhizae formation and sometimes terminate symbiosis. Interestingly, although nitrogen level is high in exposed soil treated with polluted water, the mycorrhizal symbiosis increased at each pollution level with increasing stage of harvesting. This may be explained on the basis of VA mycorrhizal adaptability to new conditions which results from irrigation with water pollution in the present study.

Our findings could not demonstrate a relationship between tissue N/P ratios and root colonization and this findings supports that reported before [8]. On the other hand these findings differs from that of [48]. Who suggested that VA mycorrhizal infection is positively related to the plant nitrogen concentration at high N/P ratio. When the N/P ratio is low, the VAM infection rate would depend on phosphorus availability.

Mycorrhizal dependency has been defined by [19] as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility". Morphological properties, plant biomass as well as other plant parameters have often been used as indicator for mycorrhizal dependency [49]. Evidence from our results indicates on mycorrhizal dependency for plant dry mass decreased at low and moderate pollution levels (10 & 20%), while it increased at high pollution level (40%). Based on these data it is conceivable to conclude that cowpea plant tolerance and its growth are depend on mycorrhizal symbiosis when grown in soils irrigated with polluted water. Furthermore, the results

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possibility of re-use of untreated industrial waste effluents in agricultural purposes along with VA mycorrhizal infection to the growing plants, especially, for the plants, were only shoot system are use by animal and human.

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تحسين نمو وتحمل نبات اللوبيا للرى بمخلفات مصانع الأسمدة بواسطة الفطريات الميكوريزية الداخلية (جلومس فاسكيلاتم)

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يهدف هذا البحث إلى إمكانية إستخدام مياه الصرف الملوثة الناتجة من مصانع الأسمده بعد تخفيفها دون معالجة فـى رى وزراعة نبات اللوبيا ، وذلك بإستخدام الفطريات الميكوريزية بحقن النبات بفطرة جلومس فاسكيلاتم. وقد أظهر البحث النتائج التاليـــة :-

١-أوضحت النتائج أن الفطريات الميكوريزية تلعب دورا كبيرا فـى زيـادة كـلا مـن معدل النمـو النسـبى وإنتـاج المـواد الجافة وتكوين العقد البكتيرية والمحتوى الصبغى ومدى تحمل النباتات للتربة الملوثة وذلك فى النباتات المصابة بهذه الفطريات عن الغير مصابه.

٢-أظهرت النتائج أن العناصر المعدنية والتى تشمل الفوسفور والنيتروجين والصوديوم والماغنسيوم والنحاس والكوبلت والزنك والحديد توجد بتركيزات كبيرة فى النباتات النامية فى التربة الملوثة عن مثيلاتها الغير ملوثـة. وأيضـا توجـد هذه العناصر دائماً بتركيزات أعلى فى النباتات المصابة بالفطريات الميكوريزية عن مثيلاتها الغير مصابه.

٣-بالرغم من زيادة تركيز العناصر وبخاصة الثقيلة منها فـى النباتـات المصابـة بالفطريـات الميكوريزيـة عن مثيلاتها الغير مصابة إلا أن نمو وتحمل هذه النباتات كان أفضل من مثيلاتها الغير مصابة.

٤-أظهرت هذه الدراسة أن نسبة الزيادة فى تركيز العناصر الثقيلة والتى تشمل الكوبلت والزنـك والحديد فـى جذور النباتات المصابة تكون أعلى من مثيلاتها فى المجموع الخضرى لهذه النباتات ، وأيضـا أثبتت التجارب زيـادة المحتوى الفوسفاتى لهذه النباتات المصابة ، ممايعطى مؤشرا واضحاً على كيفية عمل الفطريات الميكوريزية فى زيادة تحمل نبات اللوبيا بـالرى بالمياة الملوثة وذلك عن طريق تخزين وتراكم هذه العناصر الملوثة فـى جذور النبـات وهيفـات الفطـر نفسـه بالإضافة إلى تحسين المحتوى الفوسفاتى للنبات.

٥-أثبتت النتائج أن زيادة مستوى التلوث يعمل على تثبيط الإصابة بالفطريات الميكوريزية ولم تظهر النتـائج علاقـة ثابتة بين معدل الإصابة ونسبة النيتروجين إلى الفوسفور فى أنسجة النبـات ، وكذلـك أشـارت النتـائج إلـى زيـادة إعتمـاد النبات على الفطريات الميكوريزية وبخاصة عند مستويات التلوث الكبيرة.