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THE USE OF Glomus fasiculatum, A MYCORRHIZAL FUNGUS TO OVERCOME THE EFFECT OF THE INDUSTRIAL WASTES ON THE GROWTH OF COWPEA

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ABSTRACT

The effect of preinoculation with the mycorrhizal fungus *(Glomus fasiculatum)* on growth and tolerance of cowpea plants irrigated with different concentrations of waste effluents drained from fertilizers' industry 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 the polluted water. Mycorrhizal association improved not only the all above parameters but also aided in overcoming the detrimental growth effect of the polluted irrigation water. The 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 those of nonpolluted ones. Results emphasized that vesicular arbuscular (VA) mycorrhizal symbiosis can increase metal tolerance of cowpea plants. The percentage of VA mycorrhizal infection showed marked reduction with raising the pollution level. The 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 have taken three main directions, which are: development of the upper Nile projects, efficient use of all available water resources inside the country, and the 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 (Azab *et al.* 1989)

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 will be strongly toxic to crops, inhibit the formation of mycorrhizae, and consequently affect the growth and yield of the host plant (Chao and Wang 1990). 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 (Heggo *et al.* 1990, Wiemken and Ineichen 1992, and Ishac *et al.* 1994). The role of mycorrhizal symbiosis in plant growth and metal tolerance was proved by the work of many investigators (Blal *et al.* 1990, Dixom 1990, Hejne *et al.* 1992, Jindal *et al.* 1993). 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 confirmed that different types of mycorrhizae can greatly vary in their effect on promoting host metal tolerance. In some cases, VA mycorrhizae were able to provide a measure of protection against metal toxicity (Gildon and Tinker 1988), but in other cases, they increased the uptake of metals, whereby reducing growth (Killham and Firestone 1983). Under metal-contaminated field conditions, VA mycorrhizae may either fail to form, or the mycobiont involved many changes (Harris and Jurgensen 1977). However, root colonization and spore numbers of VA mycorrhizal fungi in the metal polluted field were relatively high compared with unpolluted field (Weissenhorn *et al.* 1995). On the other hand it was showing that, high nitrogen levels arrest and prevent mycorrhizal formation and sometimes terminate symbiosis (Slankis 1974).

The aim of the present work was to study the effects of the VA mycorrhizal fungus, *Glomus fasiculatum* (Thaxter sensu Gerdemann) on the growth and metal tolerance of cowpea (*Vigna sinensis* L.) plants irrigated with waste effluents liberated from Talkha fertilizers' factory, Dakahlia Governorate, Egypt.

MATERIALS AND METHODS

Experimental design:

Two mycorrhizal inoculation treatments were tested using four levels of polluted waste water (0, 10, 20 and 40% diluted with tap water) provided from Talkha fertilizers' factory. The chemo-physical analyses of the polluted waste effluents used in these experiments are listed in (Table 1). These treatments were replicated, several times for a total of 96 pots

The soil:

The soil used in the pot experiments consisted of clay : sand (2 : 1 v/v) with available phosphorus 21 $\mu g.g^{-1}$, total phosphorus, 150 $\mu g.g^{-1}$; total nitrogen 0.16%, and pH 7.2. The soil was sifted through a 4 mm-mesh sieve to remove large vegetative materials and then heated at 100°C in an oven for 2 h to eliminate any indigenous VA mycorrhizal fungi. The soil was subsequently dried and potted into plastic-pots (18 cm diameter).

Mycorrhizal treatment:

The mycorrhizal inoculum consisted of onion root fragments colonized by *G. fasiculatum* (mycorrhizal infection = 72%) from stock pot cultures of this fungus. The inoculum was placed 3 cm below the surface of the soil before sowing to produce mycorrhizal-infected plants. For the non-mycorrhizal treatment, plants were supplied with filtered washing of the inoculum to supply the same microflora other than the mycorrhizal fungus.

Table 1: Chemical analysis of polluted waste water librated from fertilizers' factory of Talkha, Dakahlia Governorate

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 ($\mu g.g^{-1}$)	25.0
Chloride ($\mu g.g^{-1}$)	102.0
Total suspended compounds ($\mu g.g^{-1}$)	83.0
Conductivity ($\mu mol.cm^{-1}$)	1451
pH	9.8

Growth conditions of the host plants:

Seeds of cowpea were surface sterilized with 7% calcium hypochlorite for 20 min., subsequently washed with distilled water, and kept for 4 days in sterilized moist vermiculate in plastic dishes to germinate. 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 normal conditions of light and temperature (22 ± 2°C). Mycorrhizal and non-mycorrhizal plants were carefully watered with equal amount of the suggested four concentrations of the polluted waste water (as previously mentioned) to maintain soil moisture near field capacity. Four replicates were used for each treatment. Cowpea plants from each treatment were harvested after 5, 7 and 9 weeks from planting.

Parameters measured:

a. Growth parameters:

At each harvest date, 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 (Jackson 1958). Total nitrogen was determined by Kjeldahl method (Jackson *et al.*1973), K and Na were assayed by using spectrophotometer, while other elements including Mg, Ca, Zn, Cu, Co and Fe were determined by atomic absorption technique (Allen *et al.* 1986). Photosynthetic pigments (Chlorophyll a, b & carotenoids) of plant leaves were extracted and determined by the method of Harborne (1984).

Mycorrhizal dependency (MD) and relative field mycorrhizal dependency (RFMD) of mycorrhizal plants were calculated according to Gerdemann (1975) at each pollution level after 5, 6 and 9 weeks, respectively as follow:

MD = dw of M plant / dw of NM plant X 100.

RFMD = dw of M plant – dw of NM plant / dw of 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* (1995) at each harvest date as follow:

Ti = Plant dw. at pollution level / plant dw of the control X 100

b. Estimation of VA mycorrhizal infection:

Random root samples were cleared and stained according to Phillips and Hayman (1970). The percentage of mycorrhizal infection was determined by the method of Trouvelot *et al.* (1986).

c. Statistical analysis:

The effect of both waste water and the VA mycorrhizal infection on the measured parameters was analyzed using two factorial analysis of variance (ANOVA) (Hicks 1982). 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 water pollution by the method of Crow *et al* (1960).

RESULTS

Growth changes

The ecological ramifications of VA mycorrhiza increased cowpea plant tolerance to the pollution, which was best assessed by evaluating the effects on plant growth (Table 2). Cowpea plants exposed to different concentrations of polluted water showed variable changes in relative growth rate and dry matter accumulation. At the concentration of 40% polluted water, the relative growth rate and dry weight of either mycorrhizal or non-mycorrhizal plants were reduced to about 50% less than the equivalent control (0% polluted water) at all harvest times except after 5 weeks post planting (first harvest). The mycorrhizal plants, however, recorded higher growth rates and dry weights when compared to the non-mycorrhizal plants. The nodulation of roots of mycorrhizal and non-mycorrhizal plants was reduced with increasing the concentration of water pollution (Table 2). But, the number of nodules produced by the VA mycorrhizal cowpea was about two times greater than that produced by non-mycorrhizal plant at each pollution level at the different harvest dates. The results presented in (Table 2) also revealed that the leaf area of the mycorrhizal plants was higher than that of non-mycorrhizal ones at all levels of polluted water and for all harvests. However, in general, the leaf area of both mycorrhizal and non-mycorrhizal plants was reduced by increasing the concentration of water pollution.

Table 2: Effect of different concentrations of waste water on growth measurements of mycorrhizal and non-mycorrhizal cowpea plants

Weeks after planting	Waste water (%)	Mycorrhizal state	Relative growth rate	Dry weight (g)	Number of nodules per plant	Leaf area cm^2/plant	Tolerance indices
5	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 (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 (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	-	0.007	0.508	8.0	30.1	0.55
		+	0.032	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. B. Control: Tap water used for irrigation

The absolute values of tolerance indices of cowpea plants were raised by their association with the mycorrhizal fungus, *G. fasiculatum* (Table 2). This was particularly marked at the second harvest. The tolerance indices of mycorrhizal plants were significantly higher than that equivalent non-mycorrhizal plant at each level of pollution.

2. Pigment content:

The data in table 3 show that the total pigment content of mycorrhizal and non-mycorrhizal plants were generally reduced when using polluted water at the different harvestings. However, the values obtained for the mycorrhizal plants remained greater than those of the non-mycorrhizal plants. The lowest value of the total pigment contents was recorded at 20% polluted water after both first and second harvests, while in third harvest this value was observed at 10% pollution level. At the end of the first and third harvestings, chlorophyll a & b decreased with increasing water pollution. However, the carotenoid content behaved oppositely with increasing the level of pollution.

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

Table 3: Effect of different concentrations of waste water on pigments' content of mycorrhizal and non-mycorrhizal cowpea plants

Weeks after planting	Waste water (%)	Mycorrhizal state	Chlorophylla	Chlorophyllb	Carotenoid pigment	Total pigment
5	0 (Control)	-	14.21	8.05	1.07	23.33
		+	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 (Control)	-	15.24	11.27	3.49	30.0
		+	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 (Control)	-	5.13	4.42	4.27	10.8
		+	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

3. Elements content:

The data in Table 4, show that the concentration of different elements detected in cowpea plants treated with different levels of polluted water were significantly higher than those of the untreated control plants. Similarly, the concentrations of such elements in mycorrhizal plants were higher than those of the non-mycorrhizal ones. The results revealed also that phosphorus content (P) of mycorrhizal and non-mycorrhizal plants, decreases with increasing the level of pollution compared with the control especially after 7 and 9 weeks from planting. The plant content of both nitrogen (N) and sodium (Na) decreased with increasing the level of water pollution compared with the control treatment at the first harvest in both mycorrhizal and non-mycorrhizal plants. However, the N and Na contents of mycorrhizal and non-mycorrhizal plants show higher values in the second and third harvests with uprising of water pollution.

Low content of potassium ions (K) was detected with increasing pollution levels at all harvests in both mycorrhizal and nonmycorrhizal plants. On the other hand, magnesium (Mg) and calcium (Ca) contents were not affected by increasing the pollution level of the irrigation water. The content of both N and Na in the cowpea plants decreased with increasing the level of water pollution compared with control treatment at the first harvest whether in mycorrhizal or non-mycorrhizal plants. However, N and Na contents of mycorrhizal and non-mycorrhizal plants showed higher values in the second and third harvests with rising of water pollution.

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

Copper (Cu) and cobalt (Co) contents increased by increasing pollution levels in irrigation water in both mycorrhizal and non-mycorrhizal plants at the three harvest stages. In addition, zinc concentration decreased in the mycorrhizal plant, but increased in the non-mycorrhizal plants with rising pollution of irrigated water after 5 weeks(Table 4).

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

The ratio of heavy metals (Cu. Co, Zn & Fe) contents in the roots to their contents in the whole plant increased with the increase of pollution level. The percentages of Co and Zn in mycorrhizal. plant roots were generally higher than those of mycorrhizal plant shoots in polluted soil. On the other hand, high percentage of Cu (>50%) in mycorrhizal plant roots were observed at higher pollution levels (20 & 40%) after 7 weeks from planting.

4. Nitrogen and 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 cowpea plants at each pollution level. Nevertheless, these ratios sometimes did not relate to the level of pollution. The rate of mycorrhizal root colonization (%M) decreased with rising the level of water pollution (Table 5). In addition, the mycorrhizal infection was increased with aging of the plant. The MD as well as RFMD decreased by raising the pollution level up to 20%. Thereafter, MD and RFMD started to increase (Table 5).

5. Effect of mutual interaction of water pollution and mycorrhizal infection on cowpea plants

According to a two-factorial ANOVA, the levels of water pollution had a highly significant effect ($P < 0.01$) on the relative growth rate, nitrogen and copper contents of cowpea plants(Table 6). Moreover, it also significantly affected ($P < 0.05$) the dry weight, the number of nodules, the N/P ratio as well as zinc and cobalt contents of grown cowpea plants. On the other hand, mycorrhizal infection showed a highly significant effect ($P < 0.01$) on the relative growth rate and the number of nodules of treated plants. Also, the mycorrhizal infection had a significant effect ($P <. 0.05$) on the dry weight, the total pigments content, and phosphorus, nitrogen, magnesium , calcium and zinc contents of plants as well as N/P ratios. The interaction (water pollution levels x mycorrhizal infection) showed significant effects on the dry weight, the 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.

Table 4 : Mineral and heavy metal contents of mycorrhizal and non-mycorrhizal cowpea plants treated with various levels of waste water

Weeks after planting	Waste water (%)	VAM state	Mineral contents in whole plant						Heavy metal content in whole plant in μg. g^{-1} dry weight							
			mg.g^{-1} Dwt		mmole.g^{-1} Dwt		mmole.g^{-1} Dwt	mg.g^{-1} Dwt	Cu^{++}		Co^{++}		Zn^{++}		Fe^{++}	
			P	N	Na^{+}	K^{+}	Ca^{++}	Mg^{++}	Total	%R	Total	%R	Total	%R	Total	%R
5	Control 0.0	-	4.4	17.6	0.80	1.76	0.22	6.76	54.0	31	16.7	27	95.2	26	81.2	15
		+	5.6	23.6	0.90	1.59	0.27	7.00	61.7	41	33.3	29	157.1	31	126.0	21
	10%	-	2.5	15.6	0.50	1.65	0.24	6.70	58.5	33	49.9	31	128.4	24	125.0	20
		+	4.2	23.1	0.46	1.58	0.29	6.91	71.2	46	83.3	47	141.3	49	139.4	28
	20%	-	2.1	14.6	0.56	1.49	0.22	6.76	60.0	35	55.3	43	128.6	30	129.0	24
		+	3.9	24.8	0.46	1.19	0.22	6.83	74.0	42	90.0	51	139.3	50	149.8	36
	40%	-	1.8	16.6	0.59	1.53	0.31	6.63	73.1	29	63.3	36	116.4	40	91.8	36
		+	3.7	22.6	0.46	1.33	0.28	6.94	84.1	46	96.7	55	121.7	53	112.2	43
7	Control 0.0	-	1.1	9.37	0.40	1.69	0.29	6.10	38.6	43	33.3	28	42.2	30	74.8	25
		+	2.5	13.2	0.50	1.76	0.31	6.46	46.3	44	66.7	32	54.9	34	102.0	28
	10%	-	1.2	14.5	0.57	1.69	0.31	6.68	61.7	36	33.3	33	154.0	25	132.2	27
		+	2.8	22.8	0.56	1.70	0.33	6.89	69.3	49	70.0	57	168.0	47	146.0	35
	20%	-	1.4	16.6	0.82	1.52	0.31	6.79	58.0	41	56.9	32	175.0	28	138.6	33
		+	3.3	32.7	0.61	1.87	0.31	6.91	70.3	59	80.0	58	186.0	50	162.0	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	76.3	50	83.3	59	153.6	52	123.4	45
9	Control 0.0	-	1.5	8.5	0.36	1.82	0.27	6.50	23.2	32	39.4	36	16.5	32	63.6	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
	10%	-	1.6	15.4	0.58	1.34	0.28	6.82	38.6	39	53.6	43	103.0	40	53.6	31
		+	3.1	29.5	0.57	1.61	0.29	6.90	46.3	42	93.6	59	125.0	51	139.4	42
	20%	-	1.9	18.0	0.67	1.39	0.31	6.81	40.8	45	59.5	45	116.0	35	124.0	21
		+	3.7	26.3	0.59	1.59	0.30	6.89	55.2	46	96.7	60	134.0	54	149.6	45
	40%	-	2.1	19.2	0.79	1.53	0.28	6.83	45.3	34	74.6	41	66.0	40	79.2	38
		+	4.6	56.3	0.63	1.7	0.28	6.93	86.3	41	113.5	61	81.0	62	105.2	53.6

% R: The percentage of heavy metals in root = amount of heavy metals in root / amount of heavy metals in whole plant X 100

Table 5: Nitrogen/phosphorus ratio of plant tissues (N/P), % of mycorrhizal infection (%), mycorrhizal dependence (MD) and relative field of mycorrhizal dependence (RFMD) of infected cowpea plant by *G. fasiculatum* irrigated with various levels of polluted water.

Weeks after planting	10% Polluted water				20% Polluted water				40% Polluted water			
	N/P ratio	%M	MD	RFMD	N/P ratio	%M	MD	RFMD	N/P ratio	%M	MD	RFMD
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	18.0	1.7	0.40
9	9.2	35	1.3	0.23	7.1	35	1.2	0.15	14.2	22.7	1.5	0.34

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

Measurements	Polluted water	Mycorrhizal infection	Polluted water and mycorrhizal infection
Relative growth rate (%)	8.14**	6.94**	1.32
Dry weight (g/plant)	4.61*	4.32*	1.95*
Number of nodules / plant	5.31*	10.21**	2.16*
Total pigment ($mg.g^{-1}$ Dwt)	2.89	3.63*	1.17
Phosphorus content ($mg.g^{-1}$ Dwt)	0.63	3.51*	1.72
Nitrogen content ($mg.g^{-1}$ Dwt)	9.13**	3.15*	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 ($\mu g.g^{-1}$ Dwt)	4.63*	3.42*	1.53
Iron content ($\mu g.g^{-1}$ Dwt)	2.19	2.61	1.8
Copper content $\mu g.g^{-1}$ Dwt)	7.46**	1.17	1.56
Cobalt content ($\mu g/g^{-1}$ Dwt)	4.2*	2.57	2.13*
F. ratio at 0.05	3.41	2.96	1.83
F. ratio at 0.01	7.35	5.26	3.16

- **Significant at 0.05 level**
- **** Significant at 0.01 level**

6. The 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" revealed that the relative growth rate, the number of nodules and the N/P ratio exhibited a significant positive correlation with mycorrhizal infection (Table 7). In contrast, the zinc content of cowpea plant had a significant inverse correlation with the VA mycorrhizal infection at the three levels of polluted water (10, 20 and 40%). However, N/P ratio as well as nitrogen content of plants showed a significant positive correlation with mycorrhizal infection only at 20 and 40% water pollution. On the other hand, mycorrhizal infection was inversely correlated with phosphorus and cobalt contents of plants at 10% water pollution, while at 20% water pollution, calcium content of plants was positively correlated with mycorrhizal infection. In addition, mycorrhizal infection induced positive correlation with the dry weight, but negatively correlated with copper content of plants at the pollution level of 40%. Furthermore, mycorrhizal infection was positively correlated with the relative growth rate, the number of nodules, the N/P ratio and potassium content of cowpea plants. In the control, there was a negative correlation with phosphorus, sodium and zinc contents (Table 7).

Table 7: Correlation coefficient between mycorrhizal infection and plant measurements at different levels of water pollution

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 content	-0.67*	-0.52*	-0.31	-0.26
Nitrogen content	-0.51	-0.58*	0.69*	0.77*
N/P ratio	0,74*	0.67*	0.63*	0.72*
Potassium 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
Zinc 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

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 included increase in the soil elements N, P, K, Zn, Cu, Mn, Co and Fe as well as other heavy metals (Abd El-Naim and El-Awady 1989). 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(Arar 1993 and Abd El-Naim 1994). In this connection, our results revealed that the relative growth rate as well as dry weight of cowpea plants were significantly ($P < 0,05$) 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 the concentration of 40% polluted water.

In the present study, the mycorrhizal infection improved the relative growth rate, dry weight and tolerance indices of cowpea plant growing in soil exposed to different concentrations of polluted water, compared to the nonexposed to different concentrations of polluted water, compared to the non-mycorrhizal plants. This finding agrees with previous results (Chao and Wang 1990, Meenakumari and Nair 1992, Biswas 1993, and Thiagarajan and Ahmad 1993a) and suggests that management of VA mycorrhizal symbiosis may not only improve growth but also help in overcoming the detrimental growth effects of the polluted irrigation water.

In this investigation, nodule formation by cowpea plants showed significant sensitive responses ($P < 0.05$) towards polluted water, where its numbers decreased as pollution level increased. This may be attributed to the high nitrogen content in the polluted water and this correlation was proved previously by Hussein *et al.* (1994). However, the effect of water pollution on the 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. The beneficial role of the VA mycorrhiza in nodule formation by pea plants was previously reported by Seikhon *et al.* (1992), Thiagarajan *et al.* (1992), and Thiagarajan and Ahmad (1993 a & b).

The present study demonstrated that the reduction in chlorophyll a & b and the total pigment contents as well as the leaf area of cowpea plants were induced by rising the level of pollution. However, these measurements values were higher in the mycorrhizal plants than the non-mycorrhizal ones. In this connection, evidence from the previous studies of Abd El-Naim (1994), Abd El-Naim et al. (1987), Jones and Hutchinson (1988), and Leake *et al.* (1990) indicated that polluted water may increase heavy metal accumulation in exposed plants. 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 plants.

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 than those of unexposed plants (control). This finding confirms the results of Chao and Wang (1990) and Abd El-Naim (1994). They found that irrigation of farm soil with polluted water increased their macro-and micro element contents, which led consequently to more accumulation of these elements in the growing plants. However, some of these elements may cause nutritive or morphological problems to the plants.

In the present investigation, it is of interest to note that, although the heavy metals; including Cu, Co, Zn and Fe; contents of mycorrhizal plants were much higher in the mycorrhizal plants than in the non-mycorrhizal ones, the mycorrhizal plants showed better growth than that of non-mycorrhizal ones. These results emphasize that VA mycorrhizal may increase metal tolerance of the cowpea plants. Similar observations have been reported by Gerdemann (1975), Jones and Hutchinson (1986 &1988), Leake *et al.* (1990), and Brown and Wilkins (1985).

Two main mechanisms for the effect of mycorrhiza on host metal tolerance have been reported. The first was proposed by Bradley *et al.* (1982) who suggested that metals may bind to the interfacial matrix found between the hyphae in ericoid mycorrhizae. This binding would then either, reduce further movement of the metal through the mycorrhiza 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 reported by Leake *et al.* (1990), Bardley et al. (1981), and Chalot *et al.* (1995). Moreover, it was found that the mycobionts did not affect the translocation of copper to the shoots of cowpea, which was in agreement with the finding of Jones and Hutchinson (1986). The presence of metal-binding mechanism is conjecture, however, until information is obtained on metal localization in fungal and host tissues.

The second mean by which infection by mycorrhizal fungi may affect metal tolerance is through improved phosphorus nutrition (Gildon and Tinker 1988). Evidence from the present study indicates that VA mycorrhizal infection significantly increased the concentration of phosphorus in cowpea plant tissues at various levels of water pollution, compared with the non-mycorrhizal plants. Our results support the previous works by Gerdemann (1975), Champawat (1990), and Lynd and Ansman (1994). They found that phosphorus uptake is usually enhanced in mycorrhizal plants than non-mycorrhizal ones. 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 of 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 plants under a long period of irrigation with polluted water. Similar observations have been reported by Slankis (1974), Alexander and Fairley (1983), Syliva and Neal (1990), and Aguilera *et al.* (1993). They showed that high nitrogen levels arrest and prevent mycorrhizae formation and sometimes terminate symbiosis. Interestingly, although nitrogen level was high in the exposed soil treated with polluted water, the mycorrhizal symbiosis increased at each pollution level with increasing stage of harvesting (later harvests). This may be explained on the basis of VA mycorrhizal adaptability to new conditions which results from irrigation with polluted water in the present study.

Our findings could not demonstrate a relationship between tissue N/P ratios and root colonization with mycorrhizal, which agreed with the results of Hejne *et al.* (1992). On the other hand these findings disagreed with those obtained by Hepper (1983) 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 VA mycorrhizal infection rate would depend on phosphorus availability.

Mycorrhizal dependency has been defined by Gerdemann (1975) 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 indicators for mycorrhizal dependency (Plenchette 1991). Evidence from present results indicated that 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 depending on mycorrhizal symbiosis when grown in soils irrigated with polluted water. Furthermore, the results confirm the possibility of the re-use of untreated industrial waste effluents in agricultural purposes along with VA mycorrhizal colonization to the growing plants, especially for the plants where only the above ground product is used by animal and human.

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إستخدام فطر الميكوريزا (جلوماس فاسيكيولاتم) للتغلب على تأثير الرى بمخلفات مصانع الأسمدة على نمو نباتات اللوبيا

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فى الوقت الحالى أصبح الماء سلعة نادرة فى كثير من دول العالم ، ويتوقع العلماء أنه فى المستقبل القريب سوف يعيش العالم عصر أزمة المياه. ولذلك تبذل الحكومة المصرية جهودا كبيرة فى المحافظة على المياه وزيادة المصادر المائية وسعت بالفعل إلى تحقيق ذلك فى ثلاثة اتجاهات رئيسية كان أحدها هو محاولة إعادة استخدام مياه الصرف بأنواعها المختلفة. وفى ظل ذلك تبلورت فكرة هذا البحث ليكون خطوة فى هذا الاتجاه ، حيث هدف البحث الحالى إلى إمكانية استخدام مياه الصرف الملوثة الناتجة من مصانع الأسمدة بعد تخفيفها دون معالجة فى زراعة ورى نبات اللوبيا وذلك باستخدام الفطريات الميكوريزية بحقن النبات بفطرة جلوماس فاسيكيولاتم.

وقد أظهرت النتائج أن الفطريات الميكوريزية أدت دورا فعالا فى زيادة معدل النمو النسبى للنباتات المعاملة بها وأيضا زيادة المادة الجافة وتشجيع تكوين العقد البكتيرية المثبتة للنيتروجين الجوى وكذلك زيادة المحتوى الصبغى وذلك تحت ظروف الرى بمخلفات مصانع الأسمدة مقارنة بالنباتات الغير ميكوريزية (الغير ملقحة بالفطر الميكوريزى). وقد أظهرت التحليلات أن النيتروجين والفوسفور والصوديوم والماغنسيوم والنحاس والكوبلت والزنك والحديد متواجدة بتركيزات أعلى فى النباتات النامية فى التربة الملوثة سواء كانت تلك النباتات ميكوريزية أو غير ميكوريزية. وبالرغم من زيادة تركيز العناصر وبخاصة الثقيلة منها فى النباتات الميكوريزية عن مثيلاتها الغير ميكوريزية إلا أن تحملها للتلوث كان أعلى مع تراكم هذه العناصر فى هيفات الفطر نفسه فيحمى بذلك النبات من تركيزاتها الضارة. وقد تركزت عناصر الكوبلت والزنك والحديد فى جذور النباتات المعرضة للتلوث مقارنة بنسبها فى المجموع الخضرى. ومن ناحية أخرى فقد أدى زيادة مستوى التلوث إلى تثبيط الإصابة بالفطريات الميكوريزية ولم تسفر النتائج عن وجود علاقة بين معدل الإصابة بالفطر الميكوريزى وبين نسبة النيتروجين إلى الفوسفور (N/P ratio) فى أنسجة النبات.