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Impacts of Certain Biofertilizers on Cowpea Plants in Sludge Amended Sandy Soil

H. Abdel Karem[1], S.M.A. Radwan[2] and M. Attia[2]
[1]National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, P.O. 29. Cairo, Egypt
[2]National Research Centre, Dokki, Cairo, Egypt

Abstract: Sludge from El-Gabal El-Asfer treatment plant was taken to minimize the population densities of certain microbial groups of hygienic significance throughout the treatments of gamma radiation and lime. Ten kGy dose level of gamma radiation and 20 percent lime were the optimum treatments to reduce the microbial load of sludge namely, total bacterial counts, total sporeformers, *Enterococcus faecalis*, total coliform, *Aeromonas hydrophila* and total fungi. A pot experiment was conducted to evaluate the role of arbuscular mycorrhizal fungi (AMF) and *Pseudomonas fluorescens* as a biological agent in controlling certain soil borne diseases and reducing the toxicity of heavy metals comparing with 20 percent lime and 10 kGy gamma radiation using 4 percent sludge application and its effect on yield of cowpea components. treatments with gamma radiation, liming and biofertilizers reduced certain soil borne diseases, moreover liming and biofertilizers reduced the concentration of heavy metals in shoots and grains of cowpea plants. However, the application of lime at the rate of 20 percent with sludge exerted negative effect in all studied parameters as compared to control (NPK) or sludge alone. The greatest values of number and fresh weight of nodules were observed under biofertilizers and gamma radiation treatments. Results revealed that significant effects on dry weight of roots and shoots as well as grain yield production of cowpea plants treated biofertilizers and gamma radiation at dose level 10 kGy under sludge application.

Key words: sludge, radiation, lime, biofertilizers, cowpea, sandy soil

Introduction

Application of sludge to agricultural soil is widely practiced means of soil inexpensive waste disposal and improvement of soil physical properties and nutrient status (Sauerbeck, 1987). Sludge can nevertheless, contain considerable amounts of heavy metals that persist in the soil long after application as well as contamination with enteric pathogens and parasites (Juste and Mench, 1992).

Radiation is a new technology for the treatment of sewage and sludge. It has been amply demonstrated during the last two decades, that it holds a great potential for the conservation. Radiation technology provides a safe approach to sludge recycling without introducing any environmental problem (Lessel, 1985).

The effectiveness of lime stabilization in controlling pathogens depends on maintaining the pH at levels that kill microorganisms and inhibit their growth. This process reduces pathogenic bacteria and viruses by over 90 percent (Ahlstorm *et al.*, 1984). Liming is a widely recommended strategy to reduce mobility and plant availability of soil contamination with heavy metals (Smolders *et al.*, 1999).

During the last few decades numerous microorganisms have been shown to exert beneficial effects on plant development include mycorrhizal fungi and other plant growth-promoting rhizobacteria (PGPRs), such as rhizobia and pseudomonads (O'Gara *et al.*, 1994). The various microorganisms found routinely in the rhizosphere and known to contribute to soil fertility and crop yield by stimulating plant growth or by reducing the damage from soil borne plant pathogens (Kloepper *et al.*, 1989).

On the other hand, mycorrhizal colonization has been shown to be delayed, reduced and even eliminated by high concentrations of Zn, Cu, Ni and Cd (Koomen *et al.*, 1990; Leyval *et al.*, 1991). However, only a few studies have been conducted on interactions between arbuscular mycorrhizae (AM) and heavy metals of different origins in soil.

The objective of this study was to evaluate the role of gamma radiation, liming and arbuscular mycorrhizal (AM) fungi and/or *Pseudomonas fluorescens* as a biological agent in controlling certain soil borne diseases and reducing the toxicity of heavy metals using 4 percent sludge application and its effect on yield of cowpea components.

Materials and Methods

Microbiological examination was carried out to study the changes in the population densities of certain microbial groups of hygienic significance throughout the gamma irradiation and lime treatments of sludge. Analysis carried out according to the methods and media given by APHA, AWWA and WPCF (1992), comprised the determination of aerobic plate counts on peptone-yeast extract agar. MPN of total coliform on Lauryl tryptone broth and confirmed by brilliant green lactose bile broth, MPN of *Enterococcus faecalis* using azide dextrose broth and confirmation was made by Pfizer Selective *Enterococcus* agar. *Aeromonas hydrophila* group was determined on MacConkey trehalose ampicillin agar (Kaper *et al.*, 1981). Total fungi was counted on malt extract agar (Galloway and Burgess, 1952).

Table 1: Physical and chemical characteristics of soil used

Soil character(%)	Values	Soil character(%)	Values	
Sand	75.02	Total-N%	0.049	
Silt	14.38	Total-P %	0.0022	
Clay	8.40	Available-P ppm	0.0006	
Texture	Sandy	Heavy metals	Values	
			Total	Available
$CaCO_3$ %	1.46	Fe ppm	290.00	72.00
pH	7.80	Zn ppm	12.30	3.65
EC (mmhos cm^{-1})	0.25	Mn ppm	7.80	2.03
O.C. %	0.40	Cu ppm	1.26	0.26
		Pb ppm	1.02	0.018

An experiment was conducted to study the effect of radiation on different microbial groups prevailing in sludge. Representative samples were exposed to increasing doses of gamma radiation ranging from 2 to 10 kGy using a ^{60}Co irradiation source (Gamma Chamber 4000 A-India, located at National Center for Radiation Research and Technology with dose rate 1 kGy/ 32.2 min at the time of the experiment). Liming was used as a chemical treatment to study its effect on microbial counts. Samples treated with 5, 10, 15 and 20 percent lime.

Table 2: Chemical characteristics and elements content of sludge in El-Gabal El-Asfer

Character	Values	Character	Values
pH	6.73	Co ppm	32.10
EC (dS cm^{-1})	5.71	Available elements	
O.C. (%)	18.24	N ppm	35
Total elements		P ppm	21
N%	1.57	Zn ppm	272.00
P%	1.24	Mn ppm	33.00
Zn ppm	1319.00	Cu ppm	30.00
Mn ppm	381.00	Ni ppm	3.41
Cu ppm	329.00	Cd ppm	0.39
Ni ppm	91.00	Pb ppm	25.00
Cd ppm	14.21	Co ppm	0.73
		Pb ppm	439.00

Table 3: Root-rot incidence of cowpea plants grown in sludge amended sandy soil under different treatments

Treatments	Diseased plant %	Plant protection
Control (NPK)	12.0 d	0
Sludge(S)	11.5 d	4
Irradiated S	2.1 c	83
S + Lime	1.6 b	87
S+ Ps.	1.2 b	90
S+ AM fungi	1.5 b	88
S+ Ps. + AM	0.8 a	93

Table 4: The effects of biofertilization, radiation and lime under sludge application on the fresh and dry weights of cowpea plants as well as fresh weight of nodules

Treatments	Fresh weights (g)		Dry weights (g)		Fresh weights of nodules
	Root	Shoots	Root	Shoots	
Control (NPK)	4.40 b	34.07 c	2.68 b	4.82 b	0.382 a
Sludge(S)	5.29 c	31.11 b	3.14 bc	5.98 c	0.469 b
Irradiated S	5.92 d	31.58 b	3.19 bc	5.91 c	0.522 c
S + Lime	3.19 a	20.13 a	1.73 a	3.42 a	0.331 a
S + Ps.	6.72 e	36.08 d	3.53 c	6.39 cd	0.559 c
S + AM fungi	9.49 f	37.28 d	4.69 d	7.15 d	0.613 d
S + Ps. + AM	7.10 e	33.67 c	3.65 c	6.36 cd	0.574cd*

Means followed by the same letter within a columns are not significantly different at p= 0.05 (Duncan's), S = Sludge, Ps. = *Pseudomonas fluorescens,* AM = arbuscular mycorrhizal fungi.

A pot experiment was conducted in the greenhouse at the National Research Centre, Dokki, Egypt. A sandy soil was collected from El-Gabal El-Asfer, air dried, passed through a 4 mm seive and packed (12 kg pot^{-1}) in a sufficient number of plastic pots. The physical and chemical characteristics of soil were reported in Table 1. A complete randomized block design was used with six replicates. The treatments were as follows:

1. Control (NPK)
2. Sludge at the rate of 4%

Table 5: Effect of sludge application on the concentration of N, P and heavy metals in cowpea shoots under different treatments

Treatments	N%	P%	Heavy metals (ppm) Fe	Mn	Zn	Cu	Pb	Cd
Control (NPK)	5.20 d	0.24 bc	557 a	283b	95 b	14 a	85 c	12.3 e
Sludge	4.77 c	0.19 ab	773 e	315 c	97 bc	25 b	60 b	9.8 d
Irradiated S	4.20 c	0.25 c	631 c	353 d	144 e	50 c	55 ab	8.67 c
S + Lime	3.83 b	0.16 a	549 a	260 b	105 cd	22 b	65 b	6.52 b
S + Ps.	4.07 c	0.23 bc	665 c	274 b	100 bc	22 b	56 ab	9.2 cd
S + AM fungi	4.47 c	0.25 c	728 d	320 c	114 d	24 b	50 a	5.9 a
S + Ps. + AM	5.53 e	0.27 c	914 f	217 a	78 a	22 b	50 a	6.7 b*

Means followed by the same letter within a columns are not significantly different at p= 0.05 (Duncan's), S = sludge, Ps. = *Pseudomonas fluorescens,* AM = arbuscular mycorrhizal fungi

Table 6: Effect of sludge application on the concentration of N, P and heavy metals in cowpea grains under different treatments

Treatments	N%	P%	Heavy metals (ppm) Fe	Mn	Zn	Cu	Pb	Cd
Control (NPK)	6.00 bc	0.28 bc	216 bc	55.7 d	66.9 c	13.0 b	49.7 e	7.6 d
Sludge	4.67 a	0.20 a	177 a	45.6 c	59.4 a	13.6 bc	34.8 c	7.6 d
Irradiated S	5.83 b	0.20 a	264 d	40.5 b	60.1 a	19.1 d	29.8 b	5.0 b
S + Lime	4.50 a	0.20 a	175 a	36.1a	59.4 a	10.1 b	24.8 a	4.8 b
S +*Ps.*	6.53 cd	0.24 ab	227c	44.3 c	64.9 b	13.0 b	39.1 d	7.0 cd
S + AM fungi	5.07 a	0.26 bc	225 c	33.6 a	62.2 ab	14.8 c	34.8 c	6.7 c
S +*Ps.* + AM	6.80 d	0.28 bc	210 b	33.6 a	55.1 a	5.0 a	24.9 a	4.2 a*

Means followed by the same letter within a columns are not significantly different at p= 0.05 (Duncan's), S = sludge, *Ps.* = *Pseudomonas fluorescens,* AM = arbuscular mycorrhizal fungi

Table 7: The effects of different treatments on the yield of cowpea plants (g)

Treatments	No. of bodes $plant^{-1}$	Weight of bodes (g $plant^{-1}$)	Weight of grains (g $plant^{-1}$)	Weight of 50 grains
Control (NPK)	6 bcd	6.44 c	4.05 b	8.76 b
Sludge	4 ab	4.46 a	4.80 cd	9.16 bc
Irradiated S	6 cd	7.29 d	5.44 d	9.60 cd
S + Lime	3 a	4.51 a	2.33 a	7.80 a
S + *Ps.*	5 bcd	5.37 b	4.16 bc	8.69 b
S + AM fungi	7 d	8.56 e	6.52 e	10.11 d
S + *Ps.* + AM	6 cd	7.80 d	5.64 de	9.95 cd*

Means followed by the same letter within a columns are not significantly different at p= 0.05 (Duncan's), S = sludge, Ps. = *Pseudomonas fluorescens,* AM = arbuscular mycorrhizal fungi

3. Irradiated sludge at the rate of 4 percent
4. Lime at the rate of 20 percent from sludge
5. Sludge at the rate of 4 percent and inoculated with *Glomus* sp.
6. Sludge at the rate of 4 percent and inoculated with *Ps. fluorescens*
7. Sludge at the rate of 4 percent and inoculated with *Glomus* sp. and *Ps. fluorescens*

In chemical fertilized (NPK) treatment, superphosphate and potassium sulphate was broadcasting on the soil before planting at rate of 200 and 50 kg fed^{-1}, respectively. Ammonium sulphate as a source of nitrogen was added at the rate of 45 kg fed^{-1} in two equal doses, 21 and 45 days. Some chemical characteristics and elements content of sludge were reported in Table 2. Sludge at the rate of 4 percent and lime at the rate of 20 percent were well mixed with soil and uniformly packed in the pots. Six seeds of cowpea (*Vigna unguiculata* L.) cv. Dokki 331 were sown into each pot. The seed beds of all pots were inoculated with a liquid inoculum of *Bradyrhizobium* sp. (cowpea group). Plants were thinned to four plants per pot after germination.

Mycorrhizal inoculum consisted of root, hyphal, spores and growth media from a pot culture of onion plant which was infected with *Glomus* sp. originally isolated from Egyptian soils and grown for 4 months in pot culture contained (peat: vermiculite: perlite mix 1:1:1 by volume). The inoculum material contained 275 spores g^{-1} oven dry bases in addition to the infected roots pieces (the infectivity 10^4 propagola). Mycorrhizal inoculation was done by planting the seed over a thin layer of the mycorrhizal inoculum material at the time of sowing at rate of 50 mg $plant^{-1}$.

Five days old culture of *Ps. fluorescens* grown on KB culture containing 10^8 viable cell ml^{-1} from a 48h old was used as a liquid inoculant by adding 5 ml to each pot in *Pseudomonas* sp. treatments.

Rhizosphere soil samples were collected up to 80 days at 20 days intervals from each of treatment for microbiological analysis. Total spore numbers were counted in nematode counting dish under the low power of dissecting microscope (Kormanik and McGraw, 1982).

The percentage of root infection with AM fungi was evaluated using the magnified intersect method described by McGonigle *et al*. (1990). Counts of *Ps. fluorescens* and rhizobia were enumerated 20, 40, 60 and 80 days under different treatments. The fresh weights of cowpea nodules were also recorded after 45 days from planting. Cowpea plants were taken to examine root-rot symptoms in all treatments after 3 weeks from emergency. Disease incidence was calculated as percentage of infected plants related to total plants per plots.

Plant samples were taken at flowering stage to determine fresh and dry weights of shoots plant, N; P content and heavy metals (Fe, Mn, Zn, Cu, Pb and Cd). At maturity stage, plants were harvested from each pot for yield measurements. Total N, P and heavy metals content in seeds were analyzed after wet oxidation using atomic absorption spectrophotometer.

Results were analyzed statistically by using the SPSS (Statistical Package for the Sciences System). Spores and microbial counts data were transformed by log X. the percent root colonization data was transformation by arc sine of the square root. Duncan's Multiple Range Test did mean separation among the treatments.

Results and Discusion

Efficiency of radiation and lime in reducing the microbial load in sludge: An experiment was conducted to study the efficiency of radiation in reducing the microbial load in sludge. The effect of increasing levels of radiation on the test organisms is illustrated in Fig. 1. The increasing doses of radiation was accompanied with corresponding decrease in microbial populations of sludge samples. Total viable counts and sporeforming bacteria initially present in counts of 2.1×10^7 g^{-1} and 1.3×10^4 g^{-1} were decreased with the increase of radiation dose to attain few cfu g^{-1} after exposure to 8 and 6 kGy respectively and complete eradication was achieved at 10 and 8 kGy dose level respectively. However, Farooq *et al*. (1993) reported that the average values of original densities of total bacteria in raw sewage were 10^5 cfu g^{-1} and exposure to 5 kGy reduced the counts by 4 log cycles. Abdel Karem and Waite (1997) stated that a dose of 6 kGy was required to decrease the total bacterial counts from 6×10^5 to few cells. Radiation with 4 kGy was quite sufficient to cause a complete elimination of *E. faecalis* and faecal coliform which were present initially in 10^3- 10^5 g^{-1} respectively. *A. hydrophila* and total fungi being found in the range of 10^4 cfu g^{-1} could not be detected after exposure to 2 and 6 kGy respectively. Harsoyo *et al.* (1992) and Farooq *et al*. (1993) mentioned that total coliform counts were 10^4cfu g^{-1} and complete elimination was achieved by using 5 kGy. Whereas, Abdel Karem and Waite (1997) reported that total coliform, *E. faecalis* and *A. hydrophila* initially present in 10^3-10^4 cfu g^{-1} could not be detected after irradiation with 2, 4 and 1 kGy respectively. On the other hand, the effect of lime concentrations on the microbial load of sludge was studied (Fig. 1). Regarding the changes in counts of microbial groups in sludge due to addition of lime with different levels, total viable counts present initially in 10^7 cfu g^{-1} was reduced 2 log cycles after 5 percent level of lime and reached 10^2 cfu g^{-1} with 20 percent lime addition. As to sporeforming bacterial counts were 10^4 cfu g^{-1} and reduced 1 log cycle at 5% lime and decreased with the increase of lime level to attain only few cfu g^{-1} after 20 percent lime. Faecal coliform and *A. hydrophila* initially present in 10^5-10^4 cfu g^{-1} could not be detected any more after addition of lime 10 percent. Lime addition with 15 percent was quite sufficient to cause a complete elimination of *E. faecalis* and total fungi, which were present initially, 10^3-10^4 cfu g^{-1} respectively. The effectiveness of lime stabilization in controlling pathogens depends on maintaining the pH at levels that kill microorganisms and inhibit their growth. This process reduces pathogenic bacteria and viruses by over 90 percent (Ahlstorm *et al*., 1984).

Survival of *Rhizobium*, *Pseudomonas* and percentage of mycorrhizal infection and spore numbers under different treatments: Counts of rhizobia and *Pseudomonas* in the rhizosphere of cowpea plants were at low densities as shown in Fig. 2. Generally, rhizosphere of cowpea plants contained always lower counts of rhizobia and *Pseudomonas* under lime application in comparison with other different treatments. This trend may be due to the increase in the soil pH at levels that kill these microorganisms or inhibit their growth. However, biofertilization with *Pseudomonas* enriched each of rhizobia and *Pseudomonas* in the rhizosphere of cowpea plants but those inoculation with AM fungi alone or combination with *Pseudomonas* sp. appeared the lowest numbers comparing with the inoculation with *Pseudomonas* alone. Similar results were reported by Paulitz and Linderman (1989). These attributed to colonization of root by mycorrhizal fungi may alter root exudation and thus indirectly affect bacterial growth in the rhizosphere (Garbaye, 1991). At the 40th-day of planting, counts of rhizobia in the rhizosphere ranged between 9×10^4 to 7×10^7/g, while those of *Pseudomonas* ranged between 3×10^4 to 6×10^6/g. It appeared that inoculation of cowpea plants with biofertilizers were proliferate of both bacteria compared to other treatments. Densities of the two bacteria decreased thereafter until the end of the experiment.

Inoculation of cowpea plants with AM fungi markedly increased the percentage of mycorrhizal infection and spore numbers (Fig.3). On the other hand, *Pseudomonas* had slight effect on mycorrhizal infection but increased mycorrhizal spore numbers than inoculated with *Glomus* alone. These results could be due to the high microbial activity in rhizosphere of mycorrhizal plants and on rhizoplane limits the root exudates recovered from roots (Azaizeh *et al*., 1995) and subsequent competition between

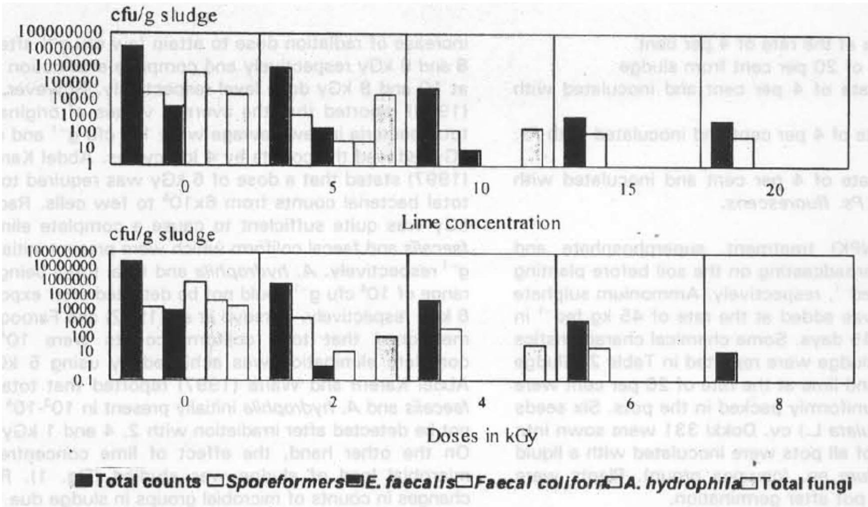

Fig. 1: Effects of gamma radiation and lime concentration on microbial counts in sludge

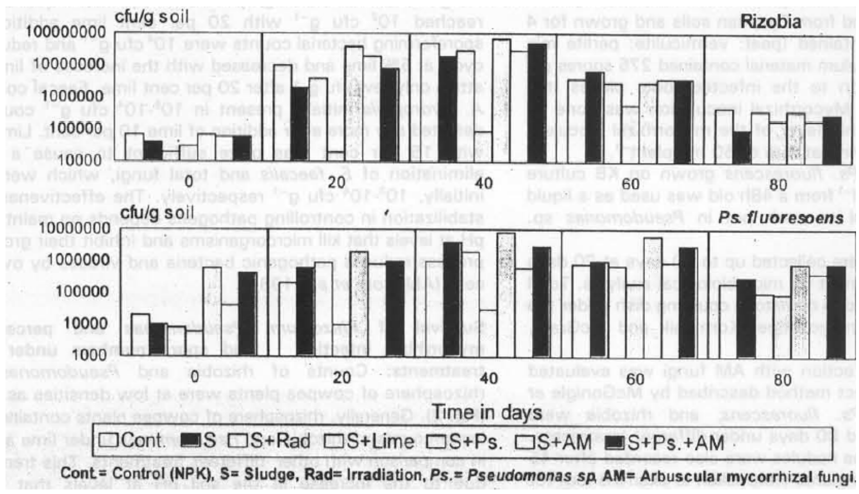

Fig. 2: Effect of different treatments on *Ps. fluorescens* and rhizobia counts in the rhizosphere of cowpea plants

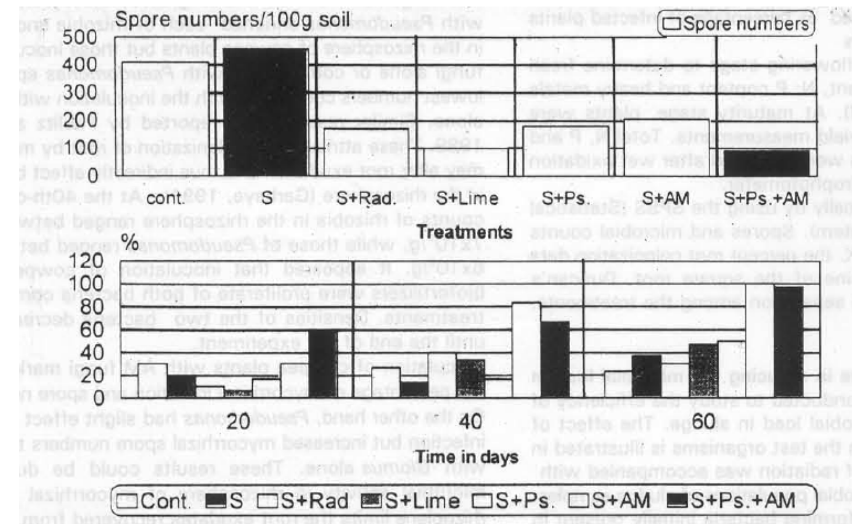

Fig. 3: Effect of different treatments on mycorrhizal formation in cowpea plants

bacteria and AM fungi for those sources (Christensen and Jakobsen 1993). On the other hand, percentage of mycorrhizal infection and spore numbers were present at low densities in the rhizosphere of cowpea plants grown in control treatments of each amended with full NPK or sludge alone but their increased in the plants amended with irradiated sludge. These attributed to some indications that soil microbes might be involved in the inhibition of spore germination and root colonization in non-sterile soil (Schonbeck, 1995).

The potential of sludge amended with 20 percent lime or treated with 10 kGy dose level of gamma radiation suppress the development of certain soil-borne pathogens. Sludge amended with lime or treated with gamma radiation increased plant protection by 87 and 83 percent respectively, in comparison with NPK treatment as shown in Table 3. The use of *Pseudomonas* or AM fungi as inoculant under sludge application increased the plant protection by 90 and 88 percent respectively, compared to control treatment. Dual inoculation cowpea plants with *Pseudomonas* and AM fungi increased plant protection by 93 percent as comparison with NPK treatment. These microorganisms are recommended for application as biological control against many soilborne plant pathogens (Papavizas and Lumsden 1980; Al-Raddad,1995; Liu *et al*., 1995; Matsubara *et al*., 1995).

Role of radiation, lime and biofertilizer on sludge and their impacts on cowpea plants: Data presented in Table 4 show that biofertilization either with AM fungi or *Pseudomonas* induced significant increases in the fresh and dry weights of roots and shoots of cowpea plants as compared to non-biofertilized plants. The increases of fresh and dry weights of cowpea plants grown in different treatments ranged between 5.98 to 21.57 percent and 32.27 to 57.87 percent of the control, respectively, as a result of dual biofertilization with *Pseudomonas* and AM fungi. Colonization of roots by AM fungi has been shown to improve productivity of numerous crop plants in soils of low fertility (Jeffries and Rhodes, 1987). This response is usually attributed to enhanced uptake of immobile nutrients such as P, Zn, Cu and N uptake (Nelsen, 1987; Faber *et al*., 1990; Kathari *et al*., 1990; Attia and Badr El-Din, 1998). On the other hand, *Pseudomonas* has beneficial effects on disease suppression, growth and nutrient availability to plants and induction of systemic disease resistance. These effects ultimately lead to improvement of root health and plants. As regards the fresh weights of nodules in cowpea plant, highly significant differences were calculated between AM fungi in the presence and absence of *Pseudomonas* and non-mycorrhizal treatments under sludge application or NPK treatment.

2-N, P and metals concentration of shoots and grains of cowpea plants: Considering the effect of sludge application on N, P and heavy metals concentration of cowpea shoots (Table 5) and grains (Table 6) showed that N and P concentration in shoots and grains were increased with AM fungi and/or *Pseudomonas* sp. As well as gamma radiation treatments under sludge application as compared to sludge alone. These increases might attributed to the role of AM fungi in increase the rate of N_2-fixation and it can assimilate and translocate ammonium (Abdel-Aziz *et al*., 1997), as well as, the role of AM fungi in increasing the roo surface area and thus enhance P-uptake, especially where soil P is limiting (Attia, 1999). In addition to effect of gamma radiation, the antagonistic effect of AM fungi and/or *Pseudomonas* sp. against the soil borne pathogens with affected plant growth and consequently uptake of nutrients from soil.

On the other hand, concentration of Mn, Zn, Cu, Pb and Cd were significantly lower for shoots and grains of cowpea plants treated with mycorrhiza and liming than uninoculated plants (Table 5, 6). Metal retention in mycorrhizal root systems can be attributed to a surface complexation of heavy metals with cystein containing ligands of fungal proteins. Therefore, AM fungi infection of the roots may act as a filter system for toxic metals in highly contaminated soils and may play a role in the resistance of plants to heavy metals (Dehn and Schuepp, 1990).

It is worthy to state that liming generally decreased the heavy metals absorpation and their translocation to plant shoots. These results are in accordance with that reported by Cordovil *et al*. (1999) and Oborn *et al*. (1999).

Results presented in Table 7 revealed that biofertilizers especially AM fungi and gamma radiation at dose level 10 kGy in the presence of 4 percent sludge gave greater number of bodes, weight of bodes and grains, as well as weight of 50 grains as compared to chemical fertilization or other treatments under the same rate of sludge application. These results might be due to the importance of AM fungi in enhancement of plant growth and possibly N_2-fixation as well as the better utilization of essential macro and micronutrients as well as gamma radiation in controlling the soil borne diseases. AM fungi has stimulating effects mainly due to the increased uptake of P and improvement of rhizobial in the rhizosphere of cowpea plants (Badr El-Din and Moawad, 1988). On the other hand, the lowest studied yield parameters were obtained by lime application in the presence sludge followed by sludge alone. These results might attributed to decrease the uptake of macro- and micronutrients by plants as a result increase soil pH by liming.

It could be concluded that the using of biofertilizers or gamma radiation under sludge application were resulted the greatest yield of cowpea plants and recycling of sludge as a organic fertilizer can safe the environment clean.

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