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Effect of Functionally-strengthened Fertilizers on Garlic Growth and Soil Properties

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Ammonium- and potassium-loaded zeolite (NK-Z) and other four kinds of environmental friendly fertilizers/agents were applied to characterize their effectiveness on garlic (*Allium sativum* L.) growth and soil amelioration. Selenium dioxide (SeO_2) and germanium dioxide (GeO_2) liquid treatments significantly increased selenium (Se) and germanium (Ge) contents in garlic stems, garlic cloves and clove peels. In soil treated with ZBFC, Se contents in garlic stems, cloves, and clove peels was 13.89-, 12.79-, and 10.96-fold higher, respectively, than in the controls. The inorganic contents of plants grown in soil treated with functional strengthened fertilizers were also higher than in plants grown in control soil. Soil treated with arbuscular mycorrhizal fungi (AMF) agents exhibited significantly greater spore density and root colonization rate than in untreated soil. The density of chitinolytic microorganisms in soil treated with colloidal chitin was also significantly higher than in untreated soil. The cation exchange capacities (CEC) in ZAFC-, ZBFC-, and ZBF-treated soils was 16.05%, 8.95%, and 8.80% higher than in control soil 28 weeks after sowing.

Key words: Ammonium- and potassium-loaded zeolite, Environmental friendly fertilizer, Garlic, Crop growth, Soil amelioration

Introduction

Garlic is a widely used food ingredient. Both scientific and herbalist traditions support the notion that garlic in various forms can provide extraordinary health benefits. It significantly reduces the risk of cardiovascular disease and certain kinds of cancer. Taking garlic in certain dosages can help protect human cells from oxidation, free radicals and certain types of radiation. Garlic is an effective immune booster and has anti-bacterial, anti-viral and anti-fungal properties (Sovova and sova, 2004).

Tremendous anecdotal evidence supports the invaluable role that garlic has played in the treatment of many diseases throughout history. Ip (1995) compared the effects of two batches of garlic powder with marked differences in their level of selenium (Se) enrichment, and found that the anti-cancer activity of the high-selenium garlic was likely to be due to the effect of Se which is an important part of a molecule in the body that protects blood cells from certain damaging chemicals. Elevated Se intake may be associated with reduced cancer risk. Large clinical trials are now planned to test this hypothesis (Rayman, 2000). The anticarcinogenic activities of some Se forms against colon, lung, skin and other types of cancer have been demonstrated (Stratton et al., 2003). Germanium has also gained popularity in recent years for its reputed ability to improve immune system function in cancer patients (Baselt, 2008).

The non-toxic, biodegradable and biocompatible properties of chitin have great potential in many food, pharmaceutical, and biotechnological applications. For the evaluation of chitinases, insoluble substrates such as tritiated solid chitin, colloidal chitin or chitin covalently coupled to different dyes (chitinred, chitin-azure) can be used (Linden et al., 2000). Depending on the substrate used, the assays are radiometric or photometric. Colloidal chitin was used in this study to determine its influence on the reproduction of chitinolytic microorganism in the rhizosphere.

The rhizosphere harbors a large number and diversity of soil microorganisms, some of which are also able to colonize roots endogenously. With the exception of damaging pathogens, plants may benefit from rhizosphere microorganisms either directly, as in the case of symbionts such as mycorrhizal fungi, or indirectly by the antagonistic

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activity of beneficial micro-organisms toward plant pathogens (Li and Liu, 2007; Li et al., 2010b; Diedhiou et al., 2003). The main effects of AM fungi are in the competition for nutrient sources and space, as well as the prevention of juvenile penetration, as non-pathogenic fungi produce nematocide substances and/or directly parasitize different stages of the nematode life cycle (Liu and Chen, 2007; Hallmann and Sikora, 1996).

Seaweed is a generic name for various types of algae, which have many commercial and industrial uses. In terms of soil structure it does not add a great deal of bulk, but its jelly-like alginate content helps to bind soil crumbs together, and it contains all soil nutrients (0.3% N, 0.1% P, 1.0% K, plus a full range of trace elements) and amino acids. In this study, seaweed concentrated liquid extract was made and applied to the soil as a kind of organic fertilizer.

Soil organic matter is considered a key attribute of soil quality (Gregorich et al., 1994), and soil quality is a key element of sustainable agriculture. The application of Se and germanium (Ge) liquid agents, AMF, seaweed organic fertilizer, colloidal chitin, and NK-Z were helpful to establish a sustainable agriculture.

In our study, Se and Ge liquid agents were applied to soil to study the relationship between external soil application and garlic content. In addition, other kinds of environmental friendly fertilizers (EFF) such as ammonium- and potassium- Loaded zeolite (NK-Z), colloidal chitin, arbuscular mycorrhizal fungi (AMF), and seaweed liquid fertilizer were also applied to the soil.

Materials and Methods

Production of NK-Z The natural zeolite rock was crushed and sieved to a size fraction of 1-2 mm and was loaded with NH_4^+ and K^+ by soaking in 1M ammonium sulfate ($(NH_4)_2SO_4$) and 1M potassium chloride (KCl), respectively, for 10 days (Li et al., 2010a). The NH_4^+- and K^+-loaded zeolites were rinsed with water until the electrical conductivity (EC) of the supernatant was <0.5 mSm^{-1} (Perrin et al., 1998). NK-Z containing 2% N and 3% K were obtained.

Mass production of AMF agent The growth medium was a mixture of sterile sand and soil (2:1, v/v) containing 1% organic matter it had a pH of 6.0. Sudan grass (*Sorghum sudanense*) were used as host. Plants were grown under greenhouse conditions and natural illumination for 6 months and watered with tap water as needed. AMF spores were collected from the rhizosphere soil of each potted host plant, and counted using the wet-sieving method (An et al., 1990). The colonization of host plant roots was assayed by using a modified method originally described by Brundrett et al. (1984).

Production of colloidal chitin Colloidal chitin was prepared from purified chitin according to the method of Roberts et al. (1998) with minor modification. Chitin powder (200 g) was added slowly into 3.5 L HCl (37%, W/V) and vigorously stirred for 30 min at 60℃. The suspension was filtered using glass wool and poured into a plastic bucket filled with 200 L water and stored overnight for deposition. The supernatant was poured off and 200 L water was added once per day for about a week until the pH reached 7. The upper supernatant was poured off and the remaining 0.3% colloidal chitin solution was obtained.

Production of seaweed fertilizer The oceans contain various valuable biological resources; although algae are considered to have low economic value, they can serve as a fertilizer with high macro- and micronutrient content. We produced seaweed fertilizer using the following procedure: First, the self-poking arrangement was prepared to mix the seaweed with water and other kinds of chemical fertilizers. The seaweed used in this experiment was *Undaria pinnatifida* (Harvey) suringer. The seaweed was cut into pieces and placed into the self-poking arrangement with the same volume of water and stirred at 80°C. Two kinds of seaweed fertilizers were produced: (1) urea, potassium chloride, and ammonium phosphate were added 10 days after stirring to make a liquid fertilizers containing N-P-K=6.69-3.66-4.63% (NPK^+-S); (2) liquid seaweed solution without chemical fertilizers (NPK^--S).

Production of Se and Ge liquid agent Se dioxide (SeO_2) and Ge dioxide (GeO_2) mother liquor (1000 mg kg^{-1}) was prepared. The mother liquor was diluted when applied to crops.

Tested crops and farming methods Garlic (*Allium sativum* L.) was selected as the test crop. The soil

Table 1. The characteristics of experimental soil.

pH	EC	OM	Avail. P_2O_5	Exch. Cation K	Exch. Cation Ca	Exch. Cation Mg	CEC
(1:5)	dS m^{-1}	g kg^{-1}	mg kg^{-1}	---------------------------- $cmol_c$ kg^{-1} ----------------------------			
5.7	0.81	28	643	1.84	4.9	2.1	10.8

characteristics were tested before the beginning of the experiment (Table 1). Five kinds of EFF were used (NK-Z, Se and Ge liquid fertilizer, AMF, seaweed fertilizer, and colloidal chitin) in this study.

There were 4 treatments in this experiment. (1) Compound fertilizers applied to the soil as basal and additional fertilizers (Control). (2) Compound fertilizers as basal fertilizers, with NK-Z as the additional fertilizer (ZAF). (3) NK-Z as basal fertilizers, NPK^+-S as additional fertilizers, plus colloidal chitin, AMF, SeO_2 and GeO_2 solution were also applied into the soil as environmental friendly agents (ZBFC). (4) Compound fertilizers as basal fertilizers, NK-Z as additional fertilizers, plus colloidal chitin, AMF, SeO_2 and GeO_2 solution and NPK^--S as environmental friendly agents (ZAFC). All samples were replicated 3 times and each experimental plot contained 280 samples for a total number of 840 samples for every treatment.

The experiment area for every plot was 3.2 m^2. In the control treatment, 250 g compound fertilizer (N-P-K= 11-10-8 kg $10a^{-1}$) were added to the soil. In the ZAF treatment, 100 g compound fertilizer (N-P-K=11-10-8 kg $10a^{-1}$) were added into the soil as basal fertilizer, 4165 g N-Z and 1432 g K-Z were added as additional fertilizer at the same time with the application of basal fertilizer before seedling transplanting. In the ZBFC treatment, 1.4 kg AMF was mixed to the soil before garlic sowing. 1500 g N-Z, 500 g K-Z, and 67.5 g superphosphate (P=20%) were added into soil as basal fertilizer, 67 mL NPK^+-S was applied as additional fertilizer for 10 times, 33 mL SeO_2 mother liquor, 33 mL GeO_2 mother liquor and 2 liter colloidal chitin were applied into soil as EFF for 10 times. In the treatment of ZAFC, 1.4 kg AMF inoculum were mixed into the soil before garlic sowing, and then compound fertilizer 100 g (N-P-K=11-10-8 kg $10a^{-1}$) were added into the soil as basal fertilizer, 4165 g N-Z and 1432 g K-Z were added as additional fertilizer, 67 mL NPK^--S, 33 mL SeO_2 mother liquor, 33 mL GeO_2 mother liquor and 2 liter colloidal chitin were applied into soil as EFF for 10 times. Black color mulching film was used to cover each treatment plot. In the ZAFC and ZBFC treatments, seaweed liquid fertilizer, SeO_2 GeO_2 and colloidal chitin were applied to the crop by drip irrigation.

The inorganic constituents and heavy metal contents in garlic stems, garlic cloves and clove peels were tested using the NIAST method (2000). Plant tissues were ground into powder and 0.5 g was placed in a 50-mL Falcon tube in 10 mL nitric acid (70%). The mixture was heated at 90°C until the liquid became transparent. The liquid was filtered using Whatman No. 6 filter paper and brought to 50 mL with distilled water. The contents of Ca, K, Mg, Na, Cu, Fe, Mn, and Zn were tested using ICP. The contents of Se and Ge were tested using ICP/MS. (ELAN DRCE, Perkin-Elmer, USA).

The soil and garlic roots were sampled 20, 22, 24, 26, and 28 weeks after sowing. AM fungi spore density, garlic root colonization, Se Ge contents, microorganism contents, and cation contents in soil were tested. The cation contents and CEC of the experimental soils were tested using the NIAST method (2000).

The chitinolytic bacterial content of the soils were tested using the Streak-plate method. Sample soil liquids were diluted into 100-fold and 1000-fold and cultured on chitin agar medium at 28℃ in incubator. Colony forming units (CFU) were calculated after 72 hours culture.

Results and Discussions

Garlic growth responses Zeolite as a soil amendment can increase the production of many kinds of vegetables, but its benificial effects may be expected only in poorer soils such as those of low CEC or high in sand, where the need for improvement in nutrient retention and moisture holding capacity is greatest (Wiedenfeld, 2003). We also described the effectiveness of NK-Z on the growth of hot pepper (Li et al., 2010a).

In this study, the growth characteristics of garlic were investigated on harvest time (Table 2). In the treatments applied with EFF, garlic stem height were significantly higher than that applied without EFF. In

Table 2. The characteristics of garlic on harvest day.

Treatments†	Plant height	Leaf length	Leaf No.	Plant weight	Bulb height	Bulb diameter	Bulb weight
	mm	cm		g	mm	mm	g
Control	40.58	48.04	5.98	53.58	46.15	56.80	66.98
ZAF	42.71	52.80	6.11	57.37	47.79	59.71	66.01
ZBFC	43.10*	52.24	6.13*	57.99	48.35	58.01	67.10
ZAFC	43.38*	53.69	6.07	62.69	49.58	61.01	73.12*
$LSD_{0.05}$	2.378	9.750	0.610	2.792	4.195	4.662	2.439

†Control: compound fertilizers applied into a soil as basal and additional fertilizers. ZAF: compound fertilizers as basal fertilizers, NK-Z as additional fertilizers applied into the soil. ZBFC: NK-Z as basal fertilizers, NPK-S as additional fertilizers, colloid chitin, AMF, organnic Se, organnic Ge were also applied into the soil as environmental friendly agents. ZAFC: compound fertilizers as basal fertilizers, NK-Z as additional fertilizers, colloid chitin, AMF, organnic Se, organnic Ge were also applied into the soil as environmental friendly agents. The same as below.

Table 3. The inorganic constituents and heavy metal contents in garlic stem.

Treatments	CaO	K_2O	MgO	Na_2O	Cu	Fe	Mn	Zn	Ge	Se
	---------- % ----------				---------- mg kg^{-1} ----------				------ μg kg^{-1} ------	
Control	2.086	3.133	0.366	0.148	7.37	168.90	41.37	17.01	11.40	27.60
ZAF	2.057	3.200	0.369	0.159	8.57	184.80	41.43	17.87	15.47	48.83
ZBFC	2.186	3.396*	0.402	0.166*	8.81	201.23	56.63	18.98	39.17*	383.30*
ZAFC	2.128	3.513*	0.365	0.170*	8.10	230.03*	51.50	18.44	35.70*	355.20*
$LSD_{0.05}$	1.235	1.144	0.051	0.005	0.79	40.12	12.60	3.94	3.86	49.80

Table 4. The inorganic constituents and heavy metal contents in garlic clove.

Treatments	CaO	K_2O	MgO	Na_2O	Cu	Fe	Mn	Zn	Ge	Se
	---------- % ----------				---------- mg kg^{-1} ----------				------ μg kg^{-1} ------	
Control	0.10	1.14*	0.08	0.09	7.43	54.33	10.50	35.63	5.70	68.87
ZAF	0.10	1.09	0.09*	0.09	8.23	54.46	10.04	35.53	8.10	68.90
ZBFC	0.11*	1.08	0.08	0.09	6.19	49.26	9.81	39.76	11.67*	880.83*
ZAFC	0.10	1.05	0.08	0.08	6.42	52.60	12.07	35.03	12.33*	876.13*
$LSD_{0.05}$	0.002	0.048	0.002	0.001	0.53	7.48	3.17	3.55	2.30	159.58

the ZBFC treatment, the leaf number was significantly higher than in the control. The individual bulb weight in ZAFC was 73.12 g which was 9.17% higher than in the control. Interestingly, we also found that there were no significant differences in garlic growth characteristics between the ZAF and control treatments. The results verified Wiedenfeld's point and revealed the effectiveness of EFF.

Inorganic constituents and heavy metal contents in garlic Inorganic constituents (Ca, K, Mg, Na) and heavy metal contents in garlic stems were tested 28 weeks after sowing (Table 3). The KO_2 and Na_2O contents in garlic stems were significantly increased after applied with EFF, but Cu, Mn, and Ze contents did not differ from the control. The Se and Ge contents increased tremendously with treatments applying SeO_2 and GeO_2. In the ZBFC treatment, Se and Ge contents were 13.89- and 3.44-fold higher than in the control.

Cu, Fe, Zn, and Mn are essential microelements for the human body, but toxicity can occur with overdose. These elements in garlic clove were tested (Table 4). The results indicated that EFF did not change the Cu, Fe, Zn, and Mn concentrations in garlic cloves. In the ZBFC and ZAFC treatments, Ge contents in garlic cloves were 2.05- and 2.22-fold higher than in the control. The Se concentrations were 880.83 μg kg^{-1} and 876.13 μg kg^{-1}, which was 12.79- and 12.72-fold higher

Table 5. The inorganic constituents and heavy metal contents in garlic clove peel.

Treatments	CaO	K_2O	MgO	Na_2O	Cu	Fe	Mn	Zn	Ge	Se
	%				mg kg^{-1}				μg kg^{-1}	
Control	0.61	0.98	0.14	0.09	4.18	106.43	20.19	33.07	9.03	22.67
ZAF	0.58	0.99	0.14	0.09	4.93	71.80	23.15	30.97	4.47	30.40
ZBFC	0.65	1.00	0.14	0.10	5.22	79.07	20.07	31.63	23.27*	248.37*
ZAFC	0.56	1.00	0.13	0.09	6.19	146.20	17.47	33.83	20.40*	226.17*
$LSD_{0.05}$	0.093	0.075	0.003	0.001	1.01	60.66	4.01	3.55	2.94	41.54

Table 6. Ge and Se contents in soil 20 and 28 weeks after sowing.

Treatments	20 W†		28 W	
	Ge	Se	Ge	Se
	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}
Control	12.09	40.17	12.302	36.665
ZAF	12.17	40.52	10.853	37.288
ZBFC	15.24*	42.11*	15.077*	41.092*
ZAFC	15.64*	42.33*	15.100*	48.150*
$LSD_{0.05}$	0.98	1.31	1.949	2.839

†20 w: 20weeks after sowing. 28 w: 28 weeks after sowing.

Table 7. AMF spore density and plant root colonization rate.

Treatments	Spore density	Root colonization rate
	spores 30 g^{-1} fresh soil	%
Control	88.7 ± 9.2	12
ZAF	95.3 ± 15.9	15
ZBFC	146.3 ± 20.4	23
ZAFC	176.3 ± 25.4	25

than in the control. The recommended dietary allowances (RDA) for selenium for adults, both male and female, are 55 μg day^{-1} with TUL of 400 μg day^{-1} (Goldhaber, 2003). Se levels ranging from 0.3 to 0.5 mg kg^{-1} was reported in different varieties of onions grown in Japan (Noda and Taniguchi, 1983). In this experiment, Se contents in garlic clove was about 0.9 mg kg^{-1} in the treatments applied with SeO_2.

The inorganic constituents and heavy metal contents in garlic clove peels were also tested (Table 5) and the result indicated that EFF did not affect these elements except for Ge and Se. In ZBFC and ZAFC treatments, Se contents were 248.37 μg kg^{-1} and 226.17 μg kg^{-1} in garlic clove peels.

Yun et al. (2003) reported that the selenium content in lettuce leaf increased with the application of Se in hydroponic solution. The results in our study indicate that Se and Ge contents in garlic stems, garlic cloves and garlic clove peels were increased with the application of SeO_2 and GeO_2. Numerous studies have shown that at low concentrations, Se exerts a beneficial effect on growth and stress tolerance of plants by enhancing their antioxidative capacity (Kong et al., 2005). In garlic stems, the macro elements, K_2O and Na_2O especially, increased significantly with the application of EFF, but in garlic clove, CaO and MgO contents were increased while K_2O contents were decreased with the application of EFF.

Changes of soil properties Ge and Se contents in soil were tested 20 and 28 weeks after sowing (Table 6). After 28 weeks, Se in ZBFC- and ZAFC-treated soils were 41.09 μg kg^{-1} and 48.15 μg kg^{-1}, which was 12.1% and 31.3% higher than in the control. In ZAF-treated soil, the Se was also higher than in the control, although not significantly. The Ge contents were also higher in ZBFC- and ZAFC-treated soil than in the ZAF and control soils 20 or 28 weeks after sowing. The contents of Se and Ge are in a lower level because of the sandy loam earth condition, but the Se and Ge contents remarkable increased because of the application of SeO_2 and GeO_2. It indicated that selenium contents in tea leaf and soil were significantly increased because of the direct application of selenium (Hu et al, 2002). Plant foods are the major dietary sources of selenium in most of the countries throughout the world. The selenium content of the soil where plants are grown or animals are raised might influence the content of selenium in food. The soil used in this experiment was sandy loam, the results indicate that Se and Ge contents in sandy loam soil were increased because of the application of SeO_2 and GeO_2. Compared with the addition that Se and Ge in garlic, the result also demonstrated that the application of Se and Ge was more available for plant uptake than that for soil.

AMF agent was applied in the ZBFC and ZAFC treatments, and spore density and crop root colonization were tested (Table 7). The spore number per 30 g soil was 176.3 ± 25.4 in the ZBFC treatment, which was 98.8% higher than in the control. The root colonization rate was 25% in ZBFC. AMF spores were also discovered in the control and ZAF treatments because AMF are naturally ubiquitous and it was difficult to find a field without AMF. Spore density in ZAF-treated soil was higher than in the control (Fig. 1), which may be due to the zeolite improvements to the soil environment, which benefit AMF reproduction. The spore density and root colonization in ZBFC- and ZAFC-treated soils and plant roots were significantly higher than that in ZAF and control because of the application of AMF agent. AMF hyphae, vesicles, and arbuscules in roots are shown in Fig. 2. Liu et al. (2006) discussed the relationship between colonization potential and inoculum potential of arbuscular mycorrhizal fungi. Our study also demonstrated the positive correlation between colonization potential and inoculum potential. The spore density and root colonization in ZAF were higher than in control, because of the zeolite supplied a suitable environment for the growth of mycorrhizal fungi.

Chitinolytic microorganisms in the soils treated with colloidal chitin were higher than in soils that were not treated with colloidal chitin (Fig. 3). The application of zeolite with cellulose was more effective in increasing microbial biomass than zeolite or cellulose alone (He et al., 2002). In our study, the chitinolytic microorganisms log value was 3.1 in ZBFC and ZAFC treatments from 22 to 28 weeks after sowing because the application of colloidal chitin facilitates reproduction of chitinolytic microorganisms. There were no significant differences

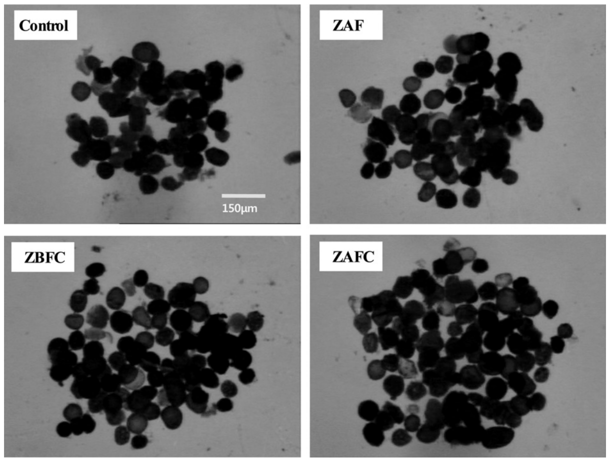

Fig. 1. AM fungi spores in soils of different treatment (Scale bar: 150 μm).

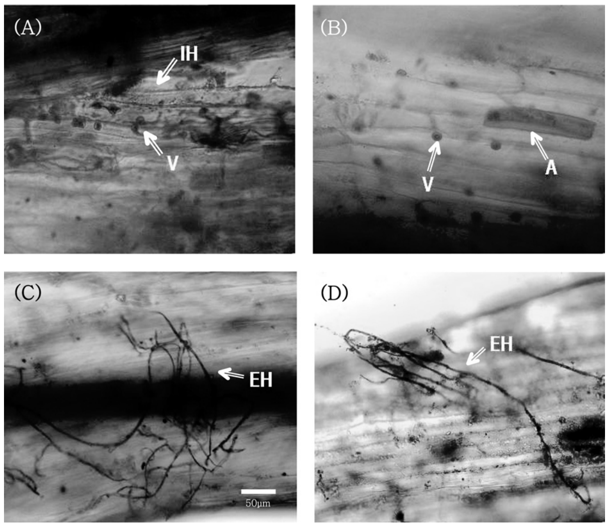

Fig. 2. Hyphae, vesicle and arbuscule in garlic roots. IH: Internal Hyphae, EH: External Hyphae, Ver: Vesicle, Arb: Arbuscule.

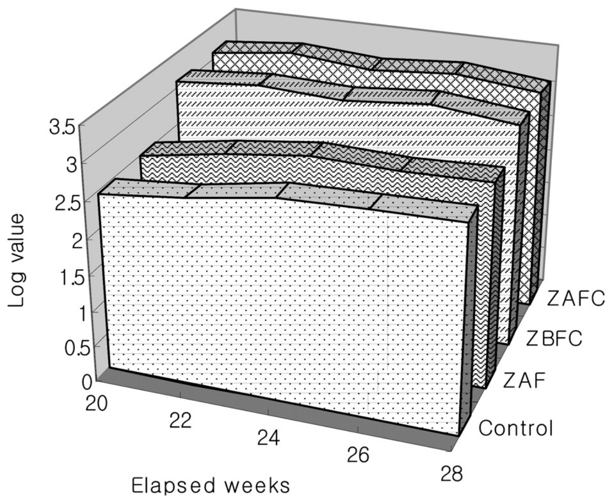

Fig. 3. Changes in density of chitinolytic microorganism in experimental soil.

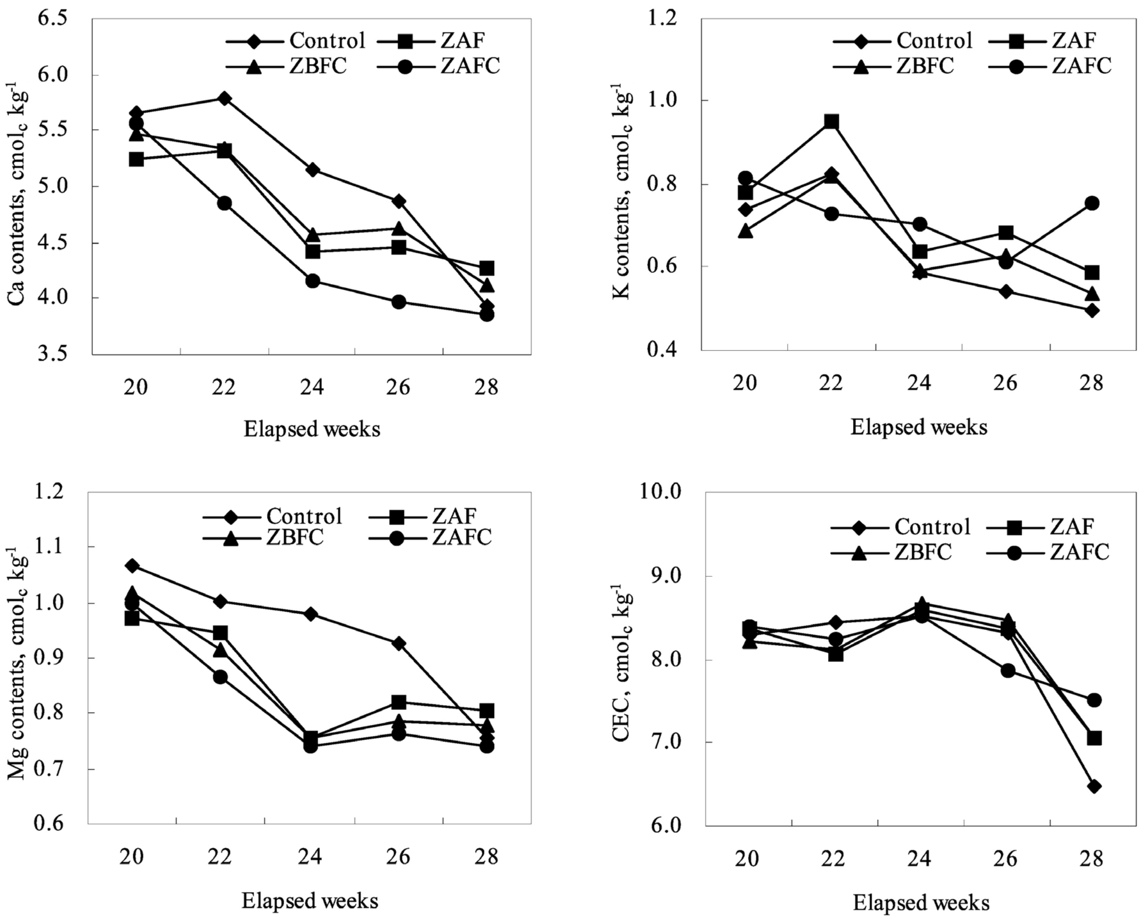

Fig. 4. The dynamic of exchangeable cation and CEC in soil.

between ZBFC and ZAFC. The log value of chitinolytic microorganisms in Control and ZAF soils also had no significant differences and the levels were raised from 2.5 to 2.8 at the period of 20 and 28 weeks after sowing. The results indicate that colloidal chitin significantly improved the abundance of chitinolytic microorganisms in soil.

The changes of cations (Ca, K, and Mg) and CEC in experimental soils were tested (Fig. 4). Compared with the control, CEC and the amounts of Ca, K, and Mg were significantly higher in ZAF 28 weeks after sowing, possibly due to the high CEC level and cation adsorbtion capacity of natural zeolite. Applied with NK-Z and of the 4 EFF can also keep the CEC high in comparison to the control 28 weeks after sowing.

Conclusion

We took 5 kinds of EFF as soil amendments to determine their effects on soil amelioration and crop growth. The results indicate that these EFFs can improve crop growth and the soil environment. The garlic weight in ZAFC-treated soil was 9.17% higher than in the control. The plant height was also significantly higher with the application of EFF.

The soil spore density and root colonization were significantly higher with the application of AMF agent, which can help crops adsorb nutrients and improve disease resistance. The log value of chitinolytic microorganisms were maintained at about 3.1 in soils treated with colloidal chitin, which was significantly higher than in soils that were not treated with colloidal chitin. In the soil treated with SeO_2 and GeO_2 liquid agents, the Se and Ge contents were 13-fold and 3-fold higher than in the control. Our result was very important and meaningful because the anti-cancer activity of garlic has been attributed to Se. The results also show that the cation contents and soil CEC were high because of the application of EFF.

In this study, we have confirmed the comprehensive effectiveness of EFF on reducing nutrient leaching, improving crop growth, and ameliorating the soil environment. In the following research, the percentage of organic and inorganic Se and Ge that can be adsorbed by humans and the individual effectiveness of different kinds of environmental fertilizer should be researched in detail respectively.

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