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Single and joint toxicity of cypermethrin and copper on Chinese cabbage (*Pakchoi*) seeds

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ABSTRACT

The single and joint effects of Cu^{2+} and cypermethrin (CPM) on the seed germination and the elongation of root and shoot of *Pakchoi* were investigated. The results showed that in solution low concentrations of Cu^{2+} could accelerate the germination rate of *Pakchoi*, whereas high concentrations of Cu^{2+} could inhibit it. CPM could strongly inhibit the germination of *Pakchoi* in solution. However, in the joint toxicity effect, CPM reduced the phytotoxicity of Cu^{2+} on the germination of *Pakchoi* seeds under solution conditions. In the single-factor experiments and joint effect tests of CPM and copper on the seedling growth, it was found that there were significant liner relationships between concentrations of pollutants and root elongation ($P<0.05$). Copper and CPM had synergic effects on root elongation of *Pakchoi* in solution cultivation test. However, in soil culture test, these synergistic effects were not significant ($P<0.05$). Meanwhile, the joint toxicity was more dependent on the effect of copper than that of CPM. The toxicity of the pollutants to seed germination, shoot and root elongation is in the following sequence: root elongation > shoot elongation > germination rate.

1. Introduction

Copper is an essential element for all organisms at low concentrations. While with the frequent use of fungicide Bordeaux mixture (mixture of $CuSO_4$ and $Ca(OH)_2$) in orchard, the content of copper in the orchard soil increased even to pollution level [1–3]. Since heavy metals are not degradable, the accumulation of copper in the soil may have long effects on the plants growing on the soil. It has been reported that excess amounts of copper may inhibit photosynthesis of plants and cause chlorosis of leaves [4].

On the other hand, synthetic pyrethroid insecticides are extensively used in place of organochlorine, organophosphorus insecticides and carbamates to control pests [5]. Cypermethrin (CPM), (RS)-alpha-3-phenoxybenzyl-2,2-dimethyl-(1R,1S)-*cis*,*trans*-3-(2,2-dichlorovinyl)-cyclopropane carboxylate, is a commonly used pyrethroid pesticide in urban and agricultural environments, and its half-life in soil is 30 days, ranged 2–8 weeks [6,7]. It is a highly active synthetic type II pyrethroid and is considered as a good pest control agent in agriculture, horticulture, and aquatic systems with low toxicity to nontarget animals [8]. The single toxicity of CPM on organisms has been studied a lot [9–11,6]; however, joint toxicity of CPM and heavy metals has been studied little, especially on the plants. In some orchards of China, CPM is frequently used as pesticide to control pest. The excess use of CPM cannot only cause residue in the soil, but also even lead to potential combined pollution of copper and CPM. Synergistic interactions of pollutants can introduce serious eco-toxicological problems [12]. Therefore, investigation of the joint effect of the pesticide and copper on the plant is very essential.

Seed germination and root elongation tests have been used as short-term phytotoxicity test to provide valuable information about inhibition, enzyme activation, cell expansion, respiration and other parameters. These types of bioassays have several advantages over other toxicity tests and are suitable as a stand-by test method as well as a rapid tool to evaluate the ecological risk of xenobiotics [13]. Thus, in this study, Chinese cabbage (*Pakchoi*) was chosen as a target testing plant to examine the single and joint effects of CPM and Cu^{2+} on seed germination, shoot and root elongation.

2. Materials and methods

2.1. Materials

All reagents used in this study are analytic grade. Seeds of *Pakchoi* were commercially available from Jinnong Seeds Co. Ltd. in Jiangsu. A commercial formulation of CPM (CAS No. 52315-07-8) was supplied by Zhenhua Chemical Factory, Shandong Province, and its molecular structure is shown in Fig. 1. Stock solutions of

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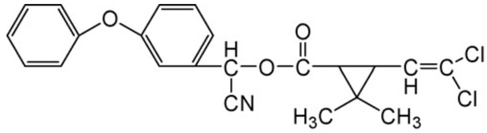

Fig. 1. Molecular structure of CPM.

test chemicals were prepared with deionized water. It was also used as the control and diluents. Three replicates were set for each concentration.

The test soil samples were collected from the land near Dashahe orchard in Jiangsu Province, China. Bordeaux mixture has been applied since the orchard established 50 years ago. Soil samples were air dried at room temperature and then passed through a 2-mm sieve. The physicochemical prosperities of soil were: OM, 1.04%; CEC, 6.8 cmol/kg; pH (H_2O, 1:1, w/v), 8.26; total N, 0.044%; total P, 0.047%; total K, 0.0044%; total Cu, 4.14 mg/kg.

2.2. Seed germination

Seed germination was tested on filter papers placed in Petri dishes and moistened the filter papers with 5 mL toxicants solution. In the single toxicant tests, concentrations of CPM were set as 0, 5.6, 8.4, 11 and 17 mg/L and the tested concentrations of Cu^{2+} were 0, 1, 3, 5, 7 and 9 mg/L, respectively. In the joint tests, the treatments were set up by keeping concentrations of CPM 8 mg/L and with the variation of the Cu^{2+} concentration 0, 1, 3, 5, 7 and 9 mg/L. Controls were obtained by moistening the filter papers with 5 mL deionized water. 15 seeds were placed in each dish, covered by the lid, and incubated in the dark at $25 \pm 2\,^{\circ}C$ and the proportion of seeds that had germination after 48 h was counted. The seeds were considered to be germinated when plumule was ≥2 mm.

2.3. Tests of shoot and root elongation in solution

After 48 h germination and initial growth period, the seedlings were gently removed from culture dish and washed, after measuring the germination, 10 seedlings were transferred to vitreous pots (90 mm × 150 mm) containing various levels of the toxicants.

In the single toxicant tests, concentrations of CPM were set as 0, 5.6, 8.4, 11 and 17 mg/L and the tested concentrations of Cu^{2+} were 0, 1, 3, 5, 7 and 9 (or 10) mg/L, respectively. In the mixture toxicant tests, the treatments were set up according to the results of single-factor experiments, tested concentrations of CPM were 8 and 16 mg/L which matched the inhibitory rate of root elongation as 60% and 100%, respectively; the tested concentrations of copper were 0, 1, 3, 5, 7 and 9 mg/L.

The experiments were carried out in natural sunlight (12 h light/12 h dark photoperiod), a day/night temperature of 25/16 °C. Seeds were submerged in various level toxicant solutions. When the length of the growing root cultured in the control solution without pesticide and heavy metal reached 20 mm, the exposed experiments were completed. The shoot and root elongation of all the treatments were measured and calculated

2.4. Tests of shoot and root elongation in soils

50 g soil amended with various concentrations of test chemicals was put into the culture dish and then 15 seeds were scattered on the soil. Water capacity in the soil was adjusted to 60% of the water holding capacity. The concentrations of single-factor tests were set up as 8, 16, 32, 50 and 64 mg/g for CPM and 100, 150, 200, 250 and 300 mg/g for Cu^{2+}. The treatments of pollutant mixture were set up according to the results of single-factor tests, the tested concentration of CPM was 50 and 100 mg/kg, tested concentration of Cu^{2+} was 100, 150, 200, 250 and 300 mg/kg, respectively. Other procedures were similar to the solution culture experiments.

All the treatments for the above tests of germination, shoot and root elongation were set up in triplicate.

2.5. Statistical analysis

Statistical analysis including calculation of average values, standard deviation (S.D.) and regression was performed on the data obtained in the tests with Microsoft Excel and SPSS 12.0. The multiple comparison procedure (LSR test) was used to compare the effect of copper and pyrethroid interaction on germination, root length and shoot height and significance was set at $P < 0.05$. The significant difference for the germination data was examined by using *t*-test with 95% confidence interval of the difference.

3. Results and discussion

3.1. Effects on seed germination

The effects of various Cu^{2+} concentrations on the seed germination of *Pakchoi* are shown in Table 1. The germination rate was 60–80% for solution cultivation tests. Low concentrations of copper could enhance the germination rate of *Pakchoi*, whereas high concentrations could exert inhibition. According to *t*-test, 60% and 63% of germination rates show significant difference from the data average. Therefore, ≥7 mg/L Cu^{2+} in solution inhibited seed germination obviously. In low concentrations, copper is a kind of microelement nutrition for the plant, so that the little of copper addition might accelerate the germination of *Pakchoi*. While in high concentrations, copper could induce toxicity effect on the plant, so that the

Table 1
The effects of Cu^{2+} and CPM on the germination of *Pakchoi* seeds for 48 h exposure in solution

Pollutants	Cu^{2+}					
Concentration (mg/L)	CK[a]	1	3	5	7	9
Germination (%)	79	70	80	77	63	60
S.D.	0.018	0.025	0.01	0.03	0.02	0.02
Pollutants	CPM					
Concentration (mg/L)	CK	5.6	8.4	11	17	
Germination (%)	73	62	42	42	10	
S.D.	0.09	0.26	0.26	0.26	0.14	
Pollutants	Cu^{2+} + CPM					
Concentration (mg/L)	CK + CK	1 + 8	3 + 8	5 + 8	7 + 8	9 + 8
Germination (%)	70	70	90	90	70	70
S.D.	0.23	0.14	0.2	0.24	0.15	0.29

[a] CK means control test, the concentration of toxicants was 0.

Table 2
Relationships between inhibitory rate of root elongation (RI) and shoot elongation (SI) and concentration of added copper

Experiments	*Pakchoi*	Regression equations[a]	*r*	*P*	ID_{50}[b]
Solution	Root	$RI = 12.08 + 10.43C_{Cu}$	0.95	<0.05	3.636
	Shoot	$SI = 29.10 + 2.560C_{Cu}$	0.97	<0.05	8.164
Soil	Root	$RI = -2.104 + 0.1896C_{Cu}$	0.895	<0.05	274.8
	Shoot	$SI = 6.85 + 0.097C_{Cu}$	0.682	>0.05	444.8

[a] C_{Cu} was the tested concentration of copper, mg/L or mg/kg.
[b] ID_{50} was concentration of Cu^{2+} correspond to 50% inhibitory rate of RI or SI.

Table 3
Relationships between inhibitory rate of root elongation (RI) or shoot elongation (SI) and concentration of added CPM

Experiments	*Pakchoi*	Regression equations[a]	*r*	*P*	ID_{50}[b]
Solution	Root	$RI = 6.57 + 5.77C_{CPM}$	0.97	<0.05	7.527
	Shoot	$SI = -18.64 + 6.99C_{CPM}$	0.94	>0.05	9.820
Soil	Root	$RI = -3.68 + 0.27C_{CPM}$	0.72	<0.05	198.8
	Shoot	$SI = 13.53 + 0.059C_{CPM}$	0.26	>0.05	618.1

[a] C_{CPM} was test concentration of CPM, mg/L or mg/kg.
[b] ID_{50} was concentration of CPM correspond to 50% inhibitory rate of RI or SI.

germination rate is inhibited by the high concentration of copper. As for CPM in solution culture, it reduced the germination of *Pakchoi* markedly (Table 1). Seed germination rate decreased with the concentration increase of CPM, from 73% for the CK to 10% for 17 mg/L of CPM, which illustrates that the toxicity effect of CPM on seed is evident.

The joint toxicity effect of Cu^{2+} and CPM on the germination of *Pakchoi* seeds was different from that of single effects of Cu^{2+} and CPM. From Table 1, it can be seen that the germination rate ranged from 70% to 90% which is higher than that tested by exposing to single Cu^{2+} at the same level except 0 and 1 mg/L Cu^{2+}, which suggests that CPM reduced the phytotoxicity of Cu^{2+} on the germination of *Pakchoi* seeds under solution conditions. CPM could combine with Cu^{2+} in the solution. Therefore, when added with CPM, most of Cu^{2+} formed complex with it, and the phytotoxicity was reduced.

3.2. *Effects on seedling growth*

3.2.1. *Single-factor effect of Cu*

The influence of Cu^{2+} on the root elongation is shown in Fig. 2A. In solutions, the root elongation in the treatment below 1 mg/L Cu^{2+} was not different from the control test. From 1 mg/L Cu^{2+} onwards, the root elongation decreased with the increasing Cu^{2+} concentration. The inhibition degree on shoot elongation of Cu^{2+} was lower than that on root elongation. ID_{50} of shoot was over 2 times higher than that of root (Table 2). Meanwhile under soil conditions, ID_{50} of shoot was also higher than that of root. That was to say the shoot of *Pakchoi* seeds showed less sensitivity to soil contamination than in solution, and the inhibitory rate of the shoot was less than that of root. The following explanation may account for this phenomenon: some plant roots can accumulate elevated concentrations of Cu^{2+} and prevent translocation of metals to the growing parts of the plant [14]. Therefore, although the concentration in the solution is relatively high, seedling growth of *Pakchoi* is not affected in a certain concentration range.

Coefficient of correlation (*r*) was used to characterize the linear relationship between concentration of Cu^{2+} and inhibitory rate of elongation. For dose–response relations of root elongation, *r* value is 0.95 in solution and 0.895 in soil; whereas for dose–response relations of shoot elongation, *r* value is 0.97 in solution and 0.682 in soil. The corresponding regression equations can be expressed as those listed in Table 2. It can be seen that the correlation between the inhibitory rate and the concentration of Cu^{2+} is lower in soil than that in solution. Therefore, soil can alleviate the phytotoxicity of Cu^{2+} on the plant in some degree. This may be due to the interactions between soil ingredients and Cu^{2+}, such as sorption of organic matter and some inorganic colloid to Cu^{2+}.

3.2.2. *Single-factor effect of CPM*

In solution test, root elongation of *Pakchoi* decreased greatly with the increasing concentration of CPM, whereas in soil conditions, root elongation was hardly affected by the concentration of CPM (Fig. 2B). The effects of CPM on the shoot elongation of *Pakchoi* were similar to that on root elongation.

The relationships between inhibitory rate of root elongation (RI) or shoot elongation (SI) and concentrations of added CPM are shown in Table 3. Under solution conditions, phytotoxicity of CPM on *Pakchoi* had close correlation with the concentration of added CPM, and the coefficients of correlation (*r*) reached 0.97 for the root and 0.94 for the shoot, respectively, which indicated that CPM had direct phytotoxicity on *Pakchoi*. In soil, the correlation between phytotoxicity on *Pakchoi* and concentration of added CPM decreased generally, being 0.72 for the root and 0.26 for the shoot, respectively. This may be due to the difference of characteristics of solution and soil. Under solution conditions, there was no organic matter to adsorb CPM, so CPM could be easily adsorbed or distributed in organic matters in the root, and afterwards, CPM was easily distributed to the growing parts of *Pakchoi* with transpiration flow. Therefore, the ID_{50} in root and shoot was very close to each other, and was 7.527 and 9.820, respectively.

While in soil, CPM can also be adsorbed or distributed by soil organic matter [15], so the phytotoxicity of CPM on *Pakchoi* is determined by the competing sorption between soil organic matter and root. If the sorption of root is stronger than that of soil organic matter, CPM may be strongly adsorbed or distributed in the root; otherwise, it may be mostly adsorbed or distributed by the soil. From Table 3, it can be seen that the existence of soil obviously affected the distribution or adsorption of CPM to the root. The coefficient of correlation is 0.72, which is much lower than that in solution conditions, and ID_{50} is much higher than that in solution conditions. Under soil conditions, CPM nearly had no phytotoxicity on *Pakchoi*; whereas in solution conditions, it had obvious effect on the growth of shoot. The coefficient of correlation was 0.26 in soil, much lower than that in solution; and ID_{50} in soil was 618.1, more than 60 times higher than that in solution. This indicates that under soil conditions, the plant can endure much higher concentrations of CPM than in solution. The reason may be that organic matter contained in the soil inhibits the translocation of CPM to the growing parts of the plant; on the other hand, since the soil contains more microelement and macro-elements nutrition than the solution, they can improve the endurance of the plant in stressing conditions, and also the plant can adjust itself at biochemical level to adapt the stressing condition [16,17].

To summarize the results of the experiments above, it was concluded that the toxicity of copper on *Pakchoi* was weaker than that of CPM in solution under the single-factor pollution tests, while it was reversed in soil cultivation. It may be that under solution conditions, copper can be available by the plants at low concentrations, and only at high concentrations, can it show phytotoxicity; but it is not true with CPM. Therefore, the phytotoxicity of copper on *Pakchoi* has a turning point around 3 mg/L, while that of CPM dose not. However, in the soil, things are different, and the reason may be that strong partition and adsorption ability of CPM with soil organic matter decreases its bioavailability and phytotoxicity, so *Pakchoi* can endure high concentrations of CPM.

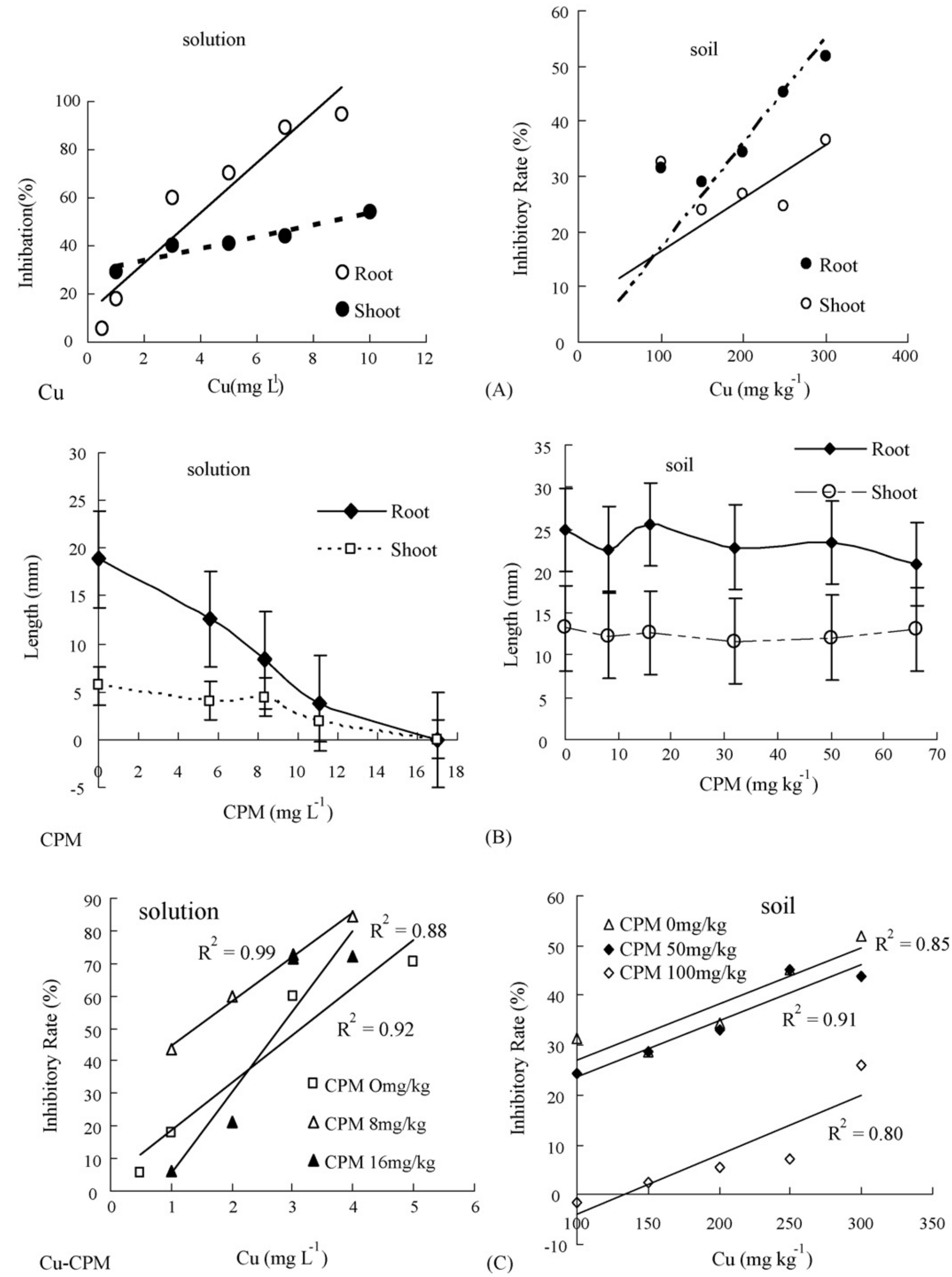

Fig. 2. Single and joint effects of Cu^{2+} and CPM on the root and shoot elongation of *Pakchoi* in solution and soil (measured after 48 h cultivation).

3.2.3. Joint effect of copper and CPM

No matter in solution or in soil, there was a positive linear relationship between the inhibitory rate of root elongation and the concentration of Cu^{2+} with adding CPM ($P < 0.05$). As shown in Fig. 2C, the inhibition curves of joint effects mostly located above that of single effect tests of Cu in solution tests, while in soil, joint toxicity inhibition curves located below that of Cu alone.

The corresponding regression equations can be expressed as those listed in Table 4. According to the equations, in solution tests, the joint toxicity of copper and CPM on root was markedly significant, in which ID_{50} was lower than that of single effect tests ($P < 0.05$). It can also be seen that with the increase of CPM concentration, the root of *Pakchoi* can endure higher concentrations of copper. This indicated that the joint effects of CPM and copper were very complicated; in low concentrations, CPM might alleviate the toxicity of copper, whereas in higher concentrations, it might have direct toxicity on the root of *Pakchoi*. But in soil tests, the situation varied. For joint toxicity of CPM and copper on root elongation, ID_{50} was higher than that of single effect tests of copper. This indicated that under soil conditions, the toxicity of copper and CPM was reduced by each other, and *Pakchoi* could endure higher concentrations of CPM and copper than that of single effect of CPM and copper. This fact demonstrated that in soil conditions, CPM could change the surface characteristics of the soil by distribu-

Table 4
Relationships between inhibitory rate of root elongation (RI) and concentration of added copper in the presence of CPM

Experiments	CPM (mg/L or mg/kg)	Regression equations[a]	r	P	ID_{50}[b]
Solution	0	$RI = 14.7C_{Cu} + 3.63$	0.96	<0.05	3.15
	8	$RI = 13.6C_{Cu} + 31.3$	0.98	<0.05	1.27
	16	$RI = 24.7C_{Cu} - 19.0$	0.95	>0.05	2.79
Soil	0	$RI = 0.114C_{Cu} + 15.6$	0.92	<0.05	301.8
	50	$RI = 0.112C_{Cu} + 12.6$	0.95	<0.05	334.1
	100	$RI = 0.120C_{Cu} - 16.1$	0.89	<0.05	550.8

[a] C_{Cu} was test concentration of Cu in the presence of CPM.
[b] ID_{50} was concentration of Cu correspond to 50% inhibitory rate of RI.

tion/absorption to the ingredients of the soil (such as organic matter and clay), which made the soil could adsorb more copper and reduce its bioavailability. As Inaba and Takenaka [18] pointed out that the low concentrations of the synthetic chelators and humic acid could decreased the toxicity and bioavailability of Cu. In this study, *Pakchoi* could endure higher concentrations of copper with the presence of high CPM concentration in soil conditions.

In comparison, based on the inhibitory rates, the toxicities of the pollutants to seed germination and shoot, root elongation are in the following sequence: root elongation > shoot elongation > germination rate.

Independent cypermethrin and fenvalerate exposure had been reported to induce chromosomal aberrations and micronucleus formation in *Allium* root meristem cells [19]. It had also been found that the interaction of two different groups of chemicals (diuron and deltamethrin) seems to change the mode of action [20], resulting in the induction of typical ultrastructural alterations. When the cells were exposed to individual compounds, the alterations were not induced, which were considered to be the synergistic action mode of two components. The growth inhibition test in this study also indicates synergistically interaction of Cu and CPM. The possible mechanism of action of two components is that the structure of plasma membrane and the cell wall is abnormal at treatment of CPM with its microfibrillar arrangement appeared, which results in the increase of the opportunities of copper ions to enter the root system and to go up to the shoot. On the other hand, the formation of complex of Cu–CPM could decrease the bioavailability of CPM or Cu and phytotoxicity induced by the complex component decreased. Therefore, joint effects of chemicals and heavy metals may depend on the ratio of the components.

4. Conclusion

In this study, the single and joint effects of Cu^{2+} and CPM on seed germination, elongation of root and shoot of *Pakchoi* were investigated. The results showed that low concentrations of Cu^{2+} could accelerate the germination rate of *Pakchoi*, while in high concentrations could inhibit that. CPM could strongly inhibit the germination of *Pakchoi*. In the single-factor experiments and joint effect tests of CPM and copper, there were significant liner relationships between concentrations of pollutants and root elongation ($P < 0.05$). Copper and CPM had synergic effects on root elongation of *Pakchoi* in solution cultivation test. However, in soil culture test, these synergistic effects were not significant ($P < 0.05$). Meanwhile, the joint toxicity was more dependent on the effect of copper than that of CPM. The toxicity effects of the cooper and CPM to seed germination and shoot, root elongation are root elongation > shoot elongation > germination rate.

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