Application of Active EM-Calcium in Green Agricultural Production — Case Study in Tomato and Flue-cured Tobacco Production

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1. Introduction

EM (Effective Microorganisms) technology has long been widely applied to agricultural production in China. EM contains more than 80 kinds of beneficial microorganisms, including yeast, lactic acid bacteria, actinomycetes and photosynthetic bacteria (Shao et al., 2013; Zhou et al., 2008). They can quickly decompose organic matter, metabolize antioxidant substances and inhibit the proliferation of harmful microorganisms (Higa, 1997). EM was supposed to have positive effects on reforming soil nematode community structure (Hu and Qi, 2013a), increasing crop yield and improving soil properties (Daly and Stewart, 1999; Khaliq et al., 2006).

High calcium content in plants has been associated with increased resistance to diseases (Berry et al., 1988), not merely the well-known tomato blossom-end rot (BER) (Sonneveld and Voogt, 2009), calcium nutrition significantly affects the resistance of tomato seedlings to the bacterial wilt caused by Ralstonia solanacearum (Yamazaki, 1995), other diseases were also reported to be suppressed by high calcium concentration in host plants (Almeida et al., 2009; Berry et al., 1988; Chiasson et al., 2005). BER is a major physiological disorder in tomato that creates up to 50% losses (Taylor, 2004), besides the inducing factors of high temperature, high salinity (Adams, 1993), BER has long been considered to be a Ca-deficiency disorder, and its incidence increases in cultivation at low Ca concentrations (Raleigh, 1944), however, most tomatoes contain little calcium (Ca^{2+}), uptake and translocation of cationic nutrients including Ca^{2+} in plants plays an essential role in the physiological processes (Chung et al., 2010). According to

early studies, the exogenous calcium supplied through tomato roots (Hall, 1977; Sachan and Sharma, 1981) or through leaves (Eraslan et al., 2007; Freitas et al., 2012) by the spraying methods can both increase the Ca uptake of tomato plants and enhance the immunity to diseases.

Flue-cured tobaccos are important industrial crop significant to the national economy for China. In southwest China, most of the rain falls during the early and middle growth stages of flue-cured tobacco, and periodic drought often happens at the later growth period of flue-cured tobacco, this will not enable the upper leaves maturing normally, resulting in a poor availability of upper leaves, so it is important to discover technologies for the drought resisting and the tobacco quality and yield improvement (Hou et al., 2012; Hou et al, 2013). Water-retaining agent has been applied in many areas (Kumar and Dey, 2011; Truax and Gagnon, 1993). However, in studies of flue-cured tobacco cultivation, application of the water-retaining agent is still in a research vacant. At present, many studies have reported the application of EM in agricultural production (Hu and Qi, 2013a, b; Javaid, 2010; Khaliq et al., 2006). EM can quickly decompose organic matter, metabolize antioxidant substances and inhibit the proliferation of harmful microorganisms (Daly and Stewart, 1999; Daming, 1999; Heo et al., 2008).

In conclusion, many studies (Heo et al., 2008; Hu and Qi, 2013b; Khaliq et al., 2006) have been undertaken on the application of EM as base fertilizer or as a component of base fertilizer, however, there has been little published concerning using EM as foliage fertilizer, especially making EM into calcium nutrient solution. In recent years, our team invents a new product based on EM technology and names it as Active EM-Calcium. Active EM-Calcium is prepared by special fermentation process with lime and EM, which contains chelating calcium (Ca) and microorganisms in the solution. In order to verify the effect of Active EM-Calcium on tomato and flue-cured tobacco production, two researches were carried out from 2012 to 2013.

2. Effects of EM-Calcium on production of greenhouse tomato

2.1. Materials and methods

2.1.1. Test site

The greenhouse experiments were carried out in plastic greenhouse during 2012 at the Vegetables and Flowers Institute of Nanjing (SW of China, lat. 31°43′ N, long. 118°46' E) in a well stirred heavy lay loam. The local climate is subtropical monsoon, with average annual rainfall of 1106.5mm, temperature of 15.7℃ and humidity of 81%. Affected by the No. 1211 (international numbering) severe tropical storm "HAIKUI", temperature of experimental field in early August saw a drop of 10℃-14℃, mixed with heavy winds and rains, but due to the short continuance, greenhouse crops were little influenced by the storm. Average temperature during the experiment period was 21.9℃ (May), 25.7℃ (June), 29.8℃ (July), 27.9℃ (August), 22.3℃ (September) respectively, which was 1.0℃, 0.9℃, 1.5℃,

0.6℃,-1.0℃ higher than the same period in recent years. Peak temperature was 37.9℃ recording on July 29th. The experimental soil properties were as follows: organic matter 14.34 g kg^{-1}, available nitrogen 104.17 ppm, available phosphorus 26.48 ppm, available potassium 184.70 ppm.

2.1.2. Experimental design

Lab experiment: 28 g lime was accurately weighed in a 100 ml volumetric flask, then added DI water to dissolve and kept constant volume to 1000 ml, and the 2% Ca^{2+}suspension was available by shaking the mixture well. Then the prepared calcium suspension was mixed with different volume EM, molasses (EM and the molasses were supported by EMRO Limited Company, Nanjing Branch) and DI water to make up 5 treatments with three replications. The ingredients of treatment T1-T5 were displayed as Table 1. These sealed EM-Calcium mixtures would be fermented for 3-6 days in a orbital shaker with constant temperature, they were taken out and setted aside when the precipitates were basically dissolved. The fermented treatment with highest calcium solubility was diluted to keep the active EM-calcium solution with 2.0‰ Ca^{2+}concentration.

Treatment	2% Ca^{2+} suspension (ml)	EM (ml)	Molasses (ml)	DI water (ml)	Theoretical Ca^{2+} concentration
T1	45	25	25	205	3.0‰
T2	60	30	30	180	4.0‰
T3	75	40	40	145	5.0‰
T4	90	45	45	120	6.0‰
T5	105	50	50	95	7.0‰

Table 1. The components of EM-calcium solution with different contents.

Field experiment: the six week old tomato seedlings ("*21st Century Crown*") were transplanted to the experiment fields on June 7th, conventional field management were carried out fairly among the treatments, no additional light, heat, or CO$_2$ were provided. The experimental field was ploughed several times and fertilized with 700kg hm^{-2} compound fertilizer (N: P$_2$O$_5$: K$_2$O=1:2:2) in May. Irrigation systems for tomato were accorded with the local farming practices. At 8:30 every morning, micro sprayers were adopted to spray the active EM-calcium solutions 2 ml once in four days on different tomato organs. The treatments were set based on the sprayed organs, including spraying root, spraying flower, spraying leaves near the newborn fruits, spraying one week old fruit, spraying three week old fruit, and a control, hereafter referred as SR, SF, SL, SO, ST and CK respectively. A plastic film was used to keep apart the other parts when spraying one tomato organ.

2.1.3. Measurements

When tomato was ripened, two fruits were harvested from one plant, and about 10 g tomato flesh per fruit was taken along the longitudinal axis (24 fruits per treatment in total) then homogenized for the following measurements. Different forms of Ca in the tomato fruits were extracted and determined by adopting Ohat Y's method (Ohat Y, 1970), including Ca nitrate and Ca chloride, water soluble organic Ca, Ca pectate, Ca phosphate and Ca carbonate, Ca oxalate, Ca silicate, hereafter the above Ca forms were recording successively as Alc-Ca, H_2O-Ca, NaCl-Ca, HAC-Ca, HCl-Ca, Res-Ca based on the extraction solvent type. Other quality indexes were evaluated by common testing methods (AOAC, 1990; Wang et al., 2011; Yang et al., 2012): Vitamin C content was measured by the 2, 6-dichloroindophenol titrimetric method; soluble sugar was measured by the anthrone method; soluble protein was measured by the Coomassie brilliant blue method; nitrate content was measured by the ultraviolet spectropho-tometry method. For each treatment, thirty tomato fruits with a red or orange color were collected randomly to determine the basic morphological parameters, containing long diameter (L-diameter) and short diameter (S-diameter). Fruit weight was calculated from the total number and weight of fruits harvested.

2.2. Results

2.2.1. Calcium solubility in LAB experiment preparation of EM-Calcium mixtures

According to the survey results from the measured Ca^{2+} concentration, the Ca solubility was calculated and showed in Table 2. Results indicated that the measured Ca^{2+} concentration of T5 and T4 was significantly higher (P<0.05) than that of T1, T2, T3. The Ca solubility of T4 reached the peak value of 89.5%, which was 22.17%, 31.00%, 9.90%, 9.79% higher than that of T1, T2, T3, and T5 respectively. Therefore T4 was selected as the optimum formula applying to the following field experiment on account of high calcium solubility. The eventually diluted T4 solution used for the field experiment was acid, with the pH value of 4.7.

Treatment	Theoretical Ca²⁺ concentration	Measured Ca²⁺ concentration	Solubility
T1	3.0‰	2.02‰d	67.33%
T2	4.0‰	2.34‰c	58.50%
T3	5.0‰	3.98‰b	79.60%
T4	6.0‰	5.37‰ab	89.50%
T5	7.0‰	5.58‰a	79.71%

Means within a column followed by the same letter are not significant at P<0.05 (Duncan's multiple range test).

Table 2. Theoretical and measured Ca^{2+} concentration after 6 days fermentation

2.2.2. Forms of Ca in fruit

The Ca accumulation in tomato fruits can directly reflect the effect of EM-calcium solution. Fig. 1 gave the content of different forms of Ca in tomato fruits with different treatments. According to the survey results, the EM-calcium application was mainly beneficial for the accumulation of Alc-Ca, H_2O-Ca, NaCl-Ca, and HAC-Ca, and which had no obvious effects on the HCl-Ca and Res-Ca accumulation.

Ca pectate (NaCl-Ca) was the main Ca form presented in the ripe tomato fruits, occupied more than 75% of total Ca content, as the figures shown. The NaCl-Ca content of SR, SF, SL, SO was significantly higher ($P<0.05$) than that of CK, and the increases of SL were the most obvious, closely followed by SO, which implied that SL and SO were conductive to the accumulation of NaCl-Ca. While no significant difference ($P>0.05$) for NaCl-Ca content was observed between ST and CK. Water-soluble calcium The EM-calcium application also had great influences on the accumulation of water-soluble Ca including the Alc-Ca and H_2O-Ca in tomato fruits. Based on the results, SL increased the content of Alc-Ca and H_2O-Ca most significantly ($P<0.05$) by 2.19 and 0.71 multiples respectively in comparison with CK. Besides, SR, SF, SO and ST increased Alc-Ca content significantly ($P<0.05$), and SR, SF increased H_2O-Ca content significantly ($P<0.05$), compared to CK. The H_2O-Ca content in the tomato fruits of SO and ST was significantly different ($P<0.05$), but there were no significant differences between that of SO and CK, either ST and CK.

HAC-Ca content of SL and SO was significantly higher ($P<0.05$) than that of CK by 65.68% and 36.58% respectively, and the differences among CK, SR, SF and ST were not significant ($P>0.05$). Dissimilarly, HCl-Ca content in SF, SL, SO was 24.77%, 29.05%, 10.05% lower than that in CK, and the effects of ST and SR on the variation of HCl-Ca content were not obvious. Res-Ca accounted for the smallest share of total Ca, except for ST, no significant differences of which were found among the treatments ($P>0.05$), indicating that EM-calcium application had little effects on the Res-Ca accumulation in tomato fruits.

2.2.3. Fruit quality

Fig. 2 showed some quality indexes of tomatoes observed with different spraying methods. Vitamin C (L-ascorbic acid) is essential for all living plants where it functions as the main hydrosoluble antioxidant (Lima-Silva et al., 2012). SF, SL, SO increased the vitamin C of tomato fruit significantly ($P<0.05$) compared to CK, the increases of SL was most obvious,with the value of 25.38%. However, SR and ST did not affect the content of vitamin C greatly.

Tomato taste quality is largely determined by the contents of soluble sugar (Dorais, 2001). According to the results, the soluble sugar content of SL and SO was significantly higher ($P<0.05$) than that of CK by 9.65% and 7.20% respectively, but there were no significant differences ($P>0.05$) among that of CK, SR, SF and ST.

SL obtained the highest content of soluble protein, which was significantly higher ($P<0.05$) than that in CK. SO took the second place, slightly lower than SL but no significant differences
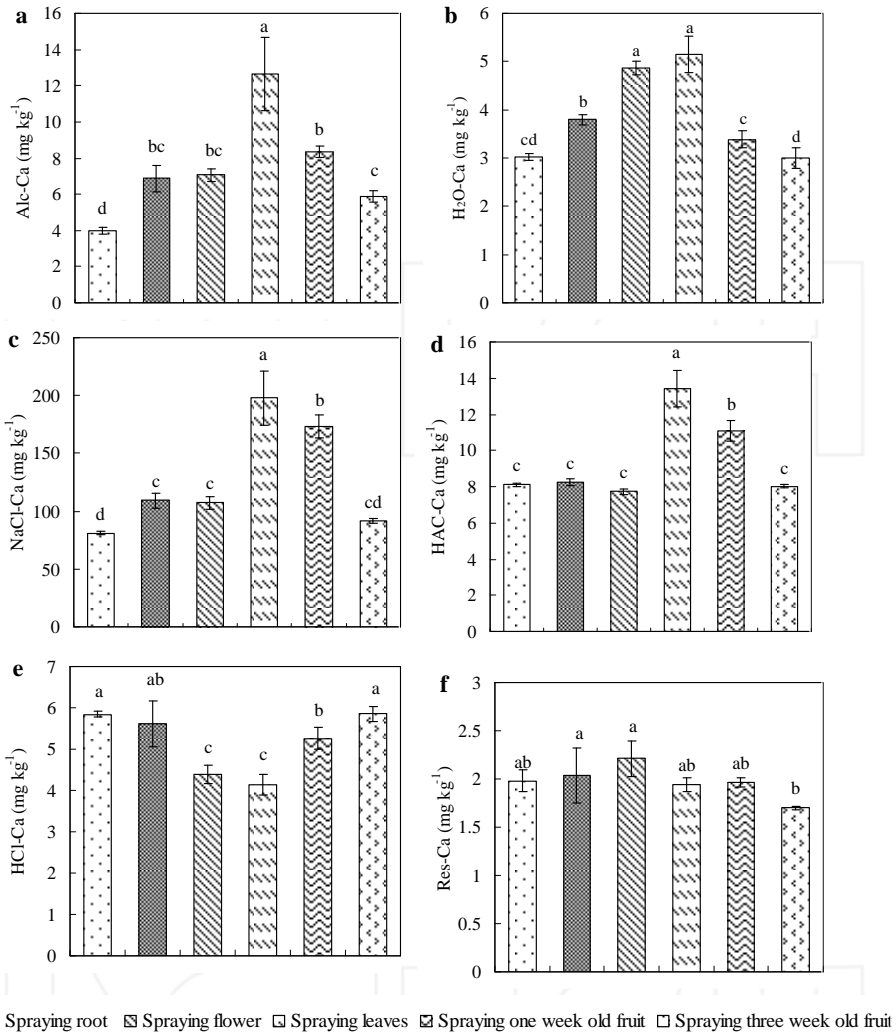
Figure 1. The accumulation of different forms of Ca in tomato fruits after the application of EM-calcium solution on different tomato organs. Columns with the same letter represent values that are not significantly different at the 0.05 level of probability according to the Duncan's multiple range test. Each value is the mean ± SD (n=3). The treatment symbols are the same as the experiment design.

(P>0.05) were found between them. SF and ST had little effects on the content of soluble protein in tomato fruits. Compared to CK, the nitrate content in the other treatments was significantly lower (P<0.05), and SL was particularly apparent. In terms of these basic quality indexes of tomato fruits measured, SL was suggested as the preferable treatment since the above quality indexes of SL were all at the satisfactory levels.
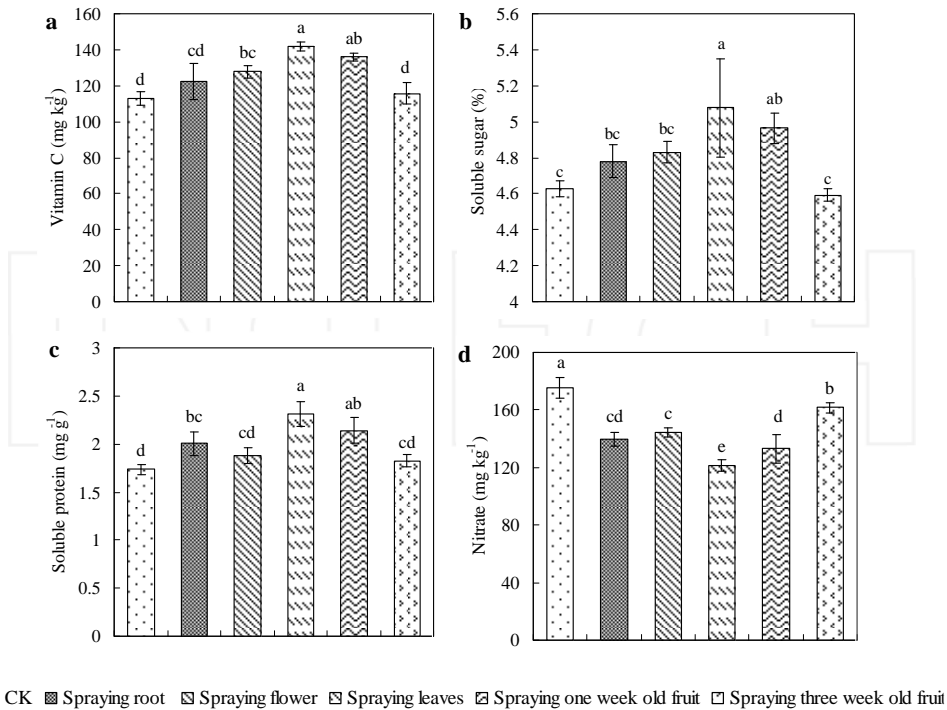
Figure 2. Effects of EM-calcium application on some routine indexes of tomato quality. Columns with the same letter represent values that are not significantly different at the 0.05 level of probability according to the Duncan's multiple range test. Each value is the mean ± SD (n=3). The treatment symbols are the same as the experiment design.

2.2.4. Fruit morphology, yield, and BER incidence

Fruit appearance is the first quality trait to consumers and determined by fruit size, shape and color (Labate, 2007). The tomato fruit size, yield and BER morbidity harvested from different treatments were displayed in Table 4. Results show that different treatments had little effect on the fruit size, maximum D-value of L-diameter, S-diameter, individual fruit weight was 0.46cm, 0.29cm and 17.8g/fruit respectively, which was found between SO and CK, this implied that the fruit size was mainly determined by the genetic cultivar but has few matters with exogenous Ca application. The highest tomato yield of 72.28 t ha^{-1} was found in SL, followed by SO, CK obtained the lowest yield of 50.26 t ha^{-1}, which was 22.02 t ha^{-1} lower compared to SL. Overall, SL processed obvious advantages in improving the tomato yield among the treatments. After applied with EM-Calcium, the BER incidence of tomato fruits was well controlled to different extents, according to the results. SL obtained the lowest BER incidence of 9.42% compared to the other treatments, followed by SR, recording as 10.28%. While ST had relatively less influences on the controlling of BER incidence, only 2.23% lower than CK.

Treatment	Fruit size			Yield (t ha⁻¹)	BER incidence (%)
	L-diameter (cm)	S-diameter (cm)	Weight (g/fruit)		
CK	6.18	4.66	116.70	50.26	22.81%
SR	6.42	4.90	128.60	56.42	10.28%
SF	6.43	4.91	128.76	60.84	18.51%
SL	6.60	4.94	133.85	72.28	9.42%
SO	6.64	4.95	134.50	68.09	13.22%
ST	6.40	4.86	126.11	53.38	20.58%

Table 3. Effects of EM-Calcium solution application on tomato fruit size, yield and BER incidence

2.2.5. Ca accumulation in main parts of tomato plant

Ca accumulations in main parts of tomato plant with different EM-Calcium treatments were displayed in Fig. 3; Ca content in upper leaf of SO, ST was significantly higher ($P<0.05$) than that of the other treatments, however, which of SL was slightly lower compared with CK, this indicated that spraying EM-Calcium on tomato leaves may change the migration path of calcium in the leaves; Similar laws were also found in the Ca accumulation of lower leaf, Ca content in lower leaf of SL was significantly lower ($P<0.05$) than that of the other treatments (except CK). According to the results from Ca content in upper leaf and lower leaf, it could be also inferred that SO had the most significant impact on the Ca accumulation of tomato leaves.

ST increased the Ca accumulation of root most significantly, and no significant differences ($P>0.05$) were observed among SF, SL and SO. Ca contents of stem were obviously lower than that of other plant organs, and the differences of which among the treatments were relatively less, Ca content in the stem of SO was significantly higher ($P<0.05$), and there were no significant differences ($P>0.05$) among the other treatments.
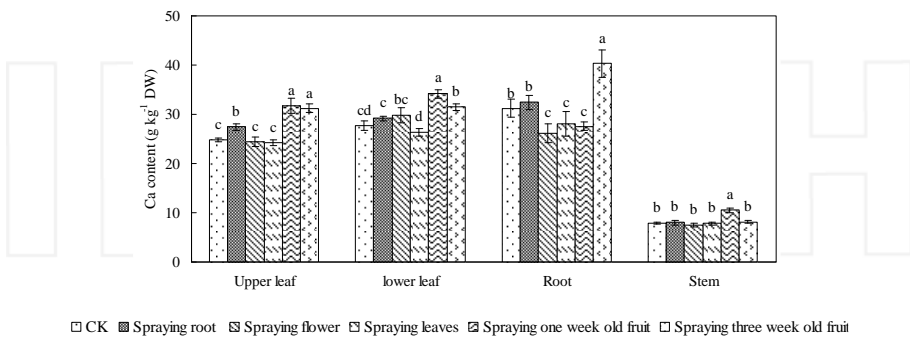
Figure 3. Effects of EM-calcium application on the Ca accumulation in main parts of tomato plants. Columns with the same letter represent values that are not significantly different at the 0.05 level of probability according to the Duncan's multiple range test. Each value is the mean ± SD (n=3). The treatment symbols are the same as the experiment design.

2.3. Discussions

Calcium is well known for having regulatory roles in metabolism and sodium ions may compete with calcium ions for membranebinding sites (Tuna et al., 2007), and is also known to bind to phospholipids and proteins on the membrane surface, which is required to maintain proper membrane structure and integrity (Jones and Lunt, 1967; Suzuki et al., 2003), thus to some degree, high calcium levels can protect the cell membrane from the adverse effects. In view of the important role calcium played, much emphasis was put on the exogenous Ca application for plants. In the choice of Ca nutrient solution for tomato, materials such as $CaSO_4$ $2H_2O$ (Hall, 1977), $CaNO_3$ $4H_2O$ (Eraslan et al., 2007; Murillo-Amador et al., 2006), $CaCl_2$ (Dong Cai-xia, 2001; Schmitz-Eiberger et al., 2002; Xu et al., 2010), CaO (Almeida et al., 2009; Asiegbu and Uzo, 1983) had been selected as Ca resources for different studies. Before preparing the Ca solution in this study, the activity of Ca ions and cost of the calcium solution were mainly taken into consideration during the selection process of materials, we tried to mixed the gypsum with EM previously, while it was found that the solubility of gypsum in EM was not satisfactory compared to that of lime, the lime was finally selected as the Ca resource. Before the experiment we also concerned about whether the Ca^{2+}suspension made by lime would change the survival environment of the acid-loving effective microbes, results proved later that the pH of the mixed liquor fallen back to suitable levels after several days' fermentation. Under microbial actions, the solubility of calcium in EM obtained a satisfactory result (maximum Ca^{2+}solubility of 89.50%), while with the increasing application of lime, EM, and molasses, the dissolving capacity of calcium presented a decline trend, it was predicted that the measured Ca^{2+}concentration of the EM-Calcium solution would stay in a certain value and the solubility of calcium would decrease with the increasing of raw materials. According to the results of the lab experiment, the mixture of Ca^{2+}suspension/ EM/ molasses/ DI water with a volume ratio of 3: 1.5: 1.5: 4 was recommended to practice.

Recent studies tented to adopt foliar-sprayed method on the exogenous Ca application for tomato plants (Eraslan et al., 2007; Gezerel, 1986; Murillo-Amador et al., 2006); there were also experiments (Tabatabaie et al., 2004) about the use of solutions of different concentrations applied to different parts of tomato root system. This experiment showed that the foliar-sprayed method was most beneficial for the Ca accumulation of tomato fruits according to Table 4, spraying EM-Calcium solution on plant leaves increased the Alc-Ca, H_2O-Ca, NaCl-Ca, HAC-Ca contents of tomato fruits more significantly than spraying on the other organs, increment of NaCl-Ca content (calcium pectate) was especially notable, this might be related to the increases of soluble pectin in tomato fruits with the ripening of tomatoes (Ashraf M, 1981). Spraying the calcium nutrition on surface of fruits such as litchi, sweet cherry and grape proved not to be an effective way (Combrink, 1995; Huang et al., 2008; Koffmann, 1996), while for tomato fruits in this experiment, results showed that EM-Calcium application had no significant effects on the Ca increment in old tomato fruit, while which had significant effects on the Ca increment in young tomato fruit.

Vitamin C acts as an antioxidant in plants and its levels are responsive to a variety of envi-ronmental or stress factors, for example light, temperature, salt and drought, atmospheric pollutants, metals or herbicides (Singh et al., 2012), and which was reported having positive

correlation with potassium (K) supply (Marin, 2009), Bangerth (1976) observed an increase in vitamin C content of tomato fruits treated with calcium chloride. In this experiment, we do not exclude the Ca factor when explained the improvement of tomato quality, while EM was more likely to be the main factor, since EM had been reported to have effects on enhancing crop photosynthesis, increasing crop protein contents, and improving crop quality (Daming, 1999; Shousong, 1998). Another evidence to support this speculation was that the soluble sugar correlated negatively with calcium (Beckles, 2012). Taken as a whole, EM-Calcium application significantly improved the fruit quality of tomato (evaluation indexes including vitamin C, soluble sugar, soluble protein, and nitrate); meanwhile, foliar-spray proved to be a preferable method when supplying EM-Calcium in this study.

The BER incidence may induced by the stresses in the root zone, such as salinity, soil water stress, NH_4^+ toxicity and oxygen withholding (Saure, 2001; Tachibana, 1991), although the impact mechanisms are not fully understood, these factors were considered either directly or indirectly related to Ca^{2+} deficiency: Tuna et al.(2007) reported that the exogenous Ca^{2+} application significantly improved growth and physiological variables affected by salt stress, from another perspective, Adams (1990) showed that increasing the salinity above 4mS cm^{-1} by addition of major nutrients would reduce Ca content; According to some studies (Albahou, 1999; Žanić et al., 2011), the increased proportions of NH_4^+ in standard nutrient solution were often associated with severity of blossom-end rot known as a physiological disorder of tomato fruit, and Siddiqi (2002) suggested that the NH_4^+ presence with a percentage of 10% in total N reduced Ca^{2+} accumulation; The BER occured commonly when the soil moisture content was deficit or fully adequate (Adams, 1992, 1993), and there was a minimum rate of transpiration relative to leaf growth rate below which calcium deficiency symptoms were occured (Hamer, 2003); Tachibana (1991) also reported that withholding the oxygen supply to roots at night was a cause of tomato BER, which greatly inhibited the absorption of Ca. The negative correlation between Ca nutrient supply and BER incidence was also found in this study, similar to many other studies (Besford, 1978; Mestre et al., 2012; Olle M, 2009). It was concluded here that the BER incidence of different EM-Calcium treatments was 2.23%-13.39% lower than that of CK.

Fig. 5 showed the yield of marketable tomatoes, which was significantly increased by the treatments except ST, Gezerel (1986) reported the application of foliar fertilizer containing calcium of 0.2% increased the tomato yields and fruit weight. Mayer (2010) inferred that the effects of EM preparation on crop yield increasing could be related to nutrient inputs by EM carrier substrates. Hu and Qi (2013b) reported that long-term effective microorganisms application could promote crop growth and increase yields and nutrition. In this study, we deduced that EM alone had no obvious effects on crop yield, while it could enhance the availability of nutrient supplied. Mestre et al.(2012) observed a negative and significant correlation between fruit yield and BER (r=-0.810), our results showed that the BER incidence had significant correlation with total tomato yield (r=-0.736) and marketable tomato yield (r=-0.862).

Early study (del Amor and Marcelis, 2006) reported that Ca concentration of tomato plant was significantly reduced by low-Ca supply (0.5 meq L^{-1}) compared with the nutrient standard

solution (9 meq L^{-1}), and with 14 days' low-Ca application, Ca concentration in all plant organs (leaves, stems and roots) was reduced by approximately 70% compared to control plants; we reported that 2.0‰ Ca application increased the Ca accumulation in upper leaf, lower leaf, root, stem to maximum rates of 28.09%, 23.50%, 29.15%, 33.34% compared to no-calcium treatment, the causes of the difference were probably related to the nutrient supply methods, calcium supply through roots increased the Ca content of tomato fruits by increasing which of other organs, while calcium spray on leaves or young fruits increased Ca content of tomato fruits by changing the Ca migration, the evidence to support this speculation in this study was that the foliar spray increased the Ca content of fruits but decreased which of leaves. Dong (2001) guessed that a "Ca-attracted" center was formed in the spray organ when spraying Ca nutrient (CaCl$_2$), Ca^{2+} was attracted to the center and then migrated from the center to the organ which needed Ca most. However, the migration mechanism about Ca migration with exogenous Ca application needed to be examined by ^{45}Ca tracing technique (Behling et al., 1989; Yamauchi et al., 1986).

3. Effects of EM-Calcium on production of flue-cured tobacco

3.1. Materials and methods

3.1.1. Test site

The experiments were carried out in a plastic sheet covered greenhouse from March 2013 to September 2013 in the Vegetables and Flowers Institute of Nanjing (latitude 31°43' N, longitude 118°46'E), China. The average annual rainfall is about 1106.5mm, with the rainy season from the end of June to the middle of July, and the average yearly temperature is approximately 15.7℃ and average humidity is about 81%. The soil characters were: pH 5.68, 14.47 g/kg organic matter, 28.5 mg/kg available P, 153.84 mg/kg available K, 1.3 8g/kg total nitrogen.

3.1.2. Experimental design

K326 was chosen as the flue-cured tobacco plant material, with young plants elaborately cultivated in made-in-order seedling trays; then they would be transplanted into the lysimeters when they grew 6 expanded leaves. The planting density was 12 plants per treatment, with the line spacing of 0.8m and plant spacing of 0.6m. After that, conventional field management was conducted in the first week.

For simulating the water stress in growing stages, the irrigation amount was designed as 400mm during the whole growth stage. Irrigation waters of root-extending stage, vigorous stage and maturity stage in this experiment were assigned as 40%, 20%, 40% for the total water consumption respectively, and they were irrigated 6 times in each growth stage. Tobacco dedicated fertilizers (provided by the Institute of Guizhou Tobacco Science, N: P$_2$O$_5$: K$_2$O=1:2:3) were applied according to a proportion of basal dressing: topdressing=7:3, the latency time of topdressing was 26 days after transplanted.

Detailed experimental treatments regarding the water-retaining agent amount and EM-calcium content were shown in Table 4. EM-calcium with 2.0‰ Ca^{2+}concentration was prepared as 2.2.1.

Treatment	Ca^{2+} concentration	EM-Ca amount (ml/time)	Intervals of spraying (days)	MP3005 (g/plant)	Growth stage
CK	---	---	---	---	---
P1	1‰	2	3	0	Root extending (R)
P2	1‰	2	3	0	Vigorous (V)
P3	1‰	2	3	0	R+V
P4	1‰	2	3	30	R
P5	1‰	2	3	30	V
P6	1‰	2	3	30	R+V

Table 4. Experimental design

3.1.3. Measurements

3 representative tobacco plants were sampled for each replication. At harvest time, the lower leaves, middle leaves and upper leaves were collected orderly, killed by 105℃ high temperature and toasted to the constant weight (Hou et al., 2012; Maomao Hou, 2013). Weight of dry tobacco leaves was measured and recorded to calculate the total yield.

IWUE was calculated by the formula (Aujla et al., 2005; Ünlü et al., 2011):

$$IWUE = \frac{Y}{I}$$

Where, *IWUE* (kg/m^3) was the irrigation water use efficiency; *Y* was the tobacco dry yield (kg/hm^2); I was the irrigation amount (m^3/hm^2).

3.2. Results

3.2.1. Agronomic characters of flue-cured tobacco

Fig. 4 showed the changes of total area of flue-cured tobacco leaves in single plant with days after transplanted. As shown in the Figure 4, during 45~77 days, flue-cured tobaccos in P4 grew more satisfactory compared to that in other treatments, and the leaf area of single plant in T4 reached a higher value of 36897.9 cm^2 in 77 days. However, P2 showed a poor performance in leaf area enhancement, recording as 32110.1 cm^2 only. From the view of leaf area enhancement, the effects of water-retaining agent treatments were obviously better than those with no water-retaining agent. Under the same application amount of water-retaining agent, the effects of EM-calcium spray in root-extending stage were better than those in vigorous stage and root-extending+vigorous stage. The results were different from the expectation, it was maybe that the EM-calcium spray blocked the leaf stoma after long-time application. On the whole, P4 was supposed to be the better treatment from a pure view of leaf area increasing.
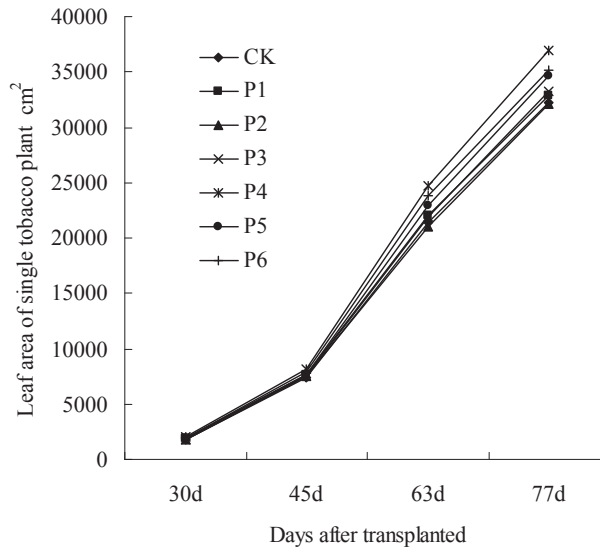
**Figure 4.** Leaf area increasing with days after transplanted

Table 5 displayed the plant height and stem girth with days after transplanted under different treatments. As shown from the Table 5, no obvious differences were found among the treatments, this was mainly because the fertilizer application was equal in different treatments. At 77 days after transplanted, the plant height of different treatments were recorded as 105.4~122.0 cm and the plant stem girth were around 6.9~7.4 cm.

Treatment	Height (cm)				Stem (cm)			
	30d	45d	63d	77d	30d	45d	63d	77d
CK	9.2	38.7	84.5	109.9	3.1	4.3	6.0	7.0
P1	10.5	39.6	86.2	110.6	3.2	4.3	6.3	7.2
P2	10.2	35.8	83.9	105.4	3.1	4.0	5.9	6.9
P3	11.1	41.2	88.0	113.9	3.3	4.4	6.2	7.3
P4	12.8	43.9	97.4	122.0	3.7	4.8	6.5	7.4
P5	11.4	41.2	91.8	108.5	3.4	4.5	6.3	7.1
P6	12.3	42.5	96.9	119.8	3.4	4.6	6.6	7.2

Table 5. Plant stem girth and height with days after transplanted

3.2.2. Chlorophyll content of tobacco leaves

Fig. 5 showed the dynamic changes of chlorophyll content in tobacco leaves under different treatments more visually, chlorophyll content in tobacco leaves was decreased with a higher rate at the later growth stage. The chlorophyll content of tobacco leaves with water-retaining agent was slightly lower than that with no water-retaining agent, at 63 d after transplanted, the chlorophyll content of tobacco leaves under different treatments was recorded as 1.69 mg/g-1.85 mg/g; at 87 days after transplanted, the chlorophyll content of tobacco leaves was in the lowest level of 0.97 mg/g-1.12 mg/g.
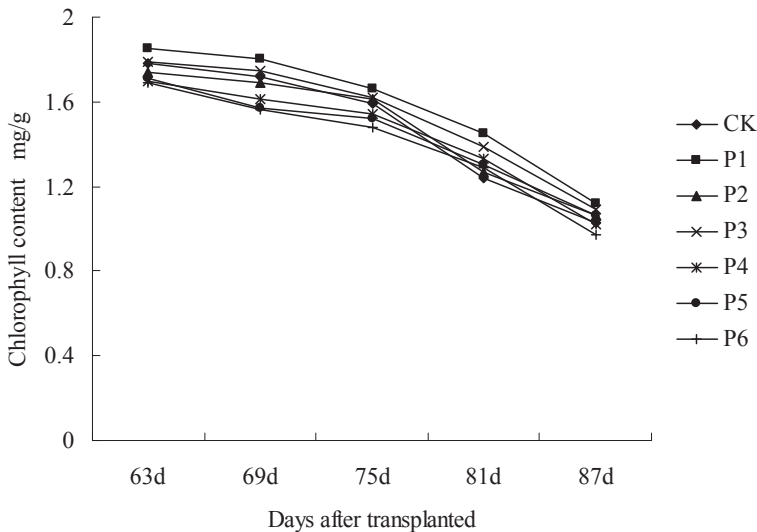
Figure 5. The changes of chlorophyll content in tobacco leaves varying with days after transplanted

3.2.3. Dry matter accumulation

Table 6 showed the dry matter accumulation of different tobacco organs and the total yield under different treatments. From the table it was found that the dry matter amount of leaf and stem and root in P4 was the highest, recording as 73.6 g, 88.1 g and 164.9 g respectively. The dry matter amount of leaves with water-retaining agent treatments were 4.99%-14.28% higher than that with no water-retaining agent application. Under the same amount of water-retaining agent application, EM-calcium spray in root-extending stage was much better than that in other stages. Dry matter amount of leaf in P1 and P4 was higher than that of other treatment, and the tobacco yield of P4 was highest, recording as 2473.5 kg/hm^2, followed closely by P6, which of P2 was the lowest.

Treatment	Root		Stem		Leaf		Whole Plant(g)	Yield (kg/hm²)
	Dry matter(g)	Proportion (%)	Dry matter (g)	Proportion (%)	Dry matter (g)	Proportion(%)		
CK	63.2	21.76%	82.9	28.55%	144.3	49.69%	290.4	2164.5
P1	62.1	21.01%	85.4	28.89%	148.1	50.10%	295.6	2221.5
P2	58.7	21.43%	75.4	27.53%	139.8	51.04%	273.9	2097.0
P3	64.5	22.13%	80.5	27.62%	146.5	50.26%	291.5	2197.5
P4	73.6	22.54%	88.1	26.97%	164.9	50.49%	326.6	2473.5
P5	67.7	22.76%	78.3	26.32%	151.5	50.92%	297.5	2272.5
P6	68.7	22.50%	77.9	25.52%	158.7	51.98%	305.3	2380.5

Table 6. The dry matter accumulation of different tobacco organs and the total yield under different treatments.

3.2.4. Nutrient absorption

Calcium is well known to maintain proper membrane structure and integrity and plays important roles in crop development and disease control (Almeida et al., 2009; Berry et al., 1988; Evans, 1953; Hall, 1977). In flue-cured tobacco cultivation, calcium helps to coordinate the physiology function of tobacco plant, making the tobacco plant root system stronger, growing vigorously and harvesting timely. Ca^{2+} has promoting effects for the growth of tobacco seedlings and can improve the drought-resistant ability of tobacco seedlings. In addition, the high calcium content in tobacco leaves delays the maturity of tobacco, characterized by stiffness and hardness of the tobacco leaves, thus the use value of tobacco leaves is decreased, excess calcium may also cause disorder of some microelements in tobacco plants and produce toxic impacts; while calcium deficiency can lead to the deformity of tobacco plants and generate a spoon-shaped reverse disease of tobacco leaves. As was shown in Fig. 6, the exogenous application of calcium significantly increased the calcium content in tobacco leaves, and the effects of spraying in root-extending stage were more satisfactory than those in other stages, this may be related to that the water stress in maturity affected negatively the effects of EM-calcium.

3.2.5. Irrigation water use efficiency

Fig. 7 showed the *IWUE* of flue-cured tobacco plants with different treatments. It could be seen that the differences of *IWUE* among the treatments were more significant, P4 obtained the highest *IWUE* of 0.618 kg/m³, followed by P6, and *IWUE* value of T2 was the minimum, recording as 0.524 kg/m³. Since the equal irrigation amount among the treatments, *IWUE* presented a positive relationship with the flue-cured tobacco yield.
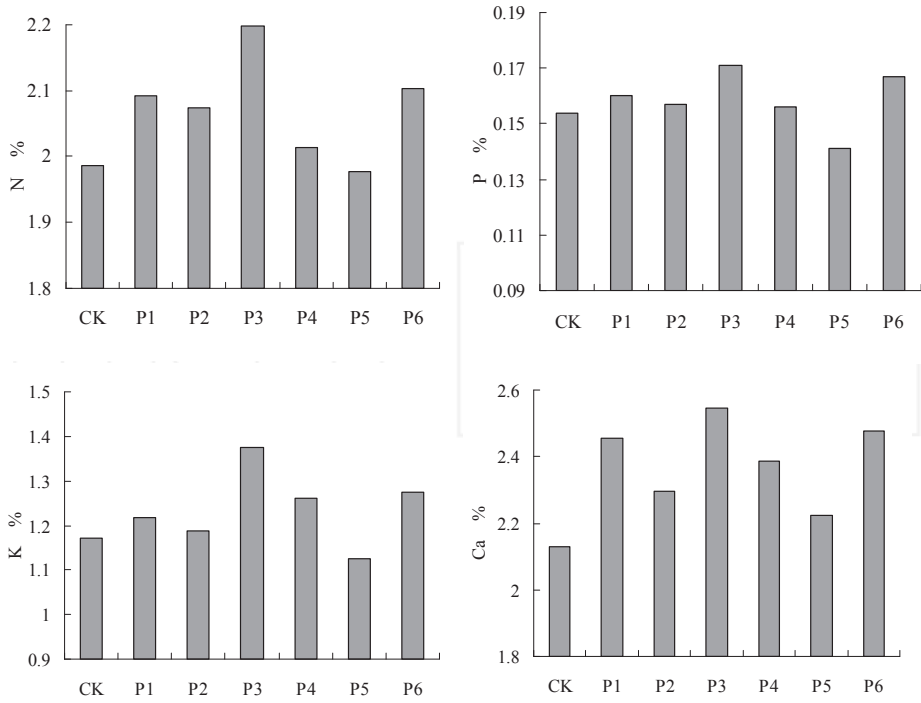
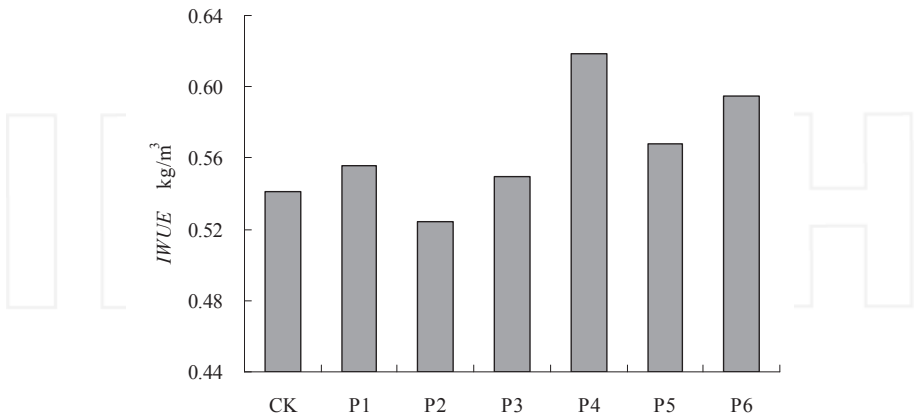**Figure 6.** Nutrient absorption of tobacco leaves with different treatments

Figure 7. Irrigation water use efficiency with different treatments

3.2.6. Optimal selection of the best management scheme

Table. 7 showed the evaluation indexes of different treatments including the tobacco yield, *IWUE*, Ca content in tobacco leaves and the cost, among these indexes, the cost was calculated based on the price list of raw materials provided by EMRO Limited Company, Nanjing Branch. Table. 7 showed that the advantages and disadvantages of each treatment were distinct, taking P1, P2 and P3 as example, although the cost of them was lower, the yield and *IWUE* were lower than those of P4,P5 and P6; Ca content of tobacco leaves with P3 treatment was the highest, reaching 2.546%, but the yield of P3 was not in the highest level. Therefore, a scientific evaluation method was needed here to select the optimal management scheme.

Treatment	Yield (kg/hm²)	IWUE (kg/m³)	Ca (%)	Cost (USD/plant)
CK	2164.5	0.54	2.131	0
P1	2221.5	0.56	2.454	0.0033
P2	2097.0	0.52	2.297	0.0033
P3	2197.5	0.55	2.546	0.0067
P4	2473.5	0.62	2.387	0.0167
P5	2272.5	0.57	2.224	0.0167
P6	2380.5	0.60	2.478	0.0200

Table 7. Evaluation indexes

Modeling approach was shown below (Chen and Li, 2010; Chou et al., 2012):

Supposing that there were n evaluation indexes and m schemes, m schemes corresponding with n indexes obtained the following matrix:

$$R = (r_{ij})_{m \times n}$$

Where; r_{ij} is the jth evaluation index of the ith scheme. To r_j, there was information entropy:

$$E_j = -\sum_{i=1}^{m} p_{ij} \ln p_{ij}, \ (j=1, 2, 3, \dots n)$$

And P_{ij} were calculated as the formula:

$$p_{ij} = r_{ij} \Big/ \sum_{i=1}^{m} r_{ij}$$

The entropy value of jth index was:

$$e_j = \frac{1}{\ln m} E_j, \ (j=1, 2, 3, \dots, n)$$

The objective weight of jth index was:

$$\theta_j = (1 - e_j) \Big/ \sum_{i=1}^{n} (1 - e_j), \quad (j = 1, 2, 3, \ldots, n)$$

It was clear that:

$$0 \le \theta_j \le 1; \quad \sum_{j=1}^{n} \theta_j = 1$$

This study took the subjective information into the calculations, the comprehensive weight could be obtained by combining the subjective weight w_1, w_2, $w_3 \ldots w_n$ of the decision makers with the objective weight θ_j ($j=1,2,3,\ldots,n$):

$$\alpha_j = \theta_j \overline{\omega}_j \Big/ \sum_{j=1}^{n} \theta_j \overline{\omega}_j, \quad (j = 1, 2, 3, \ldots, n)$$

Recording the optimum value of each row as r_j^*, normalize the elements in the matrix, and r_j^* value was varied with the index characters. The indexes could be divided into two classes: The profitable indexes and the damnous indexes, which were "the larger the better" and "the smaller the better", listing as follows:

$$d_{ij} = \begin{cases} \dfrac{r_{ij}}{r_j^*}, & r_j^* = \max\{r_{ij}\} \\ \dfrac{r_j^*}{r_{ij}}, & r_j^* = \min\{r_{ij}\} \end{cases}$$

The entropy coefficient value (A better management scheme would obtain a higher entropy coefficient value) of each treatment could be calculated by:

$$\lambda_i = \sum_{j=1}^{n} \alpha d_{ij}, \quad i = 1, 2, 3, \ldots, m.$$

The calculated objective weight of the tobacco yield, *IWUE*, Ca content in tobacco leaves and the cost were 0.2142, 0.2135, 0.2003, and 0.3720. However, since the strict requirement on water saving, the subjective weight of which was assigned as 0.4, 0.4, 0.15, 0.05 respectively. Fig. 8 showed the entropy weight coefficient of different treatments, based on the principle of entropy weight coefficient "the higher the better", P4 was supposed to be the best scheme, in other words, 30 g/plant MP3005 water-retaining agent combined with EM-calcium spray (‰1 Ca^{2+}) during the root-extending stage of flue-cured tobaccos was the optimal management scheme, and the interval of spraying time was 3 days with 2 mm each time on the back side of tobacco leaves. Additionally, entropy weight coefficient value of P1 and P4 were similar, but the mechanism was different, P1 tended to obtain a lower cost, and P4 tended to obtain a higher yield and *IWUE*.
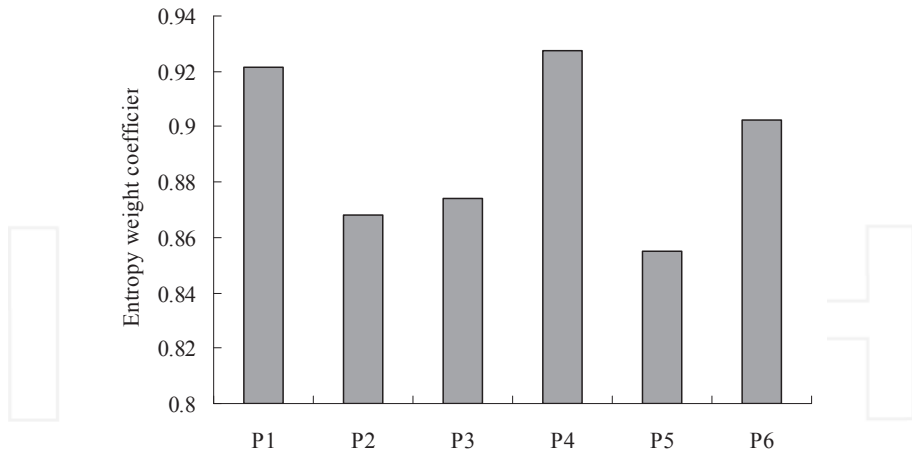
Figure 8. Entropy weight coefficient of different treatments

3.3. Conclusions

Treatment P4 (spraying 1‰ EM-Ca amount 2 ml with 30 g MP3005 per plant once in 3 days during tobacco root extending stage) obtained the highest flue-cured tobacco yield of 2473.5 kg/hm^2, followed by P6, and yield of P2 was lowest, recording as only 2097.0 kg/hm^2. The irrigation water use efficiency of P4 was highest, reaching 0.618 kg/m^3. Exogenous Ca supply significantly increased the Ca content in tobacco leaves. The evaluation results of entropy weight coefficient evaluation model showed that P4 was the best management scheme, that was to say, 30 g/plant MP3005 water-retaining agent combined with EM-calcium spray (1‰ Ca^{2+}) during the root-extending stage of flue-cured tobaccos was the optimal management scheme, and the interval of spraying time was 3 days with 2ml each time on the back side of tobacco leaves.

4. General conclusions

The research results showed that **Active** EM-Calcium could promote crop growth, improve the yield and disease resistance of crops. Main conclusions could be drawn as below:

1. The application of **Active** EM-Calcium increased the Ca accumulation in upper leaf, lower leaf, root and stem to maximum rates of 28.09%, 23.50%, 29.15%, 33.34% compared to no-calcium treatment. The BER incidence of **Active** EM-Calcium treatments was lower than that of CK. The BER incidence had significantly negative correlation with total tomato yield (r=-0.736) and marketable tomato yield (with no BER) (r=-0.862).

2. Exogenous Ca supply significantly increased the Ca content in tobacco leaves. Treatment P4 (spraying 1‰ EM-Ca amount 2 ml with 30 g MP3005 per plant once in 3 days during tobacco root extending stage) obtained the highest flue-cured tobacco yield of 2473.5 kg/hm^2, and the highest irrigation water use efficiency with 0.618 kg/m^3.

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