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DEGRADATION OF CYPRODINIL ON SPINACH BY EO WATER IN COMBINATION WITH SUPERSONIC TREATMENTS

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Abstract

The improved degradation rate of cyprodinil on surface of fresh spinach by EO water is reported. The spinach was treated with DI water, EO water, H_2O_2, EO water/H_2O_2, DI water/super sound, EO water/ super sound, H_2O_2/super sound and EO water/H_2O_2/super sound. The EO water/ supersonic treatment improved the degradation rate of cyprodinil significantly and have the highest degradation rate. Single factor experiments were used to improve the degradation rate. Pelatorate (ACC), volume of EO water, supersonic time and pH were found to affectthe degradation rate of cyprodinil. The increase of ACC, volume of EO water and supersonic time could increase the degradation rate. EO water at pH 2.8 was the optimal condition to remove the cyprodinil. The degradation rate of cyprodinl was 82% at ACC 100 mg/l, 100 ml of EO water, pH 2.8 and 15 min supersonic time. Multiple treatments of spinach also improved the degradation rate of cyprodinil.

Introduction

Cyprodinil (cyclopropyl-6-methyl-N-phenyl-2-pyrimidinamine, a pyrimidinamine fungicide has been developed and introduced by Novartis Crop Protection AG to the market (Vaquero-Fernándezet *al.* 2008), to protect the fruits and vegetables from a wide range of pathogens.

Residual behavior of the pesticide on edible portion of vegetables and fruits has drawn substantial interests (Maddalena *et al.* 2007, Pan *et al.* 2009). Studies on the degradation of the cyprodinil on vegetables (Pose-Juan *et al.* 2006, Maddalena *et al.* 2007) and soil (Pose-Juan *et al.* 2011, Liu *et al.* 2011) have been reported in literature. Cabras *et al.* (1997) studied the fate of cyprodinil from the treatment on vine to the production of wine and found that grape processing into wine caused considerable residue reduction with cyprodinil. The degradation rates of cyprodinil on grapes, spinach, and snap peas by electrolyzed water have also been studied (Qi *et al.* 2015).

Electrolyzed water is generated by electrolysis of 0.1% aqueous solution of sodium chloride in a container separated to anode and cathode compartments by a polystermembrance, producing acidic water (EO water) and alkaline water (ER water), respectively (Park *et al.* 2009).

The available chlorine content could reach more than 10 ppm in acid water with at pH 2.3-2.7 (Kim *et al.* 2000). The EO water treatment is of particular interest and become a non-heating method of sterilization. The EO water has been shown to effectively inactivate foodborne pathogens such as *Escherichia colii* O157: H7, *Salmonella enteritidis, Listeria monocytogenes, Bacillus cereus* and *Salmonella* species (Kim *et al.* 2003, 2004, Stevenson *et al.* 2004). It has been reported to reduce the germination of molds (Buck *et al.* 2002) and have a suppression effect on hepatitis B virus (Morita *et al.* 2000). The EO water has also been evaluated for its ability to reduce pathogenic bacteria on fresh vegetables and fruits (Koseki *et al.* 2004, Guentzel *et al.* 2008). When the use of EO water treatment has been extended in the food industry, the EO water also can

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remove the pesticide on fresh food. Lin *et al.* (2006) found that 15 min EO water treatment with ACC 50 mg/l achieved 38.2 and 34.7% reductions of methamidophos and dimethoate, respectively on snap beans. Qi *et al.* (2016) has found that EO water at ACC 120 mg/l with 15 min treatment achieved reductions of 37.1, 31.5, and 49.4% on grapes; 59.2, 43.8, and 85.7% on spinach; 66.5, 50.0, and 73.0% on snap peas for diazinon, cyprodinil, and phosmet, respectively. However, the removal rate of cyprodinil by EO water is relatively low. The purpose of this work is to combine other treatments with EO water to improve the removal rate of cyprodinilon the surface of spinach.

Materials and Methods

Cyprodinil was obtained from Sigma-Aldrich (St. Louis, MO, USA) and has a purity of over 99.9%. Magnesium sulfate anhydrous (USP grade) and sodium chloride (ACS grade) were provided by Amresco (Solon, OH, USA). Acetonitrile was of HPLC grade and collectedfrom EMD Millipore Corporation (Billerica, MA, USA). Primary secondary amine (PSA) was procured from Agilent Technologies (Santa Clara, CA, USA). LC/MS grade formic acid was obtained from Fisher Scientific (Fair Lawn, NJ, USA) and with a purity of 99.5%). ACS grade hydrogen peroxide, 30% (w/w), was of reagent grade from (Fair Lawn, NJ, USA).

Cyprodinil working solutions (80 mg/l) were prepared in acetonitrile and stored in amber glass bottles at $4^{o}C$. HPLC grade water was obtained with a Sybron/Barnstead NANO pure II system.

EO water were produced from 0.1% aqueous NaCl solution with an electrolyzed water generator (Primacide P-5000, Primacide, UT, USA). When a stable reading was achieved, the EO water was collected and the pH of EO water was measured using an Accumet pH meter (AR50, Fisher Scientific, Pittsburgh, PA, USA). The pH of EO water was adjusted to what by 1N HCl or 1N NaOH. The available chlorine content (ACC) of EO water was determined using a DPD-FEAS titrimetric method (Hach Co., Loveland, Colo., USA) and Deion water (DI water) was used to dilute EO water to the target values of ACC.

The young spinach leaves placed in clear plastic containers were purchased from local grocery store and kept at 4ºC for use within 24 hrs. The spinach did not contain the cyprodinil to be tested. Spinach leaves portions (10 g) were individually spray inoculated with 1 ml working solution of what using a 1 ml micropipette, and the spinach leaves were air dried for 2 hrs in a fume hood at room temperature.

Each of 10 g spinach leaves containing cyprodinil was soaked in 100mL DI water, EO water, hydrogen peroxide solution and EO water containing hydrogen peroxide, and then treated with supersonic method immediately for 10min, namely as DI water/ supersonic treatment, EO water/ supersonic treatment, H_2O_2/supersonic treatment, EO water/H_2O_2/supersonic treatment, respectively. For the comparison, each of the 10g spinach leaves containing cyprodinil was soaked in 100mL DI water, EO water, hydrogen peroxide solution and EO water containing hydrogen peroxide, namely as DI water treatment, EO water treatment, H_2O_2 treatment, EO water/H_2O_2 treatment, respectively, for 10 min. The concentration of hydrogen peroxide and ACC were 100 mg/l.

After the treatment, all spinach samples were immediately transferred on filter paper to be dried for about 2 hrs at room temperature before pesticide extraction. Aliquot of solution (10 ml) was transferred into 50 ml screw-capped tube as water phase.

The removal rate of what with DI water treatment was calculated by following equation:

$$\text{Removal rate} = \frac{C_t - C_d}{C_d} \qquad (1)$$

where, C_t is the concentration of cyprodinil on spinach after the treatment except DI water and C_d is the concentration of cyprodinil on spinach after treatment by DI water.

The treatment was tested by combining the EO and supersonic vibration for different treatment time (1, 5, 10, 15 or 20 min) at different pH (2.3, 2.8, or 3.4) and at different volumes of the EO water (60, 80, 100, 120 ml) and at different ACC (60, 100 and 140 mg/l). After the desired supersonic treatment, the spinach was transferred on filter paper immediately to be dried before pesticide extraction. Untreated spinach samples withcyprodinil were analyzed to determine the initial cyprodinil concentration.

The degradation rate of cyprodinil was calculated by the following equation:

$$\text{Degradation rate} = \frac{C_l - C_b}{C_b} \qquad (2)$$

where, C_l is the concentration of cyprodinil on spinach after the treatment; C_b is the concentration of cyprodinil on spinach without treatment.

Cyprodinil containing spinach was treated with EO water at pH 2.8, 100 mg/L of ACC, 60 ml of volume for 5 min supersonic exposure, for one, two and three times, namely as T1, T2 and T3, respectively. After each treatment, the spinach was quickly separated with EO water after the desired supersonic time and new EO water was added. For comparison, the spinach samplescontaining cyprodinil was treated with180 ml of EO water, 15 min supersonic time, pH 2.8 and 100 mg/l of ACC were also tested, and was designated as T4. The degradation rate of cyprodinil was calculated by the Eq. 2.

Ten g spinach and 50 ml DI water were transferred to a blender (Hamilton Beach, Mexico) and was homogenized into puree. Then, 10 g subsamples from the blended puree were transferred to a 50 ml screw-capped tube as cyprodinil phase.

Acetonitrile (10 ml) was added into the tube with water or cyprodinil phase, followed by vigorous shaking for 1 min on a vortex mixer. Then 1 g NaCl and 4 g $MgSO_4$ were added to the tube and was shaken vigorously for 1 min and centrifuged at 4,000 rcf for 3 min. After centrifugation, 5 ml aliquot of organic layer was placed in a 10 ml tube with 300 mg of $MgSO_4$ and 100 mg of PSA followed by vigorous shaking for 1 min. Then about 2 ml of supernatant was filtered through a 0.2 µm nylon syringe filter (Restek, Bellefonte, PA, USA) into a HPLC autosampler vial and then injected for UPLC-MS/MS analysis (Anastassiades *et al.* 2003).

The cyprodinil was determined by an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). A triple-quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) with an ESI source operated in positive ion mode was employed. An acquity UPLC Bridged Ethylene Hybrid (BEH) C18 reverse-phase column 1.7 µm (2.1 × 50 mm) was employed, fitted with an Acquity UPLCI-Class System (Waters Corp., Milford, MA, USA). Mobile phase A was acetonitrile and mobile phase B was 0.1% formic acid in HPLC grade water. The flow-rate was 1 mlymin. B concentration gradient used was 0 - 5 min, 0%; 5 - 10 min, 0 - 30%; 10-110 min, 30 - 00%; 110 - 120 min, 100%. The injection volume was 3 µl, and the flow-rate was 0.3 ml/min with linear gradient elution. The concentration gradient was 0 to 0.1 min, from 10 to 35%; 0.1 to 4.0 min, 35 to 90%; 4.0 to 4.1 min, from 90 to 10%; 4.1 to 6.0 min, holding at 10%.

The column temperature was kept at 40ºC. The mass spectrometer settings used were: capillary voltage 3.0 kV, cone voltage 35 V, source temperature 120ºC, and desolvation temperature 350ºC. High purity nitrogen was used for desolvation and nebulization. The nebulizer gas flow rate was set at 50 L/hrs while the desolvation gas flow rate was set to 500 l/hrs. The concentration of cyprodinil was calculated by five-point external standard curves.

Each treatment with 3 replicates were taken for cyprodinil analysis and averaged to take representative cyprodinil concentrations. Means and standard deviations were obtained using Excel (Micro software, USA). Duncan's multiple range test was chosen for one-way analysis of variance (ANOVA)as PRO ANOVA procedure to compare means using spss software 22.0 (IBMCorp., Armonk, New York, USA). A $p \leq 0.05$ was considered to be significantly different.

Result and Discussion

Fig. 1 shows the removal rate of cyprodinil on surface of spinach compared to the DI water by different treatments and Table 1 shows the cyprodinilthat remained in the treated solution. Although hydrogen peroxide usually was used as oxidant, it seems that it had little effect on the cyprodinil on surface and in treated solution. The removal rate of cyprodinil on spinach by hydrogen peroxide was almost the same to that by DI water. Combing with supersonic treatment, the DI water can remove almost 10% higher than DI water did alone, and more cyprodinil was transferred into the treated water. During treatment with EO water alone, the removal rate was also 10% higher than that of DI water, and the cyprodinil in the treated EO water cannot be determined. With the hydrogen peroxide or/and supersonic treatments, the removal rate of cyprodinil on the spinach by EO water was 50% higher than that by the treatment of EO water alone, and the removal rate was highest by EO water combining with supersonic treatment, which almost reached 58%. Moreover, the cyprodinil also cannot be determined in those treated water, which mean the cyprodinil transferred from spinach to solution was reacted with EO water and completely degraded in this experiment situation. This is in accordance with the results obtained in Qi's study (Qi *et al.* 2015) that the EO water can degrade the cyprodinil.

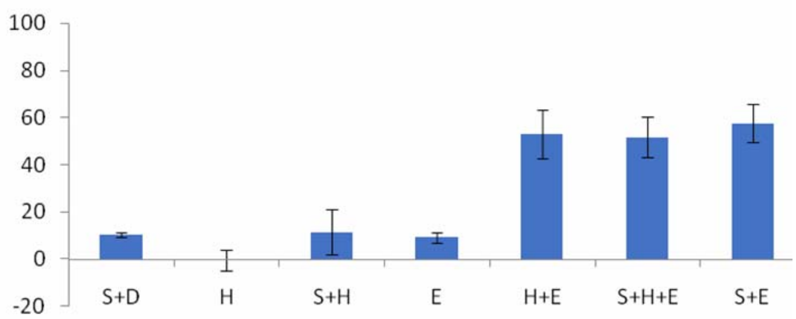

Fig. 1.Removal rate of cyprodinil changing with the different treatment method.

S+D, DI water/supersonic treatment, H, H_2O_2 treatment, S+H, H_2O_2/supersonic treatment, E, EO water treatment; H + E, H_2O_2/supersonic treatment, S + H + E, H_2O_2/EO water/supersonic, S + E, EO water/ supersonic treatment.

From the above experiment, one can know that the supersonic treatment could significantly help improving the removal rate of the cyprodinilfrom surface of spinach. Hence, EO water combined with supersonic treatment was carried out in the following experiments to improve the degradation rate of cyprodinil on spinach.

Fig. 2 showed the degradation rate of cyprodinil on spinach after 15 min supersonic treatment and EO water (100 ml) at pH 2.80 with different ACC. When the ACC increased from 0 to 100 mg/l, the degradation rate changed significantly from 10% to 81% ($p \leq 0.05$). No significant ($p> 0.05$) difference of degradation rate was found by increasing ACC from 100 to 140 mg/l. These are consistent with the results of Qi *et al.* (2015) that ACC was shown to be significant factors in removing cyprodinil on spinach and higher ACC of EO water always resulted in higher pesticide reductions.

Qi *et al.* (2015) also found that cyprodinil was less sensitive to EO water degradation than diazinon and phosmet. The degradation rate of cyprodinil on spinach (compared to sample without treatment) was 43.8% in 15 min treatment with ACC 120 mg/l by EO water treatment alone. Combined with supersonic treatment, the degradation rate reached 82% with the same treatment.

Table 1. The cyprodinil transferred from spinach to treated water phase.

	D	S+D	H	S+H	E	H+E	S+E+H	S+E
Concentration (ppm)	9.12×10^{-2}	1.66×10^{-1}	1.11×10^{-1}	1.45×10^{-1}	-	-	-	-

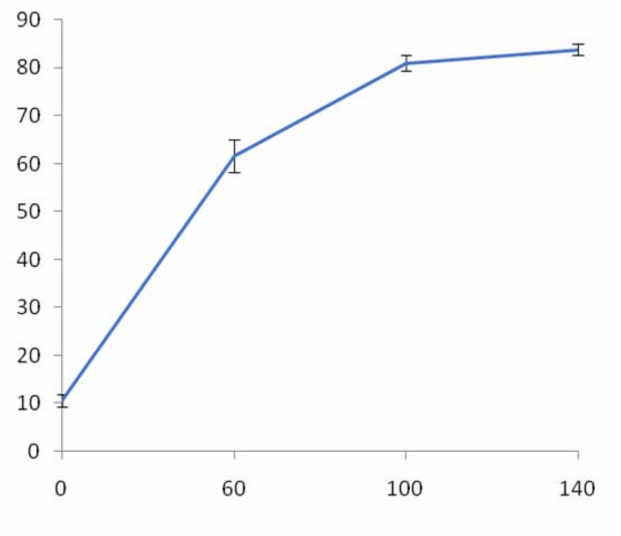

Fig. 2. Degradation rate of cyprodinil changing with ACC.

Fig. 3 shows the degradation rate of cyprodinil on spinach after 15 min supersonic time and 100 mg/l ACC at pH 2.80 with different volume of EO water as well. When the volume increased from 60 to 120 ml, the degradation rate increased from 73 to 82%. Then 60 ml of EO water was chosen as the lowest volume, because 10 g spinach could be soaked into the EO water completely. However, the spinach could float on the water, especially with the increase of volume, some glass circles had to be put on the spinach to make the spinach soaked into the EO water. Considering the degradation rate of cyprodinil and cost of treatment, 100 ml of EO water was chosen.

Supersonic treatments for 1, 5, 10, 15 and 20 min with EO water at ACC 100 mg/l and pH 2.8 were applied, and the degradation rate of cyprodinilis presented in Fig. 4. There was no significant difference ($p > 0.05$) in degradation rate when supersonic time was increased from 1 to 10 min. When the treatment time increased from 10 to 15 min, the degradation rate increased significantly ($p \leq 0.05$) from 53 to 79%. Supersonic time of 15 and 20 min had no significant ($p > 0.05$) degradation rate. The highest degradation rate was also achieved with the longest supersonic time (20 min).

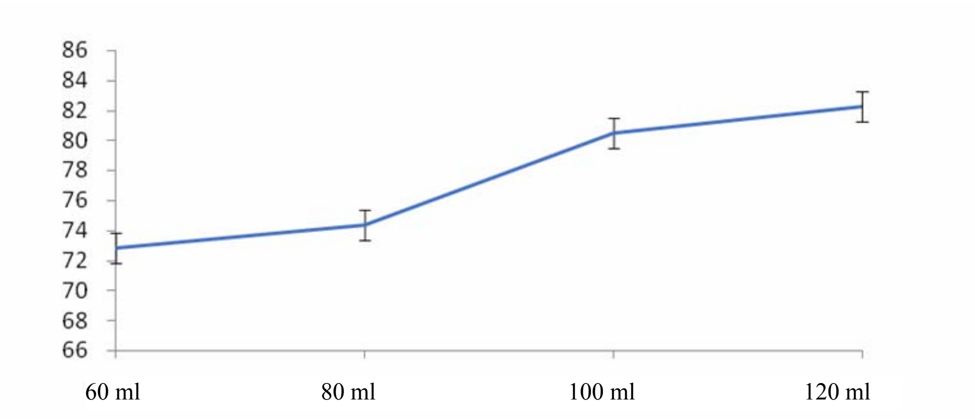

Fig. 3.Degradation rate of cyprodinil changing with the volume of EO water.

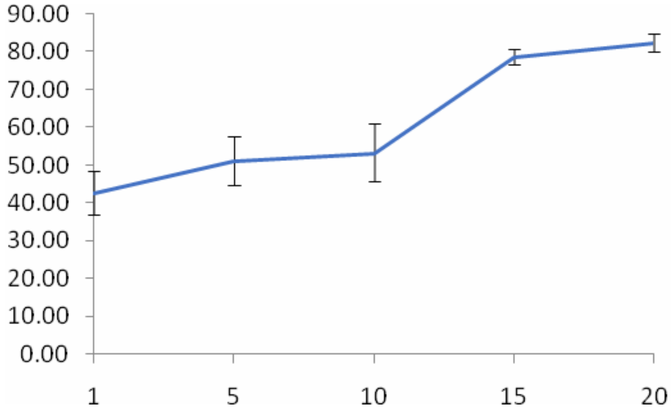

Fig. 4.Degradation rate of cyprodinil changing with supersonic time.

The supersonic cleaner usually used as degasifying device, may affect the ACC of EO water, which was a significant factor in degrading the cyprodinil. Hence, the change of ACC (Without spinach in the EO water) with the supersonic time is presented in Fig. 5. For comparing, the change of ACC (with the spinach in EO water) with the supersonic time and the change of ACC in EO water with the same time at room temperature without supersonic treatment were also shown in Fig. 4. There were no significant differences in ACC in the EO water between with and without supersonic treatment. It meant that supersonic treatment did not affect the ACC in EO water. However, ACC decreased with the treatment time when there was spinach in the EO water. It seems that ACC was rapidly inactivated by organic materials. Although ACC decreased with supersonic time, the degradation rate still increases with the treatment time.

The effect of pH on degradation rate of cyprodinil on spinach is shown in Fig. 6. There was no significant difference in pH 2.3 and pH 3.4, where the degradation rate of cyprodinil was 81%. This was consistent with the results obtained in the Qi's study (Qi *et al.* 2015), which showed that the 2.8 was the optimal pH to degrade the cyprodinil by EO water.

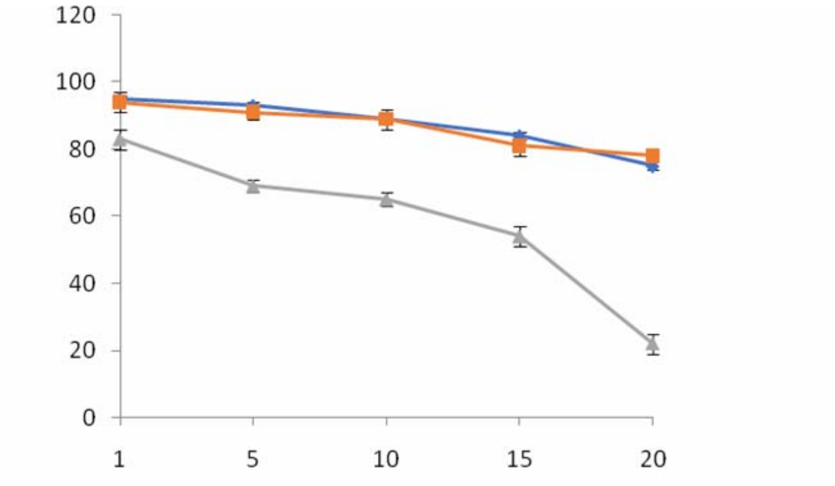

Fig. 5. ACC changing with treatment time

▲EO water with supersound, ■ EO water without supersound and ▲ Spinach in the EO water with supersound.

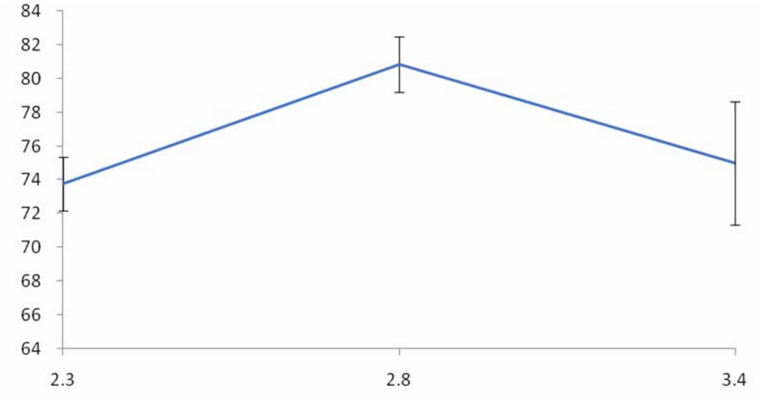

Fig. 6. Degradation rate of cyprodinil changing with pH of EO water.

There was significant difference ($p \leq 0.05$) in degradation rate among T1, T2 and T3, which was 72, 82 and 89%, respectively, indicating multiple treatments of spinach by EO water improved the degradation rate of cyprodinil (Fig. 7). The degradation rate of cyprodinil treated with 180 ml EO water and 15 min supersonic time was 85%, and was hihger than T1, but was lower than T2. It was not significantly different from the T2, but it was significantly different from T3. Hence, 180 ml EO water and 15 min supersonic time could be divided as three times to treat the spinach to improve the degradation rate of cyprodinil and save the EO water. However, it also needs more time to separate the EO water and spinach.

T1, T2 and T3 were the spinach containing cyprodinil treated with EO water at pH 2.8, 100 mg/l of ACC, 60 ml of volume and 5 min supersonic time, for one, two and three times, respectively. T4 was the spinach containing cyprodinil treated with EO water at pH 2.8, 100 mg/l of ACC, 180 ml of volume and 15 min supersonic time.

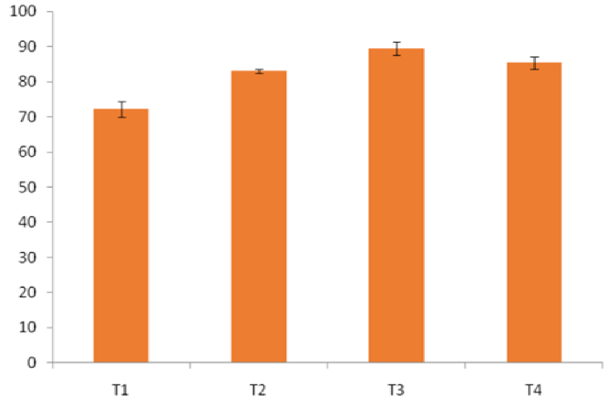

Fig. 7. Degradation rate of cyprodinil changing with multiplied treatments.

EO water could degrade the cyprodinil on surface of spinach, but the degradation rate was not high enough to remove all pesticide. When EO water was combined with supersonic treatment, the degradation rate improved significantly. The pH, volume of EO water, ACC, supersonic time and all affected the degradation rate, and pH 2.8 was the optimal condition to remove the cyprodinil. Higher volume of EO water and ACC and longer supersonic time could achieve the higher degradation rate, but the increase of ACC, volume of EO and supersonic time would make the degradation rate reaching certain limit. Multiple treatments of spinach with EO water also could improve the degradation rate of cyprodinil.

With 15min supersonic time, the removal rate of cyprodinil on the spinash was 82.0% at ACC 100 mg/l, 100 ml of EO water, pH 2.8. The results indicated that the combination of EO water with supersonic treatment could be used as a great potential method to treat the pesticide residues on the fresh produces after harvest.

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