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Cypermethrin Residues in Fresh Vegetables: Detection by HPLC and LC-ESIMS and their Effect on Antioxidant Activity

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Abstract
Cypermethrin, a synthetic pyrethroid, is used as pesticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. Fresh vegetables are of the focal points of this study, since they are a significant source of phenolic antioxidants. In this study, we applied the HPLC and LC-ESIMS techniques to detect cypermethrin residues in five fresh vegetables, when applicable with illegal doses by some farmers. For this purpose, samples of fresh bio-vegetables were bought and divided into three groups, one of which was treated with an illegal dose of cypermethrin. All samples (blank, treated with legal and illegal doses) were then cut into small pieces, frozen and lyophilized. Freeze-dried samples were extracted with ethyl acetate, filtered and evaporated till dryness. Interestingly, comparison of antioxidant activity of organic fennel, cabbage, and celery samples which were not exposed to pesticide treatment with treated samples (propylgallate positive control having 100% antioxidant activity) showing a reduction of antioxidant activity for the treated samples which was very obvious in the cabbage sample but slighter in fennel and celery samples. These results indicate a lower nutritional quality of the treated vegetables with illegal doses.

Keywords: Cypermethrin; residues; vegetables; antioxidant activity; HPLC; LC-ESIMS.

1. Introduction
Pesticides are substances or mixtures of substances intended for preventing, destroying, repelling or mitigating pests. A pesticide may be a chemical substance having antimicrobial or disinfectant properties, a biological agent (such as a virus or bacteria), or a device used against pests. Pests include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes, and microbes that destroy property, spread disease, act as vectors for diseases or cause a nuisance. The word "pesticide" is an umbrella term for all insecticides, herbicides, fungicides, rodenticides, wood preservatives, garden chemicals and household disinfectants that may be used to kill some pests. Since pesticides varies in identity, physical and chemical properties, it`s therefore logical to have them classified and their properties studied under their respective groups. Synthetic pesticides are classified based on various ways depending on the needs [1].
The chemical classification of pesticides is by far the most useful classification to researchers in the field of pesticides and environment and to those who search for details. This is because, it is from this kind of classification that gives the clue of the efficacy, physical and chemical properties of the respective pesticides, the knowledge of which is very important in the mode of application, precautions that need to be taken during application and the application rates. Based on chemical classification, pesticides are classified into four main groups namely; organochlorines, organophosphorous, carbamates and pyrethrin and pyrethroids [2].
Pyrethroids are acknowledged of their fast nocking down effect against insect pests, low mammalian toxicity and facile biodegradation. Although the naturally occurring pyrethrins are effective insecticides, their photochemical degradation is so rapid that their uses as agricultural insecticides become impractical. The synthetic analogues of the naturally occurring pyrethrins (pyrethroids) were developed by the modification of

pyrethrin structure by introducing a biphenoxy moiety and substituting some hydrogens with halogens in order to confer stability at the same time retaining the basic properties of pyrethrins. The most widely used synthetic pyrethroids include permethrin, cypermethrin and deltamethrin [3].
Cypermethrin ($C_{22}H_{19}C_{l2}NO_3$) (fig 1), is a synthetic pyrethroid class of insecticide. It is commonly used to control various pests, including moth pests of cotton, fruit, and vegetable crops [4]. It is also used for crack, crevice, and spot treatment to control insect pests in stores, warehouses, industrial buildings, houses and apartments, greenhouses, laboratories, ships, rail-cars, buses, trucks, and aircrafts. It may also be used in nonfood areas in schools, nursing homes, hospitals, restaurants, hotels, and food processing plants [5]. Consumers expect a product to be free of pesticides (or low concentrations) and other contaminants [6]. The pesticides must undergo extensive efficacy, environmental, and toxicological testing to be registered by governments for legal use in specified applications. The applied chemicals and/or their degradation products may remain as residues in the agricultural products, which becomes a concern for human exposure [7].
We report in this study the effect of over doses applications on the antioxidant activity of some green vegetables.

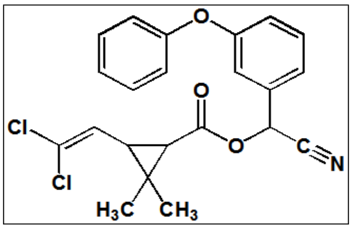

Figure 1: Molecular Structure of Cypermethrin $C_{22}H_{19}Cl_2NO_3$; Mol. Wt.: 415 g/mol.

2. Experimental method

2.1. General Experimental Procedures.

ESI/MS was conducted on a Finnigan LCQ Deca mass spectrometer. Solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements. HPLC analysis was carried out on a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S). Routine detection was at 235, 254, 280, and 340 nm. The separation column (125 × 4mm, L × i.d.) was pre-filled with Eurospher-10 C18 (Knauer, Germany), and a linear gradient of water (adjusted to pH 2 by addition of H_3PO_4) and methanol.

2.2. Sample collection, preparation and extraction.

Fresh bio-lettuce samples (figure 2) were bought from supermarket. Bio-lettuces were divided into three groups; the first one was kept as a control, the second one was treated with a legal dose of cypermethrin and the last one was treated with the dose used by the farmers for 24h.

Figure 2: Samples of green fresh organic vegetables (fennel, cabbage, and celery).

All samples were transferred in -80 °C and then lyophilized for 24h (figure 3). Every group was extracted separately with ethyl acetate three times. The extract was then dried and submitted to HPLC.

2.3. Analytical high pressure liquid chromatography (HPLC)

Analytical HPLC was used to separating, detecting, and quantifying the constituents, as well as to evaluate the purity of isolated compounds. The solvent gradient used started with MeOH:nanopure H_2O (10:90), adjusted to pH 2 with phosphoric acid, and reached to 100 % MeOH in 35 minutes. The autosampler injected 20 µL sample. All peaks were detected by UV-VIS photodiode array detector. In some cases, special programs were used. HPLC instrument consists of the pump, the detector, the injector, the separation column and the reservoir of mobile phase. The separation column (125 × 2 mm, ID) was pre-filled with Eurospher-100 C18 (5 μm), with integrated pre-column (Knauer, Berlin, Germany). LC/UV system specifications are described as follows.


(a) **(b)**

Figure 3: scheme of all samples frozen at – 80 ^{o}C and lyophilized over night to reduce water content in the samples (a) preparing them for extraction with organic solvents (b).

2.4. Liquid chromatography mass spectrometry (LC/ESI-MS)

High pressure liquid chromatography is a powerful method for the separation of complex mixtures. If a mass spectrum of each component can be recorded as it elutes from the LC column, quick characterization of the components is greatly facilitated. Usually, ESI-MS is interfaced with LC to make an effective on-line LC/MS. HPLC/ESI-MS was carried out using a Finnigan LCQ-DECA mass spectrometer connected to a UV detector. The samples were dissolved in water/MeOH mixtures and injected to HPLC/ESI-MS set-up. For standard MS/MS measurements, a solvent gradient that started with acetonitrile: nanopure H_2O (10:90), adjusted with 0.1 % HCOOH, and reached to 100 % acetonitrile in 35 minutes was used.

2.5. Radical scavenging (DPPH) assay

Extracts, fractions and/or pure compounds were evaluated for their ability to function as free radical scavengers [8 – 9]. The qualitative test was performed with a rapid TLC screening method using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Analytical TLC on Silica gel 60 F254 plates were developed under appropriate conditions after application of 10 μL of each test compound solution (1 mg/mL), dried and sprayed with DPPH solution (0.2% w/v, MeOH); 5 min later active compounds appeared as yellow spots against a purple background. The purple stable free radical 2,2-diphenyl-1-picrylhydrazyl was reduced to the yellow colored diphenylpicryl hydrayzine. An ester of gallic acid; propyl gallate (E310) was used as a standard antioxidant.

The quantitative assay was carried out at room temperature as described in 2007 by Tsevegsuren *et al*.[8]: 10 μL of a methanolic solution of the test compound(s) were added to 490 μL of a 100 μM DPPH solution in MeOH. Serial concentrations, ranging from 50 to 300μg, were prepared and analyzed in triplicate. 490 μL of 100 μM DPPH solutions in MeOH plus 10 μL of propyl gallate, 100 μM solutions were used as positive control. 490 μL of 100 μM DPPH solution plus 10 μL of MeOH were used as blank. The absorbance at 517 nm was determined after 30 min incubation and the percentage of DPPH reduction was calculated. The difference between a DPPH blank solution and the positive control was taken as 100% antioxidative activity.

3. Results and discussion

3.1. Detection of cypermethrin using HPLC and LC/ESI-MS

The proposed methods (HPLC and LC/ESI-MS) were applied to analysis of Fresh bio-lettuce samples. All studied samples gave positive results. Figure 4 shows the HPLC analysis of blank and vegetables samples which contained cypermethrine. As it can be seen, the positive finding of this insecticide was confirmed by LC/ESI-MS (figure 5).

Our method indicates that the extraction made by ethyl acetate was efficient in detecting the pesticide within complex extracts. Indeed, several methods were proposed for monitoring pesticide residues and it was found that the extraction with ethyl acetate is very efficient [10-13].

Finally, our method appears very sensitive, simple, and able to determine very small amounts of synthetic pyrethroids in some vegetables. And because there are similarities between the chemical compounds of the family of insecticides, it is possible to use this method for other pyrethroid insecticides. It therefore appears, as a technique of great importance in the analysis of pesticide residues that may be present in vegetables and fruits.

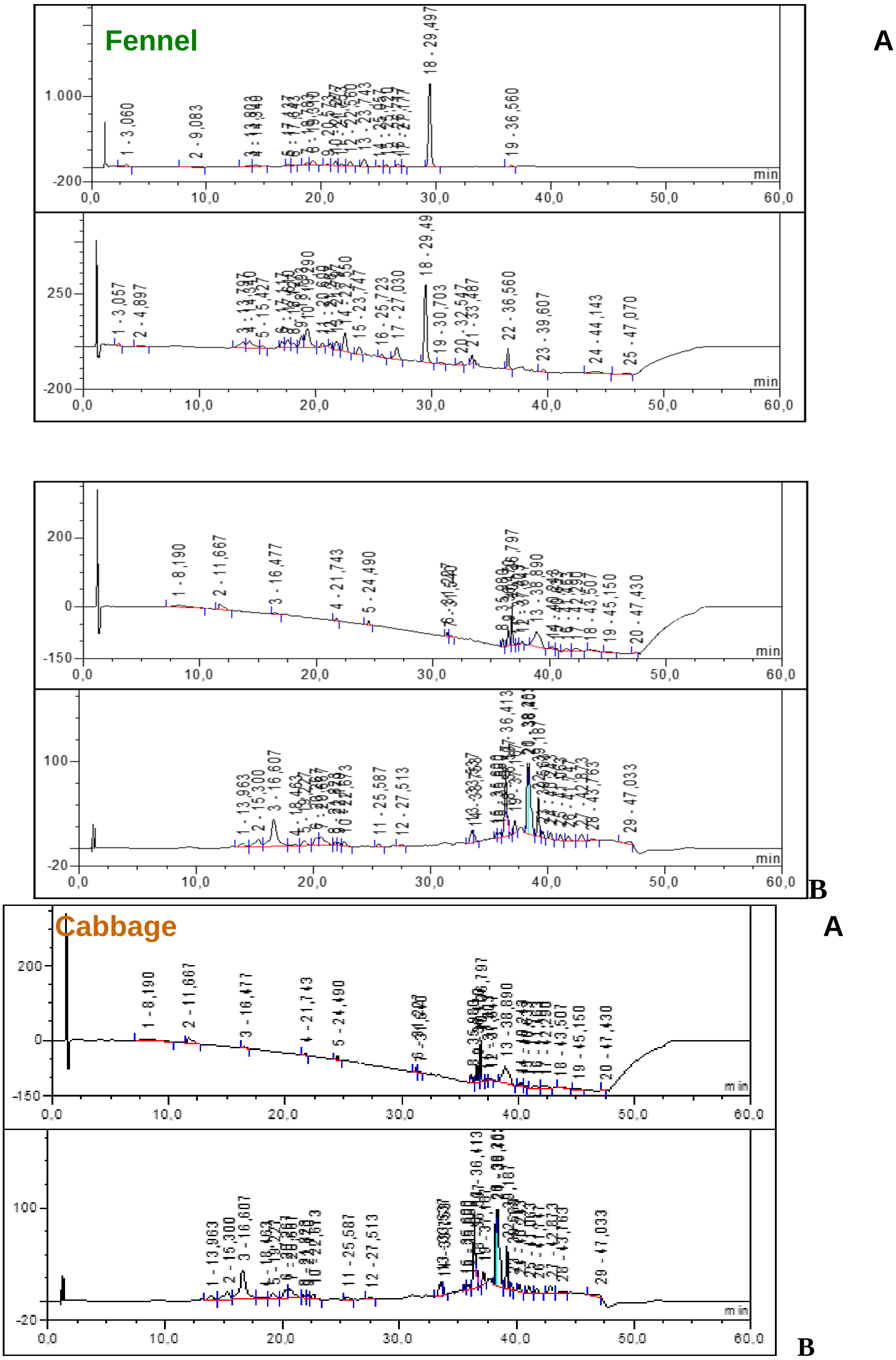

Figure 4: UHPLC analysis of vegetable Samples; **A**: Blank vegetable samples without pesticide treatment **B**: Samples treated with an illegal dose of cypermethrin**.**

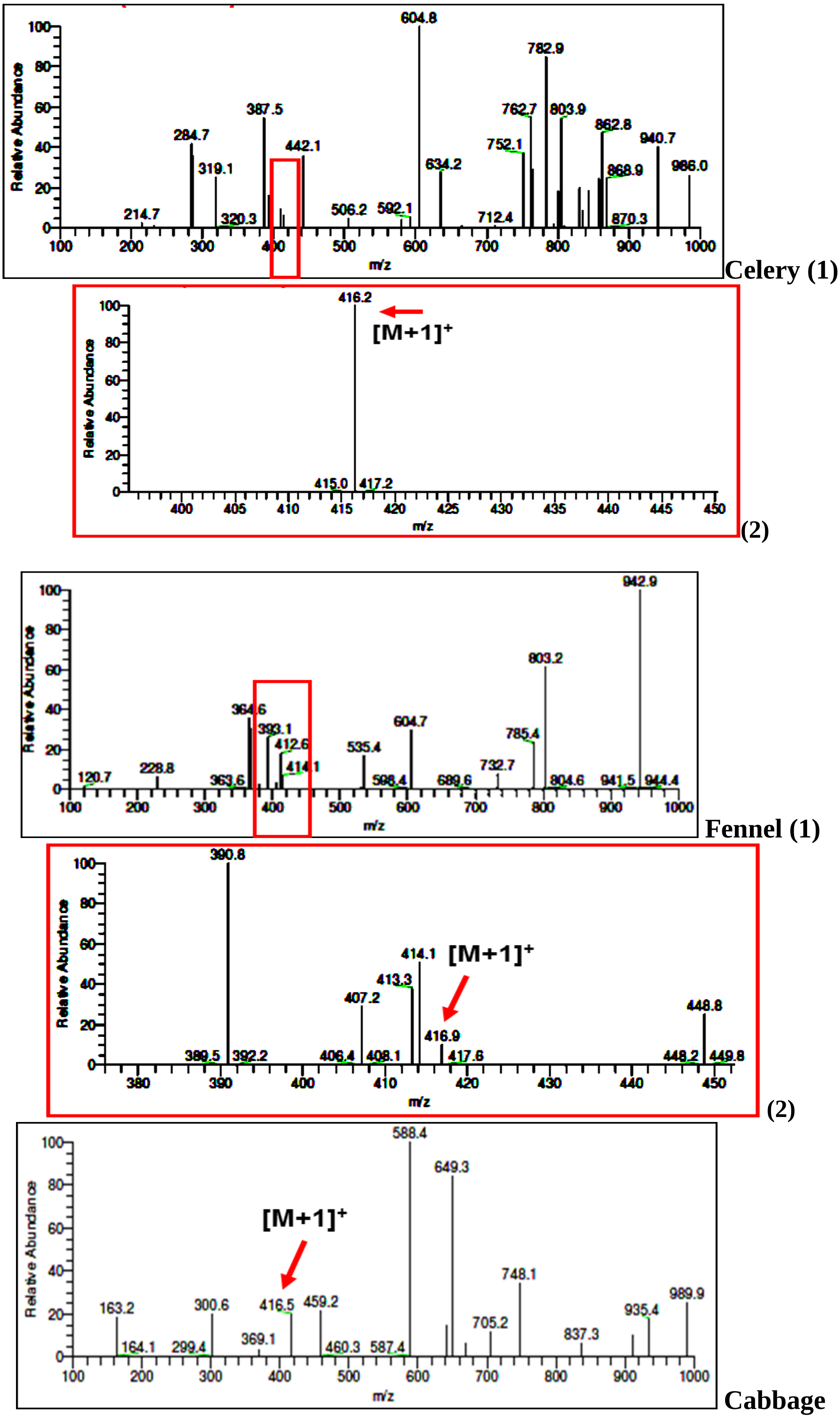

Figure 5: Detection of cypermethrin residues in vegetable samples treated with an illegal dose of the pesticide using LC-ESIMS analysis. 1: ESIMS of cypermethrin treated vegetable samples. 2: Expansion of MS region showing $[M+1]^+$ of cypermethrin.

3.2. valuation and comparison of antioxidant activity of vegetables

Antioxidants are present in the plants and their products at high concentrations up to several grams per kg [14]. In general, the content is higher in the skin of fruit and vegetables. Increased consumption of fruits and vegetables is one of the best ways to increase your intake of antioxidants. In addition, the health of the digestive tract may have a significant effect on the bioavailability of antioxidants. In fact, most flavonoids, including many antioxidants are glycosylated compounds, which reduce the bioavailability [15].
A very few studies have been conducted on the antioxidant content of foods. The content of polyphenols in vegetables from bio and sustainable agriculture is certainly higher than that of vegetables that are not subjected to any stress, such as is the case of those from conventional and hydroponics (using pesticides and others) [16]. So, our search would confirm this, in fact a non-proper use of pesticide (cypermethrin in this case) caused a decrease in the antioxidant activity of the studied vegetables and especially for cabbage, celery and fennel.
All bio vegetables (B) samples were studied for their antioxidant activity in vitro by the DPPH assay. The assay results revealed that these samples have a potent antioxidant activity (figure 6).

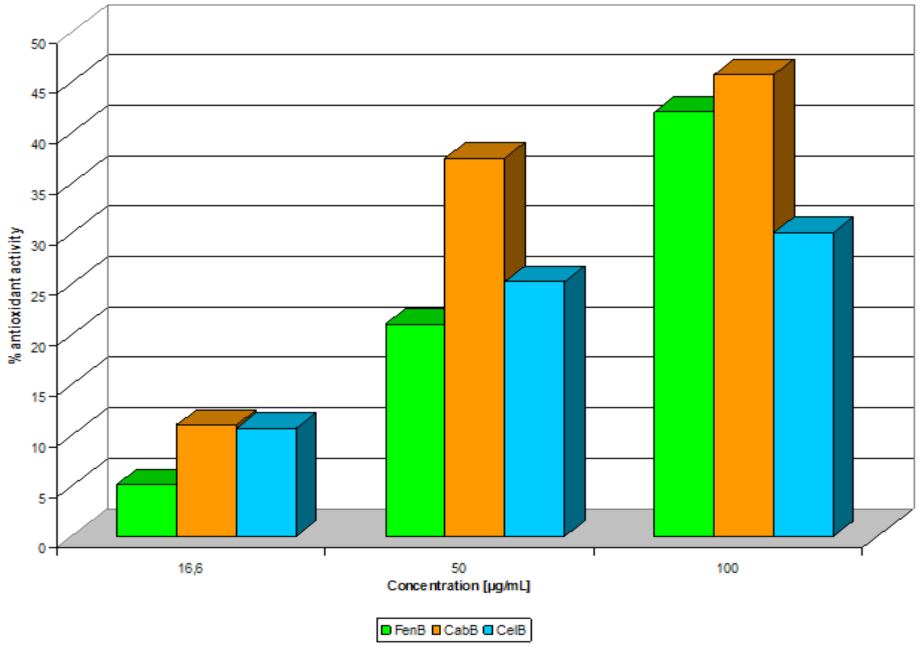

Figure 6: Evaluation and comparison of antioxidant activity of organic fennel (Fe), cabbage (Ca) and celery (Ce) samples with regard to propylgallate as positive control (100% antioxidant activity). **B** without insecticide treatment.

The comparison of antioxidant activity of organic fennel, cabbage, and celery samples which were not exposed to pesticide treatment with treated samples (propylgallate positive control having 100% antioxidant activity) (figure 7) shows a reduction of antioxidant activity for the treated samples.
For cabbage, the treatment effect is more pronounced because the antioxidant activity shows a greater decrease compared to other vegetables. Fennel shows a slight decrease in its antioxidant activity which is probably explained by antioxidant activity rather "robust" and that despite the presence of insecticide residues that keep its vegetable antioxidant capacity but which remains low compared to the untreated vegetable. For celery, antioxidant activity less than it is for fennel, shows the same behavior but we noticed a few different concentrations at 50 and 100µg/ mL. These results indicate a lower nutritional quality of the treated vegetables.
Similarly, Ismail et al [17] have found high rates βcarotène and vitamin C in some vegetables like tomatoes, kale, lettuce and Chinese cabbage. In another study, Maria et al [18] have noted that there is no difference between bio-products and products treated with pesticides as to the total antioxidant activity, they noted that antioxidant activity of vitamin C and flavonoids is superior in bio-beet.

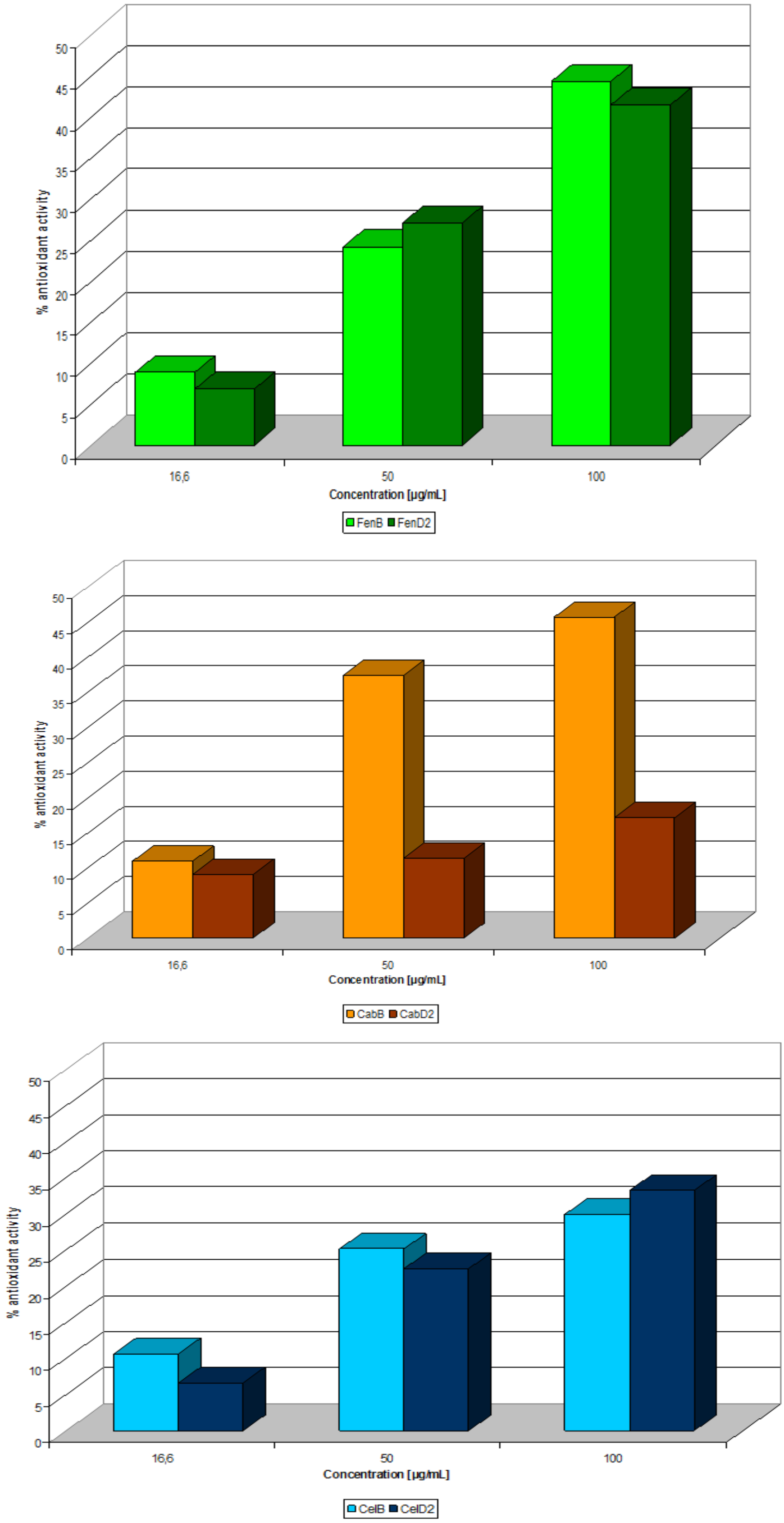

Figure 7: Comparison of antioxidant activity of organic fennel (Fe), cabbage (Ca), and celery (Ce) samples which were not exposed to pesticide treatment with treated samples (propylgallate positive control having 100% antioxidant activity). **B**: blank; **D2** samples treated by insecticide

Ren H, et al. [19] have five grown vegetables (Chinese cabbage, qing-gen-cai, green onions, spinach and green peppers) under conventional and organic conditions, and then tested to determine the polyphenol content. The vegetables were fertilized with water-soluble chitosan as a soil and foliar spray. Conventional vegetables were grown in a nearby field and treated with intensive fertilizer and pesticide. In the case of green onions, Chinese cabbage and qing-gen-cai, the antioxidant capacity of vegetables was 20 to 50% higher than those of conventional vegetables. The study also demonstrated that organic vegetables have a shelf life much longer and they taste better than conventional vegetables (containing pesticides among others).
At the end, although pesticides help protect our vegetables during growth as well as during storage, but many people are concerned about these products. They may remain in or on foods as small amounts of pesticide residue after application.

Conclusion

Cypermethrin, a synthetic pyrethroid, is used as pesticide for fresh vegetables which constitute significant source of phenolic antioxidants. In this study, we applied the HPLC and LC/ESI-MS techniques to detect cypermethrin residues in 3 fresh vegetables, when applicable with illegal doses by some farmers. In all samples, treated with cypermethrin doses, residue of insecticide was detected. Comparison of antioxidant activity of organic fennel, cabbage, and celery samples which were not exposed to pesticide treatment with treated samples (propylgallate positive control having 100% antioxidant activity) shows a reduction of antioxidant activity for the treated samples which was very obvious in the cabbage sample but slighter in fennel and celery samples. These results indicate a lower nutritional quality of the treated vegetables with illegal doses.

References

1. Drum, C. Soil Chemistry of Pesticides, PPG Industries, Inc. USA (1980).
2. Buchel, K. H. Chemistry of Pesticides, John Wiley & Sons, Inc. New York, USA (1983).
3. Manahan, S. E. *Fundamentals of Environmental Chemistry*, Second Edition, Lewis Publishers, USA (2001).
4. R. T. Meister, Farm Chemicals Handbook, Meister Publishing Company,Willoughby, Ohio, USA, (1992).
5. Anonymous, "US Environmental Protection Agency," Pesticide Fact Sheet Number 199, Cypermethrin, US PA, Office of Pesticide Programs, Registration Div.,Washington, DC, USA., Barlow S., (1989).
6. Thomas G. Nagel, *Journal of agroalimentary processes and technologies*, 17 (2) (2011) 113 – 115.
7. Rohan Dasika, Siddharth Tangirala and Padmaja Naishadham, *Journal of Environmental Chemistry and Ecotoxicology*, 4(2) (2012) 19-28.
8. Tsevegsuren N., Edrada R. A., Lin W., Ebel R., Torre, C., Ortlepp S., Wray V., and Proksch P. *J. Nat. Prod*. 70 (2007) 962 – 967.
9. Oktey, M., Culcin, I., Kufrevioglu, O.I. *Lensmittel-Wissenshaft und-Technologie*, 36 (2003) 263-271.
10. Berrada, H, Fernandez, M., Ruiz, M.J., Molto, J.C and Manes, *Food Addit. And Contam*, 23 (2006) 674-682.
11. Cajka, T and Hujslova, J. J. Chromatogr-A, 1058 (2004) 251-261.
12. Ferrer, I, Garcia-Reyes, J.F., Mezara, M., Thurman, E.M and Fernandez-Alba, A-R, *J. Chromatogr-A*, 1082 (2005) 81-90.
13. Sharif, Z, Che Man, Y.B Hamid, N.S.A.H. and Keat, CC. *J. Chromtography*. A, 1127 (2006) 254-261.
14. Daniel, O., Meier, M. S., Schlatter, J. et Frischknecht, P. *Environ. Health Perspect*. 107 Suppl. 1 (1999) 109-114.
15. Scalbert, A. and Williamson, *G. J Nutr.* 130 (8S Suppl) (2000) 2073S-2085S.
16. Manach, C., Scalbert, A., Morand, C., Remesy, C. et Jimenez, L., *Am. J. Clin Nutri*. 79(5) (2004) 727-747.
17. Ismail A, Fun CS. *Malaysian J. Nutr*., 9 (2003) 31-39.
18. Maria R.M Rossetto, Fabio Vianello, Suraya Ad. Rocha & Giuseppim P.P. Lima. *Afr.J. Plant Sci*. 3 (11) (2009) 245-253.
19. Ren H., Endo, H et Hayashi, T. *Journal of the science of Food and Agriculture* 81(15) (2001) 1426-1432.

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