A STUDY ON THE EFFECT OF SONICATION ON THE REDUCTION OF PESTICIDE RESIDUES IN CABBAGE

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Abstract : Pesticides, which are created explicitly to kill living organisms like insects, plants, fungi etc. represent an important class of pollutants for food, soil and surface water resources. Nowadays, the use of these toxic organic and inorganic compounds has increased mainly because of the substantial development in the agricultural sector and also due to increasing demand of food. An increased usage of these pesticides has resulted in an elevation of pesticide residual in every food we consume. Recent consumer awareness has increased the sale of organic foods but they are generally more expensive than their conventional counterparts. Rinsing can make foods safe for consumption, however some pesticides are systemic, i.e. they are taken up by the plant's roots and accumulate into the fruit or vegetable flesh so they can't be washed off. Therefore, it is critical to examine and reduce the levels of pesticide residues in fruits and vegetables as people are very frequently exposed to them. This study is undertaken to assess the effectiveness of the conventional hot water treatment and then to compare this effect of hot water treatment with sonication treatment on the removal of systemic pesticide residue from cabbage. Pesticide residues were extracted from the samples and the analysis was performed using High Pressure Liquid chromatography (HPLC) technique. Results show that sonication treatment is more effective than the regular hot water wash in removing the pesticide residues. Data obtained could then be used for estimating the potential health risks associated with the exposures of these pesticides and the treatments that can be given to fruits or vegetable to reduce the content of these pesticides in the food chain.

Key words: atrazine, cabbage, difenoconazole, pesticide residue, sonication,

1. INTRODUCTION:

Fruits and vegetables constitute and important part of the human diet mainly because of the vitamins and minerals they provide which are necessary in order to keep the body healthy. However, an increase in the population requires an increase in the agricultural productivity which can be achieved by several ways, one of which includes the use of fertilizers and pesticides. There are various benefits of pesticides and India, being a country majorly dependent on agricultural sector for its economy has derived maximum benefits from the use of pesticides. Food grain production has seen a tremendous rise because of the use of these artificial chemicals along with various other techniques [1].These chemicals which include pesticides, have had a major role in this increased yield as these chemicals reduced the losses from weeds diseases and insects that could have affected the yield as well as the quality of the crop. Fruits and vegetables crops are infested by pests and diseases during various stages of growth, harvest and storage which leads to decrease in the amount of substantial yield and thus considerable economic losses. In India, the crop losses due to insect's are around 15.7% at present. In terms of monetary value, Indian agricultural sector currently suffers loss of about US$ 36 billion annually [2]. Therefore, in order to obtain a significant increases in the crop yield and better economic margin, pesticides are extensively used in agricultural production to check or control pests, diseases weeds and other plant pathogens that would otherwise, destroy or affect the quality of the crop.

Cabbage (Brassica oleracea var capitata), is an important vegetable, grown in more than one season and having a high nutritive value. However, the crop is usually attacked by many insect pests like aphids, white flies and several species of lepidopterous larvae during its growth and development. Some other insects include, the Cabbage Looper, which feed on areas between leaf veins, the diamondback moth, which ravenously feeds on the leaves of cabbage, etc. [3]. The activities of these pests and diseases can partially or completely destroy the tissues and defoliate the crops.

Table I: Nutritional value of cabbage leaves, nutrients per 100g of edible portion

NUTRIENT	CONTENT / 100 g
Water	90
Calories	23.0
Protein	1.5
Fat	1.0
Carbohydrate	24.0
Fiber	28.0
Calcium	0.5
Phosphorus	0.1
Iron	0.5
Thiamine	0.1
Riboflavin	0.7
Niacin	0.7
Ascorbic acid	40
B- carotene	0.3

Source: (Rice et al., 1993)

To protect the crops from pests that can afflict the production, farmers often spray their crops with hazardous insecticides, fungicides and herbicides at different application rates and frequencies. The majority these are applied directly to the soil or sprayed over crop fields and hence released directly to the environment. Since 1950, the use of pesticides has increased 50 folds and 2.5 million tons of industrial pesticides are now used annually [4]. This is to be normal as food scarcity and security issues particularly in developing countries are given very high priority .However, lack of proper knowledge and awareness among the farmers leads to unfettered, and careless usage of these artificial chemicals which further leads to harmful consequences.

One of the major consequence of these applied chemicals is that these chemicals and/or their degradation products may remain as residues in the agricultural products and move through the environmental streams and food chains. Other consequence involves and excessive reliance on the systematic chemicals (like atrazine and difenoconalzole) to control the pests which gives rise to a number of problems. A systemic pesticide is any pesticide that is absorbed into a plant and distributed throughout its tissues, reaching the plant's stem, leaves, roots, and any fruits or flowers [5]. Since these are absorbed inside the plant tissues, they cannot be washed off easily. This makes it difficult to get rid of such chemicals using conventional treatments like hot or cold water wash.
Human beings are mainly exposed to these pesticide residues through the ingestion of contaminated foods (such as cereals, vegetables, and fruits), which are directly treated with pesticides or are grown in contaminated fields [6]. Many diseases like cancer, liver, kidney and lung damage that have increased dramatically in the recent decades have been linked to chemical exposure [7]. Pesticides can affect adversely on the nervous system [8]. Also, the elevated levels of these chemicals in our bodies are linked to loss of weight and appetite, irritability, insomnia, behavioral disorder and dermatological problems [9].

Atrazine, IUPAC name: 2-Chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine and molecular formula: $C_8H_{14}ClN_5$ is a colourless, crystalline solid belonging to the triazine class of pesticides. It is produced from cyanuric acid chloride with ethylamine and isopropyl amine in the presence of tetrachloromethane and is a type of selective systemic herbicide that can be used both before and after the emergence of a crop [10]. It is mainly effective against grass and broadleaf weeds [11]. Atrazine, being a systemic pesticide is mainly absorbed by the plant roots after which it is transported upwards to get accumulated at the extreme growing tips of the leaves. In general, injury symptoms will be most prominent at the site where the mobile herbicides concentrate [12]. In sensitive plants the light reaction of photosynthesis is inhibited. This leads to a decline in carbon dioxide fixation [13]. The transpiration rate is also inhibited as a result of the closing of the stomata, owing to an accumulation of CO2 in the stomatal regions [14]. Photosynthesis is, however, the most sensitive process and the chlorophyll and carotene contents of plants decrease in light [15] when disintegration of chloroplasts occurs [16].This corresponds with toxic symptoms commonly associated with triazine damaged plants.However, this herbicide to be moderately toxic to humans and other animals [17]. It can be absorbed into the bloodstream through oral, dermal and inhalation exposure and symptoms of poisoning include abdominal pain, diarrhea and vomiting, eye irritation, irritation of mucous membranes, and possible skin reactions [18]. Atrazine is also a mild skin irritant. Rashes associated with exposure have been reported. Moderate to severe eye irritation can occur [19]. Exposure to large concentrations of airborne particles or droplets may cause irritation of the mucous membranes [20].

Difenoconazole, IUAC name : 1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl[1,3]dioxolan-2-ylmethyl]-1H-1,2,4-triazole and molecular formula $C_{19}H_{17}Cl_2N_3O_3$ is a broad spectrum fungicide mainly used as a spray or seed treatment and used for disease

control in many fruits, vegetables, cereals and other field crops[21]. It is mainly effective against Ascomycetes, Basidiomycetes and Deuteromycete,s including Alternaria, Ascochyta, Cercospora, Cercosporidium, Colletotrichum, Guignardia, Mycosphaerella, Phoma, Ramularia, Rhizoctonia, Septoria, Uncinula, Venturia spp., Erysiphaceae, Uredinales and several seed-borne pathogens [21]. Its mode of action is basically based on inhibiting the biosynthesis of the cell membrane ergostrol which further stops the development of the fungus [22].
Although potentially a mobile molecule it is unlikely to leach due to its low aqueous solubility. It does however have potential for particle bound transport. It is slightly volatile, persistent in soil and in the aquatic environment. There are some concerns regarding its potential for bioaccumulation and it is moderately toxic to humans, mammals, birds and most aquatic organisms.

Table II: Toxicity level of the pesticides given by WHO.

Pesticide	IUPAC Name	WHO Toxicity level
Atrazine	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine	Class III
Difenoconazole	1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl[1,3]dioxolan-2-ylmethyl]-1H-1,2,4-triazole	Class III

If these pesticides are present on the surfaces of fruits and vegetables, a substantial amount of these can be removed through washing and light scrubbing. However, systemic pesticides are actually absorbed by a plant when applied to seeds, soil, or leaves or are taken up by the plant's roots and get into the fruit or vegetable flesh so they can't be washed off thereby entering human bodies [23]. It is important to reduce these pesticide residues in fruits and vegetables in order to minimize human exposure. The objectives of this study were to determine the effect of various washing treatments with and without sonication on pesticide removal from cabbage and assess the effectiveness of a water wash on selected samples.

Materials and methods

Sample selection: Samples of cabbage were chosen due to their commercial importance and potential consumption and were procured from the local market (Bangalore, India). Samples were guaranteed in good condition. Sample with and without pesticide spray were examined for the determination of pesticide residue.

Pesticide selection: Pesticides which are common and were being used extensively for the selected sample were selected. Standards were collected RALLIS, India.

Two different classes of common pesticides were studied and used as follows:

- ATRAZINE
- DIFENOCONAZOLE

The following analytical grade chemicals are used for the experiment:
Methanol and Acetone were of HPLC grade and collected from RANKEM.

Experimental design: The samples were isolated and then spiked atrazine and difenoconalzole of a nominal concentration of 1ppm for each compound. Samples were left to absorb the pesticides under normal conditions, ensuring the whole surface was in contact with the solution. Control samples were taken as it is without spiking any pesticide.

OTHER APPARATUS: Electronic balance, Sonicator.

Procedure of residue extraction of sample:

Samples were taken in two trays. 2ml of prepared solution of 1 ppm concentration of atrazine and difenoconazole was sprayed on the sample. It was kept for drying for 20-30 minutes under normal conditions.

Treatments given:

HOT WATER: Samples sprayed with atrazine and difenoconazole were washed with hot water. Washed again with 50 ml Methanol. Methanol containing residue was taken in rotoflask.

SONICATION: Samples were taken in a beaker and 150 ml water at room temperature was poured so that the samples are fully immersed. Kept for sonication in sonicator for 10 minutes. Washed with 50 ml methanol. Methanol containing residue was taken in a

rotoflask. Evaporator was used to extract the residue from the methanol.2 ml methanol was added to the leftover residue in the rotoflask. This solution was transferred into a vial.

Analytical Technique: Residues of different pesticides were checked in vegetables samples by using the High Performance Thin Layer Chromatography Agilent HPLC-VWD HPLC system (Agilent 1260 infinity) and Chemstation for LC Software was used. The pesticide analysis data were acquired and processed using Chemstation for LC software running under Windows XP on a Pentium PC.

Results and discussions

An easy method using HPLC was used for determining two systemic pesticide residues in fruit samples.

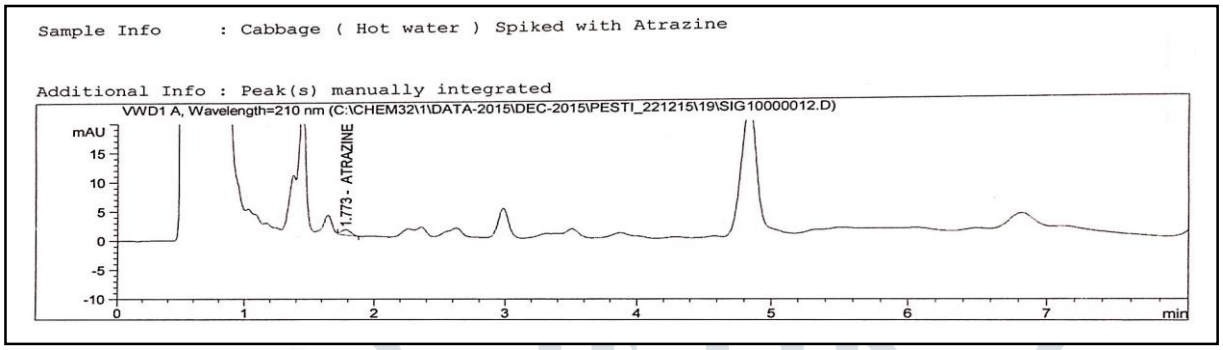


Figure I: Typical chromatogram of cabbage spiked with atrazine and treated with Hot water.

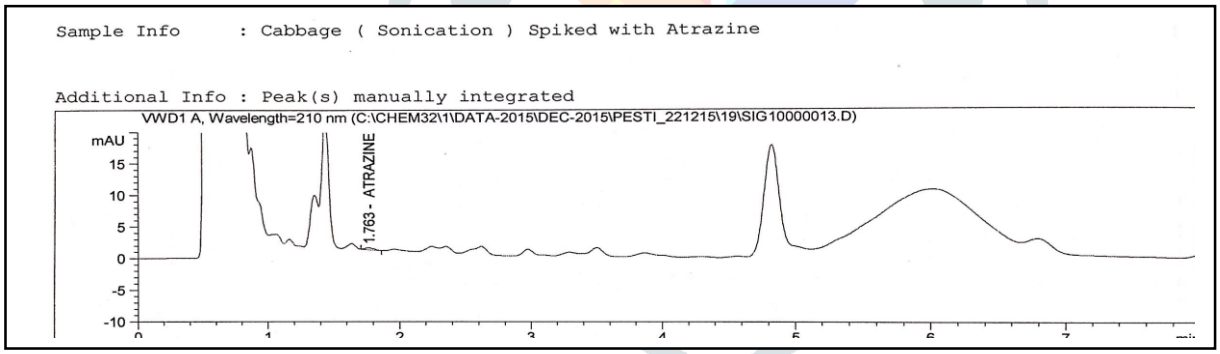


Figure II: Typical chromatogram of cabbage spiked with atrazine given sonication treatment.

For HPLC analyses, peak areas are used for quantitative calculations. The area under the peak is proportional to the amount of the pesticide which has passed the detector. The chromatograms were analyzed quantitatively and the result (in ppm) was calculated using the following formula:

Concentration (in ppm) = area/standard concentration × standard ×dilution (2 ml)

Table III: Concentration (in ppm) of Atrazine observed in the sample post treatment.

Treatment	Area	Standard Concentration	Dilution	Result
Hot water	4.38	1.0	2	0.13
Sonication	1.48	1.0	2	0.04

In the chromatogram obtained from Cabbage which were spiked with atrazine and later treated with hot water, the peak area is seen to be 4.38 and the corresponding concentration was calculated to be 0.13 ppm whereas the ones given the sonication

treatment show a peak area of 1.48 and concentration of 0.04 ppm. This shows that there is a considerable decrease (about 32%) in the pesticide residue level when the sample is given the sonication treatment.

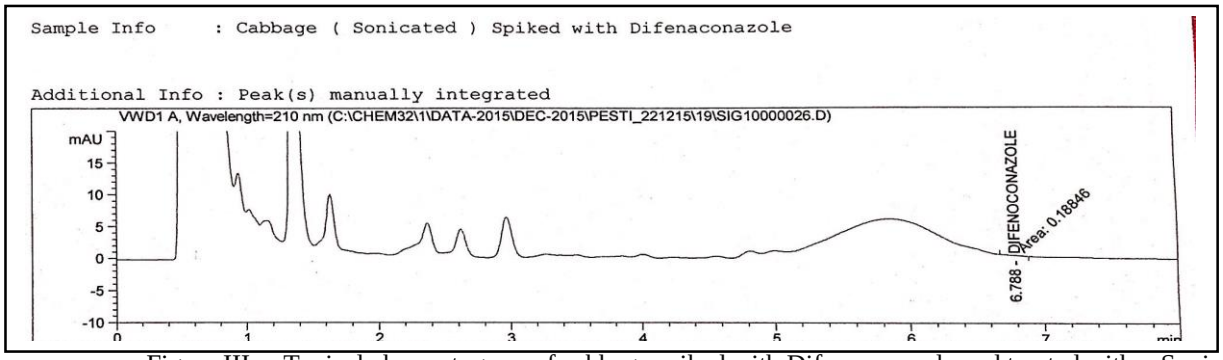


Figure III: Typical chromatogram of cabbage spiked with Difenoconazole and treated with Sonication.

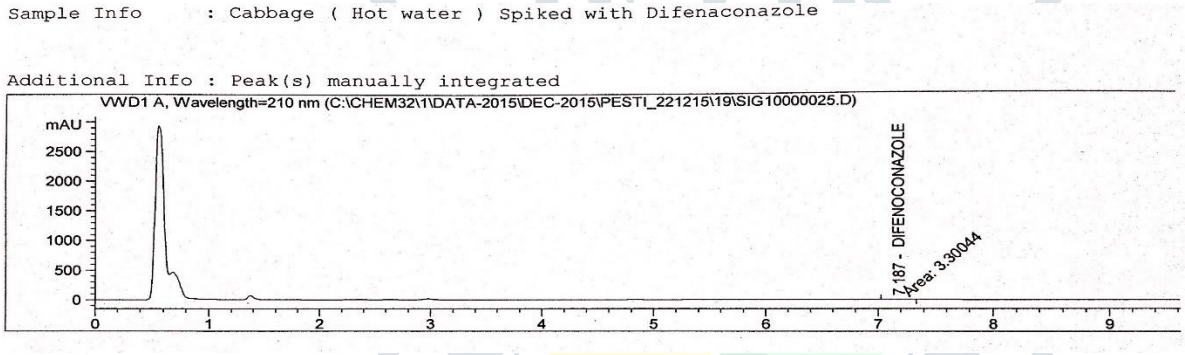


Figure IV: Typical chromatogram of cabbage spiked with Difenoconazole and treated with hot water

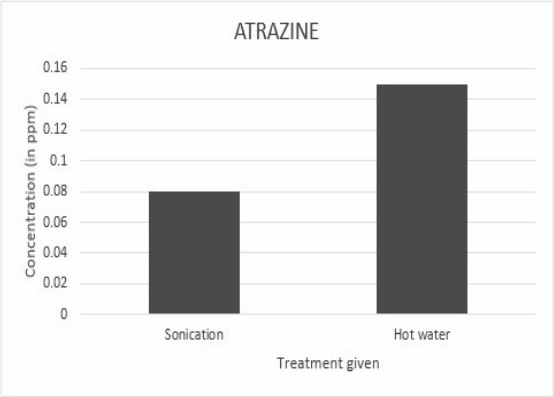
Table IV: Concentration (in ppm) of Difenoconazole observed in the sample post treatment.

Treatment	Area	Standard Concentration	Dilution	Result
Hot water	3.30	1.0	2	0.15
Sonication	1.88	1.0	2	0.08

In the chromatogram obtained from Cabbage which was spiked with difenoconazole and later treated with hot water, the peak area is seen to be 3.30 and the corresponding concentration was calculated to be 0.15 ppm whereas the ones given the sonication treatment show a peak area of 1.88 and concentration of 0.08 ppm. This shows that there is a considerable decrease (about 50%) in the pesticide residue level when the sample is treated with sonicated water.

Conclusion:

Sonication treatment, being a physical technique of food processing has proved to be a better method for extraction of systemic pesticides and thus could efficiently play an important role in preventing several adverse effects in consumers. Interactions between the matrix and the analytes can be very strong for sediments; therefore, liberating the bound fraction often requires some type of intensive physical extraction method. Also, this treatment is considered to be advantageous due to its reduced processing time with lesser energy consumption and being environmental friendly. Therefore, Sonication provides an effectual and sustainable method for extracting strongly bound chemicals from fruit tissues. Furthermore, ultrasound treatments are inexpensive, simple, reliable, and can be an effective alternative to conventional extraction techniques.



Calculated concentration of Atrazine

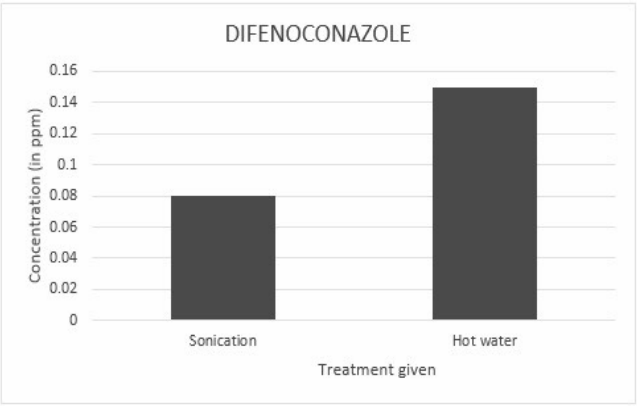


Figure VI: Calculated concentration of Difenoconazole

Figure V:

Future Prospects

A cost effective sonicator can be developed as an urban household appliance by making modifications to the laboratory model. The complete absence of such a product and the urgent need for one gives the household sonicator a great market for business as it will significantly improve food quality for the modern consumer.

References:

[1] Aktar, W., Sengupta, D., & Chowdhury, A. 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology, 2(1), 1-12. doi:10.2478/v10102-009-0001-7.
[2] Dhaliwal, G., Jindal, V., & Mohindru, B. 2015. Crop Losses due to insect pests: Global and Indian Scenario. Indian Journal of Entomology, 77(2), 165. doi:10.5958/0974-8172.2015.00033.4
[3] Ozcan, S. 2010. Viable and Rapid Determination of Organochlorine Pesticides in Water. CLEAN - Soil, Air, Water, 38(5-6), 457-465. doi:10.1002/clen.201000059
[4] Appendix 1— Some Standard Terms and Abbreviations Used in the Approval of Pesticides. (n.d.). Pesticides, 207-218. doi:10.1002/9780470995853.app1
[5] Lexmond, M. B., Bonmatin, J., Goulson, D., & Noome, D. A. 2014. Worldwide integrated assessment on systemic pesticides. Environmental Science and Pollution Research, 22(1), 1-4. doi:10.1007/s11356-014-3220-1
[6] Rodrigues, D., Carvalho, T., Sousa, A., Neto, V. S., Fechine, P., & Nascimento, R. 2011. Determination of Insecticide Residues in Vegetal Fruits. Chromatography Research International, 2011, 1-6. doi:10.4061/2011/713256
[7] Kataria, A., Trasande, L., & Trachtman, H. 2015. The effects of environmental chemicals on renal function. Nature Reviews Nephrology, 11(10), 610-625. doi:10.1038/nrneph.2015.94
[8] Keifer, M. C., & Firestone, J. 2007. Neurotoxicity of Pesticides. Journal of Agromedicine, 12(1), 17-25. doi:10.1300/j096v12n01_03.
[9] Moreland, D. E., Gentner, W. A., Hilton, J. L., & Hill, K. L. 1959. Studies on the Mechanism of Herbicidal Action of 2-Chloro-4, 6-Bis (Ethylamino)-S-Triazine. Plant Physiology, 34(4), 432-435. doi:10.1104/pp.34.4.432
[10] Shimabukuro, R. H., & Swanson, H. R. 1969. Atrazine metabolism, selectivity, and mode of action. Journal of Agricultural and Food Chemistry, 17(2), 199-205. doi:10.1021/jf60162a044
[11] Foy, C. L. 1964. Volatility and Tracer Studies with Alkylamino-s-Triazines. Weeds, 12(2), 103. doi:10.2307/4040606
[12] P. C. Nel & C. F. Reinhardt 1984 Factors affecting the activity of atrazine in plants and soil, South African Journal of Plant and Soil, 1:2, 67-72, DOI: 10.1080/02571862.1984.10634112
[13] VOSTRAL, H.J., BUCCHOLTZ, K.P. & KUST, e.A., 1970. Effect of root temperature on absorption and translocation of atrazine in soybeans. Weed Sci. 18, 115 - 117.
[14] STANGER, C.E. & APPLEBY, A.P., 1972. A proposed mechanism for diuroninduced phytotoxicity. Weed Sci. 20, 357 - 363.
[15] ASHTON, F.M. GIFFORD, E.M. & BISALPUTRA, T., 1963. Structural changes in Phaseolus vulgaris induced by atrazine. 2. Effects on fine structure of chloroplasts. Bot. Gaz. 124, 336 - 343.
[16] Hallenbeck, W.H. K.M. Cunningham-Burns. 1985. Pesticides and human health. New York: Springer-Verlag.
[17] Hayes, W.J. and E.R. Laws (ed.). 1990. Handbook of Pesticide Toxicology, Vol. 3, Classes of Pesticides. Academic Press, Inc., New York.
[18] OHS MSDS for Atrazine. 1991 (March 20).
[19] USGS. 1991 (Nov. 20). Spring sampling finds herbicides throughout Mississippi River and tributaries. U.S. Geologic Survey, Dept. of the Interior, Reston, Virgini
[20] An Introduction to Weed Biology. 2010. Herbicides and Plant Physiology, 1-26. doi:10.1002/9781444327793.ch1

[21] M., G., & L. (n.d.). Antifungal Agents: Mode of Action, Mechanisms of Resistance, and Correlation of These Mechanisms with Bacterial Resistance. Clinical Microbiology Reviews. Retrieved November 19, 1999.

[22] Xue, J., Li, H., Liu, F., Xue, J., Chen, X., & Zhan, J. 2014. Transfer of difenoconazole and azoxystrobin residues from chrysanthemum flower tea to its infusion. Food Additives & Contaminants: Part A, 31(4), 666-675. doi:10.1080/19440049.2014.882020.