

# Level of Lindane and Chlorpyriphos pesticide residues and their treatment with 3 ppm ozone in Palak and Radish vegetable samples collected from Delhi/NCR, India

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Abstract: In India, majority of the population is vegetarian, therefore the consumption and demand of vegetables is higher. To enhance the production of vegetables, pesticides are used at higher level. Therefore the population of India is at increased risk to get exposed to higher levels of pesticides. Exposure of pesticide residues at higher level has been found to affect the human health greatly. Present study was aimed to analyze the level of different pesticide residues in two vegetables namely Palak and Radish collected during winter and summer seasons of year 2016, 2017, 2018 and 2019. Overall results showed that the level of Lindane and Chlorpyriphos pesticide residues were higher as compared to FSSAI specification values (2.0 and 0.2) in majority of the vegetable samples irrespective of type, location, season and year. All the vegetable samples containing pesticide residues of Lindane and Chlorpyriphos were treated with different concentrations of aqueous solution of Ozone in order to reduce the pesticide load. We found that the 3 ppm aqueous solution of Ozone reduced the level of both Lindane and Chlorpyriphos in all vegetable samples. These findings reflected higher levels of pesticides in vegetables and present that 3 ppm ozonation can be implemented on vegetables to reduce the pesticide load at personal as well as at organizational level in order to enhance their safety for use in hospitality sectors, as well as for the consumption of native population.

Keywords: Chlorpyriphos - Lindane - Ozonation - Palak - Pesticides - Radish.

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# INTRODUCTION

Food is the most essential element of life. Consuming safe and quality food is a key to promote good health and sustain life. In addition to this, the supply of safe food also contributes to components of sustainable development such as trade, tourism, and economy. Therefore, assurance of agricultural product safety is of great importance and concern. The exponential growth in world population and food demand, globalization of food trade, change in environment and rapidly changing food systems are producing significant impact on the quality and safety of food. The Present scenario reflects that there is an exponential increase in the number of people who are at greater risk of consuming unsafe food. Emerging studies related to food safety reflect that a large number of people are becoming sick and hundreds to thousands die yearly by consuming unsafe food. According to WHO reports, unsafe food contaminated with harmful chemical substances such as pesticides are responsible for the cause and development of various diseases ranging from diarrhoea to cancers.

Pesticides are intentionally used to protect agricultural products from pests to increase the crop production and storage of their produce. However, in due course of time, pesticides get deposited and accumulated in water, soil and biota and enter into the human food chain thus posing a great threat to humans (Sharma *et al.* 2019).

The presence of pesticide residues in crops such as fruits and vegetables even after harvesting is one of the major concerns of every country including India. Several studies along the time window have been conducted to assess the level of several toxic pesticides in the fruits and vegetables produced in India.

Awasthi (1990) observed persistence of synthetic pyrethroids (fenvalerate, cypermethrin and deltamethrin) on sweet pepper. Singh & Kalra (1992) reported the contamination of residues of cypermethrin in the Brinjal. Singh et al. (1999) reported the presence of cypermethrin, fenvalerate and deltamethrin in cabbage. Chahal et al. (1992) the reported persistence of pesticides namely fenvalerate and endosulfan on okra. Ahmad et al. (1993) found contamination of pesticide residues of deltamethrin, cypermethrin, permethrin, fenvalerate and monocrotophos in okra. Awasthi (1994) reported the persistence of different pyrethroids on green chilli. Kumari et al. (2003) assessed 80 winter vegetables for pesticide contamination and found residue levels of four major chemical groups, organophosphorous with highest followed by carbamates, synthetic pyrethroids and organochlorines. Mukherjee (2003) monitored different vegetables for the estimation of thirty insecticides, fifteen organochlorineinsecticides and six organophosphorus insecticides, nine synthetic pyrethroids and two herbicides. Results revealed that 100% of vegetable samples were contaminated with pesticides however only 31% of vegetables showed the presence of pesticides above the prescribed tolerance limit. Kumari et al. (2004) monitored 84 farm gate samples of seasonal vegetables and found contamination mainly with organophosphates, pyrethroids and organochlorines. RangaRao et al. (2009) found the presence of pesticides namely monocrotophos, chlorpyrifos, cypermethrin and endosulfan in brinjal, cucumber, okra, ridge gourd and tomato. AnandaGowda & Somashekar (2012), evaluated twenty pesticides residues in fifty farmgate vegetable samples and reported that all samples were contaminated with the organo chlorines (83.5%), organophosphates (67%) and pyrethroids (55%). Neetu (2013) investigated the level of dichloro-diphenyl-trichloroethane (DDT) pesticides in vegetables, pulses and cereals collected from local markets in Delhi. Authors found that most of the samples were contaminated with DDT. Kostik et al. (2014) conducted a study on analysis of 33 pesticide residues in 168 samples of different fresh vegetables, processed vegetables, fresh fruits, processed fruits. Results showed that 98.8% of samples were contaminated with pesticides. Pathak et al. (2016) conducted as tudy to assess residue levels of pesticides namely dichlorodiphenyltrichloroethane (DDT), hexachloro-cyclohexane (HCH), endosulfan, aldrin, and dieldrin in eggplant, potato, tomato, onion, beet, spinach, and cabbage. Authors found that all almost all vegetable samples have some contamination of one or more pesticide residues. Sahai (2016) reported contamination of some pesticide residues of chlorpyriphos, endosulfan, carbaryl and piperonylbutoxide in cauliflower. Sah et al. (2018) undertook a study to evaluate the pesticide contamination in seasonal farm gate vegetables samples namely okra, brinjal, cauliflower and cabbage. Authors reported 75% of samples were found to be contaminated with different pesticides residue namely cypermethrin, chlorpyriphos, endosulphan and quinalphos. AnandaGowda et al. (2020) carried out a study involving analysis of pesticide residues in carrot samples collected from vegetable markets in five districts viz., Bangalore Rural, Bangalore Urban, Chickballapura, Kolar and Ramanagara of Karnataka, India. Authors reveal contamination of acephate pesticides in 25% in Bangalore rural and 37.5% each in Bangalore urban, Chikkaballapura, and Ramanagara districts. Chlorpyriphos residue was observed in all the sections except Bangalore Urban.

The Present study was aimed to understanding the pesticide risk by selecting two types of vegetables namely Palak (Spinaciaoleracea L. a leafy plant) and Radish (Raphanussativus L. a root vegetable). These vegetables were collected from four different locations of Delhi/NCR and were analysed to measure the concentration of different pesticides residues. Moreover, different concentrations of ozone were used to reduce the pesticide load in these vegetables.

#### MATERIAL AND METHODS

#### Requirements

GC/MS Triple Quad Equipment, 50 ml tarson centrifuge tubes, 15 ml Tarson centrifuge tubes, 100 ml volumetric flask, 100 ml measuring cylinder, Centrifuge apparatus, Vortex Mixture, Weighing Balance, Ultrasonictore, GCMS-MS, GC vial (1.5 ml), Micropipette (10–100  $\mu$ l and 100–1000  $\mu$ l).

#### Reagents

Magnesium Sulphate, Sodium Acetate, Acetonitrile, EMR- Lipid dsPE in ml tube (Agilent part no.: 5982-1010), EMR- Polish Pouch (Agilent, part no.: 5982-0102), Ethyl Acetate, Methanol, NIST certified Standard, Bond Elut sample preparation solution (Agilent Part No.: 5982-0028).

#### Standards

Gamma (HCH) Lindane, Delta (HCH), Malathion, Chlorpyriphos, Aldrin, Dieldrin, Beta-Endosulphane, www.tropicalplantresearch.com 107

## Endosulphan Sulphate, Alpha Endosulphane, o,p DDT, p&p DDT, o,p DDE, p&p DDD, p&p DDD.

#### Sample collection

Vegetable samples (Palak and Radish) were collected from four different locations of Delhi/NCR and were analysed to measure the concentration of different pesticides (Gamma (HCH) Lindane, Delta (HCH), DDT (o,p&p,p-isomers of DDT, DDE & DDD) (4,4-DDT), Malathion, Chlorpyriphos, Aldrin, Dieldrin, Beta-Endosulphane, Endosulphan Sulphate, Alpha Endosulphane). Vegetable samples were collected during the winter and summer seasons of year 2016, 2017, 2018, and 2019. Samples were divided into two groups. One group was treated with plain water and another group was treated with ozonisation (Aqueous Solution of Ozone, 3 ppm). The concentration of different pesticides was measured in vegetable samples of both the groups. Values representing measured concentration of pesticides in vegetable samples of both the groups were compared to explore the effect of 3 ppm ozonisation treatment on the concentration of pesticides. Observed values were compared with the Food Safety and Standards Authority of India (FSSAI) specification values.

## Sample Preparation

Take approx 5 gm of sample (Homogenized) in 50 ml centrifuge, add 5 gm of  $MgSO_4$  and 1.5 gm of sodium acetate in the sample then add 5 ml of 1% acetic acid in acetonitrile after that shake the sample for 10 minutes the extract the sample for 10 minutes with vortex mixture. After that centrifuge the sample at 5000 RPM for 10 minutes, and then transfer clear supernatant liquid in 1.5 ml of Bon Eluet sample preparation solution vial. Then vortex the sample for 2 min, then again centrifuge the sample on table top centrifuge on 5000 RPM. After that transfer the clear supernatant liquid into 1.5 ml GC vial.

# Methods

Gas Chromatography: An ion trap, quadrupole, time-of-flight (TOF), or other GC/MS instrument may be used with electron impact (EI) ionization, an autosampler (AS), and computerized instrument control/Data collection. Either LVI of 8  $\mu$ l for 1 g ml<sup>-1</sup> MeCN extract or 2  $\mu$ l splitlessinjection of 4 g ml<sup>-1</sup> extracts in toluene at 250°C may be used. A 3–5 m, 0.25 mm id, phenylmethyl-deactivated guard column must be used as retention gap in either case. The analytical column is a 30 m, 0.25 mm id, 0.25 µm film thickness (5% Phenyl)methylpolysiloxane (Low bleed) analytical column (DB-5 ms or equivalent). Set He head pressure on the section to be 10 psi or steady stream to be 1.0 Ml/min with frameworks equipped for electronic pressing factor/stream control. After an appropriate time for solvent delay, use an appropriate oven temperature program, for an example, starting at 75°C for MeCN extract or 100°C for toluene ramped to 150°C at 25°C/min, the to 280°C at 10°C/min, and hold for 10 min. All teammates had a lot of involvement with pesticide buildup investigation and were allowed to utilize their own insightful conditions given that pinnacle shapes were Gaussian, top widths at half statures were >5 s, and sign to-clamor proportion (S/N) of the quantitative particle for the pesticides at 10 ng  $g^{-1}$  comparable fixations in the example were >10. For subjective reason (which were not the focal point of this examination), something like 3 particle yielding relative wealth that sensibly match a contemporaneously broke down reference standard are ordinarily expected to make an analyte recognizable proof.

#### Calculation

The data chromatograms have to be acquired using the computer based Agilent technologies, Inc. 7000 D insert MSD mass data hunter software. The concentration of the unknown has to be calculated from the equation.

# Y = MX + C

Where, Y = Analyte Area; M = Slope of calibration curve; X = Concentration of anlyte; C = Y axis intercept value.

Concentration of pesticides is directly calculated by instrument itself with the help of matrix.

#### RESULTS

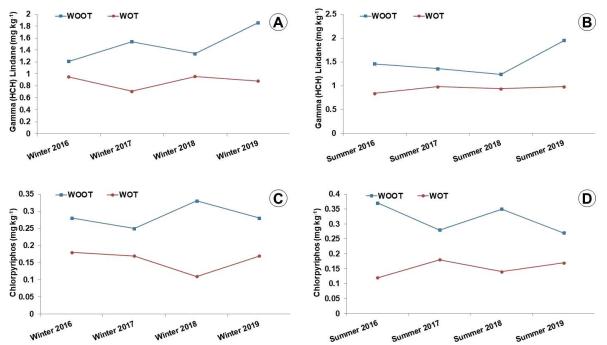
All the vegetables were subjected to four treatments (T0 - plain water, T1 - 1 ppm Aqueous Solution of Ozone, T2 - 2 ppm Aqueous Solution of Ozone, and T3 - 3 ppm Aqueous Solution of Ozone) to explore the effect of increasing levels of ozone on the levels of different pesticides. We found that 3 ppm aqueous solution of ozone was more effective than other concentrations. Therefore, 3 ppm aqueous solution of ozone was standardized and used for the treatment of different vegetable samples collected from four different locations during winter and summer seasons of year 2016, 2017, 2018 and 2019.

## Analysis of pesticides

Overall results reflected that the level of Gamma (HCH) Lindane and Chlorpyriphos were higher as compared to FSSAI specification values in the majority of the vegetable samples irrespective of type, location, season and year). The concentration of all other pesticides namely Delta (HCH), DDT (o,p&p,p-isomers of DDT, DDE & DDD) (4,4-DDT), Malathion, Aldrin, Dieldrin, Beta-Endosulphane, Endosulphan Sulphate, and Alpha Endosulphane were found below limit of quantification.

# Level of pesticides and effect of Ozonisation (3 ppm) on pesticide load of Palak samples

i. Analysis of pesticides from Location A of Delhi/NCR: Results showed that the concentration of two pesticides namely Gamma (HCH) Lindane and Chlorpyriphos was higher as compared to FSSAI specification values (1.0 and 0.2, respectively) in palak samples collected from Location-A and treated with plain water. However, the values of palak samples treated with ozonisation were found lower as compared to samples treated with plain water (Fig. 1). Ozonisation treatment with 3 ppm aqueous solution of ozone was found effective to lower down the values of Gamma (HCH) Lindane and Chlorpyriphos below FSSAI specification values of 1.0 and 0.2, respectively.



**Figure 1.** Effect of Ozonisation on pesticide namely Gamma (HCH) Lindane and Chlorpyriphos load of palak samples collected from location-A during the winter and summer of 2016, 2017, 2018 and 2019. [WOOT = Without ozonation treatment, WOT = With ozonation treatment]

- ii. Analysis of pesticides from Location B of Delhi/NCR: Results showed that the concentration of Gamma (HCH) Lindane was higher as compared to FSSAI specification value of 1.0 in palak samples collected during winter 2016, winter and summer 2017, summer 2018, winter and summer 2019 from Location-B and treated with plain water. Similarly, from the results we found that the concentration of Chlorpyriphos was higher as compared to FSSAI specification value of 0.2 in palak samples collected during winter 2016, winter and summer 2017, winter and summer 2019 from Location-B and treated with plain water. While as palak samples treated with ozonisation showed lower concentration values as compared to samples treated with plain water (Fig. 2). The values of both Gamma (HCH) Lindane and Chlorpyriphos were found less than the values of FSSAI specification values of 1.0 and 0.2, respectively.
- iii. Analysis of pesticides from Location C of Delhi/NCR: From the results we found that the concentration of Gamma (HCH) Lindane and Chlorpyriphos was higher as compared to FSSAI specification value of 1.0 and 0.2, respectively, in palak samples treated with plain water and ozonisation, irrespective of their season and year. However, the concentration values of both the pesticides were found lower (below the values of FSSAI Specification) in case of the ozonisation samples as compared to samples treated with plain water (Fig. 3).
- iv. Analysis of pesticides from Location D of Delhi/NCR: Results revealed that the concentration of both Gamma (HCH) Lindane and Chlorpyriphos was higher as compared to FSSAI specification value of 1.0 and 0.2, respectively, in palak samples treated with plain water and ozonisation, irrespective of their season and year.

However, the concentration values of both the pesticides were found lower in case of the ozonisation samples as compared to samples treated with plain water (Fig. 4). Moreover results showed that the values of both Gamma (HCH) Lindane and Chlorpyriphoswere even lower than the FSSAI specification value of 1.0 and 0.2 in all the sample treated with ozonisation.

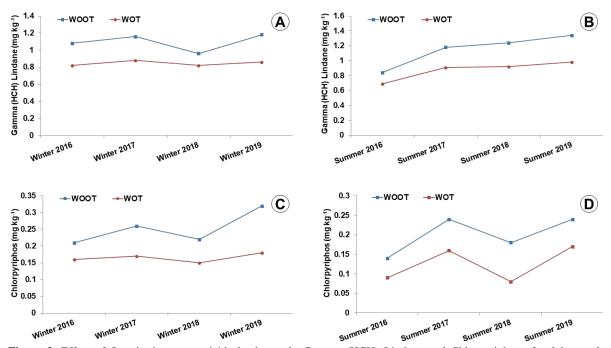
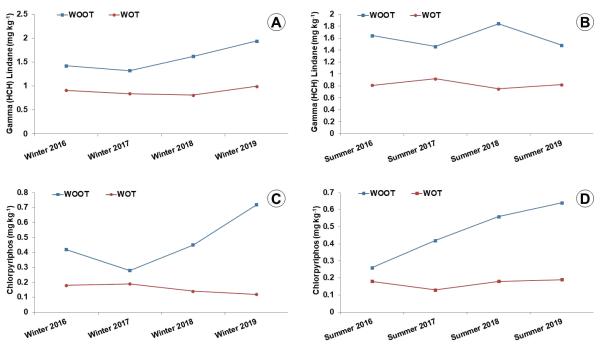


Figure 2. Effect of Ozonisation on pesticide load namely Gamma (HCH) Lindane and Chlorpyriphos of palak samples collected from location-B during the winter and summer of 2016, 2017, 2018 and 2019. [WOOT = Without ozonation treatment, WOT = With ozonation treatment.

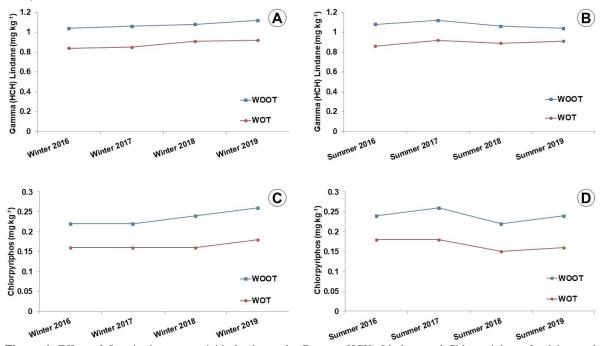


**Figure 3.** Effect of Ozonisation on pesticide load namely Gamma (HCH) Lindane and Chlorpyriphos of palak samples collected from location-C during the winter and summer of 2016, 2017, 2018 and 2019. [WOOT = Without ozonation treatment, WOT - With ozonation treatment]

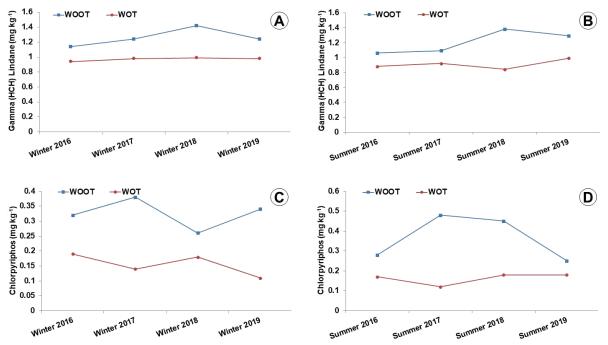
Level of pesticides and effect of Ozonisation (3 ppm) on pesticide load of Radish samples

i. *Analysis of pesticides from Location A of Delhi/NCR*: Results are presented in figure 5. Results revealed that the concentration of Gamma (HCH) Lindane and Chlorpyriphos was higher as compared to FSSAI specification value of 1.0 and 0.2, respectively, in Radish samples treated with plain water. In ozonisation treated samples, concentration values of Gamma (HCH) Lindane was found lower as compared to the FSSAI specification value of 1.0 in the samples collected during 2016, 2017 and 2019, while as the concentration

was higher in the samples collected during 2018. Further, concentration of Chlorpyriphos was lower in the samples collected during winter and summer of year 2016, winter 2018, and summer 2019 and higher in the samples collected during winter and summer of year 2017, summer 2018, and winter 2019. Moreover, results revealed that the concentration values of both the pesticides were lower (below the FSSAI specification values) in case of ozonisation treated samples as compared to plain water treated samples (Fig. 5).

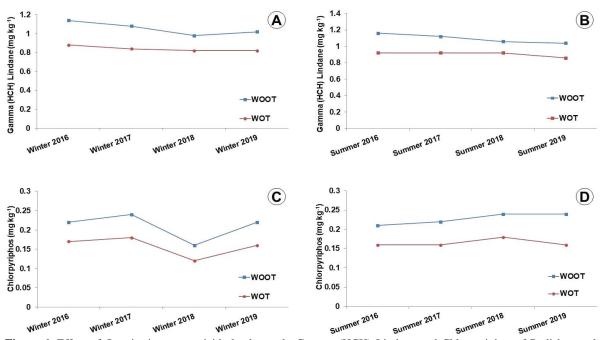


**Figure 4.** Effect of Ozonisation on pesticide load namely Gamma (HCH) Lindane and Chlorpyriphos of palak samples collected from location-D during the winter and summer of 2016, 2017, 2018 and 2019. [WOOT = Without ozonation treatment, WOT = With ozonation treatment]



**Figure 5.** Effect of Ozonisation on pesticide load namely Gamma (HCH) Lindane and Chlorpyriphos of Radish samples collected from location-A during the winter and summer of 2016, 2017, 2018 and 2019. [WOOT = Without ozonation treatment, WOT = With ozonation treatment]

ii. Analysis of pesticides from Location B of Delhi/NCR: Results revealed that the concentration of Gamma (HCH) Lindane and Chlorpyriphos was higher as compared to FSSAI specification value of 1.0 and 0.2 in all plain water treated Radish samples except the samples collected during winter 2018. In ozonisation treated samples, concentration values of Gamma (HCH) Lindane as well as Chlorpyriphos, were found lower



than the FSSAI specification values. Moreover, the concentration values of both the pesticides were lower in case of samples treated with ozonisation as compared to samples treated with plain water (Fig. 6).

**Figure 6.** Effect of Ozonisation on pesticide load namely Gamma (HCH) Lindane and Chlorpyriphos of Radish samples collected from location-B during the winter and summer of 2016, 2017, 2018 and 2019. [WOOT = Without ozonation treatment, WOT = With ozonation treatment]

iii. Analysis of pesticides from Location C of Delhi/NCR: Results revealed that the concentration of Gamma (HCH) Lindane and Chlorpyriphos was higher as compared to FSSAI specification value of 1.0 in all plain water treated Radish samples. In ozonisation treated samples, concentration values of Gamma (HCH) Lindane was found lower than the FSSAI specification value in samples collected during winter and summer 2016, and winter 2017 and higher in samples collected during summer 2017, winter and summer 2018 and winter and summer 2019. Chlorpyriphos concentration was found higher than the FSSAI specification value of 0.2 in all Radish samples except in samples collected during 2016. Comparative results showed that the concentrations of all the three pesticides were lower in the case of the ozonisation treated Radish samples as compared to samples treated with plain water (Fig. 7).

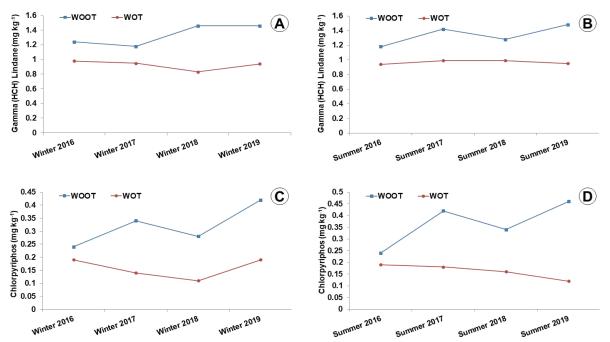
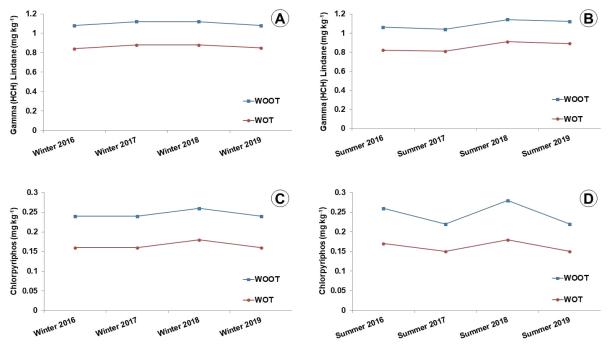


Figure 7. Effect of Ozonisation on pesticide load namely Gamma (HCH) Lindane and Chlorpyriphos of Radish samples collected from location-C during the winter and summer of 2016, 2017, 2018 and 2019. [WOOT = Without ozonation treatment, WOT = With ozonation treatment]

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iv. Analysis of pesticides from Location C of Delhi/NCR: Results showed that the concentration of Gamma (HCH) Lindane and Chlorpyriphos was higher as compared to FSSAI specification value of 1.0 and 0.2 in all plain water treated Radish samples. In ozonisation treated samples, concentration values of Gamma (HCH) Lindane as well as Chlorpyriphos were found below the FSSAI specification values. Comparative results showed that the concentration values of both the pesticides were lower in ozonation treated Radish samples as compared to the plain water treated samples (Figure 8).



**Figure 8.** Effect of Ozonisation on pesticide load namely Gamma (HCH) Lindane and Chlorpyriphos of Radish samples collected from location-D during the winter and summer of 2016, 2017, 2018 and 2019. [WOOT = Without ozonation treatment, WOT = With ozonation treatment]

## DISCUSSION

Pesticides are intentionally used to protect agricultural products from pests to increase the crop production and storage of their produce. However, in due course of time, pesticides get deposited and accumulated in water, soil and biota and enter into the human food chain thus posing a great threat to humans (Sharma *et al.* 2019). Nowadays, India among Asian countries is considered as one of the major pesticide producing countries with annual production of 90,000 tonnes. Globally, India has occupied the twelfth position in pesticide manufacturing (Sharma *et al.* 2019). Annual pesticide usage of different Asian countries reflects India at 2<sup>nd</sup> position after China. Pesticides are considered as the significant contributors to polluting air, water and soil causing severe damage to its flora and fauna. Scientific evidences have pointed out that these pesticides can harm soil ecosystem by affecting the activities of different microorganism thus affecting soil fertility (Brussaard 1997). It has also been reported that these pesticides also affect the absorption process of important mineral nutrients uptake by plants (van-der Werf 1996). Accumulation of pesticides in the ground water and drinking water is a serious concern of everyone as well as for the environmentalists.

The presence of pesticide residues in agricultural crops such as fruits and vegetables even after harvesting is one of the major concerns of every country including India. Several studies along the time window have been conducted to assess the level of several toxic pesticides in the fruits and vegetables produced in India. Many studies reported higher levels of organochlorine insecticides in fruits (Agnihotri *et al.* 1974, Kathpal 1980, Khandekar *et al.* 1982, Lakshminarayana & Menon 1975, Lal *et al.* 1989). Awasthi (1990) observed persistence of synthetic pyrethroids (fenvalerate, cypermethrin and deltamethrin) on sweet pepper. Singh & Kalra (1992) reported the contamination of residues of cypermethrin in the Brinjal. Chahal *et al.* (1992) reported persistence of pesticides namely fenvalerate and endosulfan on okra. Ahmad *et al.* (1993) found contamination of pesticide residues of deltamethrin, cypermethrin, fenvalerate and monocrotophos in okra. Awasthi (1994) reported persistence of different pyrethroids on green chilli.

In the present study we carried out the analysis of different pesticide residues (Gamma (HCH) Lindane, Delta (HCH), DDT (o,p&p,p-isomers of DDT, DDE & DDD) (4,4-DDT), Malathion, Chlorpyriphos, Aldrin, www.tropicalplantresearch.com 113

Dieldrin, Beta-Endosulphane, Endosulphan Sulphate, Alpha Endosulphane) in four different vegetable samples namely Palak, Radish, Green Chilli and Bottle gourd collected during winter and summer seasons of year 2016, 2017, 2018 and 2019. All the vegetable samples containing pesticide residues namely Gamma (HCH) Lindaneand Chlorpyriphos were treated with aqueous solution of Ozone (3 ppm) to reduce the pesticide load. We found that the aqueous solution of Ozone (3 ppm) reduced the levels of both Gamma (HCH) Lindane and Chlorpyriphos in all vegetable samples. These findings reflect that 3 ppm ozaonation can be implemented on vegetables at the personal as well as at the organizational level in order enhance their safety for use in hospitality sectors, as well as for the consumption of native population.

Applications both in commercial and research have indicated that ozone can replace traditional disinfectants with more benefits. Ozone is generally recognized as safe as a disinfectant for foods (Graham 1997).

#### CONCLUSION

Overall we found that the level of Lindane and Chlorpyriphos pesticide residues were higher than FSSAI specification values in vegetable samples collected from different locations of Delhi/NCR, India. There was no effect of type of vegetable, location of sample collection, and season and year of sample collection. Vegetable samples containing higher level of Lindane, and Chlorpyriphos were treated with a 3 ppm aqueous solution of ozone to reduce the pesticide load. We found that ozonation of 3 ppm was more effective than other concentrations in order to reduce the levels of Lindane and Chlorpyriphos pesticide residues.. In conclusion, we suggest that 3 ppm ozonation can be implemented on vegetables at personal and organizational levels to enhance their safety for use in hospitality sectors and the consumption of the native population.

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