Residual Levels of Diazinon and Benomyl on Greenhouse Mushrooms

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ABSTRACT

Background: Pesticides are one of the important sources of environmental pollution that influence human health. The aim of current study was to determine diazinon and benomyl residues levels in mushrooms grown in greenhouses.

Methods: Mushroom samples were obtained from 10 active greenhouses of Hamadan Province, Iran, every 14 days from May 2014. The absorbance of diazinon and benomyl were measured at 435nm in a Chemistry laboratory in Hamadan Branch, Islamic Azad University by spectrophotometer. Data were analyzed by one-way ANOVA and one-sample test in SPSS 20 statistical package.

Results: Diazinon residue levels in mushroom ranged from 0.026 to 0.185mg/kg. Approximately 90.0% of mushrooms were contaminated with diazinon, which was significantly more than MRL for human consumption provided by European Union (0.05mg/kg). Benomyl residue levels ranged from 0.00025 to 0.097mg/kg. Approximately 50.0% of mushrooms were contaminated with benomyl, which was significantly higher than the MRL for human consumption provided by WHO (0.01mg/kg).

Conclusion: Mushrooms of Hamedan Province, Iran, Greenhouses contamination with diazinon and benomyl is higher than international standards.

Keywords: Benomyl; Diazinon; Food Safety; Mushroom; Pesticides.

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INTRODUCTION

Environmental pollution is one of the serious predicaments of the modern world [1, 2]. During the last recent decade, it has turned into a global problem due to the significant increase in the environmental pollutants and lack of precautionary measures or observance of the environmental regulations [2-4].

Agriculture is an essential component of today's society. While agricultural products provide most of the food consumed by the world population, they also impose great pressure on global natural resources [5]. In modern agricultural practices, the use of pesticides is essential to control pests in agricultural and horticultural crops for the production of an adequate food supply for an increasing world population and control of insect-borne diseases [6]. However, it creates inevitable human exposure to their residues through different routes. Exposure of consumers to pesticide residues is undesirable because of their short-term (allergies, headaches, and nausea) and chronic (cancer, neurological disorders and reproductive disorders) impacts. The World Health Organization (WHO) has reported that roughly three million pesticide poisonings occur annually, resulting in 220,000 deaths worldwide [7, 8]. Thus, to monitor the pesticide residues in food commodities, several national and international bodies follow stringent rules to minimize the exposure of consumers to the pesticides [9].

Organophosphorus (OPPs) and organochlorinated (OCPs) pesticides have provided great benefits in the eradication of various pest diseases in agriculture and in combating the propagation of vectors transmitting lethal diseases to human [10]. Organochlorinated pesticides are lipid soluble chemicals and tend to accumulate in living

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organisms. They are primarily used to kill insects. People are exposed to organochlorinated pesticides via diet, primarily through the ingestion of foods. The most important effects of pesticides are water and soil pollutions, as well as the contamination of vegetables, fruits, milk and food products [2, 11, 12].

Human diet is largely based on plants and their products. It is essential to note that a balanced diet is essential for the maintenance of human health [13, 14]. Fruits and vegetables are originally the part of food intake which ought to lead better health for human. Accordingly, everybody is encouraged to consume more vegetables and fruits, as they are essential source of vitamins and fiber [15]. One of the beneficial methods for increasing crop output is greenhouse cultivation. Under greenhouse conditions, the use of pesticides is the easiest way to control pests and diseases, even during harvest time. The impact of pesticide residue can be traced in several diseases such as cancer, nervous system disorder, brain damage, mental diseases, tumors of central nervous system, respiratory system disorder, asthma, Parkinson's disease, abortion, infertility, and skin diseases [16].

Mushroom farming today is being practiced in more than 100 countries and its production is increasing at an annual rate of 6-7%. Present world production of mushrooms is around 3.5 million tones as per FAO Stat and is over 25 million tones (estimated) as per claims of Chinese Association of Edible Fungi. At present, Iran produces annually 40000 tons of various types of edible mushroom. A number of harmful fungi are encountered in compost and casing soil during the cultivation of mushroom. Many of these act as competitor moulds adversely affect spawn run, whereas others attack the fruit bodies at various stages of crop growth producing distinct disease symptoms [17].

Diazinon is the common insecticide for treatment of compost and casing mixture for mushroom cultivation. In addition, benomyl (benlate) is a variety of the chemical structures commercially available as fungicides for different crops and is the common fungicides recommended for the control of major fungal pathogens of mushrooms, e.g. Cladobotryum, Mycogone, Trichoderma, Verticillium (17).

Pesticide residual levels in food commodities affect the international trade, besides their direct implication on human health [18, 19]. Monitoring of pesticide residues in vegetables is a priority objective of pesticide research to avoid possible risks to human health and extensive evaluation of vegetable quality [6]. Therefore, the current study sought to determine diazinon and benomyl residues levels in mushrooms grown in Hamadan's greenhouses, Iran.

MATERIALS AND METHODS

Sample collection and preparation

Mushroom samples were obtained from 10 active greenhouses of Hamadan Province, Iran, every 14 days from May 2014. Only one type of mushroom seed was used, and drip irrigation was employed for all samples. About 500g of mushroom was taken from each row in the greenhouses randomly and they were carried in polyethylene bags to laboratory. The samples were washed with distilled water and then dried. 5kg of the sample was homogenized in a blender containing 100ml of acetone, 75ml of dichloromethane, and 15g of sodium chloride for 3min. The homogenized mixture was allowed to separate into its organic and aqueous layers. The organic phase was transferred to the beaker, and 3g of sodium sulfate was added to remove the remaining water [16].

Standard Curve

A stock solution of carbon disulfide (0.002mg/ml) was prepared in ethanol. The known volumes of the CS_2 stock solution were transferred to 25ml volumetric flasks and 15ml of the color reagent was added. The volume was completed with ethanol, and the solution was allowed to stand for 15min. The absorbance was measured in a continuous flow T80+ Double Beam UV/Vis Spectrophotometers (PG Instruments Limited; UK) at 435nm. A typical standard curve ranged from 0.38-23μg of CS_2/ml, corresponding to 0.10-6mg of CS2/kg in the food sample [20].

Determination of pesticide residue

Pesticide standards were obtained from Sigma-Aldrich®, (USA). The solutions of Cu (II) acetate monohydrate (color reagent) were prepared (0.48g in 100g of diethanolamine/1l of water) according to Caldas *et al* [20], and kept at 5°C protected from light. An acid stannous chloride solution was prepared by dissolving 31.25g of $SnCl_2H_2O$ in 500ml of concentrated HCl and bringing the volume to 2.5l. A 1.0mg/g stock mixture of diazinon and benomyl were prepared in acetone. The absorbance of diazinon and benomyl were measured at 435nm in a Chemistry laboratory in Hamadan Branch, Islamic Azad University by spectrophotometer. For all greenhouses, a control sample with no pesticide added was analyzed. No diazinon and benomyl were found in any control sample tested.

Statistical analysis

To test the differences between mean residue levels of pesticides in evaluated mushrooms, one-way ANOVA was performed (Duncan Multiple Rang Test). The mean residue levels of pesticides were compared with international standard using a one-sample test. Probabilities less than 0.05 were considered statistically significant ($p<0.05$). All statistical analyses were performed using the SPSS 20 (SPSS Inc.; Chicago, IL, USA) statistical package.

RESULTS

Diazinon residue levels in mushroom ranged from 0.026 to 0.185mg/kg (Table 1). The mean residue levels of diazinon were significantly lower than the MRL for human consumption provided by WHO (0.2mg/kg). Approximately 90.0% of mushrooms were contaminated with diazinon, which was significantly more than MRL for human consumption provided by European Union (0.05mg/kg) [16, 21]. Benomyl residue levels ranged from 0.00025 to 0.097mg/kg (Table 1). Approximately 50.0% of mushrooms were contaminated with benomyl, which was significantly higher than the MRL for human consumption provided by WHO (0.01mg/kg) [16, 21, 22].

Table 1. Diazinon and Benomyl residues (mg/kg) in mushroom samples grown in 10 greenhouses of Hamedan, Iran.

Green house	1st sampling	2nd sampling	3rd sampling	4th sampling	Statistical Mean
Diazinon (mg/kg)					
1	0.116	0.131	0.135	0.122	0.126±0.009^c
2	0.11	0.127	0.144	0.119	0.125±0.014^c
3	0.159	0.185	0.171	0.149	0.166±0.016^d
4	0.033	0.054	0.047	0.026	0.040±0.013^a
5	0.121	0.126	0.139	0.134	0.130±0.008^c
6	0.097	0.062	0.075	0.09	0.081±0.016^b
7	0.115	0.128	0.147	0.126	0.129±0.013^c
8	0.13	0.121	0.142	0.135	0.132±0.009^c
9	0.125	0.117	0.124	0.146	0.128±0.012^c
10	0.114	0.136	0.127	0.139	0.129±0.011^c
Benomyl (mg/kg)					
1	0.0054	0.0066	0.0056	0.006	0.0059±0.0005^c
2	0.0018	0.0016	0.002	0.0026	0.002±0.0004^b
3	0.054	0.036	0.042	0.028	0.040±0.011^e
4	nd	nd	nd	nd	-
5	0.0133	0.0142	0.0149	0.0136	0.014±0.0007^d
6	0.0018	0.0013	0.0014	0.0015	0.0015±0.0002^a
7	0.032	0.051	0.04	0.037	0.040±0.008^e
8	0.086	0.097	0.068	0.097	0.087±0.014^g
9	nd	nd	nd	nd	-
10	0.048	0.034	0.062	0.056	0.050±0.012^f

nd=Not Detectable; The letters “a” to “g” represent the statistical differences among mean residue concentrations of pesticides in mushroom samples ($p<0.05$)

There were significant differences within and between some evaluated brands (Table 1). The comparison of the mean residues levels of pesticides between samples revealed significant differences between samples 3, 4 and 6 for diazinon and between samples 1, 2, 5, 6, 8 and 10 for benomyl ($p<0.05$).

DISCUSSION

Residual pesticides on the foodstuffs decrease by various culinary application or with time; related to the kinds and properties of the pesticides. There is clear evidence that culinary treatments, including washing, peeling and cooking can affect the removal or degradation of the pesticide [23].

Diazinon is used in agriculture as a nematicide and insecticide against soil insects and pests of fruits, vegetables, tobacco, forage, field crops, rangelands and pasture. It is also used to keep greenhouses and mushroom houses free of flies. Diazinon is an organophosphorus pesticide with moderate mammalian toxicity, which is active against a variety of agricultural and public health pests [23]. It is readily absorbed by the gastrointestinal tract, through the intact skin, and by inhalation. It is converted to the oxygen analogue diazinon in vivo which then inhibits cholinesterase [21].

The advantages of the application of pesticides in agriculture in producing better crops must be weighed against the possible health hazard arising from the toxic pesticide residues in food. Pesticides should be applied correctly, according to good agricultural practice, using only the required amounts. Culinary applications are necessary to decrease the intake of pesticide residues. It can be concluded that the processes such as controlled dose setting for the use of these pesticides, controlled greenhouse treatments, harvest and storage processes, and culinary applications before consumption have a crucial role in the reduction of residual pesticides, which pose a serious threat to human health and the environment. We strongly suggest the need for a greenhouse crops monitoring program and a model for generating the ongoing data needed for such a surveillance programs aimed at ensuring the safety of the food supply and minimizing human exposure to toxic compounds such as pesticides and other potential contaminants.

CONCLUSION

Mushrooms of Hamedan Province, Iran, Greenhouses contamination with diazinon and benomyl is higher than international standards.

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