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ORIGINAL ARTICLE

The Effect of Fruit Harvest Time and Refrigeration on Reducing Pyridaben Acaricide Residues in Strawberry Fruits

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KEYWORDS

Sanmite;
Pesticide;
Maximum residue level;
Electron capture detector

ABSTRACT: Effect of refrigeration and different harvesting times were investigated to remove residue of pyridaben from strawberry. Transplants of Strawberry were grown in greenhouse and fruit samples were taken at 1, 4, 24, 48 and 72 hours and 7, 14 and 21 days after spraying of pyridaben (Sanmite® 20% WP) at the recommended and twice the recommended doses (0.4 and 0.8lit ha^{-1} respectively). For cooling treatment, fruit samples were refrigerated for 48 hours at 4°C. The study was done as factorial experiment in a completely randomized design (pyridaben doses and harvesting times after spraying) with three replications. Gas chromatography-electron capture detector (GC-ECD) method was used to determine pyridaben residues in strawberry fruits. The recovery rate of acaricide was 98-105% in this method and detection limit of machine was 50µg kg^{-1}. The results revealed that the maximum residual concentrations of pyridaben acaricide in strawberry fruits were observed with an average of 0.42 mg kg^{-1} for the recommended dose and 0.71mg kg^{-1} for twice the recommended dose at 4 and 24 h after application, respectively, which in twice the recommended dose was higher than the MRL (5mg kg^{-1}). Storing fruits in refrigerator for 48 hours had little effect on reducing pyridaben residues in them, and at twice the recommended dose, the concentration of pesticide in fruits was higher than that of Codex standard level.

INTRODUCTION

Many pests and diseases affect the production and storage of agricultural crops and considerably decrease the yield and quality of them. So, pesticide application is an essential part of modern farming, as it significantly reduces yield losses and maintains the quality of fruits and vegetables. Without the application of pesticide, the loss of vegetables from pest damage would exceed 50% [1]. Thousands of active ingredients and formulations of different pesticides are commercially available [2]. Pesticides are divided into different groups based on their physicochemical properties and mode of actions, which are carbamates, organochlorines, organophosphates, pyrethroids, neonicotinoids, triazine, urines, phenoxyacids, and triazoles [3]. Recently, in order to increase the production and quality of agricultural products, pesticides are widely used to reduce the effects of pests, weeds and diseases in various crops. However, the overuse application of pesticides can severely affect the environment and exposure to their residue lead to acute or chronic toxicity to humans [4, 5].

Environmental studies show that pesticide residues are found in all agricultural ecosystems, and when humans are exposed to raw and processed agricultural products contaminated with pesticide residues, these residues are dangerous to human health. These adverse health effects include headaches and nausea, metabolic disorders,

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various cancers, neurological disorders, disturbances in the reproductive system, and endocrine disruption. Children and the elderly, in particular, are more endangered by pesticides [6]. These problems have always been seriously considered by the people. So, the identification and quantification of pesticides in the food are become a public interest [7].

Many countries and organizations around the world have established maximum residue limits (MRLs) for pesticides in food to ensure food safety for consumers and to protect human health. The MRL is the maximum level of a pesticide residue (expressed in mg kg^{-1}) which is legally permitted in or on food or animal feed. Regulatory and enforcement mechanisms are also put in place by governments to monitor compliance of MRLs in foodstuffs [8]. Monitoring the pesticide residues is one of the effective ways to control the quantity of pesticides in food [9]. Because of the most vegetables and fruits are consumed raw without long storage time, they are very useful in pesticide residue monitoring programs. It is quite probable that pesticide residue levels in fresh vegetables and fruits are higher than those in other plant-derived foods [10].

To monitoring pesticide residues in agro-products, sensitive methods are necessary that determined trace amounts these compounds. Gas chromatography- mass spectrophotometry (GC-MS/MS) and liquid chromatography- mas spectrophotometry (LC-MS/MS) have become valuable techniques in multi-residue analysis with most efficient to discriminate residues at ultra-trace levels [11, 12]. Recently, the GC-MS has been accepted in the pesticide analysis field because it simultaneously allows for determination and confirmation of a large number of compounds. Additionally, it has low detection limits as a consequence of its high selectivity from the use of the selected ion monitoring [13].

Pyridaben is belongs to a new acaricide family, the pyridazine, and is one of the most important, widely-used pesticides for the control of various mite species and some insects on different agricultural crops. It was discovered in 1984 by Nissan Chemical and was commercialized in 1991 [14]. In addition, pyridaben was used in more than 32 countries since 1994 because it is Fast-acting, stable, and biodegrades relatively quickly [15]. Despite its low toxicity, laboratory studies shown that it was highly toxic to fish and invertebrates and its intensive applications may lead to undesirable side effects on human health and environmental quality. Boyd et al. [16] have reported that pyridaben can affect the activity of mitochondrial complex I in the electron transport chain. Therefore, the maximum residue limits of pyridaben in some countries have been established, such as 0.5mg kg^{-1} in Spain (peppers, 7 day pre-harvest interval) [17], 5mg kg^{-1} in China (tea) [1] and 0.05mg kg^{-1} in Iran (fruits and vegetables) [18]. Pyridaben can be detected in high water content various products by using a gas chromatography- electron capture detector (GC-ECD) method with a limit of quantification (LOQ) of 0.05mg kg^{-1}.

Two-spotted spider mite (*Tetranychus urticae* Koch, Acari: Tetranychidae) is a major worldwide pest in strawberry, and mainly damages the leaves and stems [1]. The control of this pest is difficult due to its short life cycle and high reproductivity. Although botanical acaricides and natural enemies such as mite predators have been reported to effectively control strawberry spider mite [19, 20], chemical acaricides are the primary means for the rapid and efficient control of this pest in strawberry cultivation [21, 22]. Because of Strawberry has a sweet taste, rich in vitamin C, medicinal properties and high economic benefit, it is widely cultivated worldwide [23]. Strawberry is often consumed fresh, and the contamination of acaricides may affect health risks to humans. Several studies have drawn public attention to the fact that strawberries are often contaminated by pesticides [24]. Most strawberry around the world is produced under protected environments. Excessive uses of pesticides in greenhouses lead to that crop grown under these protected environments have higher level of pesticide residue than similar crop grown in the open field [25]. This research was aimed to determine residues amount of pyridaben acaricide in strawberry fruits using Gas Chromatography Electron Capture Detector (GC-ECD) method and to evaluate the effect of harvesting time and refrigerator storing on pyridaben residues in fruits.

MATERIALS AND METHODS

Plant materials and sampling

Strawberry transplants (*Fragaria×ananassa* cv. Camaroso) were planted in plastic pots (18 cm diameter and cm 20 height) filled with perlite + cocopeat (50%+50%) and were kept in the research greenhouse of Islamic Azad University, Isfahan (Khorasgan) Branch, at 25± 2 °C, 70% RH and 16/8h light/dark frequency. The plants were irrigated every week as needed and were fertigated every 15 days with nutrient solutions until the fruit production. One week after fruit coloring, the plants were sprayed with pyridaben acaricide (SANMITE® 20%WP) at the recommended dose (0.4lit ha^{-1}) and twice the recommended dose (0.8lit ha^{-1}) by hand sprayer. Fruit samples were taken at 1, 4, 24, 48 and 72 hours and 7, 14 and 21 days after spraying. 30 minutes after sampling, samples were stored in freezer (-18°C) for next analysis. For cooling treatment, fruit samples were refrigerated for 48 hours at 4°C. Method No. 9037 of Institute of Standards and Industrial Researches of Iran about multi-point method for measuring pesticides residues in nonfat foods using gas chromatography was used in this study. Purifying method was including acetone extracting, liquid- liquid isolation with dichloromethane and purification with silica gel-active carbon column [12].

Chemicals and Reagents

The pyridaben reference standard was prepared from Nissan chemical (Tokyo, Japan) with certified purity 99%. Acetone, toluene, dichloromethane, n-hexan, sodium chloride and anhydrous sodium sulfate were obtained from Merck (Darmstadt, Germany). Active carbon, Silica gel and pentachloronitrobenzene as internal standard for GC were purchased from Sigma-Aldrich (Louis, MO, USA). All the organic solvents used were higher performance liquid chromatography (HPLC) grade. Washing solution was including dichloromethane, toluene and acetone by volume ratio of 5:1:1.

Samples preparation

One hundred gram of samples was grinded and 200ml of acetone was added to it. The mixture was homogenized for 30 seconds and then filtered. 50 ml of filtered solution was mixed with 250 ml of water, 25 of saturated sodium chloride and 50 ml dichloromethane and the mixture was shaken vigorously for two minutes. Extraction was repeated again with another 50 ml of dichloromethane and for dewatering poured on 30 grams of sodium sulfate for 30 minutes. After filtering the solution, it was dried to approximately 2 ml using a vacuum rotating evaporator and then dissolved in 10 ml of dichloromethane [12].

Purification of samples

Ten ml of obtained solution from extraction stage was added to prepared column and 5ml of dichloromethane was added to it. Obtained liquid from column was collected in a 250ml balloon. The column was washed with 140ml of washing solution. Obtained liquid was evaporated to approximately 2ml using a vacuum rotating evaporator. At the end, the obtained solution was poured in 2ml vials and dried. Collected samples were dissolved in 500µl of toluene prior to injection to device [11, 12].

Isolating and measuring the poison

Agilent (6890 GC/MS) gas chromatography equipped with electron capture detector (ECD) and HP-5 column (30 m long, 0.32 mm internal diameter, and 0.25 mm film thickness) was used for analysis the quantitative amount of pyridaben acaricide. Carrier gas was nitrogen (99.9% pure). Temperature program of machine was: 50°C for 4 minutes, increasing by 25°C per minute slope until 290˚C and four minutes in this temperature. Detector and injector temperatures were adjusted to 390°C and 270˚C respectively [12, 26].

Standard concentrations of pyridaben (0.1, 0.5, 1, 5, 10, 20, 40, 80, 150, and 250mg kg^{-1}) were prepared and injected to GC-ECD machine and the calibration curves were plotted to use in determination of various acaricide concentrations. Identification of extant poison in samples was done by comparing peak retention times of samples chromatograms with standard chromatograms. To minimize the errors of volume or method, inner standard of machine (pentachloronitrobenzene) was used. The recovery rate of method was determined by adding specific amount of pyridaben standard to normal straw berries and extracting it by mentioned method. The recovery rate of this method was 98-105% and detection limit of machine was 50μg kg^{-1} (Figures 1 and 2).

Experiment design and Statistical analysis

The study was carried out as factorial experiment in a completely randomized design with two factors (pyridaben doses and harvesting times after application) at three replications. The level of pesticide residues in the samples was calculated in mg kg^{-1} automatically using the Agilent GC software. Statistical analyses were performed using MSTAT-C v.1.42 and Excel 2010. Mean comparisons were done using Duncan multiple ranges test at 5% probability level ($P < 0.05$).

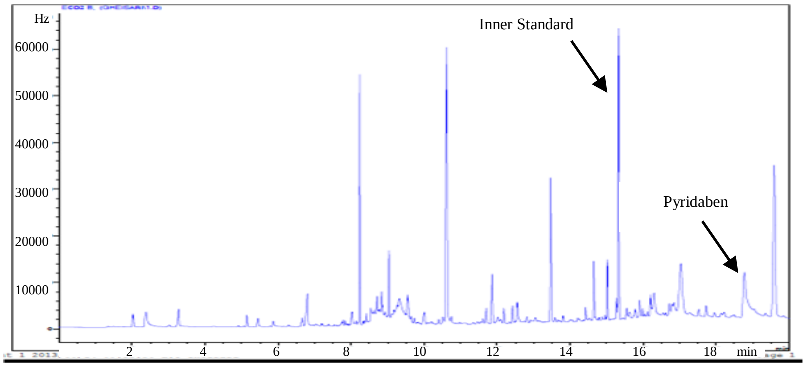

Figure1. GC-ECD chromatogram of pyridaben acaricide, one hour after application of recommended dose (0.4lit ha^{-1})

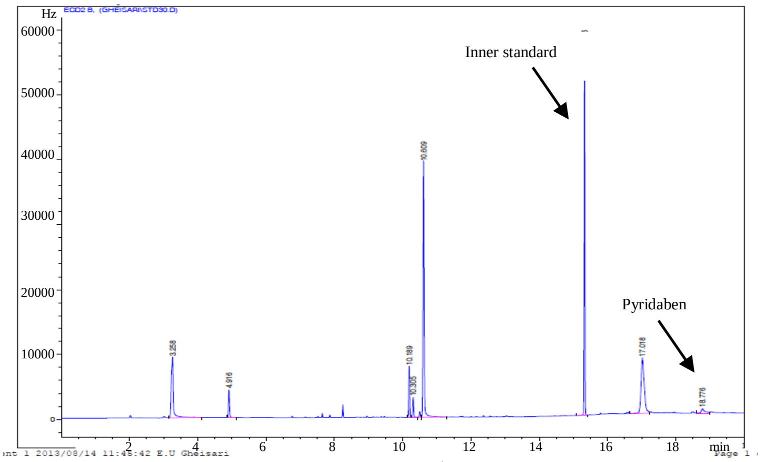

Figure 2. GC-ECD chromatogram of 30 mg kg^{-1} of pyridaben reference standard.

RESULTS

The results of variance analysis showed that there is a significant difference between the recommended dose (0.4lit ha^{-1}) and the twice recommended dose (0.8lit ha^{-1}) on pyridaben residues at the 5% probability level (Table 1).

Also, the results of variance analysis showed that the interaction effect of acaricide doses (recommended dose and twice recommended dose) and different hours of fruit harvest after foliar application is significant at the level of 1% probability (Table 2).

Table 1. Variance analysis of effect of acaricide different doses on Pyridaben residues in strawberry fruits

Sources of Variability	Degree of Freedom	Sum of Squares	P value
Pyridaben doses	1	513.521*	0.1108
Error	46	74.941	
Coefficient of Variation (%)		34.21	

* is significant at the 0.05 probability level.

Table 2. Variance analysis of interaction of pesticide doses and harvesting times of strawberry fruits on pyridaben residues.

Sources of Variability	Degree of Freedom	Sum of Squares of recommended dose	Mean of Squares of twice recommended dose	P value
Harvesting times after pyridaben spray	7	52.571	268.756**	0.0314
Error	16	31.333	43.542	
Coefficient of Variation (%)			34.21	

** is significant at the 0.01 probability level.

According to Figure 3, the highest residue concentration of pyridaben acaricide in recommended dose (0.4lit ha^{-1}) was observed four hours after spraying in all replications and for twice the recommended dose (0.8lit ha^{-1}) was related to 24 hours after spraying. Also, recommended dose showed that pyridaben residue was ascending trend for first four hours and descending trend after that while in twice recommended dose, ascending trend was observed until 24 hours and after that descending trend was started.

The trend of pyridaben residual changes at different fruit harvesting times after spraying showed that the residue of this acaricide in the twice recommended dose (except in the third week) was more than the recommended dose. Also, the remaining amount of pyridaben in none of the doses used did not reach zero even after 21 days (Figure 3). As results represented in Figure 3, the allowable residue level of this acaricide in strawberry fruits (0.2mg kg^{-1}) for EU commission, were reached in the recommended dose and the twice recommended dose at 7 and 21 days after spraying respectively.

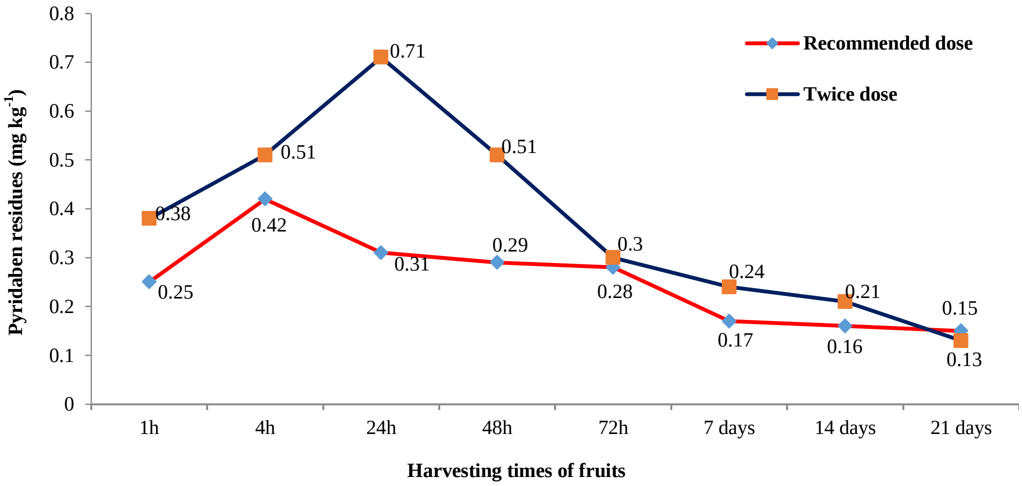

Figure 3. Residual changes of pyridaben in sprayed strawberry fruits with the recommended and the twice recommended doses at different times after application.

Comparisons of means between different fruit harvest times after acaricide spraying at the recommended dose showed that there was no significant difference between 1, 4, 24, 48 and 72 hours after spraying, in the amount of pyridaben residue. While there is a significant difference between 4 hours after spraying and 1, 2 and 3 weeks after spraying. Also, comparisons of means between different hours of fruit harvest after pyridaben spraying at the twice recommended dose showed that there was a significant difference between the mean of pyridaben residue in strawberry fruits one hour after harvest with 4, 24 and 48 hours after acaricide spray. While there is no significant difference between 1 hour and 72 hours, 1, 2 and 3 weeks after spraying (Table 3).

Table 3. Effects of harvesting times of strawberry fruits and pesticide doses on means (±SE) of Pyridaben residues.

Harvesting times after pyridaben spray	Pyridaben residues in recommended dose (mg kg^{-1})	Pyridaben residues at twice recommended dose (mg kg^{-1})
1 h	0.25±0.06 ^{ab}	0.38±0.06 ^{b}
4 h	0.42±0.08 ^{a}	0.51±0.15 ^{ab}
24 h	0.31±0.04 ^{ab}	0.71±0.25 ^{a}
48 h	0.29±0.07 ^{ab}	0.51±0.09 ^{ab}
72 h	0.28±0.06 ^{ab}	0.30±0.07 ^{bc}
7 days	0.17±0.06 ^{bc}	0.24±0.06 ^{bc}
14 days	0.16±0.05 ^{bc}	0.21±0.05 ^{cd}
21 days	0.15±0.03 ^{bc}	0.13±0.03 ^{d}

Means followed by different letters in each column are significantly different (P<0.05, Duncan).

The results of variance analysis of the effect of refrigerated strawberry fruits in different doses of acaricide on pyridaben residue show that between the recommended dose (0.4lit ha^{-1}) and twice the recommended dose (0.8lit ha^{-1}) there is a significant difference at the 5% probability level (Table 4).

Table 4. Variance analysis of effects of refrigerator storing of strawberry fruits on Pyridaben residues.

Sources of Variability	Sum of Squares	Degree of Freedom	P value
Acaricide doses	3313.500*	1	0.1108
Error	181.333	4	
Coefficient of Variation (%)		34.21	

* is significant at the 0.05 probability level.

The results of the effect of cold (refrigerated storage) on pyridaben residues in strawberry fruits showed that the average residual amount of this acaricide in 48 hours after refrigerated fruits for the recommended dose and twice the recommended dose, were 0.43 mg kg^{-1} and 1.36 mg kg^{-1} respectively. Comparisons mean of the pyridaben residue between the recommended dose and twice the recommended dose in refrigerated fruits showed a significant difference at the 5% probability level (Table 5).

Table 5. Effects of refrigerator storing of strawberry fruits on means (±SE) of Pyridaben residues

Used doses	Pyridaben residues (mg kg^{-1})
Recommended dose	0.43±0.06^{b}
Twice recommended dose	1.36±0.10^{a}

Means followed by different letters are significantly different (P<0.05, Duncan).

DISCUSSION

Measurement of pyridaben residue using gas chromatography-electron capture detector showed that the residues of this pesticide decreased over the time and refrigerated storage. Based on the results, the maximum concentration of pyridaben residues in strawberry samples was seen four hours and 24 hours after pesticide application for recommended and twice recommended doses respectively. Although the, in the first hours (one to four hours) after spraying, the amount of this pesticide in fruits was increased and at least three days are required to fall the pyridaben residues below the MRLs. This result is accordance with other studies [27, 28] on the durability of pesticides residues on cucumber; which in those, the residue of ethion was decreased more than 80% after 7 days. In other study, the maximum level of imidacloprid residues was observed one hour after application [29].

It seems that the decrease in pyridaben residue is due to decomposition (because of environmental factors such as light and temperature) and evaporation from the plant surface. So that over the time, this acaricide decomposes from the surface of the fruit tissue and less residue remains in the fruit. This result is in accordance with

Mohamed et al. [24] and Carretero et al. [30]. Accordingly, the amount of pesticide residues in agricultural products is affected by environmental conditions. Okihashi et al. [31] concluded that the pesticide residues in fruits decreased with the over the time after application. Our results are in accordance with this study.

Storing fruits and vegetables at low temperatures is another common method of processing agricultural products. In this research storing the fruit samples in refrigerator (at 4˚C for 48h) did not lead to the expected reduction in pyridaben residues. So that the average residual amount of pyridaben in 48 h after refrigerated fruits for the recommended dose and the twice the dose recommended, were 0.43 mg kg^{-1} and 1.36 mg kg^{-1} respectively. Leili et al. [27] and Naser et al. [29] reported that storage of cucumber fruits for one day in greenhouses temperature led to more reduction (about 33%) in the imidacloprid residues than refrigerated storage (about 10-20%). Our findings in this regards are consistent with them. Fruits storage at 4 ˚C had less effect on reducing of pesticide residues than environmental temperature. Therefore, refrigerated storage had little effect on reducing pyridaben residues. This may be due to that at lower temperatures, the decomposition of pesticides takes place much less than at higher temperatures. In other words, high temperature increases the degradation of pesticides, so storage in the refrigerator causes the pesticides residues to degrade later and their chemical structure to be more stable. Fenoll et al. [32] showed that pesticides residues are differently reduced by refrigerated storage, depending on the characteristics of each pesticide and their stability at different temperatures. Also, Hassanzadeh et al. [33] concluded that refrigerated storage had little effect on imidacloprid residues, which also confirmed in this study.

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Conflict of interests

The authors declare that there is no conflict of interest.

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