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Does pesticides pollution affect rice plants in the southern coastline of the Caspian Sea?

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ABSTRACT

Considering the increasing rice consumption in the world and also the excessive application of pesticides to increase production, an experiment was conducted to determine the pesticide residues and their effects on nitrogen, potassium and phosphorus content of Hashemi, Khazar and Gohar (SA13) rice cultivars, in a factorial design at randomized complete block design with three replications. In this study, rice plants were treated with three pesticides including insecticide Diazinon, herbicide Butachlor and fungicide Tricyclazole which are commonly used in the paddy fields of the southern coastline of the Caspian Sea, with standard concentrations recommended for these pesticides. The results indicated that the impacts of different pesticides on nitrogen, phosphorus and potassium contents in rice grains of Hashemi and Khazar cultivars were not significantly different. However, the nitrogen content reduced significantly in Gohar cultivar treated by Butachlor and Tricyclazole in comparison with control ($p \leq 0.01$). Determination of pesticides residues showed that Diazinon residue in white grains of Hashemi, Khazar and Gohar cultivars was lower than recommended limit determined by Codex (0.1 ppm), and also Tricyclazole amount was below the limit of detection. Therefore, using pesticides in permissible limits is strongly recommended. However, it cannot be concluded that using pesticides; even in permissible limits, does not have dangerous impacts over time on living organisms of the Caspian ecosystem.

Key words: Caspian coastline, Rice, Pesticides, Mineral elements

INTRODUCTION

Environmental pollution is one of the serious challenges in the modern world. During the last decade, due to significant increase in the environmental pollutantsand lack of precautionary measures or observance of the environmental regulations, it has become a global problem (Arjmandi *et al,* 2010). It was recognized that pesticide residues are important factors in contamination various food and endangering the biological food chains (Khani *et al*, 2011). Excessive use of pesticides in agricultural production, causing a phenomenon known as "pesticide residue" is considered an unsafe factor affecting environment as well as human health (Jahed-Khaniki *et al*, 2011). Pollutants may enter plant roots directly from contaminated soils into the roots and then transporting them to the plant body by the xylem (Chen *et al,* 2007). Pesticide pollution is the main environmental issue for rice growing areas, because pesticides in the rice field are easily enter to the open environment and may affect the quality of resources such as groundwater and surface water (Nhung *et al*, 2009; padovani *et al,* 2006; Elfman *et al,* 2011; Rahbar Hashemi *et al*, 2013). About 800 pesticides are used in the world. Among them, 211 chemical compounds have been recorded in Iran as used with different formulations and applications. However,the important issue is the amount of pesticides

and their residues in agricultural areas (Shokrzadeh *et al,* 2013). Rice is an important foodstuff throughout the world and the quality of the grain can be compromised by the presence of pesticide residues. The biggest amount of rice production takes place in three provinces of Iran including Guilan, Mazandaran and Golestan adjacent to the southern coastline of the Caspian Sea comprising 71% of the total cultivable areas of the country. Guilan is one of the provinces that has attracted much attention regarding the agricultural products, especially rice. This province has allocated 35% and 42% of paddy production and cultivation areas of the country, respectively (Peykani *et al,* 2008).

Diazinon [O,O-diethyl-O-2-isopropyl-6-methyl (pyrimidine-4-yl) phosphorothioate] is an organophosphate insecticide for agricultural applications as well as a pesticide for rice, tomato, potato and grapes (Moore & Kroger, 2010; Ghassempour *et al,* 2002). Herbicide Machete or Butachlor (N-(butoxymethyl)-2-chloro-N-(2,6-diethyl-phenyl)acetamide) is mainly used in the spring against various weeds (Arshad *et al,* 2006). Tricyclazole (5-methyl-1,2,4-triazolo [3,4-b]benzothiazole) is a common systemic fungicide used to control rice blast, especially in Asian countries (Phong *et al,* 2009; Habibzadeh *et al,* 2012).

Thus, regarding the numerous reports about the contamination of various pesticides in the rice field of the Caspian Sea coastline, this study aimed determining the residues of such toxic chemicals in the biotic environment. Moreover, we investigated the residues of pesticides in the grains of three rice cultivars and their effects on the N, P and K uptake.

MATERIALS AND METHODS

Field experimen

Field experiment was carried out at the Rice Research Institute in Rasht, Iran. The experiment was carried out in 36 plots, each with an area of 15 m^2. The area was separated by irrigation and drainage channels. A complete randomized block design (CRD) was applied with three replicates. In this study, rice plants (including Hashemi, Gohar (SA13) and Khazar cultivars) were treated in separate plots with three pesticides (insecticide Diazinon, fungicide Tricyclazole and herbicide Butachlor) commonly used in paddy fields in north of Iran. Diazinon, Tricyclazole and Butachlor were applied at recommended dose for each plot: 22.5, 0.75 gram and 6 ml, respectively. The sampling was performed after currency period of the pesticides.

Sample preparation

Measuring N, P and K concentrations

N, P and K contents of rice cultivars were determined in dried white grain of rice. Nitrogen measurement was carried out using Kjeldahl method, phosphorus measurement using spectrophotometer and the absorbance was measured using UV-Spectrophotometer at 880 nm. Calibration curve was prepared using potassium dihydrogen phosphate as standard. Total potassium was measured using Flame photometry at 766.5 nm and standards were prepared with potassium chlorideusing the method of Vijayarengan *et al,* (2012).

Determination of pesticide residues

The comminuted rice samples (25 g) was sieved to 2 mm and then put into a cone flask with 75 ml acetone. The cone flask was capped and shaken on a shaker for 1 h. The extracts were filtered with a filter paper, followed by evaporating with the rotary evaporator at 50 °C until the final volume reduced to 10 ml. The sample was transferred to a separator funnel containing 25 ml 4% sodium chloride, followed by liquid–liquid partitioning with dichloromethane two times at 25 and 15 ml, respectively. The organic phases were collected by pouring through a funnel plugged with a small piece of cotton wool overlaid by a portion of anhydrous sodium sulfate, which had been previously washed with the same solvent. The final sample was concentrated to 2 ml with a rotary evaporator at 50 °C for gas chromatography analysis (Yi & Lu, 2006).

Gas chromatographic determination

Gas chromatography was used to analyze all extracts. A Young Lin Gas Chromatograph System was coupled with a Flame Ionization Detector (FID) and fitted with a TRB-5column (length 30 mm, diameter 0.53 mm and thickness of stable phase 1.5 μm). The gas chromatograph was equipped with a split/splitless injector with electronic pressure must control. The temperature

program was as follows: For detection of Diazinon, the initial temperature was 80 °C, for 20 min, followed by 10°C min^{-1} gradually increasing to 120 °C, for 1 min, followed by 20 °C min-1 increasing to 280 °C, for 10 min. The total run time was 25 min. For detection of Tricyclazole, initial temperature was 120 °C, for 1 min, followed by 20 °C min^{-1} increasing to 280 °C, for 15 min. The operating condition was H2 gas flow, 6 $ml.min^{-1}$; injector temperature, 300°C; and detector temperature, 300 °C. The extracts, standards, and blanks were injected into the gas chromatograph in splitless mode.

The chromatogram of sample obtained under the optimized conditions was shown in Figs. 1 & 2, respectively. The retention time of Diazinon and Tricyclazole were 15.41 and 15.76 min, respectively.

Statistical analysis

Statistical analysis of the data was carried out using SAS Software (1.9) and the mean of the groups were compared using Duncan's multiple range significant differences test (P≤05).

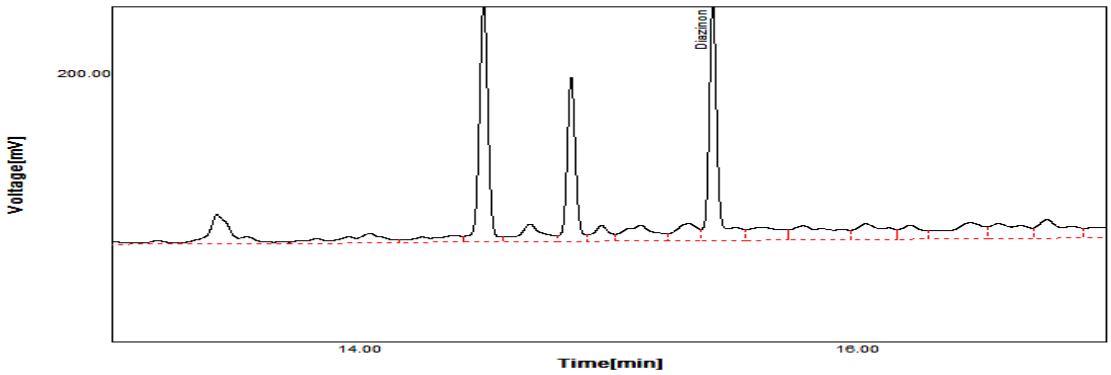

Fig. 1. Chromatogram of standard Diazinon.

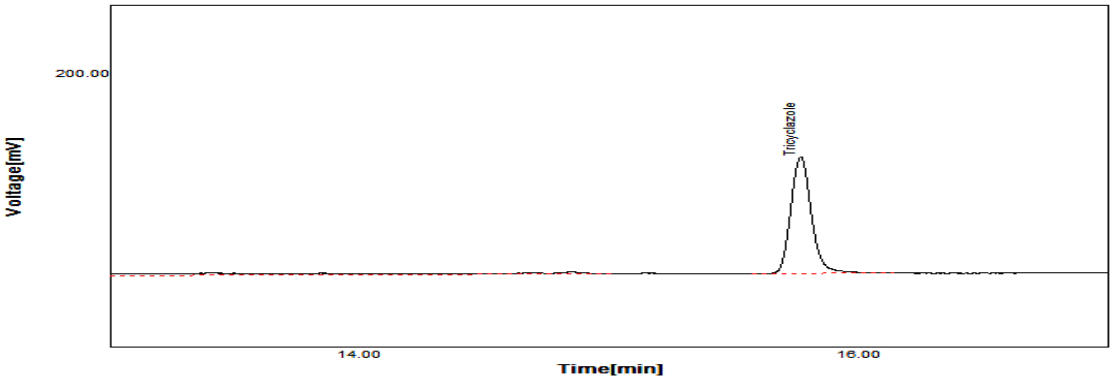

Fig. 2. Chromatogram of standard Tricyclazole.

RESULTS

Effect of pesticides on the nitrogen uptake

According to the results, all pesticides had little effects on the uptake of N in Hashemi and Khazar rice cultivars in comparison with control, but there was significant reduction of nitrogen content in Gohar (SA13) cultivar treated with herbicide Butachlor and fungicide Tricyclazole (p≤0.01) (Fig.1).

Effect of pesticides on the phosphorous uptake

Statistical analysis of data (Fig. 2) showed that there was no significant difference regarding the uptake of P in all three examined cultivars treated with pesticides. However, phosphorous content of Gohar cultivar increased in Diazinon treatment 24% and Butachlor treatment by 6% in contrast to other cultivars (Fig. 4).

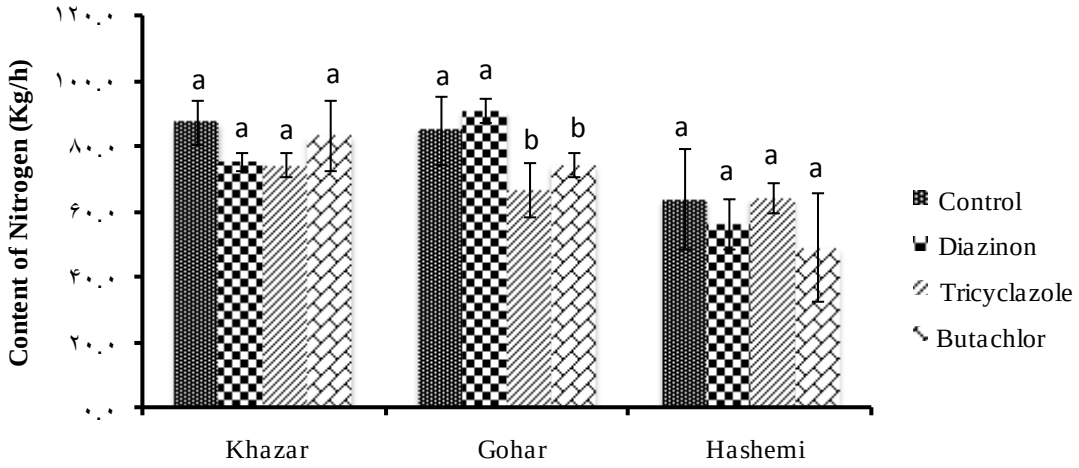

Fig. 1: Effect of Pesticide on content of nitrogen compared with control

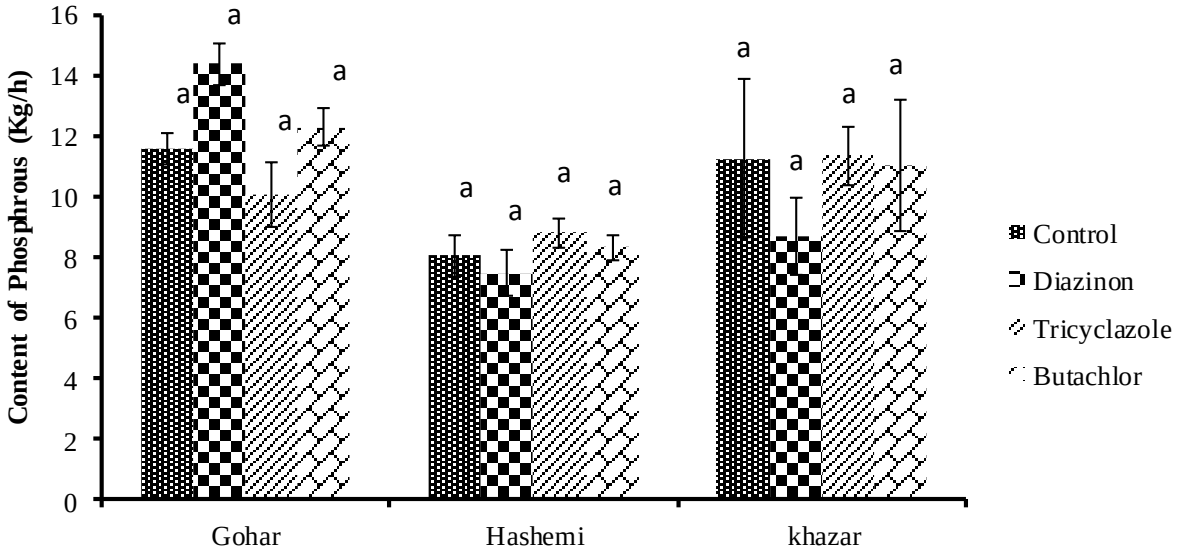

Fig. 2: Effect of Pesticide on content of phosphrous compared with control

Effect of pesticides on the potassium uptake

The results indicated that there was no significant difference ($p \leq 0.05$) in the content of K exposed to all pesticides compared with control (Table 5). However, a slight reduction was observed regarding the potassium uptake in three examined cultivars treated with all pesticides except for Diazinon treatments in which K uptake was increased by 3% in Gohar cultivar. Also, the results showed that the reduction of potassium uptake in Khazar cultivar was higher than in other cultivars.

Pesticide residues in rice grain

The residue of Diazinon in Hashemi, Gohar and Khazar ricegrain was 0.030, 0.076 and 0.012 ppm, respectively, while the residue of Tricyclazole was undetectable in all cultivars. Because of the lack of standard, Butachlor residue was not determined (Table 1).

Table 1. Determination of Diazinon and Tricyclazole in rice grain.

Pesticide	Hashemi	Khazar	Gohar
Diazinon	0.030 ppm	0.012 ppm	0.076 ppm
Tricyclazole	ND	ND	ND

DISCUSSION

Effect of pesticides on the uptake of N, P and K

Results of the present study indicated that the effects of different pesticides on nitrogen, phosphorus and potassium content of rice grain in the Hashemi and Khazar cultivars were not significantly different. However, the nitrogen content reduced significantly in Gohar variety treated with Butachlor and Tricyclazole. Anatomy, morphology and physiology of crop seedlings are all factors that determine the extent of pesticide effectiveness and crop injuries. Crop species are different in their sensitivity and responses to pesticides. Differences have been also reported among different crop cultivars in response to the same or different pesticides. In addition, cultivars are different in germination, emergence, growth and development, physiological and biochemical responses (Qasem, 2011). Qiu *et al,* (2004) observed that pesticides had little effect on the uptake of N by rice roots as compared with control. Narwal *et al,* (2001) reported that rice seeds treatment with pesticides significantly reduced the uptake of N, P and K. Huang & Ziong (2009) also reported that pesticide treatment decreased nitrate content. Meanwhile, Vijayarengan *et al,* (2012) found that the application of pesticides increased the uptake of N, P and K in Red Amaranth. The potential impact of pesticides on plant physiology depends on the crop cultivar, the active component, dosage and frequency of pesticide application. Crop plants may vary in the level of tolerance to pesticide exposure. Some pesticides within one pesticide class could reduce photosynthesis and respiration while other pesticides in the same class do not (Qiu *et al,* 2004). Different mechanisms have been suggested, how the certain concentration of pesticides impacts the physiological and biochemical activities of the plants. For instance, the presence of pesticide residues (solutes) in soil distresses the thermodynamic activity of water along with micro and macro nutrients in nearby soil (Siddiqut & Ahmed, 2006). Among numerous elements affecting plant growth, nitrogen (N) is regarded as one of the most important essential elements and a key factor that determine plant growth and productivity. Any significant alteration in N uptake and the activities of N assimilatory enzymes due to different types of environmental stresses would severely affect the growth and productivity of crops (Huang & Ziong, 2009).

In current research, significant reduction was observed in nitrogen uptake of Gohar cultivar treated by Tricyclazole and Butachlor. The presence of pesticide residues in soil distress the water potential which reduces uptake of nutrients from the surrounding soil, depolarizes the plasma membrane of the root cells (Siddiqut & Ahmed, 2006). Therefore, reduced nitrogen due to plasma membrane damage is expected.

Pesticides residue

To eliminate or reduce biotic stresses affecting plant production, farmers have to use a variety of pesticides. However, pesticides not only remain on the surface of the products, but also penetrate into the vegetables and grains (Jahed-Khaniki *et al,* 1390, Hassanzadeh *et al,* 1387, Arjmandi *et al*, 2010). Although intensive application of pesticides in rice paddy fields increases grain production, its usage has several drawbacks. Due to indiscriminate use of pesticides, residues of several pesticides can be found in rice, not only affecting the quality of rice, but also threatening human health and the environment (Joeng *et al,* 2012).

According to the results of the present stud, the residue of the Diazinon in Hashemi, Gohar and Khazar cultivars was 0.030, 0.076 and 0.012 ppm, respectively, which was lower than the Maximum residue limit (0.1 ppm) established by Codex Organization. The residue of Tricyclazole was also lower than the detection limit. It has been reported that there is relationship between the time of spraying pesticides and harvesting time and the amount of residue of pesticides in plants (Shokrzadeh *et al,* 2013).

Therefore, residues lower than permissible limits and limit of detection in this study is possibly due to the application of pesticides in the permissible limits and also theinterval of two months between spraying and harvesting time. Meanwhile, Yi & Lu (2006) reported that the residue of Probenazole in rice was undetectable at recommended level 63 d after application.

CONCLUSION

The results of this study indicated that the examined physiological parameters in all three cultivars treated with insecticide of Diazinon, fungicide of Tricyclazole and herbicide of Butachlor were not significantly different compared with control. Also, the results showed that residue of Diazinon in Hashemi, Gohar and Khazar cultivars was lower than the permissible limit established for this pesticide (0.1 ppm) by Codex Organization. Therefore, using pesticides in permissible limit is strongly recommended.

Finally, according to the results, although pesticide residues were lower than permissible limit, using pesticides over the time in the Caspian Sea coastline, even in permissible limit, can possibly be dangerous for consumers of products as well as other organisms living in this ecosystem. So, there should be more investigation in this regard to clarify the precise impacts of pesticides on various living organisms.

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تاثیر آلودگی آفتکش‌ها بر مزارع برنج حاشیه جنوبی دریای خزر

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چکیده

با توجه به افزایش روز افزون سرانه‌ی مصرف برنج در سراسر جهان و همچنین گسترش کاربرد آفت‌کش‌های مختلف جهت افزایش تولید محصولات، آزمایشی به منظور تعیین باقیمانده‌ی آفت‌کش و بررسی اثرات جانبی آن بر روی پارامترهای مختلف برنج ارقام هاشمی، خزر و گوهر (SA13)، به صورت طرح فاکتوریل در قالب بلوک‌های کامل تصادفی با ۳ تکرار طراحی شد. در این پژوهش، ارقام مورد بررسی با سه آفت‌کشی که به طور معمول (حشره‌کش دیازینون، علف‌کش بوتاکلر و قارچ‌کش تری سیکلازول) در مزارع برنج حاشیه جنوبی خزر استفاده می‌شوند، با غلظت مجاز توصیه شده برای این سموم، تیمار شدند.نتایج نشان داد که اثر آفت‌کش‌های مختلف بر میزان عناصر نیتروژن، فسفر و پتاسیم در ارقام هاشمی و خزر اختلاف معنی داری نداشت. میزان نیتروژن دانه رقم اصلاح شده‌ی گوهر، برای تیمار علف‌کش بوتاکلر و قارچ‌کش تری‌سیکلازول در مقایسه با کنترل در سطح ۱ درصد اختلاف معنی‌داری را نشان داد.نتایج حاصل از بررسی باقیمانده سموم نیز نشان داد که باقیمانده‌ی حشره‌کش دیازینون در دانه‌ی سفید ارقام مختلف برنج هاشمی، خزر و گوهر برنج کمتر از حد مجاز تعیین شده توسط کدکس (۰/۱ میلی گرم بر کیلوگرم) و قارچ‌کش تری سیکلازول نیز پایین تر از حد تشخیص بود. بنابراین، بر اساس نتایج این تحقیق، در صورت استفاده از آفت‌کش‌ها در حد مجاز، می‌توان به سلامت دانه سفید برنج ارقام بررسی شده تقریبا اطمینان داشت. گرچه نمی‌توان نتیجه گرفت که استفاده از سموم، حتی در حد مجاز، در دراز مدت روی موجودات زنده موجود در اکوسیستم خزری بی تاثیر است.

*مولف مسئول