Dissipation kinetics and risk assessment of diniconazole in raphanus sativus l.

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Abstract:

This study investigated the degradation characteristics and conducted a risk assessment of four pesticides (Diniconazole, Dinotefuran, Metconazole, and Tebuconazole) in the leaves and roots of radish. Radish was cultivated in two greenhouse fields, and samples were collected at 0, 1, 2, 3, 5, 7, and 10 days after pesticide application. Sample analysis was performed using LC-MS/MS, and the recovery rates ranged from 70.1% to 118.6%. The biological half-life of Diniconazole was found to be 6.2 days (leaf and root), Dinotefuran was 5.3 days (leaf) and 4.6 days (root), Metconazole was 9.3 days (leaf) and 3.2 days (root), and Tebuconazole was 8.0 days (leaf) and 5.1 days (root). After comparing the maximum residue limits (MRL) of each pesticide in Korea with the residues during the pre-harvest interval (PHI), Diniconazole showed a Hazard quotient (HQ) exceeding 1, indicating potential risks for true consumers. Furthermore, Tebuconazole showed an HQ of 0.3 or higher, indicating a significant level of risk.

Keywords:

Pesticides, dissipation pattern, risk assessment, *Raphanus sativus*, radish

1. Introduction:

In modern society, pesticides are considered economically crucial inputs in agriculture. They play a vital role in enhancing crop productivity and reducing time and labor costs, primarily through the advancement of herbicides. However, the residual pesticides in agricultural products due to their use are a major factor in endangering human health. Therefore, in the Republic of Korea (ROK), for agricultural products without set maximum residue limits (MRLs), the Positive List System (PLS) is applied to restrict pesticides to below 0.01 mg/kg as regulated by the Rural Development Administration (RDA). Additionally, to minimize damages to producers and consumers, ROK sets pre-harvest residue limits (PHRLs) to manage residual pesticides before cultivation. The PHRLs are determined and established using residual pesticide reduction constants and biological half-lives, and they predict residual amounts at harvest to prevent unsuitability resulting from exceeding MRLs. Therefore, establishing MRLs and PHRLs for domestic agricultural products is essential to ensure safety.

Radish (*Raphanus sativus* L.) is an agricultural product in the Brassicaceae family, and it is consumed not only for its root but also for its leaves and sprouts. Radish contains various phytochemical contents such as flavonoids, terpenes, and minerals.

2. Materials and methods:

2.1. Materials and standards:

The standard pesticides (DNC, DNT, MTC, and TBC) were purchased from Sigma Aldrich (PO, USA). The purities of the standard pesticides ranged from 98.0% to 99.8%. The pesticides used in the packaging test, including Diniconazole 5% wettable powder (Vinnari), Dinotefuran 10% wettable powder (Osine), Metconazole 20% suspension concentrate (Sallimkkun), and Tebuconazole 20% suspension concentrate (Silvaco Plus), were purchased as commercial products. HPLC-grade Acetonitrile was purchased from Fisher Scientific (Seoul, Korea), and water was purchased from J.T. Baker (Phillipsburg, NJ USA). The solid reagent, formic acid, was purchased from Honeywell (Charlotte, NC, USA), and ammonium formate was purchased from Sigma Aldrich (Saint Louis, MO, USA). The QuEChERS extraction kit (EN 15662; $MgSO_4$ 4 g, NaCl 1 g, Sodium citrate 1 g, Disodium citrate sesquihydrate 0.5 g) and QuEChERS dispersive kit (EN 15662; Magnesium sulfate 150 mg, PSA 25 mg) were purchased from CTK (Daejeon, Korea).

2.2. Greenhouse experiments:

Greenhouse experiments were conducted using cultivation sites located more than 20 km apart to account for geographical differences. The selected greenhouse facility sites were Eumseong, Chungcheongbuk-do (Field 1, 36.947427° N, 127.470263° E), and Icheon, Gyeonggi-do (Field 2, 37.309258° N, 127.406608° E). The experiments were carried out from March to April, and the radish variety used for all fields was Sangchun radish, which is generally cultivated in Korea and was purchased from a local farm (Table S2). Seeding was performed on March 8th for both Field 1 and Field 2. The experimental plots were selected with an area of 15 m^2 per replicate, consisting of three replicates for the treatment group and one replicate for the control group. According to the pesticide safety guidelines, the formulated pesticide was diluted and prepared for application (Table 1). The formulation and method that achieve the maximum dispersion amount based on the active ingredient pesticides were selected for the spray formulation. It was processed following the safety guidelines for pesticide use as in RDA in ROK. After pesticide application, samples were collected at appropriate intervals for commercial harvest: immediately after treatment (within 2 h), 1, 2, 3, 5, 7, 10, and 14 days. The collected samples were chosen to ensure a combined weight of at least 1 kg, with a minimum of 12 individual samples. Each sample was labeled with information on the treatment and collection date, placed in a polyethylene bag, and stored in an icebox for transportation to the laboratory within 24 h. In the laboratory, the transported samples were divided into leaf and root parts, weighed for sample preparation, prepped, sectioned, and stored in a refrigerator (−20 °C or below) for at least 48 h. They were then homogenized using dry ice and a homogenizer and subsequently kept in frozen storage (−20 °C or below) until further analysis.

Table. 1: Types of pesticides and application details

Pesticide	**Formulation**		**Application**				**PHI (Days)**
	Type	**AI**	**Dilution Rate**	**Spray No.**	**Interval (Days)**	**TSA**	
Diniconazole	WP	5	2000	3	7	0.2475	7
Dinotefuran	WP	10	1000	3	7	0.99	14
Metconazole	SC	20	3000	1	7	0.22	14
Tebuconazole	SC	20	3000	2	7	0.44	7

AI, Active ingredient, %, TSA, Total sprayed amount of pesticides = active ingredient content/dilution factor × application times × >application rate, g, PHI, Pre-harvest interval, WP, Wettable powder, SC, Suspension concentrate.

2.3. Standard solution:

Standard stock solutions of the four pesticides were prepared at a concentration of 1000 mg/L in acetonitrile. Working solutions at concentrations of 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 mg/L were prepared by serially diluting the stock solutions with acetonitrile. Each of the working solutions was mixed with the extract of radish leaf or root in a 1:1 ratio to prepare the matrix-matched standard solutions of 0.005, 0.01, 0.02, 0.05, 0.1, and 0.25 mg/L.

3. Results and discussion:

3.1. Method validation:

The ILOQ for pesticides in radish were 0.005 mg/L and MLOQ were 0.01 mg/kg. The calibration curves of the standard solutions were established through regression analysis of peak area within the concentration range, and the linearity within the concentration range was confirmed by the determination coefficient (r^2). . The recovery tests were conducted with three replicates from each field. The results of the recovery tests at 0.01 mg/kg, 0.1 mg/kg, and the highest concentration were all within the acceptable criteria for method validation in residue analysis during the production phase, which are recovery rates of 70–110% and a coefficient of variation within 20% . During the quantification analysis using LC-MS/MS for DNC, DNT, MTC, and TBC in radish, it was confirmed that there was no interference from other peaks in both the untreated samples and the recovery samples. The storage stability test at a concentration of 0.1 mg/kg showed recovery rates of 80–110% for all components, indicating that there was no decomposition or loss during the sample storage period.

Table. 2: Linear equation of the calibration curves for the quantification of the four pesticide residues in radish leaf and root

Pesticide	**Linear Range (mg/L)**	**Radish Leaf**				**Radish Root**			
		Field 1		**Field 2**		**Field 1**		**Field 2**	
		Linear Equation	**R^2**	**Linear Equation**	**R^2**	**Linear Equation**	**R^2**	**Linear Equation**	**R^2**
DNC	0.005–0.25	y = 240376.5527x + 356.7611	0.9903	y = 242721.8089x − 83.5911	0.9908	y = 248804.1197x + 321.0432	0.9969	y = 244659.5860x + 591.6900	0.9950
DNT		y = 285107.3727x + 1330.1962	1.0000	y = 261336.3591x + 10356.9996	0.9993	y = 254921.7192x + 1574.7351	0.9988	y = 227456.5412x + 1764.5123	0.9966
MTC		y = 919210.5027x + 1209.6831	0.9997	y = 916146.4301x + 637.5441	0.9999	y = 837243.8481x + 871.9971	0.9997	y = 828347.2924x + 818.4145	0.9999
TBC		y = 1007501x + 1291.8580	0.9999	y = 1007846.6112x + 1287.1053	0.9997	y = 962329.3438x + 775.5557	0.9999	y = 1012285.6426x − 468.7282	0.9996

During spraying, the recommended safe usage of DNC was three applications within seven days prior to harvest, DNT was three applications within 14 days prior to harvest, MTC was one application within 14 days prior to harvest, and TBC was two applications within seven days prior to harvest. Accordingly, the recommended dilution rates for spray solutions were as follows: DNC wettable powder at a dilution rate of 2000-fold with a 5% concentration, DNT wettable powder at a dilution rate of 1000-fold with a 10% concentration, MTC suspension concentrate at a dilution rate of 3000-fold with a 20% concentration, and TBC suspension concentrate at a dilution rate of 2000-fold with a 20% concentration. Based on this, the total amount of effective ingredient applied via spraying was found to be four times higher for DNT compared to DNC, 4.5 times higher compared to MTC, and 2.25 times higher compared to TBC.

The residue levels relative to the total amount of effective ingredient applied are shown in Table 1. In the leaves, MTC was 1.4 times higher than DNC, 5.6 times higher than DNT, and 1.2 times higher than TBC. In the roots, MTC was 2.4 times higher than DNC, 4.0 times higher than DNT, and 2.5 times higher than TBC. The reasons for the differences in pesticide degradation despite the TSA are generally attributed to various factors such as the physicochemical properties of the pesticide, formulation, cultivation environment, and crop cultivation characteristics.

4. Conclusions:

In the greenhouse harvest stages of radish, we investigated the decline patterns and residue characteristics of the neonicotinoid insecticide Dinotefuran and the triazole fungicides Diniconazole, Metconazole, and Tebuconazole. Based on this assessment, we conducted a risk evaluation considering pesticide toxicity. The decline patterns of residues in the two fields exhibited differences in initial residue levels when applying the approach of total sprayed effective ingredient. Despite similar cultivation conditions, the pesticides showed different half-lives. The risk assessment, using the pesticide residue levels during the respective PHI periods, indicated that all HQ values for the overall survey participants were below 1, suggesting low risks. However, for actual consumers, Diniconazole exceeded an HQ of 1, indicating a very high risk, while Tebuconazole showed an HQ above 0.3, indicating a potential risk. These findings provide fundamental data for re-evaluating and establishing domestic standards such as MRLs, PHI, or PHRLs.

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