농학석사학위논문

조 재배 중 살균제 Azoxystrobin 의 소실 및 토양 중 분해 양상

Dissipation of Fungicide Azoxystrobin in Foxtail Millet during Cultivation and Its Degradation in Soil

2018 년 8 월

서울대학교 대학원

농생명공학부 응용생명화학전공

Xiu Yuan

A Dissertation for the Degree of Master of Science

Dissipation of Fungicide Azoxystrobin in Foxtail Millet during Cultivation and Its Degradation in Soil

조 재배 중 살균제 Azoxystrobin 의 소실 및 토양 중 분해 양상

August 2018

Xiu Yuan

Applied Life Chemistry Major

Department of Agricultural Biotechnology

Seoul National University

조 재배 중 살균제 Azoxystrobin 의 소실 및 토양 중 분해 양상

Dissipation of Fungicide Azoxystrobin in Foxtail Millet during Cultivation and Its Degradation in Soil

지도교수 김 정 한

이 논문을 농학석사학위논문으로 제출함

2018 년 6 월

서울대학교 대학원

농생명공학부 응용생명화학전공

Xiu Yuan

Xiu Yuan 의 석사학위논문으로 인준함

2018 년 6 월

위 원 장 김 민 균 (인)

부위원장 김 정 한 (인)

위 원 최 양 도 (인)

Abstract

This study investigated the dissipation of fungicide Azoxystrobin on minor crop foxtail millet and in soil. All the experiments of preparation methods were used QuEChERS modified methods and analyzed by Shimadzu LC-MS/MS 8040. For minor crop field trial, Azoxystrobin 10% wettable powder (WP) was sprayed on 4 plots according to prearranged time. Method limit of quantitation (MLOQ) of Azoxystrobin and R230310 were 0.01 mg/kg in grain and straw. The linearity (r^2) of matrix matched calibration curves were ≥ 0.999. As results of samples analysis, total maximum residue amount of grain was decreased from 0.61 mg/kg (Plot 4) to 0.07 mg/kg (Plot 1) and total maximum residue amount of straw was decreased from 5.02 mg/kg (Plot 4) to 0.14 mg/kg (Plot 1). These results will contribute to establish Pre-Harvest Intervals (PHIs) and Maximum Residue Limits (MRLs) of Azoxystrobin during cultivation of foxtail millet in Korea. For the soil field trial, Azoxystrobin 10% WP sprayed 200 L/10 a for one time. For the laboratory incubation, Azoxystrobin standard solution spiked at the concentration of 0.2 mg/kg in 10 g soils which contain 75% water of field capacity and incubated at 25±2℃ in the incubator. Field and laboratory soil samples were collected according to prearranged time. The MLOQ of Azoxystrobin was 0.002 mg/kg and R234886 was 0.005 mg/kg in both soils. The linearity (r^2) of matrix matched calibration curves were ≥ 0.99. The Azoxystrobin was dissipated rapidly in field soil and the half-life of Azoxystorbin was calculated 12.38. But the Azoxystrobin was dissipated slowly in laboratory soil and the half-life of Azoxystrobin was over 90 day. The residue of soil metabolite R234886 was detected less than MLOQ in both soils.

Key Words: Azoxystrobin, R230310, R234886, Minor crop, Residue, Foxtail millet, LC-MS/MS, Dissipation, Soil, Half-life

Student Number: 2016-23084

List of Abbreviations

WP	wettable powder
EC	emulsifiable concentrate
SC	suspension concentrate
SE	suspended emulsion
WG	dispersible granule
FG	fine granule
GR	granule
PHIs	pre-harvest intervals
MRLs	maximum residue limits
ACN	acetonitrile
MeOH	methanol
EA	ethyl acetate
DCM	dichloromathane
MS/MS	tandem mass spectrometry
MLOQ	method limit of quantitation
ILOQ	instrumental limit of quantitation
HPLC	high performance liquid chromatography
ESI	electrospray ionization
DL	desolvation
QuEChERS	quick, easy, cheap, effective, rugged, and safe
NH_2	amine
GCB	graphitized carbon black

SPE	solid phase extraction
dSPE	dispersive solid phase extraction
PSA	primary secondary amine
MRM	multiple reaction monitoring
CE	collision energy
IUPAC	international union of pure and applied chemistry

List of Figures

List of Tables

Table of Contents

Introduction

Pesticides and target fungicide Azoxystrobin

In agriculture, pesticides are used as an important agricultural material because of the productivity of agricultural products and the convenience of cultivation of crops (Kim et al., 2010). Pesticides have been widely used around the world since the middle of the 20th century. On the basis of the condification of the British Crop Production Council, about 860 active substances are formulated currently in pesticide products (Tomlin, 2003; Lutz alder, 2006). The large quantity of chemicals and pesticides mixtures have been classified with insecticides, miticides, herbicides, nematicides, fungicides, molluscicides and rodenticides to use (Francisco prieto garcia, 2012).

This study target pesticide is Azoxystrobin which is a systemic, broad spectrum fungicide of strobilurin group with protective action commonly used in agriculture (Chaido lentza-rizos, 2006; Wikipedia). Azoxystrobin was present to the world market in 1996 and has since then been registered for use on a variety of crops (Kerstin gustafsson et al., 2009; Tomlin, 2000; Bartlett et al., 2002). It is a pesticide ingredient that is widely used because it dose not leave any marks on leaves or fruit even after spraying (Kim, 2010). The mode of action of Azoxystrobin is inhibit the electron transport in the respiration pathway in mitochondria, therefore interrupting all energy demanding processes in the target organisms (Kerstin gustafsson et al., 2009).

The physicochemical properties of Azoxystrobin, R230310, and R234886 are presented in Table 1 and structures are showed in Figure 1. The formulations of Azoxystrobin include suspension concentrate (SC), suspended emulsion (SE), wettable powder (WP), water dispersible granule (WG), fine granule (FG), granule (GR), and emulsifiable concentrate (EC). According to

Pesticide Residue Definitions for Agricultural Products, Azoxystrobin is sum of Azoxystrobin and R230310 (2-(6-(2-cyanophenoxy) pyrimidin-4-yloxy) phenyl)-3-methoxyacrylate]) in crop (National institute of agricultural sciences). The soil metabolism products of Azoxystrobin were R234886 in *Definition of Environmental Residues of Pesticides-3* (National institute of agricultural sciences). R230310 is an isomer and a photolysis product of Azoxystrobin. These are dived into *E* form and *Z* form (Hamdy balba, 2007). Total residue amount was calculated by following equation:

Total residue amount = Azoxystrobin residue amount + (R230310 residue amount × 1.0 [1)])

[1)]1.0 (Conversion factor) = 403.4 (Azoxystrobin M.W.)/403.4(R230310 M.W.)

Table 1. Physicochemical properties of Azoxystrobin

Property	Information		
Common name	Azoxystrobin	R230310	R234886
IUPAC name	Methyl (2*E*)-2-(2-{[6-(2-cyanophenoxy) pyrimidin-4-yl]oxy}phenyl)-3-methoxyacrylate	Methyl (*Z*)-2-(2-{[6-(2-cyanophenoxy) pyrimidin-4-yl]oxy}phenyl)-3-methoxyacrylate	(E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylic acid
CAS No.	131860-33-8	143130-94-3	1185255-09-7
Molecular formula	$C_{22}H_{17}N_3O_5$	$C_{22}H_{17}N_3O_5$	$C_{21}H_{15}N_3O_5$
Molecular weight	403.4	403.4	389.36
Boiling point	>345℃/760 mmHg	-	-
Vapor pressure	1.1×10^{-7} mPa (20℃)	-	-
K_{ow}	log P = 2.5 (20℃)	-	-

*The Pesticide Manual Seventeenth Edition (J A Turner)

https://www.trc-canada.com/product-detail/?R070240

https://www.hpc-standards.com/shop/ReferenceMaterials/Pesticides/1311773771_ZAzoxystrobinR230310.htm

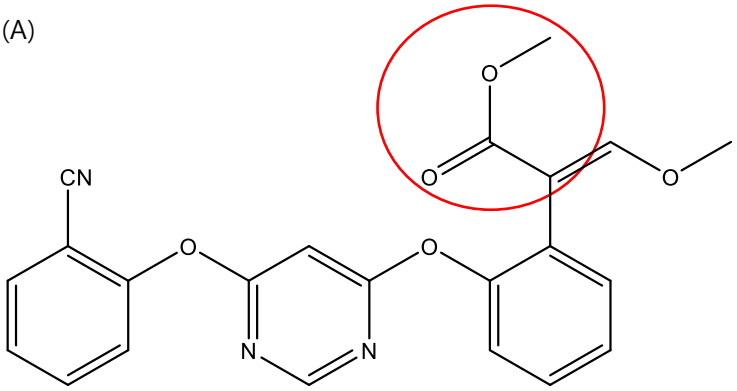
Figure 1. Structure of Azoxystrobin and metabolites

(A) Azoxystrobin

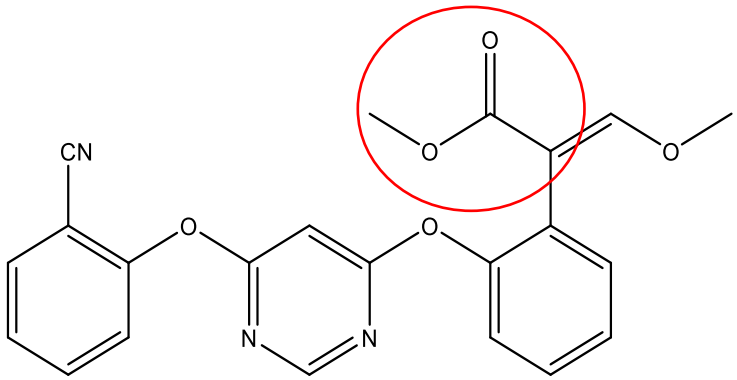
(B) R230310

(C) R234886

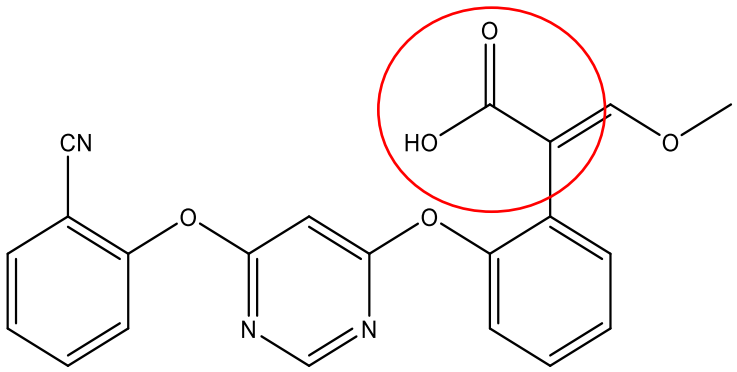
(A)



(B)



(C)



Target crop foxtail millet

Target crop was foxtail millet (*Setaria italica L.*) which is one of the earliest raised crops, widely grown in the arid and semiarid areas of Asia and Africa (Figure 2). It have common names that include Italian millet, German millet, Chinese millet, Hungarian millet, green millet. Foxtail millet is raised in 26 countries currently and it is second most cultivated species of millet. It contains proper nutritive components like starch, protein, vitamins and minerals (Nitya Sharma & Keshavan Niranjan, 2018).

There are a lot of pests occur during foxtail millet cultivation, but too little pesticides registered for foxtail millet in Korea. There are only three pesticides currently registered for foxtail millet in the *Crop Protection Guidelines* that was offered in Korea Crop Protection Association. All of them are insecticides that include fenthion EC, clothianidin SC and etofenprox EC, respectively.

This study target disease was blast of foxtail millet. Symptoms of spots appear on the leaf when it is stricken with blast of foxtail millet. They are shaped like a circle with a straw-coloured center and remnants are dark brown. The spots are 2-5 mm inner diameter that are small and sparse. If the disease is severe, the leaves shrivel up and dry (Plantwise Knowledge Bank).

Figure 2. Foxtail millet

Maximum Residue Limits (MRLs) and Pre-Harvest Intervals (PHIs)

Maximum Residue Limits (MRLs) and Pre-Harvest Intervals (PHIs) are must be established if use pesticide legally and safely. The MRLs stands for the maximum concentration of a pesticide residue legally allowed in food or feed commodity (Dugald j. maclachlan et al., 2010). In Korea, 466 ingredients of pesticide were registered in MRLs in the Ministry of Food and Drug Safety. There are 34 kinds of pesticides are registered in MRLs for foxtail millet in the Ministry of Food and Drug Safety (Table 2). The minimum and maximum values of pesticides registered in the foxtail millet were Ethoprophos 0.005 mg/kg and Chlormequat 10 mg/kg, respectively. There are 67 kinds of items are registered in MRLs of Azoxystrobin in the Ministry of Food and Drug Safety (Table 3). The minimum and maximum values of Azoxystrobin registered on dried fruits were 0.01 mg/kg and leaf beet 50 mg/kg, respectively. China has regulated 433 pesticides in MRLs of 4140 items as National Food Safety Standard for Food Additive Use (GB 2763-2016) in 13 kinds of food (Zhang, 2016).

PHIs is defined as the minimum time must pass between the last pesticide spray and the harvest. The PHIs is one of the main factors having an effect on pesticide residues in crops (Philippe tixier, 2007). Figure 3 is an example of PHIs of Azoyxstrobin 10% WP regulated in Korea (*Korea Crop Protection Association). Depending on the application disease, different pesticide formulations are regulated for use correctly and each crop has different PHIs. Pesticides have to be used only for the crops and pests that listed on the product's label and certainly obey the application rates, number of applications and PHIs indicated on the label (Onesmus kyalo mwaniki, 2017).

Table 2. MRLs of pesticides in foxtail millet

Pesticides	MRL (mg/kg)	Pesticides	MRL (mg/kg)	Pesticides	MRL (mg/kg)
Deltamethrin	0.2	Oxamyl	0.02	Indoxacarb	0.5
Dichlofluanid	0.1	Imazalil	0.05	Cyazofamid	2
Metalaxyl	0.05	Chlormequat	10	Clothianidin	0.3
Metolachlor	0.1	Thiobencarb	0.1	Pyraclstrobin	2
Methoprene	5	Parathion-Methyl	1		
Metribuzin	0.05	Permethrin	2		
Bentazone	0.2	Pendimethalin	0.2		
Bitertanol	0.1	Fenvalerate	2		
Cypermethrin	1	Fensulfothion	0.1		
Cyfluthrin	2	Fenthion	0.05		
Cyhalothrin	0.2	Phoxim	0.05		
Ethiofencarb	1	Pyrethrins	3		
Etofenprox	2	Pirimicarb	0.05		
Ethoprophos	0.005	Flufenoxuron	0.5		
Oxadixyl	0.1	Dimethomorph	2		

*Pesticides and Veterinary Drugs Information (Ministry of food and drugs safety)
(Korean Pesticides MRLs in Food; 2018; 2018) (Safety, 2018)

Table 3. MRLs of Azoxystrobin in various agricultural products

Crop	MRL (mg/kg)	Crop	MRL (mg/kg)	Crop	MRL (mg/kg)
Egg plant	0.7	Codonopsis lanceolata	0.1	Water melon	0.2
Mandarin	1.0	Balloon flower	0.1	Fresh ginseng	0.1
Citrus Fruits	10	Perilla	20	Spinach	20.0
Potato	0.1	Chinese yam	0.1	Rice	1.0
Dried ginseng	0.5	Garlic	0.1	Crown daisy	30.0
Dried fruits	0.01	Mango	0.7	Onion	0.1
Sweet potato	0.05	Melon	1.0	Leaf and stem vegetable	3.0
Hot pepper	2.0	Fig	2.0	Leaf vegetable	20.0
Dried pepper	7.0	Water parsley	5.0	Chinese magnolia vine	2.0
Pepper leaves	5.0	Wheat	0.2	Cucumber	0.5
Box thorn	10.0	Banana	2.0	Corn	0.02
Leaf beet	50.0	Cabbage	0.05	Indian lettuce	3.0
Soybean	0.5	Rubi Fructus	3.0	Burdock leaves	2.0
Jujube	3.0	Broccoli	0.05	Pome	1.0
Dried jujube	7.0	Lettuce	20.0	Ginseng extracts	0.5
Red ginseng	0.5	Red ginseng concentrate	0.5	Plum	1.0
Stone fruits	20.0	Sesame	0.1	Chwinamul	3.0
Tea	1.0	Oriental melon	0.2	Coffee bean	0.02
Kiwi fruit	1.0	Tomato	1.0	Spring onion	2.0
Dried spring onion	7.0	Parselv	20.0	Grape	3.0
Green garlic	1.0	Unripe beans	0.5	Bell pepper	2.0

Pistachio	0.5	Gourd	0.1

*Pesticides and Veterinary Drugs Information (Ministry of food and drugs safety)
(Korean Pesticides MRLs in Food; 2018; 2018) (Safety, 2018)

Figure 3. PHIs of Azoxystrobin 10% WP

살 균 제 (다 3)

이 품목은 등록회사에 따라 적용대상이 다르므로 반드시 포장지를 확인하십시오.

아족시스트로빈 수 화 제
액상수 화제
입상수화제

유효성분 : azoxystrobin ····· (수화제)10%, (액상수화제)21.7%, (입상수화제)50%
기 타 : (수화제)계면활성제, 보조제, 증량제, ㊀안정제 ····· 90%
(액상수화제)계면활성제, 증점제, 소포제, 안정제, 부패방지제, 부동제, 증량제 ··· 78.3%
(입상수화제)습윤제, 분산제, 증량제 ····· 50%
계 통 : 스트로빌루린계

【적용병해충 및 사용량】

[수화제] 상표 : 아미스타,센세이션,영일아족시스트로빈,나타나, 두루두루 저독성 · 어독성 Ⅱ급

작물명	적용병해	사용적기 및 방법	물 20ℓ 당 사용약량	안전사용기준	
				시 기	횟 수
사 과	겹무늬썩음병	6월중순부터 10일간격	20g	수확 14일전까지 사용	5회 이내
	갈색무늬병 · 역병	발병초 10일간격			
	점무늬낙엽병				
	㊀탄 저 병	6월상순부터 10일간격			
배	검은별무늬병	발병초 10일간격		수확 7일전까지 사용	
	㊀역 병				
감 귤	더뎅이병	춘지발생초 15일간격			
국 화	흰녹병	발병초 7일간격		–	–
인 삼	점무늬병	발병초 10일간격		수확 7일전까지 사용	4회 이내
장 미	검은무늬병	발병초 7일간격		–	–
감(단감포함)	둥근무늬낙엽병	6월상순부터 10일간격		수확 14일전까지 사용	5회 이내
	㊀모무늬낙엽병	6월중순부터 10일간격			
복 숭 아	잿빛무늬병	발병초 7일간격		수확 21일전까지 사용	3회 이내
포 도	노 균 병	발병초 10일간격		수확 7일전까지 사용	5회 이내
	㊀새눈무늬병	발병초 7일간격			
	㊀탄저병 · ㊀갈색무늬병	발병초 10일간격			
벼	잎도열병	발병초		수확 21일전까지 사용	
	목도열병	출수 7일전			
	잎집무늬마름병	유수형성기, 수잉기 2회			
	㊀세균벼알마름병	출수직전부터 7일간격			
	㊉흰잎마름병	발병직전 7일간격			

*Korea Crop Protection Association

Objective of study

This study is divided into two parts. Part 1 is dissipation of fungicide Azoxystrobin in minor crop foxtail millet during cultivation. First, selected target crop and pesticide. Then, conducted field test. Finally, carried out preparation step and analysis step to quantified Azoyxstrobin residues and investigated dissipation characteristics in foxtail millet grain and straw. According to this data, it could be contributed to establishment of MRLs and PHIs of Azoxystrobin in foxtail millet.

Part 2 is divided into degradation of fungicide Azoxystrobin in field soil and laboratory soil. Field soil experiment was conducted in parallel with minor crop experiment. Azoxystrobin 10% WP sprayed once to field soil and investigated the degradation of Azoxystrobin in field soil and measured the half-life. Laboratory soil artificially spiked at 0.2 mg/kg level and incubated at 25±2℃, investigated the degradation of Azoxystrobin in laboratory soil and measured the half-life.

Part 1

Dissipation of Fungicide Azoxystrobin in Minor Crop Foxtail Millet

Introduction

Background of minor crop

Minor crops and minor uses of pesticides are defined by country. According to the annual report of agriculture and forestry statistics, minor crops are defined as crops that cultivation area is less than 1,000 ha or without recorded cultivation area in Korea (Kim, 2017). Representative minor crops include mung beans, soybean, carrot, and millet etc. There are many kinds of minor crops and target pests, so registration of the target pesticide is not active and this phenomenon is a global problem (Chang-hyun ahn et al., 2014). All over the world, if the pesticide usage is too small such as minor crops, pesticide manufacturers are not able to follow the demand of pesticide use registration for these crops because of economic reasons. But there are not enough pesticides available for insect pest control. Therefore, farmers are faced with many difficulties during minor crops cultivation. In particular, the pesticide detection rate and the nonconformity rate are high in minor crops in Korea. So it is necessary to develop a solution to this problem (Kim et al., 2006; Lee, 2013). On the other hand, minor crops are at the center of the pesticide regulatory methodology that is currently evolving. Because the number of field trials is determined by major crops and minor crops. In addition, it have an effect on extrapolation of the residue data when setting the maximum residue limit (Park et al., 2009; Hur et al., 2009; Eun et al., 2005, 2006; Son et al., 2012a, 2012b; Lee et al., 2004; Lee, 2013). To solve this problem, the Rural Development Administration has been accomplished pesticide registration of authority for minor crops since March 1998 in Korea (Lee, 2013).

In the world, in order to solve this problem, the united states has been carrying out initiated by a government pesticide registration test (IR-4 project)

since 1964. The EU mutually recognized among member countries that pesticide used in minor crops and pesticide residues data and push ahead similar form extrapolation of minor crop test results. In addition, the Food and Agriculture Organization (FAO) and Codex have grouped crops which are similar with cultivation type and pesticide residue type and then mutual application with test data by representative crops in order to international mutual harmony, discussing the push ahead of crop grouping that classification of different of climatic zone region and then mutual recognition of test results for the same crop (Yim et al., 2014)

Table 4 is the representative study for Azoxystrobin residue in the crops in Korea. There are many thesis or posters for Azoxystrobin residue in different crops that crops include Grape, Indian lettuce, and Ginseng. But can’t find the study for foxtail millet. So this study can provide Azoxystrobin residue data in foxtail millet.

Table 4. Overview of studies in recent years for Azoxystrobin residue in crop in Korea

Matirx	Title	Author
Grape	Residue Patterns of Azoxystrobin and Cyenopyrafen in Grape between Rainshield and Plastic House Conditions	(Cho Rong Lee et al., 2011)
Ginseng	Residual characteristics of Azoxystrobin and Difenoconazole in ginseng	(Hyun Ho Noh et al., 2012)
Greeb garlic	Dissipation Pattern of Azoxystrobin, Difenoconazole and Iprodione Treated on Field-Grown Green Garlic	(Hye-Rim Kang et al., 2011)
Korean melon	Residual Patterns of Strobilurin Fungicides in Korean Melon under Plastic Film House Condition	(Eun Jeong Park et al., 2009)
Spinach	The Degradation Patterns of Two Pesticides in Spinach by Cultivation, Storage and Washing	(Jungmi Seo et al., 2010)

Materials and Methods

Analytical standard and pesticide for spraying

Standard material of Azoxystrobin (Purity: 98%) was purchased from SIGMA ARDRICH and R230310 (Purity: 98%) was purchased from Syngenta. Azoxystrobin 10% wettable powder (WP) Amistar from Syngenta was purchased at pesticide market (Seoul, Korea).

Standard solutions and working solution

The standard stock solution of Azoxystrobin and R230310 was prepared at the concentration of 1,000 μg/mL with acetonitrile. Then mixed Azoxystrobin 1,000 μg/mL 10 mL and R230310 1,000 μg/mL 10 mL made a Azoxystrobin and R230310 mixture 500 μg/mL 20 mL. The working solutions were prepared by serial dilution of stock solution with acetonitrile.

Analytical reagent

Analytical solvent HPLC grade ACN (acetonitrile, purity: 99.9%), EA (ethyl acetate. purity: 99.9%), DCM (dichloromethane, 99.9%), Hexane (purity: 99.9%) were purchased from Fisher Scientific Korea. Hypergrade for LC-MS MeOH (methanol, purity: 99.8%) was purchased from Merck. Acetic acid (purity: 99.7%) and formic acid (purity: 99.8%) were purchased from SIGMA-ALDRICH.

Subject crops

Foxtail millet certified seed (samdachal) was purchased from Foundation of Agri. Tech. Commercialization & Transfer on April 14th, 2017. Stored in the freezer -4℃ before seeding.

Instrumental conditions

The samples were analyzed by Shimadzu LC-MS 8040 with UHPLC Nexera (ESI positive mode). The column was Kinetex C18 (100 mm × 2.1 mm, 2.6 μm) and the oven temperature was 40℃. Mobile phases were 0.1% formic acid & 5 mM ammonium formate in distilled water (A) and 0.1% formic acid & 5 mM ammonium formate in methanol (B). 15 min gradient program set as follows: initially mobile phase B was set at 30% for 0.5 min. Then increased 30% to 90% for 6 minutes and hold 90% for 3 min. Finally decreased it to 30% for 0.5 min and hold 30% for 5.5 min. The flow rate was set at 0.2 mL/min and the injection volume was 5 μL. Desolvation line (DL) temperature was 250℃ and heat block temperature was 400℃. The nebulizing gas, drying gas was nitrogen and collision energy was used argon gas. Nebulizing gas flow was 3 L/min and drying gas flow was 15 L/min.

Preparation method

Pulverized samples measured the weight 5 g in 50 mL falcon tube and soaked with 10 mL of distilled water for 30 min. Then added ceramic homogenizers and extracted with 10 mL of acetonitrile using a shaker at 300 rpm for 5 min. After that, all samples in the falcon tube were cooling on ice and added $MgSO_4$ 4 g, NaCl 1 g, then used Mini G shaking vigorously for 1 min. Centrifuged at 3,000 rpm for 10 min. In the case of grain, transferred supernatant 4 mL and evaporated at 40℃. After that, reconstitution in 4 mL dichloromethane/hexane (10/90, v/v). SPE GCB/NH_2 cartridge (500 mg/500 mg, 6 mL) was used in the purification process and activation with 10 mL hexane. Then loading 4 mL reconstitution solution, washing with 10 mL ethyl acetate/hexane (10/90, v/v), elution with ethyl acetate/hexane (40/60, v/v). Finally dried with nitrogen gas and reconstitution in 2 mL acetonitrile, 1:1 matrix matched with acetonitrile analyzed by LC-MS/MS. In case of straw, transferred 1 mL supernatant to dSPE (PSA, GCB, $MgSO_4$) then vortexed for 1 min, centrifuged at 13,000 rpm for 5 min. Finally 1:1 matrix matched with acetonitrile and analyzed by LC-MS/MS.

Method validation

After the establishment of instrumental conditions and preparation method, method validation was performed. In method validation, Set ILOQ, MLOQ and evaluated calibration curve linearity and matrix effect. The accuracy and precision of the preparation method verified by recovery test. The pesticide-treated samples should be analyzed during the day. If it was impossible analyzed inevitable during the day, the storage stability test was carried out.

ILOQ and MLOQ

To set MLOQ (Method Limit of Quantitation) a series of standard solutions were analyzed by LC-MS/MS for set ILOQ (Instrumental Limit of Quantitation)**.** The ILOQ was set as the concentration that the signal-to-noise ratio was higher than 10. MLOQ was calculated by the equation below:

$$\text{MLOQ (mg/L)} = \frac{\text{ILOQ (ng)} \times \text{Final volume (mL)} \times \text{Dilution factor}}{\text{Injection volume } (\mu\text{L}) \times \text{Initial sample weight (g)}}$$

(Min Woo Jung, 2017)

Matrix matched calibration curve and linearity

Matrix matched standard solution of grain	**MSTD 1 (0.0025 μg/mL)**	**MSTD 2 (0.005 μg/mL)**	**MSTD 3 (0.01 μg/mL)**	**MSTD 4 (0.025 μg/mL)**	**MSTD 5 (0.05 μg/mL)**	
Matrix matched standard solution of straw	**MSTD 1 (0.0025 μg/mL)**	**MSTD 2 (0.005 μg/mL)**	**MSTD 3 (0.01 μg/mL)**	**MSTD 4 (0.025 μg/mL)**	**MSTD 5 (0.05 μg/mL)**	**MSTD 6 (0.1 μg/mL)**

Control sample matrix and a series of standard solution were diluted 2 times by matching at 1:1 ratio. Grain drew matrix matched calibration curve with 5 points and straw was 6 points. The coefficient of determination (r^2) calculated at matrix matched calibration curve.

Matrix effect calculation

Matrix effects (ME, %) was calculated by comparing the slope of matrix

matched calibration curve and solvent standard calibration curve using the following equation:

$$\text{ME}, \% = \left(\frac{\text{slope of matrix matched calibration curve}}{\text{slope of solvent standard calibration curve}} - 1\right) \times 100$$

(Min Woo Jung, 2017)

Recovery test and storage stability test

Recovery test was carried out 5 repetitions spiking on untreated samples at 2 levels (MLOQ and 10MLOQ). Storage stability test was carried out 3 repetitions spiking on untreated samples at 1 levels (10MLOQ), then stored in -20℃ freezer and analyzed with field trial samples. Grain stored in the freezer for 21 days and straw stored in the freezer for 26 days.

Field trials for foxtail millet

The field was located in 404-1, Hagil-ri, Hyangnam-eup, Hwaseong-si (Kyeonggi-do, Korea). Sowing was conducted on June 1st, 2017 (Figure 4). The field size was 43 m (length) x 8 m (width). There were four plots in field trials depending on the date of pesticide spraying day. Every plot was divided into 3 replicated plots that each area was 20 m^2. Each plot was treated with the pesticide by 2 times as follows: Plot 1 was treated at 40/30 days before harvest, plot 2 was 30/21 days before harvest, plot 3 was 21/14 days before harvest, and plot 4 was 14/7 days before harvest. To prevent cross-contamination during spraying the pesticide, the buffer zones were installed between control and treated plots, and each treated plot (Figure 5).

Azoxystrobin 10% WP weighing in 50 mL falcon tube and prepared by 1,000 times dilution with water using a pressurized 20 L sprayer. Before using the sprayer, the reproducibility test for spraying was carried out to check a constant spraying capacity and speed. The crop was treated with the diluted pesticide solution until the grain and straw were wetted sufficiently (Figure 6).

The harvest of grain and straw of millet was conducted on September 21, 2017. Pesticide-free plot (control) was first harvested to prevent contamination. Other samples in plot 1, 2, 3, and 4 were randomly collected over 2.0 kg (Figure 7). The samples were rapidly transferred to the laboratory after harvest. In case of grain, dried 2 days, then threshed and remove husks. In case of straw, cut to size of 3~5 cm before maceration. After preparation, grain and straw were macerated by food processor with dry ice. Every sample was kept in a freezer -20 ℃ in polyethylene bags (Figure 8).

Figure 4. Field trials for foxtail millet

(A) Satellite picture

(B) Sowing

(A)

(B)

Figure 5. The diagram of field trials

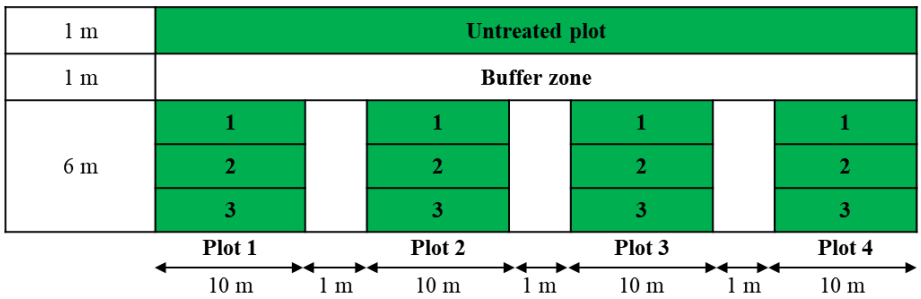
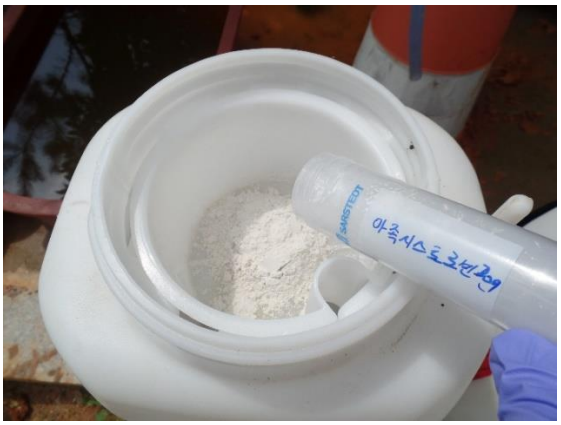

1 m
Untreated plot
1 m
Buffer zone
6 m
1
2
3
1
2
3
1
2
3
1
2
3
Plot 1
Plot 2
Plot 3
Plot 4
10 m
1 m
10 m
1 m
10 m
1 m
10 m

Figure 6. Working in the field

(A) Preparation of pesticide solution

(B) Spraying on the foxtail millet

(A)



(B)

Figure 7. Sample collection

Figure 8. Sample preparation

(A) Remove husks

(B) Cut straw

(C) Macerated with dry ice

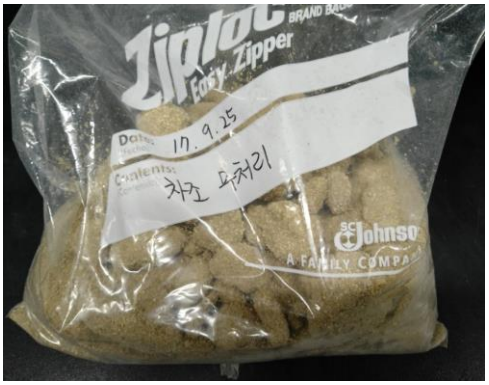
(D) Keep in polyethylene bag

(A)

(B)

(C)

(D)

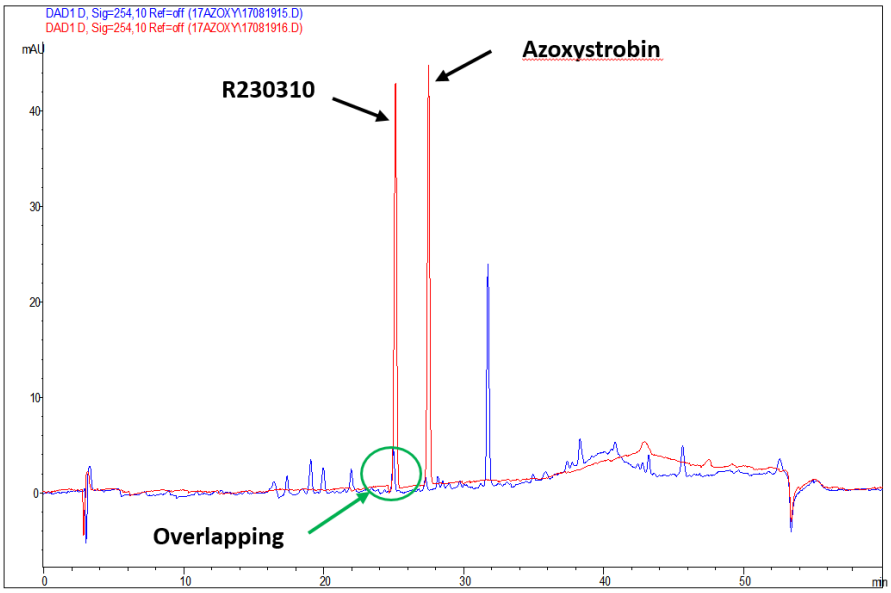

Ziploc
Easy Zipper
Date: 17. 9. 25
Contents:
차고 유처리
SC Johnson
A FAMILY COMPA

Results and Discussion

The instrument selection

By Korean Food Standards Codex general analytical method, the analytical method of Azoxystrobin is HPLC (UVD) and LC-MS/MS (positive mode). Therefore first analyzed by Agilent HPLC 1100. Several columns were used to developed instrument method such as Kinetex, YMC, Fusion-RP, Luna. The best sensitivity and separation of Azoxystrobin and R230310 was Luna 5 μ C18 (2) 100 A column. To select appreciate column, mobile phase A used distilled water and added 1% different acid to ACN used as mobile phase B such as formic acid and acetic acid. It showed good sensitivity under the different mobile phase conditions. But there was a problem, many columns and mobile phases were used, but matrix was always detection in control due to standard peak were not separated with control. So eventually changed the instrument. Figure 9 is an example of chromatogram overlapping control and standard.

Figure 9. HPLC overlapping chromatogram

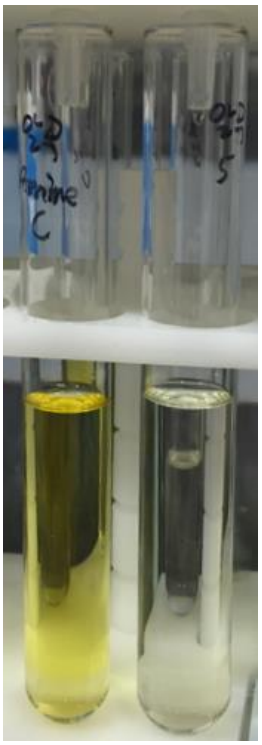

DAD1 D, Sig=254,10 Ref=off (17AZOXY\17081915.D)
DAD1 D, Sig=254,10 Ref=off (17AZOXY\17081916.D)
mAU
40
30
20
10
0
R230310
Azoxystrobin
Overlapping
0
10
20
30
40
50
min

Development of preparation method

According to Multi class pesticide multi residue methods in Korean Food Standards Codex general analytical method, extraction solvent was used 100 mL acetonitrile and partition step was used the separate funnel. So many solvents were used and many time to spend in partition step. Therefore, the preparation method used QuEChERS-based modified methods which were first published in 2003. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method is the much-applied extraction method recently for the analysis of pesticide residues in various samples (Prestes et al., 2009). By using the QuECheERS-modified method, reduced the preparation time and price.

The preparation method of extraction and partition in grain and straw were the same, but purification in grain and straw was different. In case of grain, NH_2/GCB (Graphitized Carbon Black) SPE cartridge (500 mg/500 mg. 6 mL) was used and optimized washing and elution solvent mixture ratio. NH_2/GCB was used to remove pigment stand in NH_2 (Figure 10). To purified effectively, optimized washing and elution solvent ratio. Washing solvent tested 5/95, 10/90, 15/85 and 20/80 EA/hexane. Elution solvent tested 20/80, 40/60, 60/40, 80/20. About washing solvent, ratio 10/90 was proper because it is biggest EA percentage that target pesticide was not eluted. About elution solvent, the ratio 40/60 was proper because most of the pesticides were recovered. In case of straw, dSPE (PSA, GCB, $MgSO_4$) was used because of recovery was less than 70% if SPE cartridge was used. When used dSPE, purification time was shorter than SPE and recovery was higher than SPE.

Figure 10. Compare NH_2 and GCB/NH_2 cartridge pigment purified effect



Multiple reaction monitoring (MRM) optimization and advantage

LC-MS/MS instruments operating in MRM are widely used for pesticide analysis because of their high selectivity and sensitivity (Anna Stachniuk et al., 2015). In the 2013 IUPAC recommendations, multiple reaction monitoring is defined as a "special case of selected reaction monitoring where multiple product ions are produced from one or more precursor ions" (Kermit K. Murray, 2015).

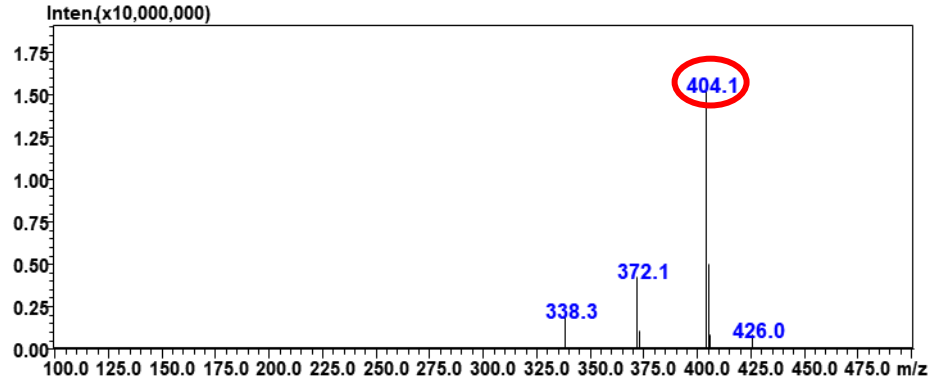
To select precursor ion for optimize MRM condition. First, observed the full scan spectrum of Azoxystrobin and R230310 not installed the column (Figure 11). Selected appropriate molecular weight for precursor ions. Both precursor ions were selected 404.1 for both components. Then optimized for method, optimized collision energy and selected product ion. After that, added MRM event to analysis method (Table 5). By selecting product ion for detection, so it showed good sensitivity and base line noise was very clean.

Figure 11. Full scan spectrum

(A) Azoxystrobin

(B) R230310

(A)


Inten.(x10,000,000)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
404.1
372.1
338.3
426.0
100.0 125.0 150.0 175.0 200.0 225.0 250.0 275.0 300.0 325.0 350.0 375.0 400.0 425.0 450.0 475.0 m/z

(B)

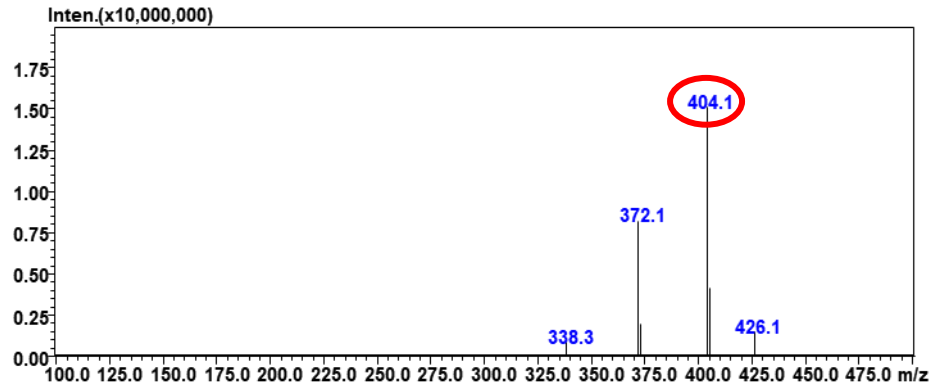

Inten.(x10,000,000)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
404.1
372.1
338.3
426.1
100.0 125.0 150.0 175.0 200.0 225.0 250.0 275.0 300.0 325.0 350.0 375.0 400.0 425.0 450.0 475.0 m/z

Table 5. MRM (Multiple Reaction Monitoring) conditions

Compound	M.W.	Ionization	Precursor ion> Product ion (CE, eV)	
			Quantifier ion	Qualifier ion
Azoxystrobin	403.1	[M+H]+	404.0>372.0 (-13)	404.0>344.1 (-24)
R230310		[M+H]+	404.0>372.0 (-15)	404.0>344.1 (-26)

ILOQ and MLOQ results and calibration curve linearity

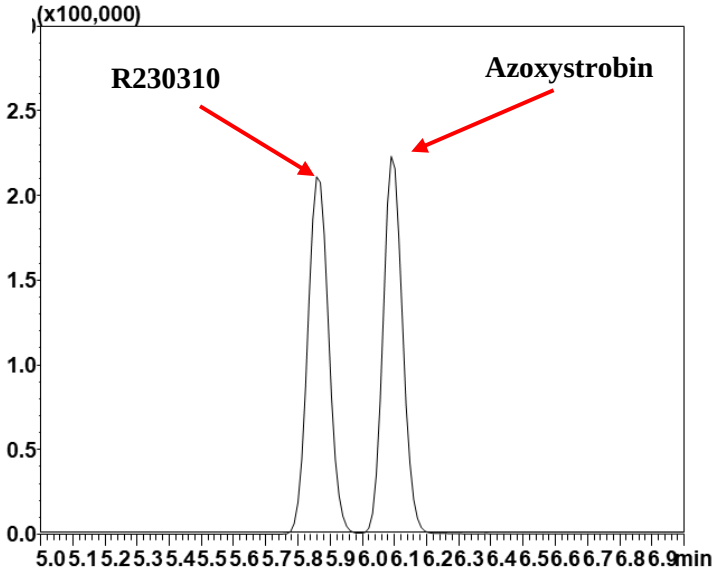
ILOQ of Azoxystrobin and R230310 were 0.025 ng (0.005 μg/mL × 5 μL) in grain and 0.0125 ng (0.0025 μg/mL × 5 μL) in straw (Figure 12). Azoxystrobin retention time was 6.1 min and R230310 retention time was 5.8 min. Calculated MLOQ according to the MLOQ equation, MLOQ of Azoxystrobin and R230310 were 0.01 mg/kg in grain and straw. Matrix matched standard calibration curves of Azoxystrobin (Figure 13) and R230310 (Figure 14) had a good linearity in samples of grain and straw. 5 point concentrations were set in grain, 6 point concentration were set in straw. The ranges were between 0.005 to 0.1 mg/kg in grain and 0.0025 to 0.1 mg/kg in straw. The regression equations were y = 3E+07x + 104459 (grain) and y = 4E+07x + 255762 (straw), respectively. Coefficients of determination (r^2) of Azoxystrobin and R230310 were over 0.999 in both samples.

Figure 12. Chromatograms of ILOQ of Azoxystrobin and R230310

(A) Grain: 0.025 ng

(B) Straw: 0.0125 ng

(A)


(x100,000)
R230310
Azoxystrobin
2.5
2.0
1.5
1.0
0.5
0.0
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

(B)

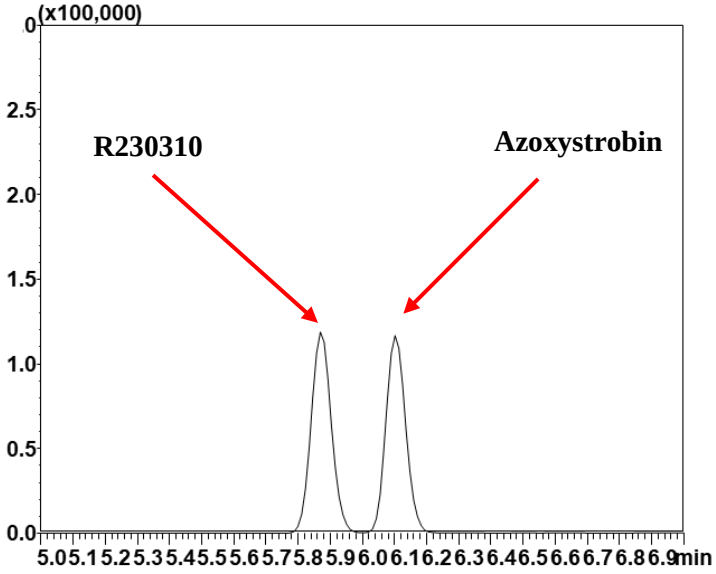
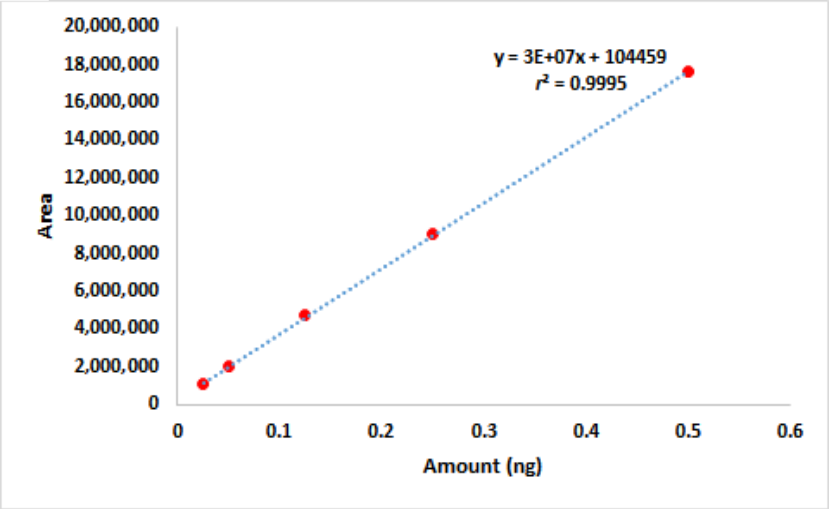

(x100,000)
0
2.5
R230310
Azoxystrobin
2.0
1.5
1.0
0.5
0.0
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

Figure 13. Azoxystrobin matrix matched standard calibration curves

(A) Grain

(B) Straw

(A)


y = 3E+07x + 104459
r² = 0.9995
Area
Amount (ng)
20,000,000
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
0
0.1
0.2
0.3
0.4
0.5
0.6

(B)

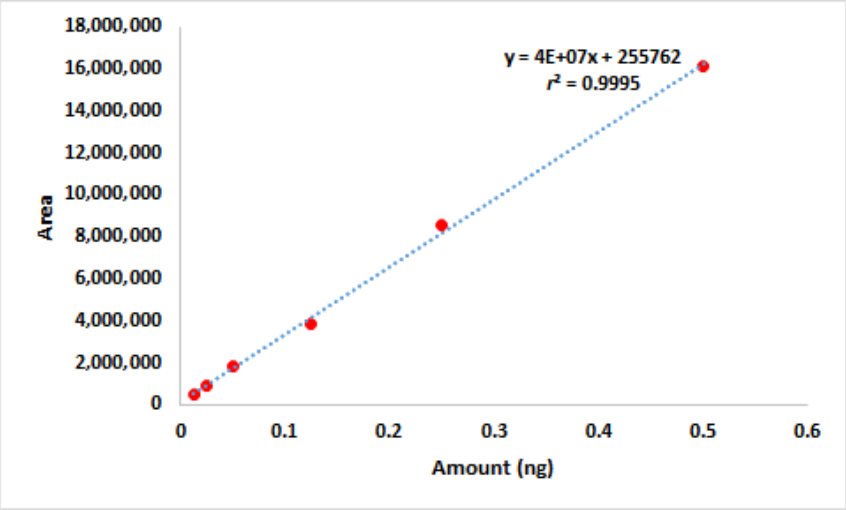
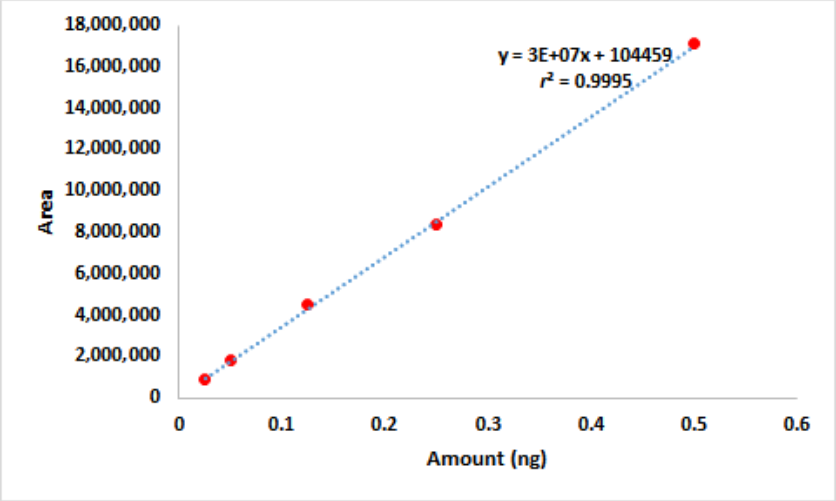

y = 4E+07x + 255762
r² = 0.9995
Area
Amount (ng)
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
0
0.1
0.2
0.3
0.4
0.5
0.6

Figure 14. R230310 matrix matched standard calibration curves

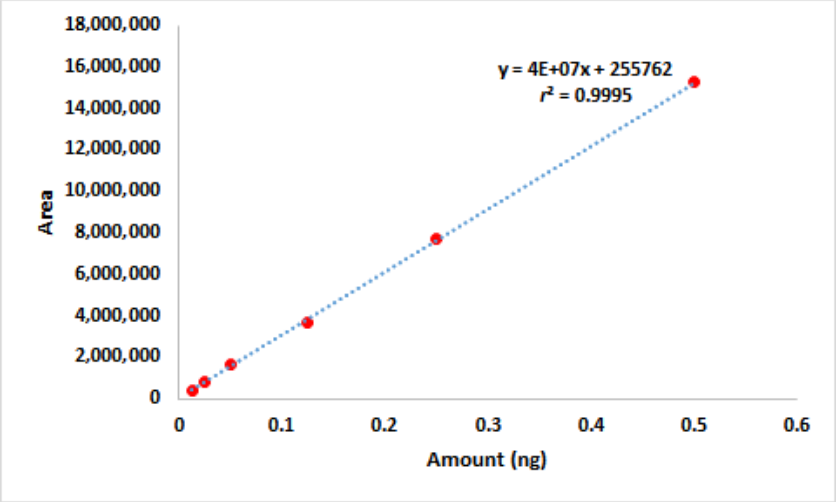
(A) Grain

(B) Straw

(A)


y = 3E+07x + 104459
r² = 0.9995
Area
Amount (ng)
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
0
0.1
0.2
0.3
0.4
0.5
0.6

(B)


y = 4E+07x + 255762
r² = 0.9995
Area
Amount (ng)
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
0
0.1
0.2
0.3
0.4
0.5
0.6

Matrix effects

Matrix effects appeared by ESI-MS that perhaps change the signal intensity of a target samples and strikingly reduce the accuracy of LC-MS/MS analysis results with ESI mode (Annelic Kruve, 2009). Matrix effects have been called the Achilles' heel of quantitative LC-MS/MS (Taylor, 2005). The term matrix is used here to represent all components in a sample with out the analyte. Matrix effects were defined by IUPAC as "the combined effect of all components of the sample other than the analyte on the measurement of the quantity" (Guilbault and Hjelm, 1989). Matrix effect as per the environment in that the ionization and ion evaporation step take place, matrix may effectively effect ion suppression or ion enhancement the efficiency of form the expected analyte ions existence the same concentrations in the interface (B.K. Matuszewski, 2003).

Based on matrix effect equation calculated matrix effect in grain and straw. The results of Azoxystrobin was -12.11% and R230310 was -4.66% in grain, Azoxystrobin was -17.32% and R230310 was -12.90% in straw. According to results of matrix effect in grain and straw, discovered ion suppression in both samples.

Recovery and storage stability test results

According to Korean Food Standards Codex pesticide residue analysis method guideline part 4, pesticide residue analysis method requires verification of the accuracy and reproducibility of the analysis as well as sensitivity. The accuracy and reproducibility of the analysis methods are generally verified by recovery test. Recovery test means evaluating the accuracy of the analysis method by comparing the analysis result with the throughput by performing the analysis method for processing and verifying a certain amount of the target component in the food and environmental sample in which the target pesticide component is not detected. In addition, according to the repeated of analytical results by levels of spiking, calculated and verified reproducibility and accuracy. The average recovery results of Azoxystrobin and R230310 in the grain at MLOQ and 10MLOQ levels were 75.6% and 92.2%, 74.6% and 82.4%, respectively. The average recovery results of Azoxystrobin and R230310 in the straw at MLOQ and 10MLOQ levels were 103.9% and 112.5%, 109.3% and 113.8%, respectively (Table 6). Two peaks were separated completely and no components detected in control at the same retention time with Azoxystrobin and R230310 (Figure 15).

The goal of storage stability test is to offer evidence, under the influence of environmental factors such as temperature, humidity, and light, how the quality of samples varies with time (BPU, 2004). The average storage stability results of Azoxystrobin and R230310 in the grain at 10MLOQ levels were 96.0% and 85.6%, respectively. The average storage stability results of Azoxystrobin and R230310 in the straw at 10MLOQ levels were 113.5% and 114.3%, respectively (Table 7). Storage stability test also good (Figure 16) and all results of recovery at 2 levels and storage stability at 1 level were satisfied with 70-120%, RSD were less than 10%.

Table 6. Recovery test results in grain and straw

Crop	Pesticides	Part	Spiking levels (mg/kg)	Recovery (%)	RSD (%)
Foxtail millet	Azoxystrobin	Grain	0.01	75.6	6.3
			0.1	92.2	3.0
		Straw	0.01	74.6	7.0
			0.1	82.4	4.7
	R230310	Grain	0.01	103.9	6.5
			0.1	112.5	3.2
		Straw	0.01	109.3	3.1
			0.1	113.8	2.7

Table 7. Storage stability test results in grain and straw

Crop	Pesticide	Storage time	Part	Spiking level (mg/kg)	Recovery (%)	RSD (%)
Foxtail millet	Azoxystrobin	21 days	Grain	0.1	96.0	3.7
		26 days	Straw		113.5	3.8
	R230310	21 days	Grain	0.1	85.6	4.4
		26 days	Straw		114.3	2.2

Figure 15. Representative chromatogram of recovery test of Azoxystrobin and R230310 in grain and straw

(A) Control (grain)

(B) MLOQ (grain)

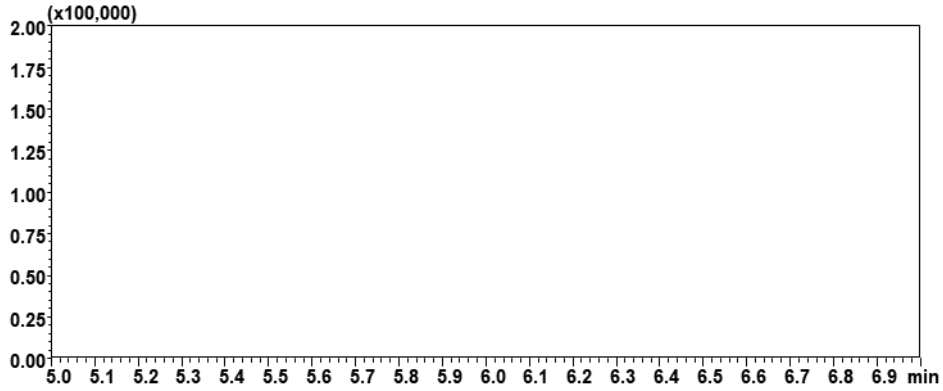
(C) 10MLOQ (grain)

(D) Control (straw)

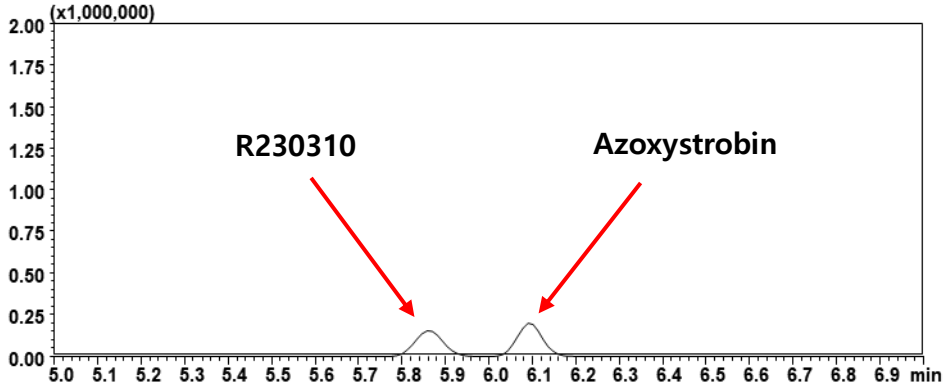
(E) MLOQ(straw)

(F) 10MLOQ(straw)

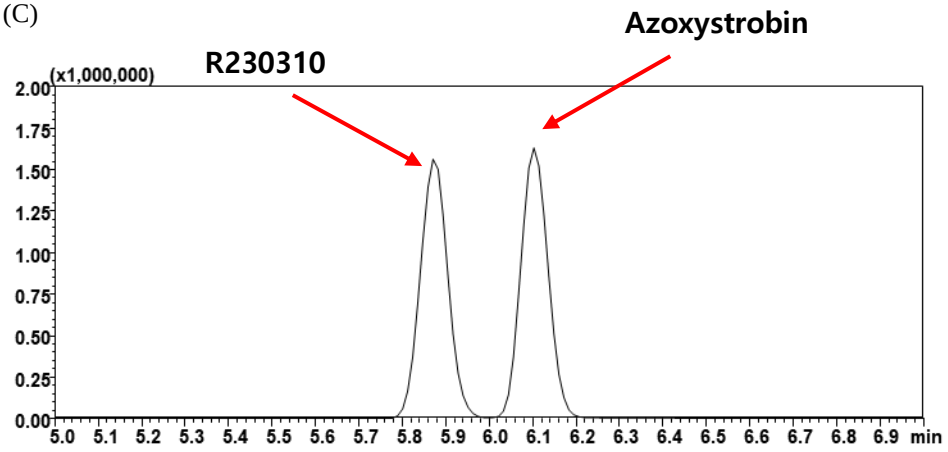
(A)


(x100,000)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

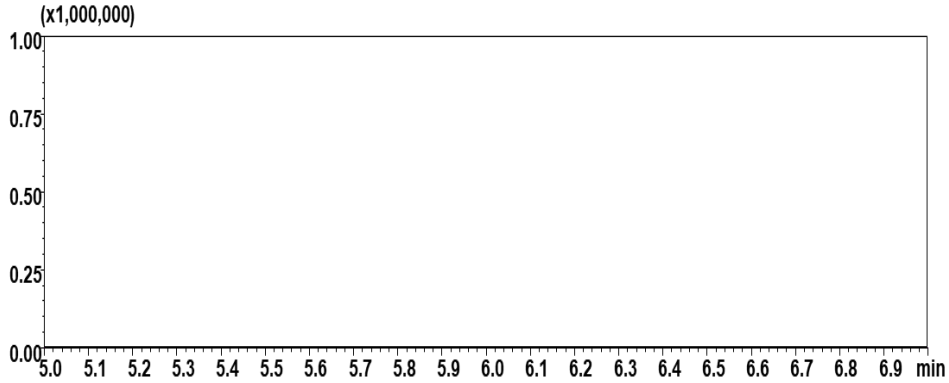
(B)


(x1,000,000)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
R230310
Azoxystrobin
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

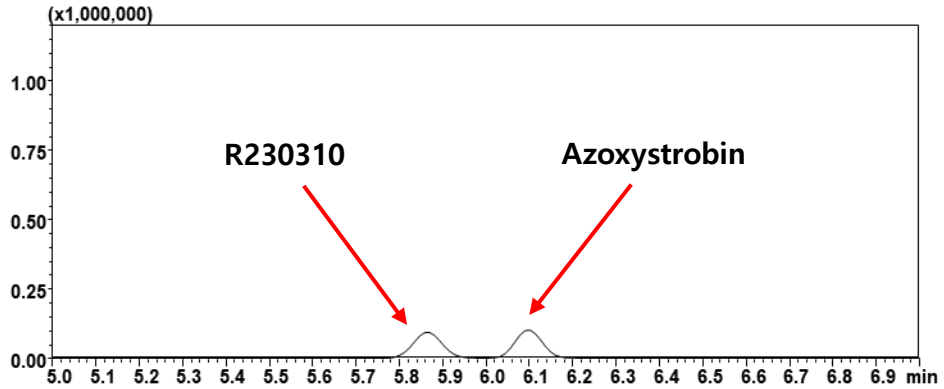
(C)


Azoxystrobin
R230310
(x1,000,000)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

(D)


(x1,000,000)
1.00
0.75
0.50
0.25
0.00
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

(E)


(x1,000,000)
1.00
0.75
0.50
0.25
0.00
R230310
Azoxystrobin
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

(F)

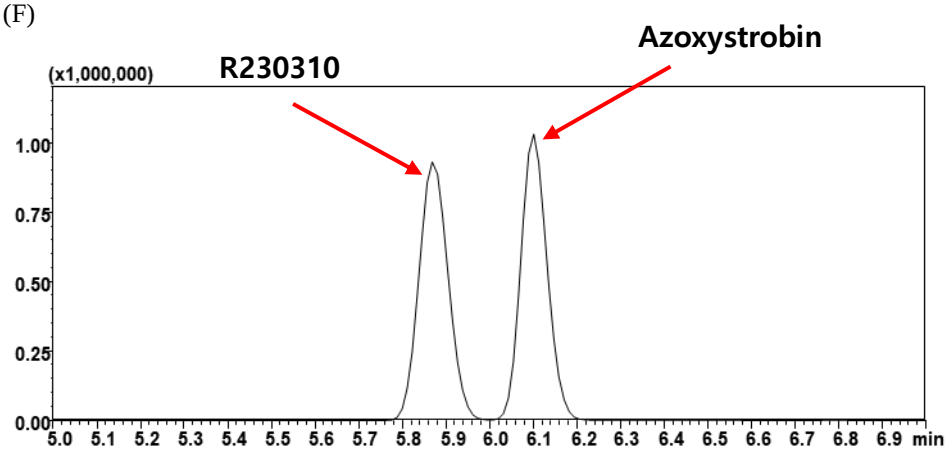

Azoxystrobin
R230310
(x1,000,000)
1.00
0.75
0.50
0.25
0.00
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

Figure 16. Representative chromatogram of storage stability test of Azoxystrobin and R230310 in grain and straw

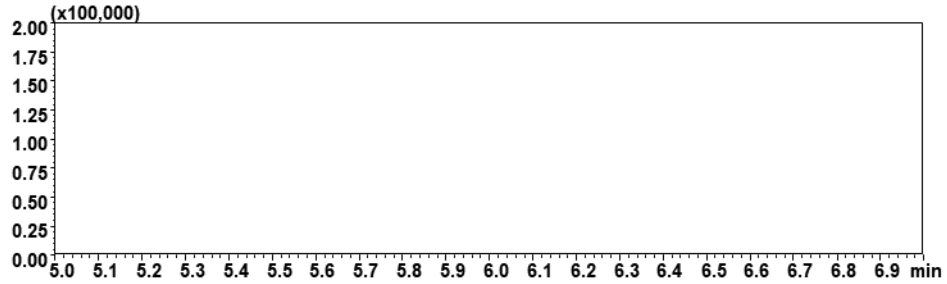
(A) Control (grain)

(B) 10MLOQ(grain)

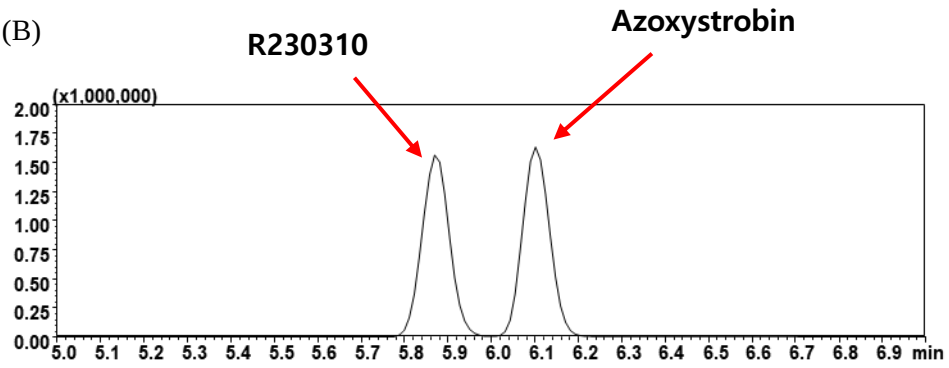
(C) Control(straw)

(D) 10MLOQ(straw)

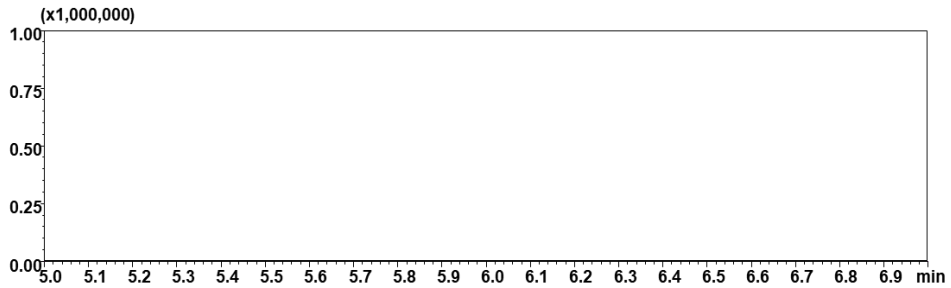
(A)


(x100,000)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

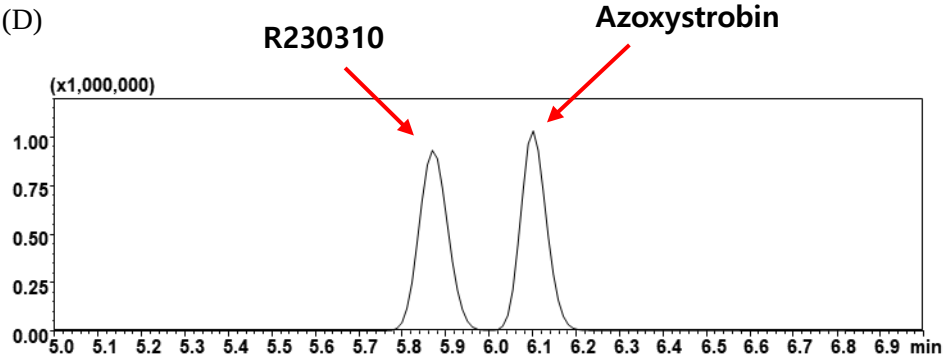
(B)


R230310
Azoxystrobin
(x1,000,000)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

(C)


(x1,000,000)
1.00
0.75
0.50
0.25
0.00
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

(D)


R230310
Azoxystrobin
(x1,000,000)
1.00
0.75
0.50
0.25
0.00
5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 min

The meteorological data during field experiment

The environmental conditions were affecting the crop persistent of the pesticide such as temperature and humidity (Table 8). Higher temperature and more extreme rainfall are having an influence on pesticide residue which saw as the major climate cause of change. When the influence of rainfall intensity on pesticide residues was assessed, temperature increases or heavy rain comes within after spraying, the pesticide residues on crops reduce faster (Michael Houbraken UGent et al., 2014). Fortunately, it didn't rain on the day of sowing and spraying.

Table 8. The meteorological data

Date	Low temperature (℃)	Highest temperature (℃)	Average temperature (℃)	The amount of precipitation (mm)
6 / 1	18.3	26.3	22.1	0.0
6 / 2	13.6	25.7	20.0	
6 / 3	15.0	25.8	19.5	
6 / 4	12.5	28.1	20.4	0.8
6 / 5	13.7	30.2	22.6	0.7
6 / 6	14.7	22.7	18.9	6.4
6 / 7	15.2	19.3	17.3	
6 / 8	15.3	26.4	19.8	
6 / 9	15.5	27.2	20.8	
6 / 10	15.8	27.6	20.8	
6 / 11	12.6	29.6	21.9	
6 / 12	17.3	27.2	21.6	0.2
6 / 13	16.4	26.3	20.4	104.2
6 / 14	17.2	26.3	20.8	2.5
6 / 15	16.2	29.0	22.1	
6 / 16	18.5	32.2	24.1	43.7
6 / 17	18.3	29.7	23.4	37.2
6 / 18	15.3	32.0	22.8	0.8
6 / 19	17.0	30.3	23.9	
6 / 20	17.9	31.8	24.8	
6 / 21	19.8	30.7	23.9	
6/ 22	20.1	30.5	24.3	
6 / 23	19.0	33.2	25.0	0.2
6 / 24	19.5	27.6	22.8	5.5
6 / 25	19.8	29.9	24.2	
6 / 26	20.0	30.3	23.2	0.2
6 / 27	20.2	30.8	24.1	1.6
6 / 28	21.3	30.6	24.9	49.2
6 / 29	20.2	31.4	25.0	3.4
6 / 30	21.4	31.2	25.2	
7 / 1	21.4	28.1	23.9	

7 / 2	23.5	26.5	24.9	85.0
7 / 3	23.5	27.8	24.5	12.4
7 / 4	22.8	31.2	26.0	82.3
7 / 5	22.7	33.6	27.0	
7 / 6	22.3	34.0	27.3	
7 / 7	24.3	28.7	26.4	6.1
7 / 8	23.7	28.2	25.6	19.3
7 / 9	22.6	31.8	26.4	28.0
7 / 10	23.2	26.1	24.8	69.5
7 / 11	22.2	32.0	26.1	1.3
7 / 12	23.2	31.9	26.3	0.0
7 / 13	21.5	32.3	27.0	
7 / 14	24.4	33.9	28.2	0.2
7 / 15	22.7	26.1	24.3	74.7
7 / 16	22.9	27.2	25.2	77.8
7 / 17	23.5	29.1	26.0	0.9
7 / 18	23.0	31.0	25.9	1.3
7 / 19	24.5	34.8	28.0	
7 / 20	24.7	34.4	28.8	
7 / 21	26.2	32.6	29.1	5.1
7 / 22	26.7	32.5	28.7	0.2
7 / 23	25.7	29.2	26.8	63.3
7 / 24	24.9	27.7	25.9	19.4
7 / 25	25.3	35.8	29.4	
7 / 26	24.3	32.2	28.1	
7 / 27	24.4	30.7	27.0	0.8
7 / 28	23.7	27.4	25.3	16.5
7 / 29	24.8	30.7	27.0	
7 / 30	23.9	32.8	27.6	
8 / 31	23.1	26.6	24.2	104.8
8 / 1	23.2	33.6	27.3	0.8
8 / 2	25.2	34.2	29.3	
8 / 3	26.2	32.7	29.2	

8 / 4	25.1	35.4	29.7	
8 / 5	27.0	36.9	31.2	
8 / 6	25.5	33.4	28.9	2.7
8 / 7	25.2	33.6	28.5	
8 / 8	24.6	32.1	27.6	0.0
8 / 9	23.8	29.6	26.5	6.6
8 / 10	23.3	25.6	24.5	11.3
8 / 11	23.5	32.0	26.0	6.9
8 / 12	23.0	32.4	27.1	
8 / 13	24.0	27.3	25.8	0.0
8 / 14	22.3	25.6	23.6	8.0
8 / 15	21.1	23.8	22.3	50.9
8 / 16	21.6	27.9	24.1	1.9
8 / 17	22.1	29.8	25.2	0.2
8 / 18	22.6	30.9	25.8	
8 / 19	22.3	28.9	25.5	13.7
8 / 20	22.1	27.0	23.8	130.8
8 / 21	23.2	28.6	25.0	22.6
8 / 22	23.0	32.1	26.9	
8 / 23	23.7	31.4	27.1	34.8
8 / 24	23.5	29.2	26.2	38.1
8 / 25	20.9	31.5	25.3	
8 / 26	17.6	29.6	23.6	
8 / 27	18.5	26.6	22.3	0.0
8 / 28	18.3	26.1	21.3	30.4
8 / 29	17.4	24.3	20.1	
8 / 30	16.0	23.6	19.2	
8 / 31	15.1	28.2	21.2	
9 / 1	15.8	29.7	22.6	
9 / 2	17.5	29.5	22.8	
9 / 3	17.6	29.1	23.1	
9 / 4	17.9	29.2	23.6	
9 / 5	19.8	23.8	21.7	

9 / 6	18.3	22.9	20.2
9 / 7	16.5	27.7	21.8
9 / 8	19.6	29.8	23.4
9 / 9	18.5	29.4	23.2
9 / 10	18.6	25.2	22.2
9 / 11	18.2	25.1	20.5
9 / 12	16.7	27.6	21.1
9 / 13	13.9	27.5	20.4
9 / 14	13.7	28.8	21.5
9 / 15	17.8	27.7	22.2
9 / 16	16.6	26.3	21.2
9 / 17	18.3	28.5	22.3
9 / 18	14.9	27.3	20.8
9 / 19	17.5	27.0	21.8
9 / 20	12.3	23.9	18.3
9 / 21	12.6	26.5	18.7

Field trial samples results

The average samples results of Azoxystrobin in the grain, plot 1 (40/30 days) was 0.07 mg/kg, plot 2 (30/21 days) was 0.25 mg/kg, plot 3 (21/14 days) was 0.26 mg/kg, plot 4 (14/7 days) was 0.60 mg/kg. In case of r230310, plot 2 was 0.01 mg/kg, other plots were less than 0.01 mg/kg (Figure 17 and Table 9). Azoxystrobin residue toward decreased as time goes on in the grain and R230310 can't compare the residue because residue amount was too low. The average samples results of Azoxystrobin in straw, plot 1 was 0.14 mg/kg, plot 2 was 0.52 mg/kg, plot 3 was 0.91 mg/kg, plot 4 was 4.76 mg/kg. In r230310, plot 1 was less than 0.01 mg/kg, plot 2 was 0.03 mg/kg, plot 3 was 0.05 mg/kg, plot 4 was 0.24 mg/kg (Figure 18 and Table 10). The residue amounts of Azoxystrobin and R230310 in the straw were higher than the residue amounts of Azoxystrobin and R230310 in the grain and also Azoxystrobin and R230310 residue toward decreased as time goes on in the straw. Total sample amounts calculated by equation (Table 11) and also total amount of residue to ward decreased as time goes on in grain and straw. According to Azoxystrobin residue study present in Table 4. Azoxystrobin generally showed a tendency to decrease over time. Therefore, similar results were obtained when compared with other papers.

Table 9. Results of Azoxystrobin and R230310 in grain

Treatment	Azoxystrobin residues in grain (mg/kg)			
	Replicated 1	Replicated 2	Replicated 3	Maximum
Plot 1	0.05	0.05	0.07	0.07
Plot 2	0.25	0.18	0.23	0.25
Plot 3	0.26	0.17	0.25	0.26
Plot 4	0.46	0.60	0.58	0.60

Treatment	R230310 residues in grain (mg/kg)			
	Replicated 1	Replicated 2	Replicated 3	Maximum
Plot 1	<0.01(0.0013)	<0.01(0.0011)	<0.01(0.0024)	-
Plot 2	0.01	<0.01(0.006)	<0.01(0.007)	0.01
Plot 3	<0.01(0.009)	<0.01(0.005)	<0.01(0.007)	-
Plot 4	<0.01(0.004)	<0.01(0.009)	<0.01(0.008)	-

Table 10. Results of Azoxystrobin and R230310 in straw

Treatment	Azoxystrobin residues in straw (mg/kg)			
	Replicated 1	Replicated 2	Replicated 3	Maximum
Plot 1	0.11	0.14	0.12	0.14
Plot 2	0.52	0.44	0.43	0.52
Plot 3	0.91	0.75	0.49	0.91
Plot 4	4.76	4.51	4.02	4.76

Treatment	R230310 residues in straw (mg/kg)			
	Replicated 1	Replicated 2	Replicated 3	Maximum
Plot 1	<0.01(0.004)	<0.01(0.002)	<0.01(0.005)	-
Plot 2	0.03	0.03	0.03	0.03
Plot 3	0.05	0.04	0.03	0.05
Plot 4	0.24	0.21	0.21	0.24

Table 11. Total sample results in grain and straw

Treatment	Total residues in grain (mg/kg)			
	Replicated 1	Replicated 2	Replicated 3	Maximum
Plot 1	0.05	0.05	0.07	0.07
Plot 2	0.26	0.18	0.24	0.26
Plot 3	0.27	0.18	0.25	0.27
Plot 4	0.46	0.61	0.59	0.61

Treatment	Total residues in straw (mg/kg)			
	Replicated 1	Replicated 2	Replicated 3	Maximum
Plot 1	0.11	0.14	0.12	0.14
Plot 2	0.54	0.47	0.46	0.54
Plot 3	0.97	0.79	0.52	0.97
Plot 4	5.02	4.73	4.23	5.02

Figure 17. Representative chromatogram of samples of Azoxystrobin and R230310 in grain

(A) 40/30 treatment before harvest

(B) 30/21 treatment before harvest

(C) 21/14 treatment before harvest

(D) 14/7 treatment before harvest

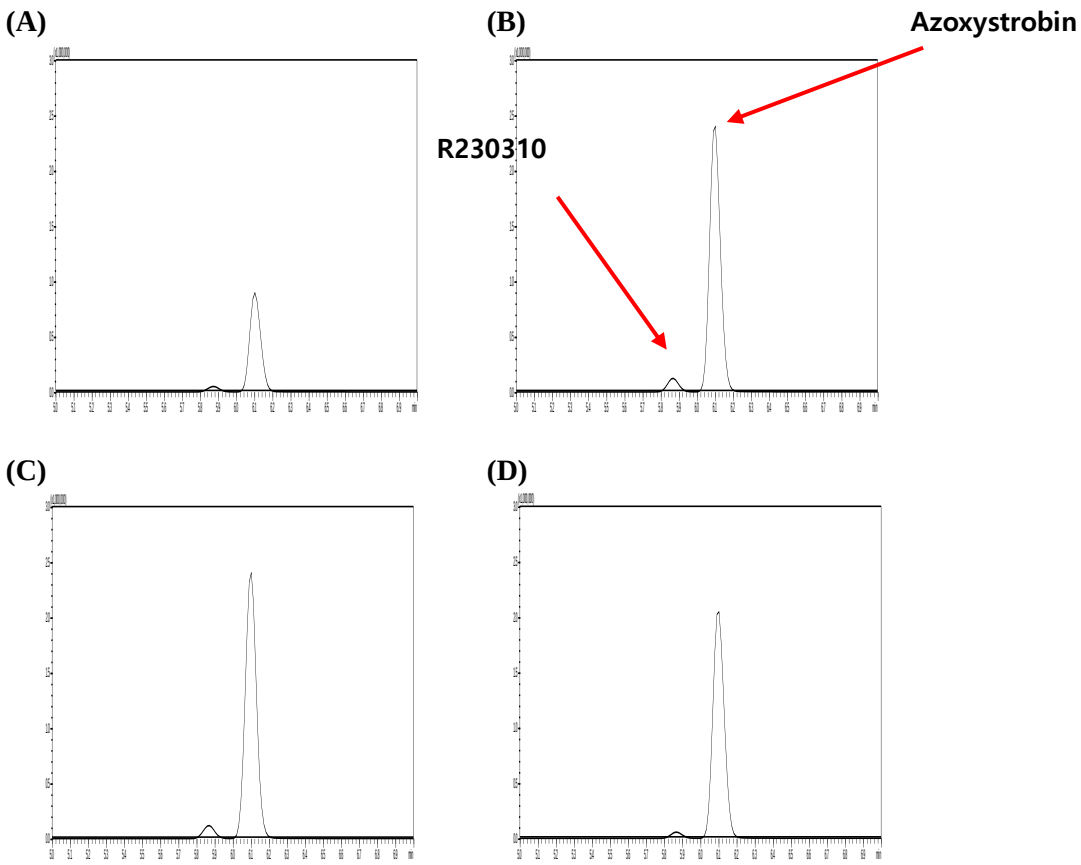

(A)
(B)
Azoxystrobin
R230310
(C)
(D)

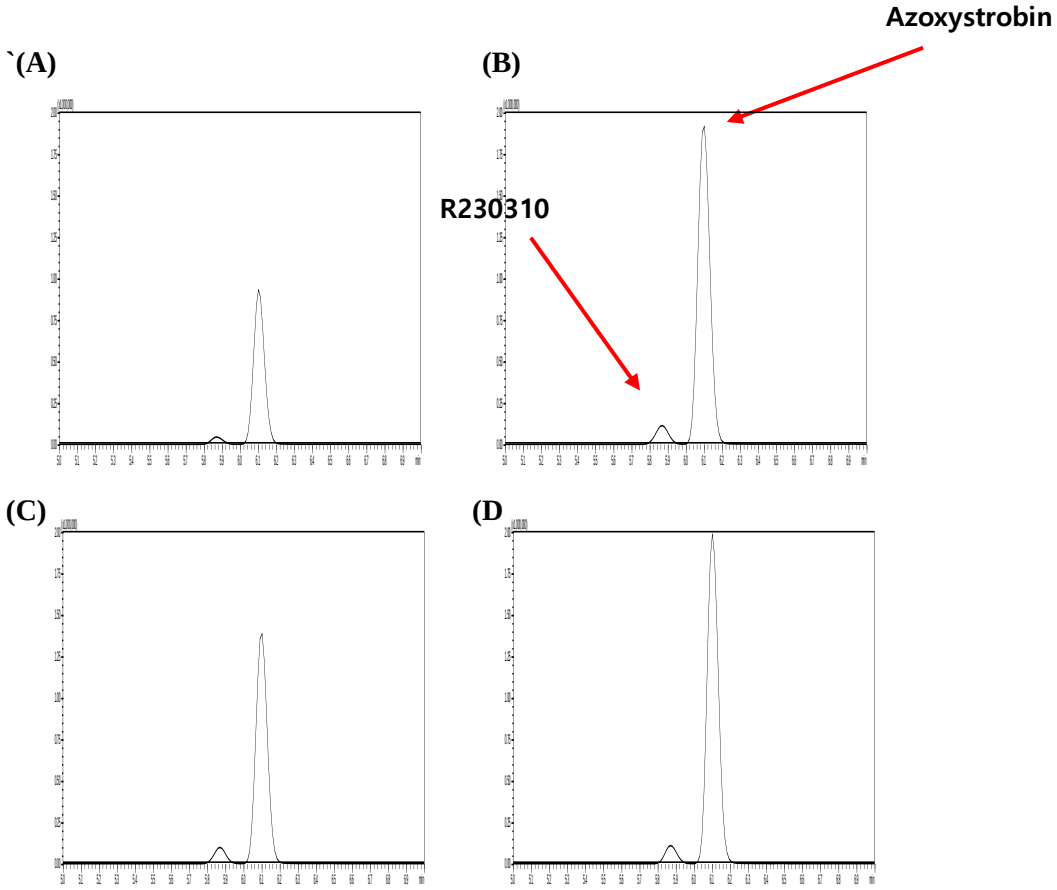
Figure 18. Representative chromatogram of samples of Azoxystrobin and R230310 in straw

(A) 40/30 treatment before harvest

(B) 30/21 treatment before harvest

(C) 21/14 treatment before harvest

(D) 14/7 treatment before harvest


`(A)
(B)
Azoxystrobin
R230310
(C)
(D

Part 2

Dissipation of Fungicide Azoxystrobin in Field and Laboratory Soil

Introduction

Background of soil residue and half life

In 2017, soil persistent problems have occurred in Korea. Potentially toxic pesticide dichloro-diphenyl-trichloroethane (DDT) was detected in eggs and chickens at a radial shape poultry farm where chickens are released to the ground. Consequently, it was a big impact on the consumption of egg and chicken for a while. Thus, the pesticides indirectly affecting human health. In the case of DDT, the half-life in the environment is known two days, but it is known that can increase to 15 years if remain in the soil. Therefore, not only crop residue is an important issue but also soil residue is a very important issue for human life.

The most important aspect of pesticide behavior in the soil is how long the pesticide remains in soil (Chae-Man Choi, 2011). Pesticide degradation is the pesticide sprayed in the environment ingredient detoxification mechanism that except the required amount of a certain period of time for medicinal effect expression, plays an important role in the behavior of pesticides in the environment (Kyeong-Seok Oh, 2000). A half-life means the time that takes times a certain amount of a pesticide decrease to half. Commonly, a pesticide will degrade 50% of the original amount after a single half-life. 25% will remain after two half-lives and about 12% will remain after three half-lives. This continues until the amount remaining is nearly zero (NPIC).

The behavior of pesticides in the soil is greatly influenced by environmental factors, but the behavior pattern varies depending on the type of pesticide, usage method, usage amount and spraying time (Byung-Jun Park, 2011). Soil properties affecting pesticide behavior include the type of clay minerals and content, organic matter content, and property, soil solution pH,

soil moisture content, soil temperature etc. These factors go through complex reactions through interactions in the soil environment, so it is very difficult to make a decision which factors are more important to the persistence of pesticides. Therefore, residue characteristics of pesticides under the field conditions are established through laboratory experiment which fixed environmental conditions and takes measures to establish during the field experiment (xue hua An, 2006).

In Korea, the pesticide half-life period is over 180 days that remain in soil and effect on soil are defined as “soil residual pesticide”, but most foreign countries defined soil residual pesticide as the pesticide that half-life period is over 1 years. Currently, the pesticides circulation in Korea in which more than 95% of the pesticides half-life is less than 100 days. Pesticides that judged as soil residual pesticide are prohibition of use (The latest pesticide science).

Table 12 was the representative study for degradation of Azoxystrobin in the field and laboratory soil. The half-life of Azoxystrobin is different in different literature. It is different half-life time between different soils and different conditions. The half-life appear in field soil is shorter than laboratory soil.

Table 12. Overview of studies in recent years for degradation of Azoxystrobin in soil

Title	Half-life	Author
Degradation profile of Azoxystrobin in Andisol soil: laboratory incubation	77 day (Lab)	(Indra Purnama et al., 2015)
Field versus laboratory experiments to evaluate the fate of Azoxystrobin in an amended vineyard soil	89, 148 day (Lab)	(E. Herrero-Hernandez et al., 2015)
Persistence of Azoxystrobin in/on Grapes and Soil in Different Grapes Growing Areas of India	8.1 day (Field)	(Vijay Tularam Gajbhiye et al., 2011)
Dissipation rates and residues of fungicide Azoxystrobin in ginseng and soil at two different cultivated regions in China	2.8 day (Field)	(Zhiguang Hou et al., 2016)

Materials and Methods

Analytical standard and pesticide for spraying

Standard material of Azoxystrobin (Purity : 98%) was purchased from SIGMA ARDRICH and R234886 (Purity: 100%) was purchased from syngenta. Azoxystrobin 10% wettable powder (WP) amistar from syngenta was purchased at pesticide market (Seoul, Korea).

Standard solutions and working solution

Standard stock solution of Azoxystrobin and R234886 was prepared at the concentration of 1,000 μg/mL with acetonitrile. Then mixed Azoxystrobin 1,000 μg/mL 10 mL and R234886 1,000 μg/mL 10 mL made a Azoxystrobin and R234886 mixture 500 μg/mL 20 mL. The working solutions were prepared by serial dilution of stock solution with acetonitrile.

Analytical reagent

Analytical solvent HPLC grade ACN (acetonitrile, purity: 99.9%) was purchased from Fisher Scientific Korea. Hypergrade for LC-MS MeOH (methanol, purity: 99.8%) was purchased from Merck. Formic acid (purity: 99.8%) was purchased from SIGMA-ALDRICH.

Soil samples

Laboratory experiment target soil samples were used already been collected from Hwaseong-si (Kyeonggi-do, Korea) and already homogenized and sieved (2 mm mesh). Field experiment target soil samples were collected from Hwaseong-si (Kyeonggi-do, Korea) on scheduled date. The physicochemical characteristics of soil were present in Table 13 (Min Woo Jung, 2017)

.

Table 13. Physicochemical characteristics of soil

Classification	pH (1:5)	Organic matter content (%)	Cation exchange capacity (meq/100 g)	Particle size distribution (%)			Field capacity (%)
				Clay	Silt	Sand	
Loamy soil	5.0	1.72	16.1	23.1	35.2	41.7	28.8

Measurement of field capacity

Field capacity is defined as the amount of water after excess water has drained away and the rate of downward movement has materially decreased (Drissa DIALLO et al., 2013). According to environmental persistence test data writing tip, soil moisture should satisfied with 60-80% of field capacity. So field capacity was measured.

Before measure the field capacity, water content of laboratory soil was determined, first weight can and labeled, then added soil 10 g into can and evaporated in oven 24 hour. Finally, weight the can contain dried soil and calculated water content by equation below:

$$\text{Water content (\%)} = \frac{M_{ws} - M_s}{M_s} \times 100 = \frac{M_w}{M_s}$$

[M_{ws}: hydric soil weight (g), M_s: dried soil weight (g), M_w: water weight (g)]

For measure field capacity, put a fixed quantity soil into can and added 3-4 mL of water and mixed, fixed rubber ring on the porous pressure plate and put the soil into a rubber ring using a spatula, pressed it with hand and fill the rubber ring with soil flat. Put the porous pressure plate into a washbowl and added water to saturated condition and left for a day (Figure 19). When soil was saturated with water, put the porous pressure plate into pressure plate extractor, the objective of this experiment is measure moisture by the pressure, so moisten the paper slightly and cover the soil to prevent the loss of water by evaporation. The pressure was set at 0.05 bar and received the water that was lost by pressure for a day. If the water is no longer trapped by the 0.05 bar pressure, weight 2-3 spoon of soil in can 3 repetitions. The soil was dried in a 105℃ oven for 24-48 hour, remove the dried soil and put in a desiccator and allow to cool for about 10 minutes and weight the dried soil. Next, set the pressure to 0.1, 0.33, 0.5 and 1 bar sequentially, and repeat the above

procedure to measure the moisture content. Field capacity refers to the water content at 0.33 bar.

Figure 19. Measurement of field capacity

(A) Added water to saturated condition

(B) Pressure plate extractor (receive the water at 0.05 bar)

(C) Cooling the soil in a desiccator

(D) Dried soil

(A)

(B)

(C)

(D)

Soil instrumental conditions

The analytical instrument used shimadzu LC-MS 8040 with UHPLC Nexera with ESI positive mode. The analytical column was kinetex C18 (100 mm × 2.1 mm, 2.6 μm) and oven temperature was 40℃. The mobile phases were 0.1% formic acid & 5 mM ammonium formate in distilled water (A) and 0.1% formic acid & 5 mM ammonium formate in methanol (B). Gradient program tested a lot, finally 15 min gradient program set as follows: Initially mobile phase B was set 5% for 0.5 min. Then increased 5% to 90% for 14 minute and hold 90% for 3 min. Finally decreased it to 5% for 0.5 min and hold 5% for 2.5 min. Flow rate was set at 0.2 mL/min and injection volume was 5 μL. Desolvation line (DL) temperature was 250℃ and heat block temperature was 400℃. Nebulizing gas and drying gas was nitrogen, flow was 3 L/min and 15 L/min. Collision energy was used argon gas.

Soil preparation method

Weight laboratory soil 8.76 g in 50 mL falcon tube and added 1.24 mL distilled water. Then added ceramic homogenizers and extracted with 10 mL of 0.1% formic acid in acetonitrile using a shaker at 300 rpm for 5 min. After that, all samples in falcon tube were cooling on ice and added $MgSO_4$ 4 g, NaCl 1 g, then used Mini G vigorously shaken for 1 min. Centrifuged at 3,500 rpm for 5 min. Transferred supernatant to 2 mL vial 1:1 matrix matched with acetonitrile analyzed by LC-MS/MS.

Weight laboratory soil 10 g in 50 mL. Then added ceramic homogenizers and extracted with 10 mL of 0.3% formic acid in acetonitrile using a shaker at 300 rpm for 5 min. After that, all samples in falcon tube were cooling on ice and added $MgSO_4$ 4 g, NaCl 1 g, then used Mini G vigorously shaken for 1 min. Centrifuged at 3,500 rpm for 5 min. Transferred supernatant to 2 mL vial 1:1 matrix matched with acetonitrile analyzed by LC-MS/MS.

Method validation

In method validation, Set ILOQ, MLOQ and evaluated calibration curve linearity and matrix effect. The accuracy and precision of the preparation method verified by recovery test.

ILOQ and MLOQ

To set MLOQ (Method Limit of Quantitation) a series of standard solutions were analyzed by LC-MS/MS for set ILOQ (Instrumental Limit of Quantitation)**.** The ILOQ was set as the concentration that the signal-to-noise ratio was higher than 10. MLOQ was calculated by equation below:

$$\text{MLOQ (mg/L)} = \frac{\text{ILOQ (ng)} \times \text{Final volume (mL)} \times \text{Dilution factor}}{\text{Injection volume } (\mu\text{L}) \times \text{Initial sample weight (g)}}$$

(Min Woo Jung, 2017)

Matrix matched calibration curve and linearity

Matrix matched standard solution of field soil	**MSTD 1 (0.0005 μg/mL)**	**MSTD 2 (0.001 μg/mL)**	**MSTD 3 (0.0025 μg/mL)**	**MSTD 4 (0.005 μg/mL)**	**MSTD 5 (0.01 μg/mL)**	**MSTD 6 (0.025 μg/mL)**
	MSTD 7 (0.05 μg/mL)	**MSTD 8 (0.1 μg/mL)**	**MSTD 9 (0.2 μg/mL)**			
Matrix matched standard solution of laboratory soil	**MSTD 1 (0.0005 μg/mL)**	**MSTD 2 (0.001 μg/mL)**	**MSTD 3 (0.0025 μg/mL)**	**MSTD 4 (0.005 μg/mL)**	**MSTD 5 (0.01 μg/mL)**	**MSTD 6 (0.025 μg/mL)**
	MSTD 7 (0.05 μg/mL)	**MSTD 8 (0.1 μg/mL)**	**MSTD 9 (0.2 μg/mL)**			

Control sample matrix and a series of standard solution were diluted 2 times by matching at 1:1 ratio. Coefficient of determination (r^2) calculated at matrix matched calibration curve.

Matrix effect calculation

Matrix effects (ME, %) was calculated by comparing the slope of matrix

matched calibration curve and solvent standard calibration curve using the following equation:

$$\text{ME}, \% = \left(\frac{\text{slope of matrix matched calibration curve}}{\text{slope of solvent standard calibration curve}} - 1\right) \times 100$$

(Min Woo Jung, 2017)

Recovery test

Recovery test was carried out 3 repetitions spiking on untreated samples at 2 levels (MLOQ and 10MLOQ).

Field trails for soil

Field trial was located in Hwaseong-si (Kyeonggi-do, Korea) and conducted as a middle-scale test concurrent with minor crop cultivation experiment. The field size was 4×16 m^2 divided into four plot that contains 3 treated plot and one untreated control plot. Buffer zones were installed between each plot to prevent cross contamination. Drainage canal was installed in center of buffer zone which length was 30 cm. The large rock and weeds were removed and set up treatment plot labelling (Figure 20).

Pesticide spraying was carried out one time. In the case of upland soil, regulated spray 200 L/10 a. So Azoxystrobin 10% WP prepared with 1,000 times dilution and sprayed 3.2 L every treated plot. Sprayed drawing S shape at a constant speed to spray evenly (Figure 21). Approximately 2 hours after spraying, every plot chose twelve sites like a Z shape to collect soil samples and mixed in polyethylene bag, every sample was collected 10 cm depth use auger and the weight of every plot samples were approximately 0.5 kg. In order to duplication, made a mark on the collection place. Samples were collected on 0, 1, 3, 7, 21, 28 days and rapidly transferred to laboratory stored at -18℃. Before analysis, used 2 mm pore test sieve pass the soil.

Figure 20. Field trials for soil

(A) Satellite picture

(B) Made drainage canal

(A)

(B)

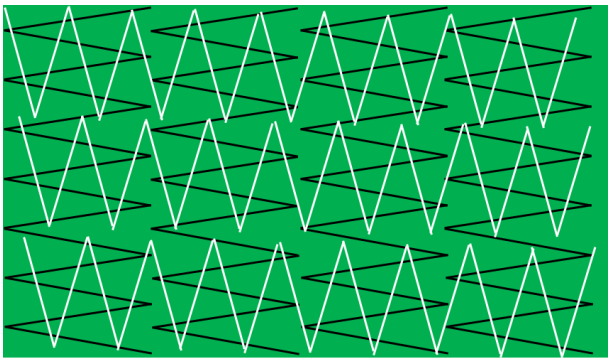
Figure 21. Spraying pesticide and method

(A) Spraying pesticide

(B) Spraying method

(A)

(B)



Laboratory soil incubation

Incubator was purchased from Hanbaek scientific co. (Figure 22). Before incubation, weight 8.76 g soil into 50 mL falcon tube and added 1.24 mL water (water content was 75% of field capacity), Azoxystrobin standard solution spiked at 0.2 mg/kg levels and labeled sample collect day that include 0 (after 2 hour), 7, 15, 30, 45, 60, 90. All tubes were cover with foil and made 5 stoma using syringe. Then all samples were incubated at 25±2℃ and all samples were collected prearranged time and stored in -18℃ (Figure 23). Incubator glass cover with foil to prevent light entering. When the water evaporated significantly, it was necessary to add water. On average, 0.15 g of water was added every day for water correction. All samples were analyzed within 15 days.

Figure 22. Laboratory soil incubator

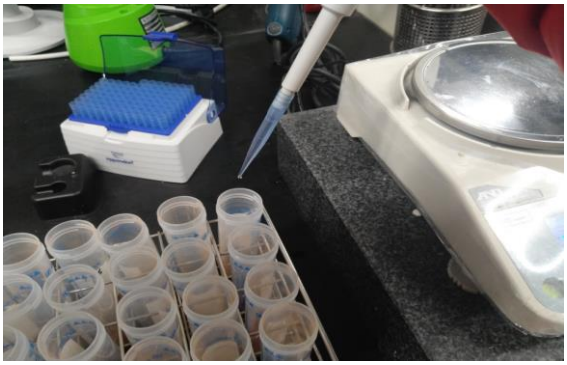

23.7
SHAKING INCUBATOR

Figure 23. Prepare incubation

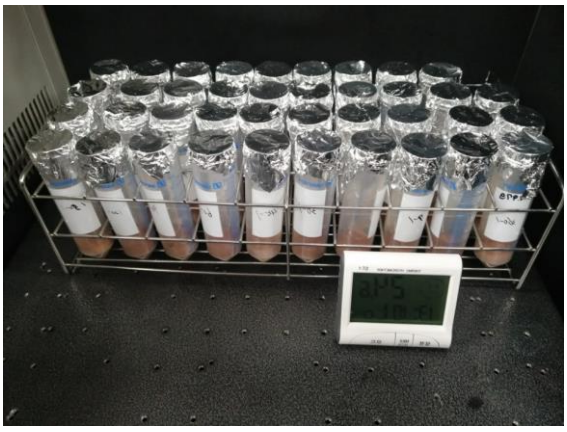
(A) Spiking at 0.2 mg/kg level

(B) Cover with foil and incubation

(A)



(B)



Results and Discussion

Preparation method optimization

Laboratory soil was used over 20 g that recommended in environmental persistence test writing tips. For convenience, optimized sample weight for use less soil, reagent and solvent. When 2 g soil was used, it should be add water every day for satisfied with field capacity of 60-80% and can't transfer 1 mL supernatant that extract with 2 mL 0.1% formic acid in ACN after centrifuge. So 10 g soil was proper. Laboratory soil was contain 6.65% water in 10 g soil. By water content equation, 10 g soil contain 0.62 g water. The soil field capacity was 28.8%. In order to set water content at 75% (60-80%) of field capacity in 10 g soil, weight 8.76 g soil and add 1.24 g water as a 10 g soil.

Field soil was optimized acid ratio in extraction solvent, 0.1%, 0.3% and 0.5% formic acid in acetonitrile were tested. When used 0.1% or 0.5% formic acid, R234886 recovery was not satisfied with 70-120%. When used 0.3% formic acid in acetonitrile, recovery was satisfied with 70-120%. So 0.3% formic acid in acetonitrile was used as extraction solvent.

Scheduled MRM

In minor crop experiment, two peaks were completely separated, but Azoxystrobin retention time and R230310 retention time were very similar. So can't display one peak in one window. In soil experiments, gradient time were set longer than minor crop gradient time. So two components were not detected on similar retention time. Therefore, used scheduled MRM display only one peak in one window. MRM conditions were present in Table 14.

Table 14. MRM (Multiple Reaction Monitoring) conditions

Compound	M.W.	Ionization	Precursor ion> Product ion (CE, eV)	
			Quantifier ion	Qualifier ion
Azoxystrobin	403.1	[M+H]+	404.0>372.0 (-15)	404.0>344.1 (-26)
R234886	389.1	[M+H]+	390.1>372.0 (-13)	390.1>344.1 (-25)

ILOQ and MLOQ results and calibration curve linearity

ILOQ of Azoxystrobin was 0.005 ng (0.001 μg/mL × 5 μL) and R234886 was 0.0125 ng (0.0025 μg/mL × 5 μL) in field and laboratory soil. Azoxystrobin retention time was 12.0 min and R234886 retention time was 11.1 min. Calculated MLOQ according to the MLOQ equation, MLOQ of azoxystrobin was 0.002 mg/kg and R234886 was 0.005 mg/kg in field and lab soil. Matrix matched standard calibration curves of Azoxystrobin and R234886 had a good linearity in field soil (Figure 24) and laboratory soil (Figure 25). The ranges were between 0.0005 to 0.2 mg/kg. The regression equations were y = 151725x + 403909 (Azoxystrobin in field soil) and y = 57496x – 6490.7 (R234886 in field soil), respectively. y = 170978x + 342686 (Azoxystrobin in laboratory soil) and y = 82487x + 47353 (R234886 in laboratory soil), respectively. Coefficients of determination (r^2) of Azoxystrobin and R234886 were over 0.99 in both samples.

Figure 24. Matrix matched calibration curve in field and lab soil

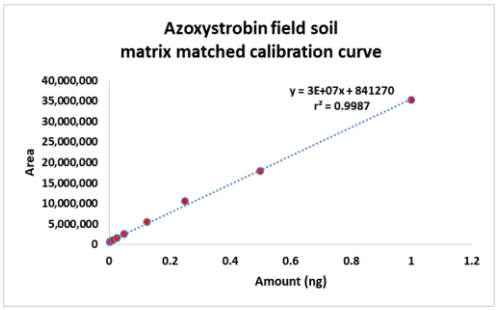
(A) Azoxystrobin (Field)

(B) R234886 (Field)

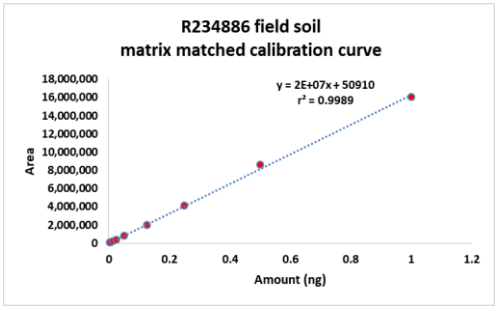
(C) Azoxystrobin (Laboratory)

(D) R234886 (Laboratory)

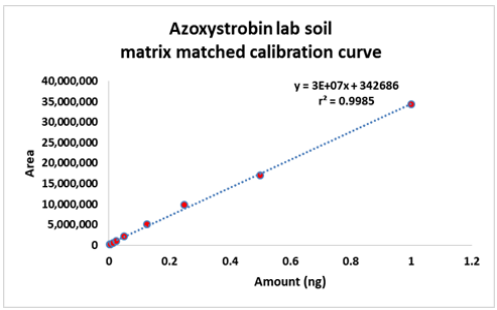
(A)


Azoxystrobin field soil
matrix matched calibration curve
y = 3E+07x + 841270
r² = 0.9987
Area
40,000,000
35,000,000
30,000,000
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
0
0.2
0.4
0.6
0.8
1
1.2
Amount (ng)

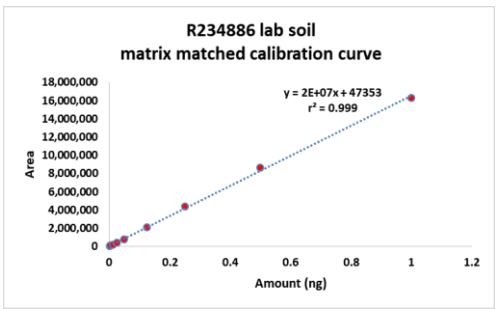
(B)


R234886 field soil
matrix matched calibration curve
y = 2E+07x + 50910
r² = 0.9989
Area
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
0
0.2
0.4
0.6
0.8
1
1.2
Amount (ng)

(C)


Azoxystrobin lab soil
matrix matched calibration curve
y = 3E+07x + 342686
r² = 0.9985
Area
40,000,000
35,000,000
30,000,000
25,000,000
20,000,000
15,000,000
10,000,000
5,000,000
0
0
0.2
0.4
0.6
0.8
1
1.2
Amount (ng)

(D)


R234886 lab soil
matrix matched calibration curve
y = 2E+07x + 47353
r² = 0.999
Area
18,000,000
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
0
0.2
0.4
0.6
0.8
1
1.2
Amount (ng)

Matrix effects in soil

Based on matrix effect equation calculated matrix effect in field and laboratory soil samples. The results of Azoxystrobin was -17.81% and R234886 was -12.78% in field soil, Azoxystrobin was -7.38% and R230310 was 25.13% in laboratory soil. According to results of matrix effect in field and laboratory soil, discovered ion suppression and enhancement in analysis.

Recovery test result

The average recovery results of Azoxystrobin in field soil at MLOQ and 10MLOQ levels were 88.2% and 108.5% and R234886 were 100.4% and 104.3%, respectively (Figure 25). The average recovery results of Azoxystrobin in laboratory soil at MLOQ and 10MLOQ levels were 87.0% and 109.4% and R234886 were 107.0% and 111.5%, respectively (Figure 26). No components detected in control at the same retention time with Azoxystrobin and R234886. All results of recovery at 2 levels were satisfied with 70-120%, RSD were less than 10% (Table 15).

Figure 25. Representative chromatogram of recovery test in field soil

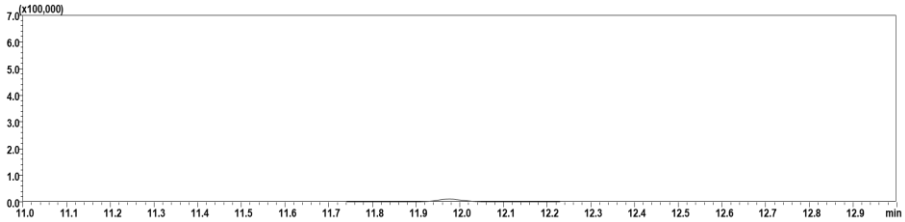
(A) Control (Azoxystrobin)

(B) 10MLOQ (Azoxystrobin)

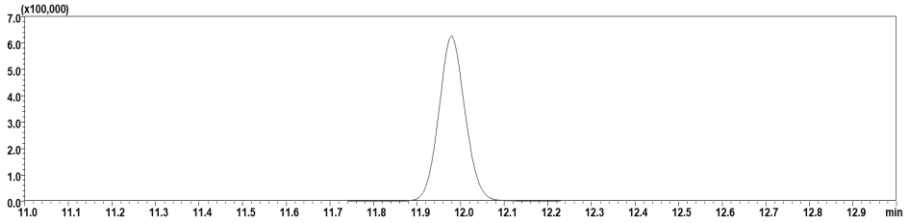
(C) Control (R234886)

(D) 10MLOQ (R234886)

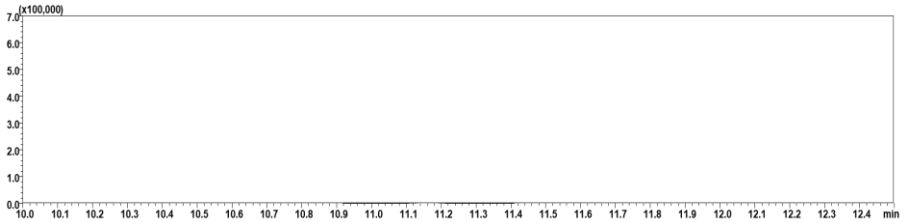
(A)


(x100,000)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
11.0 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 12.0 12.1 12.2 12.3 12.4 12.5 12.6 12.7 12.8 12.9 min

(B)


(x100,000)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
11.0 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 12.0 12.1 12.2 12.3 12.4 12.5 12.6 12.7 12.8 12.9 min

(C)


(x100,000)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
10.0 10.1 10.2 10.3 10.4 10.5 10.6 10.7 10.8 10.9 11.0 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 12.0 12.1 12.2 12.3 12.4 min

(D)

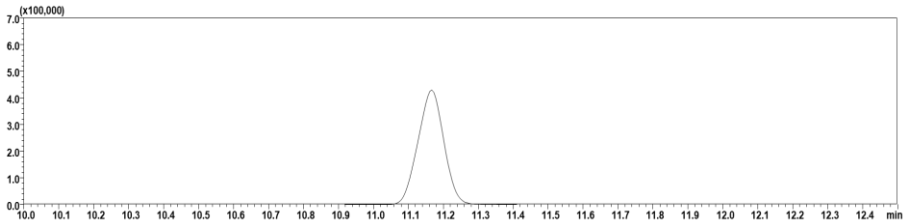

(x100,000)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
10.0 10.1 10.2 10.3 10.4 10.5 10.6 10.7 10.8 10.9 11.0 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 12.0 12.1 12.2 12.3 12.4 min

Figure 26. Representative chromatogram of recovery test in laboratory soil

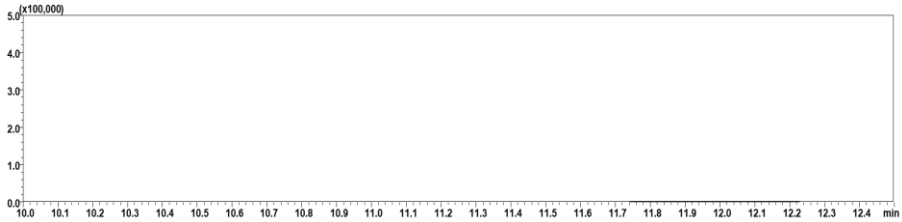
(A) Control (Azoxystrobin)

(B) 10MLOQ (Azoxystrobin)

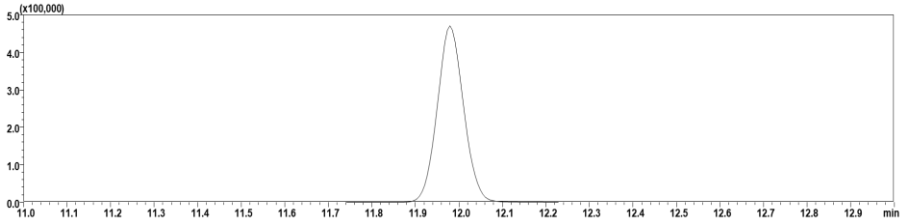
(C) Control (R234886)

(D) 10MLOQ (R234886)

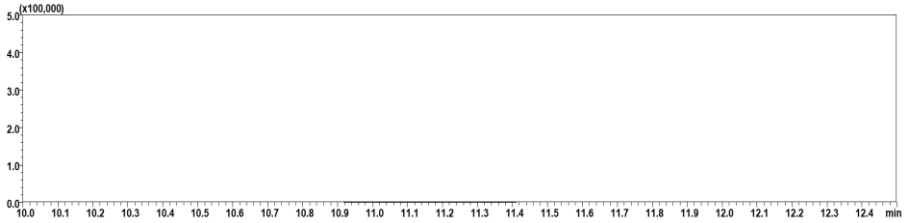
(A)


(x100,000)
5.0
4.0
3.0
2.0
1.0
0.0
10.0 10.1 10.2 10.3 10.4 10.5 10.6 10.7 10.8 10.9 11.0 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 12.0 12.1 12.2 12.3 12.4 min

(B)


(x100,000)
5.0
4.0
3.0
2.0
1.0
0.0
11.0 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 12.0 12.1 12.2 12.3 12.4 12.5 12.6 12.7 12.8 12.9 min

(C)


(x100,000)
5.0
4.0
3.0
2.0
1.0
0.0
10.0 10.1 10.2 10.3 10.4 10.5 10.6 10.7 10.8 10.9 11.0 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 12.0 12.1 12.2 12.3 12.4 min

(D)

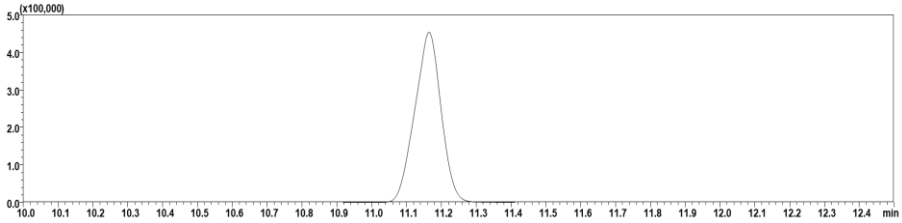

(x100,000)
5.0
4.0
3.0
2.0
1.0
0.0
10.0 10.1 10.2 10.3 10.4 10.5 10.6 10.7 10.8 10.9 11.0 11.1 11.2 11.3 11.4 11.5 11.6 11.7 11.8 11.9 12.0 12.1 12.2 12.3 12.4 min

Table 15. Recovery test results of field and laboratory soil

Pesticides	Part	Spiking levels (mg/kg)	Recovery (%)	RSD (%)
Azoxystrobin	Field soil	0.002	88.2	7.81
		0.02	108.5	5.23
	Lab soil	0.002	87.0	5.08
		0.02	109.4	1.57
R234886	Field soil	0.002	107.0	2.06
		0.02	111.5	1.69
	Lab soil	0.002	100.4	4.19
		0.02	104.3	0.95

The climate effect on pesticide degradation in soil

The factors affecting soil degradation include rainfall, wind, and sunlight (The latest pesticide science). Table 16 shows meteorological data during field trial.

Table 16. The meteorological data (12:00 am)

Date	Temperature (℃)	Humidity (%)	Average wind speed(m/s)	Soil water (%)
9 / 7	24.6	64.5	1.4	25.1
9 / 8	26.8	53.8	1.2	24.5
9 / 9	25.8	63.5	1.3	23.9
9 / 10	23.7	76.1	0.5	23.2
9 / 11	20.9	77.3	1.7	28.3
9 / 12	24.3	66.2	2.4	27.9
9 / 13	24.9	32.8	1.4	26.9
9 / 14	25.0	42.1	2.0	26.0
9 / 15	24.3	48.2	2.7	24.9
9 / 16	23.1	53.2	3.9	24.0
9 / 17	26.7	50.4	2.5	23.4
9 / 18	24.4	53.4	1.7	22.9
9 / 19	24.3	76.3	1.6	22.4
9 / 20	21.6	40.5	2.7	22.5
9 / 21	22.7	44.2	0.9	22.4
9 / 22	24.5	46.7	2.1	22.1
9 / 23	23.5	69.0	1.0	21.6
9 / 24	24.8	71.2	1.1	21.4
9 / 25	26.3	47.4	1.7	21.1
9 / 26	26.9	48.8	1.8	20.7
9 / 27	22.1	62.0	1.7	20.5
9 / 28	21.7	29.9	2.7	20.5
9 / 29	18.7	35.3	1.9	20.1
9 / 30	23.2	59.6	1.4	19.8
10 / 1	18.4	71.7	1.5	19.5
10 / 2	24.0	56.6	1.9	22.6
10 / 3	19.0	55.0	1.1	22.3
10 / 4	19.3	54.6	1.7	22.0

10 / 5	20.7	55.2	1.8	21.5

Samples results and dissipation characteristics in field soil

The average samples results of Azoxystrobin in field soil, Sampling 0 day was 150 μ/kg, Sampling 1 day was 151 μ/kg, Sampling 3 day was 107 μ/kg, Sampling 7 day was 137 μ/kg, Sampling 21 day was 51 μ/kg, Sampling 28 day was 30 μ/kg (Table 17). In the case of R234886, all average samples results were less than 5 μ/kg (Table 18). Half-life of Azoxystrobin was 12.38 day and amounts were decreased as time goes on (Figure 27). But R234886 can't compare the amounts because of residue amounts were too low.

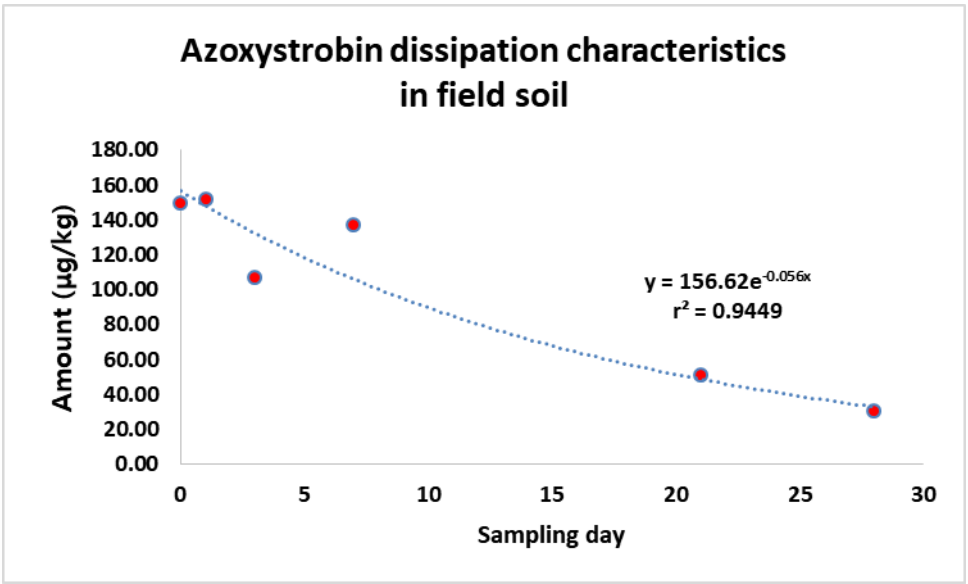
Table 17. Results of Azoxystrobin in field soil

Pesticide	Sampling day	Residual amount (㎍/kg)					Hal-life (day)
Azoxystrobin	-	1	2	3	Average	RSD	ln2/b =12.38
	0	120	134	194	150	26.32	
	1	108	142	204	151	32.16	
	3	85	114	122	107	18.19	
	7	86	70	255	137	74.82	
	21	46	49	58	51	12.25	
	28	30	34	27	30	45.79	

Table 18. Results of R234886 in field soil

Pesticide	Sampling day	Residual amount (μg/kg)				
	-	1	2	3	Average	RSD
R234886	0	0.88	0.94	1.01	0.94	6.90
	1	1.19	1.23	1.76	1.39	22.84
	3	1.11	1.18	1.30	1.19	8.03
	7	2.05	1.86	6.47	3.46	75.39
	21	1.78	1.56	1.97	1.77	11.59
	28	1.62	1.69	1.87	1.72	7.47

Figure 27. Dissipation characteristics of Azoxystrobin in field soil


Azoxystrobin dissipation characteristics in field soil
Amount (µg/kg)
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
0
5
10
15
20
25
30
Sampling day
y = 156.62e-0.056x
r2 = 0.9449

Samples results and dissipation charateristics in laboratory soil

The average samples results of Azoxystrobin in laboratory soil, Sampling 0 day was 193 μ/kg, Sampling 7 day was 188 μ/kg, Sampling 15 day was 188 μ/kg, Sampling 30 day was 182 μ/kg, Sampling 45 day was 181 μ/kg, Sampling 60 day was 176 μ/kg, Sampling 90 day was 140 μ/kg. (Table 19). In the case of R234886, all average samples results were less than 5 μ/kg (Table 20). The half-life of Azoxystrobin in laboratory soil was over 90 day (Figure 28). The results had an error because of the working solutions were degraded over time. So actual quantified values are larger. Therefore, the actual half-life was shorter than measured.

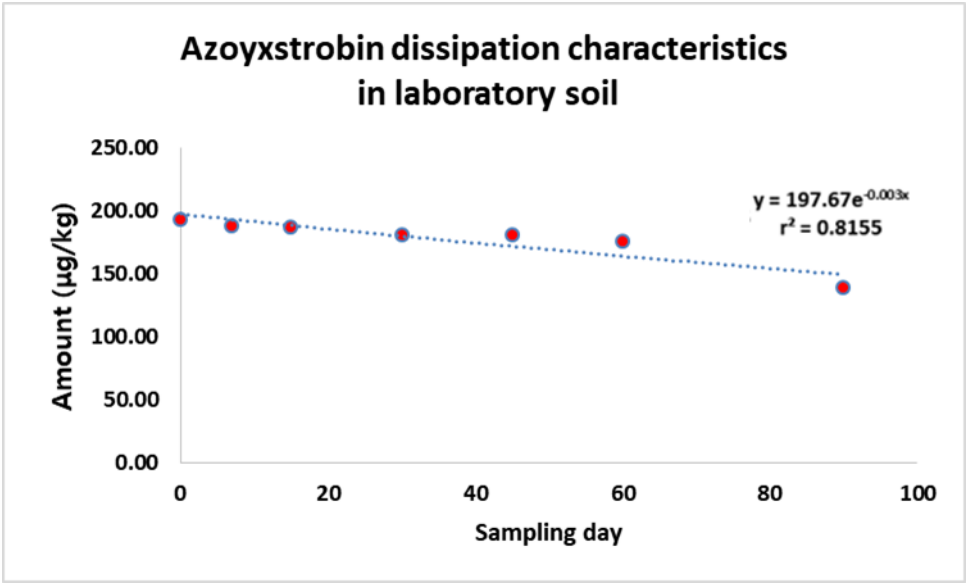
Table 19. Results of Azoxystrobin in laboratory soil

Pesticide	Sampling day	Residual amount (μg/kg)					Hal-life (day)
Azoxystrobin	-	1	2	3	Average	RSD	ln2/b = over 90 day
	0	193	195	191	193	1.03	
	7	193	190	183	188	2.72	
	15	191	188	184	188	1.87	
	30	176	182	185	182	2.53	
	45	185	179	178	181	2.10	
	60	173	180	176	176	1.99	
	90	112	161	147	140	18.02	

Table 20. Results of R234886 in laboratory soil

Pesticide	Sampling day	Residual amount (μg/kg)				
	-	1	2	3	Average	RSD
R234886	0	0.13	0.13	0.13	0.13	0
	7	0.15	0.39	0.12	0.22	67.85
	15	1.71	1.13	1.23	1.36	22.85
	30	1.95	2.06	3.23	2.41	29.39
	45	1.85	2.12	0.68	1.55	49.38
	60	1.62	1.69	1.87	1.72	7.46
	90	2.09	1.34	3.40	2.28	45.79

Figure 28. Dissipation characteristics of Azoxystrobin in laboratory soil


Azoyxstrobin dissipation characteristics in laboratory soil
Amount (µg/kg)
250.00
200.00
150.00
100.00
50.00
0.00
0
20
40
60
80
100
Sampling day
y = 197.67e-0.003x
r² = 0.8155

Conclusion

Currently, it is ridiculously lacking pesticides registered in the minor crop. So farmers are faced with many difficulties during minor crops cultivation. Part 1 of this study was carried out to register fungicide Azoxystrobin in minor crop foxtail millet. ILOQ of Azoxystrobin and R230310 was 0.0125 ng in grain, 0.025 ng in straw, MLOQ was 0.01 mg/kg in grain and straw, $r^2 \geq 0.999$ had a good linearity. Recovery test carried out at 2 levels (MLOQ and 10MLOQ), both levels of recovery were satisfied with 70~120% and RSD≤10%. Storage stability test carried out at 10MLOQ level, also satisfied with 70~120% and RSD≤10%. As results of field sample analysis, total maximum sample residue amount was decreased from 0.61 mg/kg (14/7 day treatment before harvest) to 0.07 mg/kg (40/30 day treatment before harvest) in grain. In case of straw, total maximum sample residue amount was decreased from 5.02 mg/kg (14/7 day treatment before harvest) to 0.14 mg/kg (40/30 day treatment before harvest) in straw. This results can be used as basic data to establish PHIs and MRLs for Azoxystrobin during foxtail millet cultivation.

In 2017, soil persistent problems have occurred in Korea. Therefore, not only crop residue is an important issue but also soil residue is a very important issue for human life. Part 2 of this study was carried out to investigate dissipation of Azoxystrobin in field and laboratory soil. ILOQ of Azoxystrobin was 0.001 ng, R234886 was 0.0025 ng in both soil. MLOQ of Azoxystrobin was 0.002 mg/kg and R234886 was 0.005 mg/kg in both soils. Azoxystrobin and R234886 matrix matched calibration curve both had a good linearity in both soils. Recovery test carried out at 2 levels (MLOQ and 10MLOQ), both levels of recovery were satisfied with 70~120% and RSD≤10%. The half-life of Azoxystrobin in field soil was 12.38 day. The

half-life of Azoxystrobin in laboratory soil was over 90 day. Because of rainfall, wind, and sunlight, there was a large difference in the half-life between field and laboratory soil. According to field data, it was judged Azoxystrobin didn’t have an effect on next crop cultivation.

References

Anneli Kruve, Ivo Leito, Koit Herodes* (2009). Combating matrix effects in LC/ESI/MS: The extrapolative dilution approach. *Institute of Chemistry, University of Tartu, Jakobi 2, 51014 Tartu, Estonia.*

Anna Stachniuk[1] & Emilia Fornal[1] (2015). Liquid Chromatography-Mass Spectrometry in the Analysis of Pesticide Residues in Food. Food Anal. Methods (2016) 9:1654–1665, DOI 10.1007/s12161-015-0342-0.

Byung-Jun Park* (2011). Pesticide Residue Monitoring and Environmental Exposure Assessment in Paddy Field Soil and Greenhouse Soils. The Korean Journal of Pesticide Science Vol. 15, No. 2, pp. 1 ~ 6.

B. K. Matuszewski,* M. L. Constanzer, and C. M. Chavez-Eng (2003). Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC-MS/MS. Anal. Chem. 2003, 75, 3019-3030.

CHAIDO LENTZA-RIZOS, ELIZABETH J. AVRAMIDES,* AND KALLIOPI KOKKINAKI† (2006). Residues of Azoxystrobin from Grapes to Raisins. J. Agric. Food Chem. 2006, 54, 138-141

Cho Rong Lee, Ji Hyung Hong, Jong Sung Lim and Kyu Seung Lee (2011). Residue Partterns of Azoxystrobin and Cyenopyrafen in Grape between Rainshield and Plastic House Conditions. The Korean Journal of Pesticides Science Vol. 15, No. 2, pp. 97~103

Chae-Man Choi, Dong-Hyeon Yook, Chae-Kyu Hong, Tae-Rang Kim, Young-sook Hwang, In-Sook Hwang et al., (2011). Monitoring of Residual Pesticides in Agricultural Land from the Southern Area of Seoul. The Korean journal of Pesticide Science 15(2), 2011.06, 160-165.

Drissa DIALLO[1], Adama MARIKO[2] (2013). Field capacity (FC) and permanent wilty point (PWP) of clay soils developed on Quaternary alluvium in Niger River loop (Mali). International Journal of Engineering Research and Applications (IJERA) ISSN:2248-9622 www.ijera.com

Dugald J. MacLachlan [a], Denis Hamilton [b] (2010). Estimation methods for Maximum Residue Limits for pesticides. Regulatory Toxicology and Pharmacology Volume 58, Issue 2, November 2010, Pages 208-218

Eun-jeong Park, Ju-Hee Lee, Tae-Hwa Kim, and Jang-Eok Kim (2009). Residual Patterns of Strobilurin Fungicides in Korean Melon under Plastic Film House Condition. Korean Journal of Environmental Agriculture Vol. 28, No. 3, pp. 281-288

E. Herrero-Hernandez, J.M. Marin-Benito, M.S. Andrades, M.J. Sanchez-Martin, M.S. Rodriguez-Cruz (2015). Field versus laboratory experiments to evaluate the fate of azoxystrobin in an amended vineyard soil. Journal of Environmental Management 163 (2015) 78-86

Francisco Prieto Garcia1*, 1*Sandra Y. Cortés Ascencio, 2John C. Gaytan Oyarzun,3Alejandra Ceruelo Hernandez and 3Patricia Vazquez Alavarado (2012). Journal of Research in Environmental Science and Toxicology (ISSN: 2315-5698) Vol. 1(11) pp. 279-293, December 2012.

G. G. GUILBAULT' and M. HJELM' (1989). NOMENCLATURE FOR AUTOMATED AND MECHANISED ANALYSIS. *Pure&Appl.* Chern.,Vol. 61, No. 9, pp. 1657-1664, 1989. Printed in Great Britain.

Hamdy Balba (2007). Review of strobilurin fungicide chemicals. Review of strobilurin fungicide chemicals, Journal of Environmental Science and Health Part B, 42:4, 441-451, DOI: 10.1080/03601230701316465

Hyun-Ho Noh, Jae-Yun Lee, So-Hyun Park, Kwang-Hun Lee, Jae-Ho Oh, Moo-Hyeog Im et al (2012). Residual characteristics of Azoxystrobin and Difenoconazole in ginseng. The Korean journal of Pesticide Science 16 (2). 2012.06. 131-136 (6 pages)

Hye-Rim Kang, Young-Ju Lee, Yu-Ri Lee, Guk-Tak Han, Hee-Ra Chang and Kyun Kim (2011). Dissipation Pattern of Azoxystrobin, Difenoconazole and Iprodione Treated on Field-Grown Green Garlic. Korean J Environ Agric (2011) Vol. 30, No. 4, pp. 446-452 http://dx.doi.org/10.5338/KJEA.2011.30.4.446

Indra Purnama, Farag Malhat, Piyanuch Jaikaew, Hirozumi Watanabe, Sri Noegrohati, Bambang Rusdiarso et al (2014). Degradation profile of azoxystrobin in Andisol soil: laboratory incubation. Toxicological & Environmental Chemistry, 2015. http://dx.doi.org/10.1080/02772248.2015.1015297

Jungmi Seo, Dongryong Ha, Hyanghee Lee, Musul Oh, Jongjin Park, Hyeonwo Shin, and Eunsun Kim (2010). The Degradation Patterns of Two Pesticides in Spinach by Cultivation, Storage and Washing. J. Fd Hyg. Safety Vol. 25, No. 2, pp. 91-99 (2010)

Kermit K. Murray (2015). The term "multiple reaction monitoring" is recommended. Rapid Commun. Mass Spectrom. 2015, 29, 1926–1928 (wileyonlinelibrary.com) DOI: 10.1002/rcm.7297.

Kyeong-Seok Oh, Byung-Youl Oh, Seung-Soon Park, Yang-Bin Ihm, Kee-Sung Kyung, Jae-Koo Lee (2000). Degradation of the herbicide dicamba under sunlight and in soil. The Korean journal of Pesticide Science 4(3), 2000.09, 1-6.

Kerstin Gustafssona, b* Anna Hellströmd, Henrik Kylind and Elena Gorokhovab , Eva Blidbergb, Irene Karlsson Elfgrenc (2009). Direct and indirect effects of the fungicide azoxystrobin in outdoor brackish water microco. Published in Ecotoxicology (2010) 19:431–444 DOI 10.1007/s10646-009-0428-9

Kerstin Gustafsson • Eva Blidberg • Irene Karlsson Elfgren • Anna Hellstro¨m •Henrik Kylin • Elena Gorokhova (2010). Direct and indirect effects of the fungicide azoxystrobin in outdoor. Ecotoxicology (2010) 19:431–444, DOI 10.1007/s10646-009-0428-9 brackish water microco.

Lutz Alder,1* Kerstin Greulich,1 Gu¨nther Kempe,2 and Ba¨rbel Vieth1 (2006). RESIDUE ANALYSIS OF 500 HIGH PRIORITY PESTICIDES:BETTER BY GC–MS OR LC–MS/MS? Federal Institute for Risk Assessment, Residue Analysis Unit,Thielallee 88-92, 14195 Berlin, Germany. 2Landesuntersuchungsanstalt fu¨r das Gesundheits- und Veterina¨rwesen,

Min Woo Jung (2017). Dissipation Charateristics of Insecticide Phenthoate in Minor Crop Millet during Cultivation. MS Thesis, Seoul National University, Korea.

Michael Houbraken UGent, David Senaeve UGent, Ilse Delcour and Pieter Spanoghe UGent (2014). Climate change and pesticides: (no) need for concern? Book of abstracts of the IUFoST 2014 world congress of food science and technology.

Nitya Sharma & Keshavan Niranjan (2018). Foxtail millet: Properties, processing, health benefits, and uses, Food Reviews International, 34:4, 329-363, DOI: 10.1080/87559129.2017.1290103.

ONESMUS KYALO MWANIKI (2017). DETERMINATION OF PRE-HARVEST INTERVAL FOR CARBENDAZIM USED FOR CONTROL OF FUNGAL DISEASES IN FRENCH BEANS CONTROL OF FUNGAL DISEASES IN FRENCH BEANS.ONESMUS KYALO MWANIKI 156/73912/2014.

Osmar D. Prestes, Caroline A. Friggi, Martha B. Adaime e Renato Zanella* (2009). QuEChERS – UM MÉTODO MODERNO DE PREPARO DE AMOSTRA PARA DETERMINAÇÃO MULTIRRESÍDUO DE PESTICIDAS EM ALIMENTOS POR MÉTODOS CROMATOGRÁFICOS ACOPLADOS À ESPECTROMETRIA DE MASSAS. *Quim. Nova*, Vol. 32, No. 6, 1620-1634.

Paul J. Taylor* (2005). Matrix effects: The Achilles heel of quantitative high-performance liquid chromatography–electrospray–tandem mass spectrometry. Clinical Biochemistry 38 (2005) 328– 334.

PHILIPPE TIXIER,*,† CHRISTIAN CHABRIER,† AND ERIC MALEÄ ZIEUX‡ (2007). Pesticide Residues in Heterogeneous Plant Populations, a Model-Based Approach Applied to Nematicides in Banana (Musa spp.). J. Agric. Food Chem. 2007, 55, 2504-2508.

Standort Chemnitz, Zschopauer Street 87, D-09111 Chemnitz, Germany.

Vijay Tularam Gajbhiye, Suman Gupta, Irani Mukherjee, Shashi Bala Singh, Neera Singh, Prem Dureja et al (2011). Persistence of Azoxystrobin in/on Grapes and Soil in Different Grapes Growing Areas of India. Bull Environ Contam Toxicol (2011) 86:90-94. DOI 10.1007/s00128-010-0170-2

XueHua An, WenHao An, IlBin Im, Sang Bok Lee, JongGook Kang (2006). Persistence of Fungicide Pencycuron in Soils. The Korean journal of Pesticide Science 10(4), 2006.12, 296-305.

Zhiguang Hou, Xiumei Wang, Xiaofeng Zhao, Xinhong Wang, Xing Yuan, Zhongbin Lu (2016). Dissipation rates and residues of fungicide azoxystrobin in ginseng and soil at two different cultivated regions in China. Environ Monit Assess (2016) 188:440 DOI 10.1007/s10661-016-5449-2

김인경 (2017). 소면적 작물 중 채소류의 농약잔류허용기준 설정 연구. 학위논문, 전남대학교

김미옥, 황혜신, 임무송, 홍지은, 김순선, 도정아 등 (2010). LC/MS/MS 를 이용한 국내 유통 농산물의 잔류농약 실태조사. KOREAN J. FOOD SCI. TECHNOL. Vol. 42, No. 6, pp. 664~675.

이미경 (2013). 농약의 마이너 사용에 대한 국내외 관리 및 규제 현황. 농약과학회지 Vol. 17, No. 3, pp. 231-236.

안창현, 김용훈, 엄훈식, 이광하, 류갑희 (2014). 소면적 재배작물의 약효 및 안전성 그룹화 적용 연구. 농약과학회지 18(4), 2014.12, 364-375.

임영주, 박은성, 박연기, 명인식, 이상계, 심홍식, 이인용 등 (2014). 소면적 재배작물의 농약등록관리. 한국농약과학회 학술발표대회 논문집, 2014. 10, 56-56.

정영호, 김장억, 김정한, 이영득, 임치환, 허장현 (2004). 최신 농약학. 2004 년 3 월 25 일 개정판 1 쇄 발행. 제 10-2642 호. (The latest pesticide science)

张卫锋 (2016). 食品中农药最大残留限量标准制修订研究. 1994-2018 China Academic Journal Electronic Publishing House.

4.1.2.2 mcpmm. (2013) 식품공전 잔류농약 분석법 실무 해설서. 식품의약품안전처 식품의약품안전평가원 잔류물질과.

*The Pesticide Manual Seventeenth Edition (J A Turner)

Crop Protection Guidelines of Korea Crop Protection Association

농자재평가과 국. (2017) 환경잔류성 시험자료 작성요령.

www.plantwise.org/KnowledgeBank (Platwise)

https://en.wikiedia.org/wiki/Azoxystrobin

http://biz.khan.co.kr/khan_art_view.html?artid=201708242204005&code=920100

http://npic.orst.edu/factsheets/half-life.html (NPIC)

Abstract in Korean

서울대학교 대학원
농생명공학부 응용생명화학전공
원수

현재 한국에서는 소면적 재배작물에 대한 농약등록이 그 수요를 만족하지 못하고 있고 소면적 재배작물에 등록된 농약이 턱없이 부족한 상태이다. 따라서 농민들은 소면적 재배작물의 재배에 많은 어려움을 겪고 있다. 또한 2017 년 닭을 땅에 풀어놓고 자래우는 양계장에서 계란과 닭에 DDT 가 검출되면서 토양에 대한 잔류농약 검사가 시급한 상황이다. 본 연구는 소면적 재배작물인 조의 알곡, 짚에 Azoxystrobin 10% 수화제를 적용하고 토양에서도 실외와 실내 실험을 병행하여 진행 하였으며 살균제 Azoxystrobin 및 그의 작물 대사체 R230310 과 토양 대사체 R234886 의 잔류 특성을 파악하고자 하였다. 농약의 살포는 수확을 기준으로 서로 다른 시기에 살포하여 4 개의 서로 다른 처리구로 나누어 실시되었다. 처리구 1 은 수확 40/30 일전, 처리구 2 는 30/21 일전, 처리구 3 은 21/14 일전, 처리구 4 는 14/7 일전으로 구획하여 각 처리구당 2 회 살포하였다. 실외 토양은 1 회처리로 실시 하였으며 실내토양은 표준살포농도의 working solution 을 인위적으로 토양에 첨가하여 실시하였다. 모든 분석은 LC-MS/MS (Shimadzu LC-MS 8040)으로 분석하였다. 조에서 0.0025-0.1 mg/mL 범위와 토양에서 0.0005-0.2 mg/mL 범위에서의 Matrix matched calibration curve 의 직선성은 상관계수 0.99 이상으로 좋은 직선성을 나타내었다. 작물에서의 전체잔류량을 확인한 결과, 알곡의 경우, 처리구 4 (14/7 일전)의 0.61 mg/kg 에서 처리구 1 (40/30 일전)의 0.07 mg/kg 으로 감소되었다. 짚의 경우, 처리구 4 (14/7 일전)의 5.02 mg/kg 에서 처리구 1 (40/30 일전)의 0.14 mg/kg 으로 감소되었다. 본 소면적 실험 자료는 PHIs (Pre-harvest Intervals)와 MRLs (Maximum Residue Limits)을 설정하는데 기여할수 있을것으로 판단된다. 실외 토양실험의 경우 포장구획을 나누고 Azoxystrobin 10% WP 를 200

L/10 a 농도로 살포하여 0 일차, 1 일차, 3 일차, 7 일차, 21 일차, 28 일차 회수하여 -18℃에 보관되었다. 실내토양은 포장용수량의 75%함유 수분인 토양 10 g 에 Azoxystrobin 표준용액을 0.2 mg/kg 의 농도로 인위적으로 첨가한후 25±2℃에서 incubation 하였다. 0 일차, 7 일차, 15 일차, 30 일차, 45 일차, 60 일차, 90 일차를 회수하여 -18℃에 보관하였다가 분석하였는데 실내토양 모든 시료는 15 일 이내로 분석하였다. 실외토양에서 Azoxystrobin 및 대사체 R234886 의 잔류 양상은 다음과 같다. 실외토양 dissipation equation 은 $y=156.62^{e-0.056x}$ 이었고, 상관계수는 r^2=0.9449 이었으며 이에 따른 토양 속 Azoxystrobin 의 반감기는 12.38 일 이었다. 실내토양은 천천히 분해 되었는데 반감기가 90 일 이상으로 나타났다. 실내에서의 반감기는 실외에서 보다 더 길었고 해빛, 바람, 강우 등 요인 때문에 반감기가 큰 차이를 보이는것으로 판단된다. 실외,실내 토양실험 모두 R234886 은 MLOQ 미만의 잔류량을 보였다.

주요어: Azoxystrobin, R230310, 소면적, 재배작물, 조, LC-MS/MS, 잔류, R234886, Dissipation, 토양, 반감기

학 번: 2016-23084

Acknowledgements

우선 먼저 저를 석사과정 동안 저를 지도해 주시고 행복한 실험실 생활을 할수있게 도와주신 김정한 교수님께 감사의 인사를 드립니다. 한국에서의 생활은 모든것이 낯설었지만 교수님과 실험실 맴버들의 도움으로 원만하게 생활할수 있었습니다. 또한, 농생명공학부 응용생명화학 전공 교수님이신 김수언 교수님, 최양도 교수님, 오기봉 교수님, 노희명 교수님, 김민균 교수님, 배의영 교수님, 이상기 교수님, 신찬석 교수님에게 감사의 인사를 드립니다.

실험실 생활을 하는 동안 많은 사람들의 도움을 받았습니다. 많은 도움을 주신 한예훈 박사님, 항상 묵묵히 챙겨주시는 종화형, 함께 하는 시간이 많지는 않았지만 자꾸 생각나는 은혜누나, 저를 제일 많이 이끌어 주신 정학형, 농약실 에이스 용호형, 분위기 메이커 지호형, 항상 좋은말 많이 해주시는 병준형, 저랑 같은 외국인 마이누나, 마음씨 따뜻한 정희누나, 활발한 수진선배, 좋은말 많이 해주시는 다래누나 와 동기 티격태격 혜진이, 가끔씩 챙겨주는게 너무 고마웠던 서현이, 말이야 방구야 은영이, 농약실 제갈량 락도 다들 너무 고마웠습니다. 그리고 제 토양 실험을 도와주신 토양실 지숙누나, 너무 가깝게 지내는 천연물실 철호형, 아름누나 다들 감사합니다. 그리고 도움을 줬던 제민형, 김진누나, 휘귀형, 범구형에게 감사의 인사를 드림니다.

모든 사람들이 행복한 삶을 살고 하는 일이 잘되고 건강하기를