KSPS
(사)한국농약과학회 창립20주년
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2017 International Symposium and Annual Meeting of The KSPS

Safety Management of Pesticides for Next Generation

April 06 (Thu) ~ April 07 (Fri), 2017

MVL Hotel, Yeosu, Republic of Korea

Supported by

식품의약품안전처
MINISTRY OF FOOD AND DRUG SAFETY

식품의약품안전평가원
National Institute of Food and Drug Safety Evalution

여수시
YEOSU CITY

사단법인 한국농약과학회
The Korean Society of Pesticide Science

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2017 International Symposium and Annual Meeting of The KSPS

KSPS
(사)한국농약과학회 창립20주년

2017. April 6(Thu)

12:30~13:30	Registration		Reception Desk
13:30~13:50	General Meeting		Grand ballroom(F1)
13:50~14:00	Coffee break		
International Symposium and Annual Meeting of The KSPS / Safety Management of Pesticides for Next Generation			
14:00~14:35	Technical Regulation of Pesticides in Agricultural and Food Products in the Eurasian Economic Union	Dr. Jannat Iskakova (Kazakh National Agrarian University, Kazakhstan)	Grand ballroom(F1)
14:35~15:10	"QuEChERS" Approach for Traditional/Conventional Detectors in Pesticide Residue Analysis	Dr. Abd El-Alty A.M. (Cairo University, Egypt)	〃
15:10~15:45	Pesticide Registration System in Japan -The Latest Movement -	Dr. Kazuhiko Motoba (Nihon Nohyaku, Japan)	〃
15:45~16:00	Coffee break		
16:00~16:35	Integrated Agriculture Solutions - Biotechnology and Crop Protection	Dr. Kimberly Hodge-Bell (Monsanto, USA)	〃
16:35~17:10	3R Best Practices in Pesticide Regulation	Dr. Troy Seidle (Humane Society International, Canada)	〃
17:10~17:45	Eicosanoid Biosynthesis Inhibitors as Novel Synergists for Microbial Insecticides	Dr. Yonggyun Kim (Andong National University, Korea)	〃
17:45~18:00	A message from KJPS Editor		〃
18:00~18:50	Poster Presentation & Audit		F1, F2 Lobby
19:00~21:00	Welcome Reception		Grand ballroom(F1)

2017. April 7(Fri)

Oral Presentation				
09:00~12:00	Chemistry Ⅰ	Chemistry Ⅱ	Toxicity	Biological Efficacy
	Grand ballroom Ⅰ (F1)	Grand ballroom Ⅱ (F1)	Notos (F2)	Zepiros (F2)
09:00~11:00	Poster Presentation & Audit (F1, F2 Lobby)			
12:00~12:20	Closing Ceremony			

공지사항

1. 등록안내

- 등록 장소 및 시간 : 그랜드볼룸 로비(F1), 12:30부터 접수
- 회비납부 요령
  - **참 가 비** : (발표초록 유인물비 + 간친회비 포함)
    정회원 : 100,000원 비회원 : 100,000원 준회원(학 생) : 80,000원
    (학부생) : 무료
  - **학 회 비 : 정회원 50,000원/년, 준회원(학생회원) 30,000원/년**
  - **이 사 회 비 : 100,000원/년**
  - **단 체 회 비 : 500,000원/년, 미납회비 동시 납부요망**

2. 간친회 안내

- 참석자 : 참가회비를 납부한 회원 (입장 시 학회명찰 패용 요망)

※ 학회명찰이 없을 경우 간친회 출입을 엄격히 제한하오니 꼭 지참하여 주시기 바랍니다.
· 장소 및 시간 : 그랜드볼룸, 4월 6일 19:00~21:00

3. 발표자 유의 사항

- 발표준비 : Window XP(Power Point 프로그램, ppt) 용 CD-Rom diskette 또는 이동용 USB device (Beam projector는 회의장에 설치되어 있음).
- 발표자는 발표자료를 4월 7일 각 발표장의 진행요원에게 발표자의 이름이 기재된 자료를 제출하여 주시기 바랍니다.
- 발표자는 발표시간을 준수하여 주시기 바랍니다.
- 발표시간 : 특강 및 주제발표 35분, 일반발표 15분

4. 학회 홈페이지 활용(www.kjps.or.kr)

- 회원께서는 모두 학회 홈페이지에 회원등록해 주시고 논문 검색 및 게시판을 적극적으로 활용하여 주시기 바랍니다.

5. 홍보전시 참여업체 : 13개사
- 태산사이언스, 보성과학(주), (주)성문시스텍, 써모 사이언티픽코리아(주), 에이비사이엑스코리아(유), 한국애질런트테크놀로지스, 영인프런티어(주), (주)한국분석기술연구소, (주)엘림글로벌, (주)유로사이언스, (주)애니랩, ㈜랩테크, (유)워터스코리아

※ 참여업체에 감사드리며, 회원님의 많은 방문을 부탁드립니다.

6. 광고 협찬사에 감사드립니다.

- 귀사의 광고를 한국농약과학회 홈페이지(www.kjps.or.kr)에 연결할 수 있음은 물론 『추계 초록 논문집』에 게재하고자 합니다. 이를 통하여 귀사 또는 귀사의 제품을 회원들에게 널리 알리고, 소비자들이 올바른 선택을 할 수 있도록 광고하고자 합니다.
- 한국농약산업과 학회발전에 긴요하게 사용되어지는 광고료 수입을 위하여 귀사에 광고게재 협조를 요청하오니 아래 기준을 참고하시어 광고위치를 선정하여 주시기 바랍니다. 귀사의 애정과 배려에 감사드립니다.

참고 : 초록논문집 광고료 기준

광고 위치	인쇄내용	광고료
광고용 지면 추가	전면칼라	1,500천원
앞표지, 뒤표지 내면광고	전면칼라	2,000천원
뒤표지 외면광고	전면칼라	2,500천원

▌광고 참여업체 : 15개사 ▐

농협중앙회, (주)팜한농, 한국삼공(주), (주)경농, 바이엘크롭사이언스(주), (주)농협케미컬, (주)동방아그로, (주)성보화학, ISK바이오사이언스코리아(주), (주)한얼싸이언스, (주)분석기술과미래, (주)AB솔루션 , (주)LG화학, 한국바스프(주), (유)워터스코리아

7. 문의 사항

- 학술대회관련 문의 : 김순영 사무국장
  ☏ 031-296-4088, E-mail: kjps97@hanmail.net
- 학회관련 일반사항 문의 : 유오종 총괄운영위원장
  ☏ 063-238-0824, E-mail: zzan@korea.kr

교통편 안내

1. 교통안내

대명리조트(www.daemyungresort.com)주소를 클릭하시면 자세히 오시는 길을 확인하실 수 있습니다.

[오시는 방법]

- **승용차**
  - 서울↔여수 : 서울 → 천안JC → 논산JC → 익산JC → 완주JC → 동순천IC → 여수 엠블호텔(약 4시간)
  - 부산↔여수 : 부산 → 냉정JC → 진주JC → 옥곡IC → 여수 엠블호텔(약 2시간 10분)
  - 대구↔여수 : 대구 → 서대구IC → 옥포JC → 현풍IC → 칠원JC → 광양 IC → 여수 엠블호텔(약 3시간 30분)
  - 대전↔여수 : 대전 → 익산JC → 완주JC → 동순천IC → 여수 엠블호텔(약 2시간 40분)
  - 광주↔여수 : 서광주 → 순천IC → 여수 엠블호텔(약 1시간 20분)

- **열차**
  - 용산역↔여수 엑수포역 : 2시간 50분(KTX)
  - 대전역↔여수 엑수포역 : 2시간 40분(ITX)
  - 전주역↔여수 엑수포역 : 1시간 30분(KTX)
  - 엑수포역↔엠블호텔 : "오동도 방면"정류장에서 2번 버스(약 10분)
  - 엑수포역↔엠블호텔 : 택시 약 3,000원(약 5분)

- **고속버스**
  - 서울↔여수 시외버스터미널 : 약 4시간 10분
  - 광주↔여수 시외버스터미널 : 약 1시간 50분
  - 부산↔여수 시외버스터미널 : 약 2시간 50분
  - 전주↔여수 시외버스터미널 : 약 3시간 30분
  - 시외버스터미널↔엠블호텔 : 시내버스 333번 → 엠블호텔(약 15분)
  - 시외버스터미널↔엠블호텔 : 택시 약5,000원(약 10분)

▸ 여수 MVL호텔 위치

정기총회 순서

개회

학회장 인사

회무보고

- 2017년 제20대 임원진
- 2016년 결산 및 2017년 예산(안)
- 기타 사항

폐회

연구상 시상

○ **우수 논문상**

1. 잔류성 수상자 : 권혜영 회원 (국립농업과학원)
   논문제목 : 액체크로마토그래피를 이용한 농산물중 pyribencarb와 대사물 KIE-9749의 잔류 분석법

2. 생물활성 수상자 : 이동운 회원 (경북대학교)
   논문제목 : 한국산 곤충병원성선충을 이용한 솔수염하늘소(*Monochamus alternatus*)의 생물적 방제

공로패 증정

○ **제19대 (사)한국농약과학회 김용환 회장**

2016년 추계 학술발표회 연구상 수상자

○ **우수 논문 발표상**

1. 잔류성 수상자 : 임지수 회원 ((주)팜한농)
   논문제목 : Acetamiprid 훈연제의 비닐하우스 내에서 잔류특성 및 생물활성 평가

2. 독성 수상자 : 권보원 회원 ((주)경농)
   논문제목 : 식물 2차대사산물 Synomone이 서양종 꿀벌(Apis mellifera)에 미치는 영향

3. 생물활성 수상자 : 김정은 회원 (경북대학교)
   논문제목 : 우리나라 주요 곶감 생산지의 떫은 감 병해충 발생 실태

○ **우수 포스터 발표상**

1. 수상자 : 정혜현 회원 (경북대학교)
   논문제목 : Dissipation Characteristics of Triazole Fungicides in Spinach during Cultivation and Cooking Processes

2. 수상자 : 황규원 회원 (한경대학교)
   논문제목 : Dissipation of Chlorfenapyr Residue in Cucumber and Strawberry Grown in Mongolia

3. 수상자 : 오진아 회원 (국립농업과학원)
   논문제목 : Indaziflam 및 함유품목의 환경생물에 대한 위해성평가

4. 수상자 : 이영기 회원 (국립농업과학원)
   논문제목 : 2016년 배·사과 화상병 진단 및 발생상황

5. 수상자 : 류정필 회원 (성보화학(주))
   논문제목 : *Bacillus amyloliquefaciens* 5B6균주의 고추 세균점무늬병 및 병원성 진균류에 대한 효과

Plenary Session

• April 6(Thu)

Subject : Safety Management of Pesticides for Next Generation

▌Chairman : Dr. **Su Myeong Hong**(NIAS)

- **[S-01]** 14:00 ~ 14:35
  Technical Regulation of Pesticides in Agricultural and Food Products in the Eurasian Economic Union
  Dr. Jannat Iskakova(Kazakh National Agrarian University, Kazakhstan)

- **[S-02]** 14:35 ~ 15:10
  "QuEChERS" Approach for Traditional/Conventional Detectors in Pesticide Residue Analysis
  Dr. Abd El-Alty A.M.(Cairo University, Egypt)

- **[S-03]** 15:10 ~ 15:45
  Pesticide Registration System in Japan -The Latest Movement -
  Dr. Kazuhiko Motoba(Nihon Nohyaku, Japan)

- **15:45 ~ 16:00 Coffee break**

▌Chairman : Dr. **Sung-Eun Christopher Lee**(Kyungpook National University)

- **[S-04]** 16:00 ~ 16:35
  Integrated Agriculture Solutions - Biotechnology and Crop Protection
  Dr. Kimberly Hodge-Bell(Monsanto, USA)

- **[S-05]** 16:35 ~ 17:10
  3R Best Practices in Pesticide Regulation
  Dr. Troy Seidle(Humane Society International, Canada)

- **[S-06]** 17:10 ~ 17:45
  Eicosanoid Biosynthesis Inhibitors as Novel Synergists for Microbial Insecticides
  Dr. Yonggyun Kim(Andong National University, Korea)

[SPECIAL LECTURE 1]

Dr. **Jannat Iskakova**
Kazakh National Agrarian University,
Kazakhstan

Technical Regulation of Pesticides in Agricultural and Food Products in the Eurasian Economic Union

S-01

Jannat Iskakova, Ph.D.

- Associate Professor of Food Engineering Department
- Kazakh National Agrarian University, Kazakh
- E-mail: lady.iskakova2015@yandex.kz

➢ Education

2002, Kyzylorda State University by Korkyt Ata,

2008, Graduate school in Kazakh National Agrarian University, Almaty city

2010, Protection for the degree of candidate of agricultural sciences (PhD)

➢ Experience

2008-2012 Assistance Professor in Kazakh National Agrarian University, Department of Standatdization, certification and metrology

2012-2015 Senior Lecturer in Kazakh National Agrarian University, Department of Food technology

2015- Associate Professor of Food Engineering Department in Kazakh National Agrarian University

➢ Publications

The Issue of Meat Safety in Kazakhstan - *Biosciences Biotechnology Research Asia*, Published by: Oriental Scientific Publishing Company Volume No. 12 Issue No.: SESEP Page No. 175-180.

Questions of food quality and safety regulation within European and the Eurasian Economic Union: SCIENCE AND the WORLD - *the International scientific magazine*, No. 3 (31), 2016, Volume 1

Ensuring quality and safety of meat products at the meat-processing enterprise according to the principles of HACCP//Materials I the International conference on veterinary and sanitary examination "Veterinary and sanitary aspects of quality and safety of agricultural production". Russia, *Voronezh*, on November 26-27, 2015 the 305th page 3 -

Technical Regulation of Pesticides in Agricultural and Food Products in the Eurasian Economic Union

J. Iskakova[1], N. Demidova[2]

[1]Kazakh National Agrarian University, Abay,8. Almaty. Kazakhstan
[2]Kazakhstan Institute of Standardization and Certification, Almaty, Kazakhstan

Currently wide range of food raw and food products (fruits and vegetables) are imported to Kazakhstan from the foreign countries (Kyrgyzstan, Uzbekistan, China). Many requirements are not harmonized with international ones. Therefore, it is necessary to strengthen the effective state control over the content of pesticide residues in food raw and food products imported into the territory of the Republic of Kazakhstan. Since 2000, Kazakhstan pays great attention to the protection and plant quarantine. The list of especially dangerous harmful organisms is approved by the decision of the Government. To deal with them from the state budget annually grants funds.

In the Republic of Kazakhstan is currently about 810 registered pesticides (chemicals) for different applications and uses. Every year the list of registered pesticides (toxic chemicals) updated with 15-20 new drugs that require adaptation bazaz laboratory toxicology of pesticides.

Questions of application of pesticides and monitoring of residual content in the environment, as well as carrying out monitoring of polychlorinated biphenyls and dioxins is relevant not only for Kazakhstan, but also for most countries

Since the raw material base for production in Kazakhstan there is no pesticide, pesticide formulation to 100% at the expense of imported raw materials from Russia, India, China and Sweden. To do this need a technical regulation which develops the general requirements for all pesticides, which are brought to our country.

TECHNICAL REGULATION OF PESTICIDES IN AGRICULTURAL AND FOOD PRODUCTS IN THE EURASIAN ECONOMIC UNION

Ishankova Janaar, PhD
Associate Professor

Department of Food Technology and Safety
Kazakh National Agrarian University

- The Eurasian Economic Union (EEU) is an international economic integration association, established on the basis of the Customs Union and the United Economic Space has been operating still January 1, 2015. The EEU was established in order to fully modernize, co-operate and improve the competitiveness of national economies and create conditions for sustainable development in order to improve the living standards of the population of the Member States.
- The member states of the Eurasian Economic Union are the Republic of Armenia, the Republic of Belarus, the Republic of Kazakhstan, the Russian Federation and the Republic of Kyrgyzstan (since May 6, 2015).
- The treatment of pesticides in the countries of the EAEU members is regulated at three levels:
- • Universal International agreements in field of transport and classification of hazardous substances (UN, FAO, CIS)
- • Regional International Agreements (EurAsEC)
- • National (still practically the full range of pesticides treatment is regulated)

Table 1 - International conventions and multilateral agreements ratified by Kazakhstan

Convention name	Date of ratification
European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR)	26.07.2001
Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal	04.06.2003
Development of the National Chemicals Management Plan, implementation of the SCTM	First Edition: June 2006 Second Edition: November 2009
Rotterdam Convention on the Application of the Prior Informed Consent (PIC) Procedure for Certain Hazardous Chemicals and Pesticides in International Trade	01.11.2007
Stockholm Convention on Persistent Organic Pollutants (POPs)	01.11.2007

Table 2 - Pesticides and sanitary regulations of chemicals among the country members of EAEU

Kazakhstan	Russia	Belarus	Kyrgyzstan	Armenia
1. Law of Republic of Kazakhstan "On Plant Protection", 04 of Tuesday 2011 10 Ch. 2. National Hygienic Regulations for the pesticides and toxic chemical substances" approved by Decreased Decree of MH of Rep. Kaz. 1999	1. Federal Law "Plant Protection" 04 of July 2000 N 110-FZ 2. Federal Law of Russian Federation "On the Safe Management of Pesticides and Agrochemicals" 19.07.1997, N 109-FZ	1. Republic of Belarus "Plant Protection" 07.05.2005 N 29-Z	1. Law of Kyrgyzstan Republic "About Pesticides and Agrochemicals" 27 of January 1998 N 12	1. Law of Armenia Republic "About Pesticides" 11.10.2011
List of pesticides (toxic chemicals) admitted for use in the territory of Kazakhstan on 01.12.2012 y.	Sanitary Rules and Standards and agrochemicals permitted for use in the territory of the Russian Federation	The Register of Plant Protection Products and Agrochemicals permitted for use in the territory of the Republic of Belarus, from 2012	Sanitary rules and agrochemicals permitted for use in the territory of Kyrgyzstan 27 of January 1998 N 12	The Sanitary of pesticides (toxic) agents for Trading or pesticides permitted to use in the territory of Armenia
Sanitary rules and standards	SR 1.1.1058-01 "Hygienic requirements for production of pesticides"	"Hygienic requirements for production of pesticides"	Instructions on the use, storage and transportation of pesticides in permitted pesticides, dated on 1st of 19 April 2012 N 202	
Technical Regulations of the Customs Union "On the safety of pesticides" (Date 30.10.2013 TR Customs Union) Customs Regulations "On Customs Code of the Union" (Date 30.10.2013) Unified sanitary-epidemiological and hygienic requirements for goods (goods) subject to sanitary and epidemiological supervision (control) (Decision of Customs Union Commission dated 28 May 2010 N 299)				

Pesticide situation in Kazakhstan

- • All pesticides (pesticides) listed in the List have state registration in accordance with the Law of the Republic of Kazakhstan "On Plant Protection"
- • Pesticides (toxic chemicals) not listed in the List are prohibited to import, sell, produce, use and advertise on the territory of the Republic of Kazakhstan
- • Pesticides (toxic chemicals) in the List are located in groups according to their purpose (insecticides and acaricides, fungicides, preparations for presowing seed treatment, herbicides, biological preparations, defoliants and desiccants, plant growth regulators, etc.), and within groups - in Alphabetical order by trade names.
- • The list of pesticides is coordinated with the Committee for Environmental Regulation of the Ministry of Environmental Protection of the Republic of Kazakhstan and the Committee of State Sanitary and Epidemiological Supervision of the Ministry of Health of the Republic of Kazakhstan.
- • The list is located: http://www.kz/pesticidy/list.c-ochrana-rastenij-minsx-sh-kz/ru/spisok

- Kazakhstan producers of pesticides are engaged in the formulation (production) and packaging of purchased active ingredients. Raw material base for producing (formulation) pesticides in Kazakhstan is absent, formulation of pesticides to 100% realized due to imported raw materials, which comes mainly from Russia, India, China, Sweden
- The main raw materials for producing of various types of pesticides are:
  - 2,4 D dimethylamine salt, glyphosate acid, fluroxypyr-p-ethyl, bromoxynil, metsulfuron-methyl, etc. - for herbicides;
- - imidacloprid, diflubenzuron, abamectin, alpha-cypermethrin, lambda-cyhalothrin, etc. - for
- insecticides;
  - Carbonin, thiuramid, tebuconazole, cyproconazole, etc. - for fungicides

- In the Russian Federation, using of pesticides is carried out also on the basis of the "State Catalog of Pesticides and Agrochemicals Permitted for Use on the Territory of the Russian Federation," which is issued regularly, since January 27, 2014, an updated version of this Catalog is in operation. The range of drugs used in the Russian Federation is also expanding: if in 2006 - 524 pesticide names were included in the list of permitted, then in 2016 – it increased to 3063 preparations.
- More than 370 names of pesticides and toxic chemicals are included in the "State Register of Plant Protection Products (Pesticides) and Fertilizers permitted for use on the territory of the Republic of Belarus".
- In the Kyrgyz Republic, 71 pesticides are included in the "List of chemicals and pesticides, the use of which is prohibited or severely restricted". 23 substances are listed in the National Catalog of Pesticides and Agrochemicals approved to use for 2011-2015, and controlled according to this List. Some of the highly dangerous preparations of pesticides (2,4-D, Butoxyethyl, Carbofuranum, Halvin(Ipo-Propyl), Monocrotoh, Propargit) are not currently replaceable, since there are no alternatives for biological efficacy against certain resistant pests, diseases and weeds on agricultural crops.

Sanitary measures of pesticides in Russia based on the laws:

- "Sanitary and Epidemiological Welfare of the Population"
- "Food Quality and Safety".
- Sanitarian rules and norms N 1.2.1077-01 "Hygienic requirements for storage, use and transportation of pesticides and agrochemicals"
- Sanitarian rules and norms N1.2.1330-03 "Hygienic requirements for the production of pesticides and pesticides"
- Sanitary and epidemiological welfare of the population

- The safety of pesticides produced and imported on the territory of the member states of the Eurasian Economic Union is regulated by the requirements of the Uniform Sanitary and Epidemiological and Hygienic Requirements and distributed regardless of the country of origin. These requirements are developed on the basis of country-members' legislation.
- Uniform requirements are binding for all citizens and legal entities involved in the sphere of pesticide handling.
- For violation of uniform requirements establish administrative, disciplinary and criminal liability in accordance with the legislation of country-members of the Union.
- Import and circulation of pesticides that are not registered in the State Catalog (Register) of pesticides is not allowed.

The criteria for assessing the safety of active substances of pesticides are:

- Toxicological characteristics of the active substance (acute, subacute, chronic toxicity, including the assessment of specific and long-term effects for human health (allergenicity, reproductive toxicity, teratogenicity, mutagenicity, carcinogenicity, embryo toxicity), indicating the current standards, CAC, IUPAC, registration in the REACH system;
- Equivalence of technical products (active substances) of the registered pesticide to the technical product form originating Site;
- The presence of dangerous (toxicologically significant) impurities and their metabolites - the effect on the human habitat (drinking water, air, soil), on the quality and safety of food products, using monitoring data (if available) for the content of active substances in environmental objects.

The evaluation criteria for industrial strains of microorganisms (bacteria, fungi) and ready-made forms of biological preparations are:

- The origin and conditions of cultivation of the strain, the method of its identification, dissemination of the strain;
- Pathogenicity (virulence, toxicity, toxigenicity) of bacteria, fungi in two types of laboratory animals;
- Irritating effect on the mucous membrane of the eyes; Sensitizing and immunotoxic action of microorganisms upon ingestion through the skin and upper respiratory tract;
- Limiting criteria of harmfulness in a chronic experiment;
- Influence on the processes of microbial self-purification in the aquatic environment (if necessary, rationing in water reservoirs)

The evaluation criteria for the formulations of pesticides are:

- Toxicological characteristics of the formulation components (excipients, emulsifiers, stabilizers, solvents, etc.) indicating the current standards, CAC numbers, IUPAC, registration in the REACH system, acute oral toxicity (mice, rats) - LD_{50};
- Acute dermal toxicity when applied to the skin - LD_{50}; Acute inhalation toxicity - CL_{50};
- Irritating effect on the skin and mucous membranes;
- Subacute oral toxicity (cumulative properties), coefficient of cumulating;
- Subacute cutaneous toxicity (for drugs with pronounced dermal toxicity);
- Subacute inhalation toxicity (for drugs that represent a pronounced inhalation hazard);
- Sensitizing effect;
- Chemical and physical properties of pesticides, including their volatility, stability, compatibility with other compounds, fire and explosion hazard;

These criteria are the basis for assessing the dangers of imported pesticides and in accordance with the legislation of the country members of the Eurasian Economic Union.

To evaluate the pesticide, the manufacturer or supplier must provide:

- Toxicological dossier on the pesticide (including, the characteristics of the active ingredient, the inert components and the formulation as a whole).
- Materials substantiating the hygienic standards of the active substance of the pesticide in food products, environmental objects (water, soil, atmosphere air and air in the work area.
- Justification of the allowable daily dose (ADD) of entering the active substances into the human body.
- An analytical sample of the preparation form of the pesticide in the manufacturer's packaging with the original packaging label.
- Safety data sheet (MSDS), manufacturer's specifications or declaration, describing first aid measures in cases of pesticide poisoning.
- Standard sample of the active substance of the pesticide.
- Certificate of analysis (from the manufacturer, from five batches of the drug).
- Information on the method (methods) of analytical control of a specific active substance in appropriate test materials (in food products, as well as, if necessary, in water from sources of domestic and drinking water, soil, air in the work area and the atmosphere).
- The results of segregation tests of pesticides on the territory of each country-members (based on the rate/date of segregation, the soil and climatic conditions of the region, the specific features of the development of plant diseases and pests of agricultural crops).
- Data on the study of pesticide residues in crop production and animal husbandry, economical value assessment and food organoleptic, as well as the effect of pesticides on the organoleptic properties of water and the overall sanitary conditions of water bodies.
- Results of evaluation of the risk risk of pesticide for workers and population.

Table 3 - Criteria for evaluating drugs by hazard class

Criteria for evaluation	Danger classes			
	1	2	3	4
	Extremely dangerous	Highly dangerous	Moderate dangerous	Low risk
Average lethal dose when injected into the stomach, mg / kg	Less than 50	51-200	201-1000	Over 1000
Average lethal dose when inflicted to the skin, mg / kg	Less than 100	101-400	401-2000	Over 2000
Average lethal concentration in the air, mg / m³	Less than 500	501-2000	2001-20000	More than 20000
Cumulating coefficient (1.11.1.2001, 2 months)	Less than 1	1-3	3.1-5	More than 5
Resistance (soil) TM	Time of decomposition into non-toxic components - more than 1 year	Time of decomposition into non-toxic components - 6-12 months	Time of decomposition into non-toxic components - 2-6 Months	Time of decomposition into non-toxic components within 2 months

In all types of food raw materials and food products, pesticides are controlled - global pollutants:

- Hexachlorocyclohexane (alpha, beta, gamma isomers)
- DDT and its metabolites,
- Heptachlorin.

In the grain and products of processing, mercury-organic pesticides, 2,4-D acid, its salts and esters are also monitored. 2,4-D acid, its salts and esters are also monitored in fish and processed products.

Table 4 - Maximum acceptable levels of pesticides contained in food raw materials of plant origin and products

Products	Name	Permissible levels, mg/kg
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

Table 5 - Maximum permissible levels of pesticides in grain supplied for food purposes (Technical regulation of EAEU 015/2011)

Subject of product	Name	Permissible levels, mg/kg, not more than
Grains (wheat, rye, triticale, oats, barley, millet, buckwheat, rice, corn, sorghum)	Hexachlorocyclohexane (alpha, beta, gamma isomers)	0.2 0.2
	DDT (sum) metabolites	0.01
	Heptachlorin	0.01
	Mercury-organic pesticides	Not allowed
	2,4-D acid, its salts, ethers	Not allowed
Legumes (peas, beans, chickpeas, lentils from cereal crops)	Hexachlorocyclohexane (alpha, beta, gamma isomers)	0.2
	DDT (sum) metabolites	0.01
	Mercury-organic pesticides	Not allowed
	2,4-D acid, its salts, ethers	Not allowed
Oilseeds (sunflower, soybean, cotton, flax, rapeseed, mustard, sesame, peanut)	Hexachlorocyclohexane (alpha, beta, gamma isomers)	0.2 0.2 0.2
	DDT (sum) metabolites	0.01 0.1 0.01

- The determination of pesticide residues, with the exception of global pollutants, is based on information on their use provided by the food producer (supplier) when they are imported into the territory of the member states of the Eurasian Economic Union.
- For food raw materials of animal origin, information is required on the use of pesticides for fighting ectoparasites or diseases, for treating premises, reservoirs, bee colonies with name indication. Import and turnover of food raw materials of vegetable and animal origin, which has no information on the use of pesticides, is not allowed.
- Thus, the EAEU applies maximum permissible levels (MPLs) for chlorothalonil, clofentezin, cyprodinil, kresoxim methyl, iprodione, propamocarb, pirimicarb, thiabendazole, carbendazim, famoxadone, copper compounds and lambda-cyhalothrin in accordance with international standards.

- As a basis for harmonizing sanitary legislation, the countries of the EAEU have chosen an international framework, as stated in the item 56 of the EAEU Agreement. The Codex Alimentarius standards as a result of the intellectual efforts of the world community in the struggle for food safety proved to be acceptable for these purposes

Table 6 – Comparison of the minimum permissible levels of pesticides in food established in the EEU with the norms of "Codex Alimentarius", mg/kg

№	Name of pesticide	Maximum permissible level	
		According to Codex Alimentarius	According to EEU regulations
1	Chlorinated	Norms established in 2008 Grapes - 2.0; Citrus fruits - 0.5	Grapes - 1.0; Citrus fruits - 0.05
2	Cyprodinil	Grapes - 5 (2005); Berries and other small fruits - 10 (2004); Fruit cocktail - 2 (2005)	Grapes - 2.0; Fruit (pomegranate - 1.0, Fruit (kiwi) - 2.0
3	Fenoxaprop	Potato - 0.05 (2007); Tomatoes - 2.0 (2005); Grapes - 2.0 (2007)	Potatoes - 0.05; Tomatoes - 0.2; Grapes - 0.25
4	Propamocarb	Potato - 0.3 (2007); Salad - 10.0 (2007); Radish - 1 (2007); Tomatoes - 2 (2007)	Potatoes - 0.3; Salad - 15.0; Radish - 1.0; Tomatoes - 10.0
5	Metabendazole	Potatoes - 0.5 (2005)	Potatoes - 1.0

Products	National norms of Kazakhstan (also for imported food)	National norms of Russia (also for imported food)	Technical regulations of Eurasian Economic Union	Codex Alimentarius
	Resolution of the Chief State Sanitary Doctor of the Republic of Kazakhstan No. 49	Resolution of the Chief State Sanitary Doctor of the Russian Federation No. 18	TR CU 021/2011 "About the safety of food products"	Codex
1	2	3	4	5
Chlorinated				
Chard	0.2	0.2	Not in TR of 021/2011	0.2
Cabbage	Brussels sprouts - 0.1; cabbage cabbage and other - 1.0	Brussels and Shoots sprouts - 0.1; Cabbage head and leafy other - 1.0	Not in TR of 021/2011	Brussels sprouts, Brussels Sprouts, Chinese and leaf Chinese - 0.2; 0.2
Tomatoes	0.05	0.05	Not in TR of 021/2011	0.05
Grain of cereals		–	[illegible]	–
Agrochlor				
Dimethoate (total)	0.5	0.5	[illegible]	0.5
Cabbage	0.5	0.5	Not in TR of 021/2011	0.5
Parsley	2.0	2.0	Not in TR of 021/2011	2.0

Table 4 – Comparison of standards for permissible levels, MRL, mg/kg, not more. Low-toxic substances

Products	Maximum permissible levels (MRL) in Kazakhstan (also for imported food)	Maximum permissible levels (MRL) in Russia (also for imported food)	Technical regulations of Eurasian Economic Union	Codex Alimentarius
	Resolution of the Chief State Sanitary Doctor of the Republic of Kazakhstan No. 49	Resolution of the Chief State Sanitary Doctor of the Russian Federation No. 18	TR CU 021/2011 "About the safety of food products"	Codex
1	2	3	4	5
Cereals				
Grain crops	Insecticides, fumigants, high-toxicity pesticides, pesticides prohibited in the CIS	Insecticides, fumigants, high-toxicity pesticides, pesticides prohibited in the CIS	[illegible]	[illegible]
Barley	0.05	0.1	[illegible]	0.1
Oats	0.05	0.05	[illegible]	0.05
Rice husks and husks	0.1	0.1	[illegible]	0.05
Legumes				
Legumes, peas, soybeans, beans, lentils, peas, and peas in the CIS	0.1	0.1	[illegible]	0.1
Soy	0.05	0.05	[illegible]	0.05
Soybean oil cake	0.1	0.1	[illegible]	0.05
Oilseeds				
Oilseeds, seeds and kernels of oilseeds, except for castor beans, castor seeds and castor oil seeds	0.05 0.05	0.05 0.05	[illegible]	0.1
Sunflower seeds, including seeds	0.1	0.1	[illegible]	0.1

- Special conditions are established for baby food. They, in particular, for producing meat products using food raw materials obtained using global pollutants such kind of hexachlorocyclohexane and DDT are prohibited. Their levels are controlled depending on the age of the children.
- According to the technical regulation of the Customs Union "On Food Safety" (TR CU 021/2011) there is a list of pesticides banned for use in producing of raw food intended for producing baby food:
  1. Dimethoate (in sum - dimethoate, dimethoxon sulfoxide and sulfon of dimethoate, expressed by dimethoate)
  2. Fenoxaprop (in sum - fenoxaprop, its oxygen analogue and their salt forms, expressed by fenoxaprop)
  3. Formic, expressed by the triphenyltin cation
  4. Haloxyfop (in total - haloxyfop, its salts and esters, including conjugates expressed by haloxyfop)
  5. Heptachlor and trans-heptachlor epoxide, expressed by heptachlor
  6. Hexachlorobenzene
  7. Nitrofen
  8. Omethoate
  9. Terbufos (in sum - terbufos, its sulfoxide and sulfone, expressed by terbufos)
  10. Aldrin and dieldrin expressed by dieldrin
  11. Endrin

Official report of Department of consumer rights protection

Currently, pesticide stocks in Kazakhstan pose a serious and widely recognized threat to human health and to the environment.

The number of used pesticides increases every year.

According to the official report of "Department of Consumer Rights Protection" the list of applied pesticides and mineral fertilizers in the Republic amounted to 613 titles.

Determination of residual amounts of pesticides (toxic chemicals) is carried out by laboratories of branches of the National Expertise Center for 140 indicators, which is 22.8% of the total amount of pesticides. However, the number of determined pesticides remains in low level, which is explained by the lack of methodological instructions and standards. For example, 145 names of various pesticides were used on the territory of the North Kazakhstan region, of which only 28 pesticides are identified by the laboratory, and there are no research methods for the other used pesticides.

- Totally 29166 pesticide samples were tested in the Republic for the past period of 2016, of which 17 samples did not meet regulatory requirements, otherwise the proportion of samples not conforming to regulatory documentation was 0.05%.
- To determine the residual amounts of pesticides in food products were examined 10323 samples, samples with exceeding the permissible levels were not registered. Positive findings of organochlorine pesticides were noted in the Zhambyl oblast (2 samples), in the North Kazakhstan region (3 samples), South Kazakhstan oblast (28 samples), Kyzylorda oblast (8 samples), West Kazakhstan region (26 samples).

- In 2016, 9657 samples of vegetables and fruits were analyzed for the content of residual amounts of pesticides, with samples exceeding the MRL of 0.04% (4 samples). Inadequate samples of fresh-frozen vegetables from China, were found in the Shemonaikha district of the East Kazakhstan region. In the salad "Tsarskii", fresh frozen cabbage color, cabbage broccoli, carrots was found heptachlor with a content of 0.0169-0.0775 mg / kg. Positive findings were noted in the South Kazakhstan region (206 samples), Kyzylorda oblast (5 samples), East Kazakhstan region (5 samples). Samples exceeding the permissible levels were found in melons (melons, watermelons), early cucumbers, cabbage, onions, potatoes, produced in Uzbekistan, Russia, China.

Principles of preventing contamination of food and agricultural products by pesticide residues:

- Hygienic regulation of the use of pesticides - the creation of standards, norms and rules that prevent the development of poisoning in humans and damage to the environment;
- Hygienic selection of drugs for use - replacement of dangerous less dangerous: less toxic, with less cumulative properties, less resistant, etc .;
- Improvement methods of using pesticides (use of pesticides in the form of briquettes, water-glycol solutions, concentrated suspensions, water-dispersible granules, microgranules, film-forming flowable pastes), which reduces the risk for users.

Conclusion

- Requirements for pesticides imported / produced in the territory of the country-members of the Eurasian Economic Union are approved by Section 15 (Requirements for pesticides and agrochemicals) of the Uniform Sanitary and Epidemiological and Hygienic Requirements.

- The analysis of the Unified Requirements showed that, on the whole, they correspond to the structure of the International Code of Conduct on the Distribution and Use of Pesticides (Codex). Like the Code, Uniform requirements are binding for all citizens and legal entities involved in the circulation of pesticides, while these requirements are developed on the basis of the legislation of the country-members of the Eurasian Economic Union and existing international law instruments and are aimed at ensuring maximum safety of pesticides for human and environment.

Thank you for attention!

[SPECIAL LECTURE 2]

Dr. **Abd El-Alty A.M.**
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"QuEChERS" Approach for Traditional/ Conventional Detectors in Pesticide Residue Analysis

S-02

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➢ Publications

Various extraction methods for detection of bistrifluron residues in Asian pear using high-performance liquid chromatography: Application to dissipation patterns under open-field conditions. *Biomedical Chromatography* 2016 Oct; 30(10): 1535-1540

A simple extraction method for the detection and quantification of polyoxin D, a nucleoside antibiotic, in butterbur using UPLC-MS/MS. *Food Chemistry* 2017 Apr 15; 221: 683-688

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"QuEChERS" Approach for Traditional/Conventional Detectors in Pesticide Residue Analysis

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In pesticide residue analysis, relatively low-sensitivity traditional detectors, such as UV detector, diode array detector, electron-capture detector, flame photometric detector, and nitrogen-phosphorus detector, have been used following classical sample preparation (liquid-liquid extraction and open glass column cleanup); however, the extraction method is laborious, time-consuming, and requires large volumes of toxic organic solvents. A quick, easy, cheap, effective, rugged, and safe method was introduced in 2003 and coupled with selective and sensitive mass detectors to overcome the aforementioned drawbacks. Compared to traditional detectors, mass spectrometers are still far more expensive and not available in most modestly equipped laboratories, owing to maintenance and cost-related issues. Even available, traditional detectors are still being used for analysis of residues in agricultural commodities. It is widely known that the quick, easy, cheap, effective, rugged, and safe method is incompatible with conventional detectors owing to matrix complexity and low sensitivity. Therefore, modifications using column/cartridge-based solid-phase extraction instead of dispersive solid-phase extraction for cleanup have been applied in most cases to compensate and enable the adaptation of the extraction method to conventional detectors. In GC, the matrix enhancement effect of some analytes has been observed, which lowers the LODs and therefore enables GC

to be compatible with the quick, easy, cheap, effective, rugged, and safe extraction method. For LC with a UV detector, a combination of column/cartridge-based solid-phase extraction and dispersive solid-phase extraction was found to reduce the matrix interference and increase the sensitivity. A suitable double-layer column/cartridge-based solid-phase extraction might be the perfect solution, instead of a time-consuming combination of column/cartridge-based solid-phase extraction and dispersive solid-phase extraction. Therefore, replacing dispersive solid-phase extraction with column/cartridge-based solid-phase extraction in the cleanup step can make the quick, easy, cheap, effective, rugged, and safe extraction method compatible with traditional detectors for more sensitive, effective, and green analysis.

[SPECIAL LECTURE 3]

Dr. **Kazuhiko Motoba**
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Pesticide Registration System in Japan
- The latest movement -

S-03

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➢ Publications

Species-Specific Detoxification Metabolism of Fenpyroximate a Potent Acaricide,. *Pesticide Biochemical Physiology* (2000), 67, 73-84.

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Agrochemicals Registration System in Japan
- The latest movement -

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1. Introduction

Global population reaches seven billions and is still continuously growing,while all depend on limited and rather decreasing farm land on the earth. Increasing food demand to feed increasing population has been supported by many innovations such as nitrogen fixation by Haber-Bosch process, breeding, irrigation, mechanization and so on (so called "green revolution"). Agrochemicals are regarded as one of those innovations to secure efficient and stable agricultural production.Since agrochemicalspossess biological activity in any way, it is possible that they sometimes give negative impact on non-target organism, environment and human health. To prevent possible adverse effects and facilitate replacement of obsolete molecules by safermodern molecules, government authorities defined and made stricter the law and rule to regulate agrochemicals in any countries and regions. In this lecture, regulatory system and procedure, data requirement and so on in Japan will be presented and explained focusing on the latest change.

2. Registration system in Japan (Over view)

In Japan, procedure of any agrochemicals registration is governed by "Agricultural Chemicals Control Act (Act No. 28)" established in 1948. The preamble of the law states as follows; this law intends to contribute to stable agricultural production, health of nation, and environmental protection by providing appropriate quality agrochemicals, their safe and appropriate use through defining the registration system and regulating sales and use of agrochemicals. As described in this law and related notice such as

notice 12-nousan- 8147 (Data Requirements for Supporting Registration of Pesticides), registration system, procedure, data requirement and so on were clearly defined. A precise list of data requirement is presented in the following web site; http://www.acis.famic.go.jp/eng/shinsei/index.htm. All data were submitted to Food and Agricultural Material Inspection Center (FAMIC), an agency governed by JMAFF. Then the efficacy, adverse effect, human safety, environmental safety, and other aspects of the candidate chemical to characterize its value will be evaluated by four different ministries. Four different authority, i.e., JMAFF[1)], JMHWL[2)], FSC[3)], and JMOE[4)] are responsible for effectiveness(including adverse effect), setting of human safety(MRL), ADI and aRfD, and environmental safety(setting limit value in air soil and water to reserve registration), respectively. Finally, the candidate molecule which satisfies the standards of all aspects can be registered as agrochemicals.

3. Comparison with the system in EUand US

The data requirement concerning mammalian toxicology among Europe, United States and Japan are rather similar to oneanother. For example, data requirement list are summarized in the table shown below. Many of the data requirements are concerning technical grade active ingredient (TGAI) or pure active ingredient (PAI) but some study should be conducted also with final products.

Table 1. Data requirement for pesticide registration in Japan

Toxicology	Environmental Safety
1. Acute-oral 2. Acute dermal 3. Acute inhalation 4. Dermal irritation 5. Eye irritation 6. Skin sensitization 7. Acute neuro toxicity*	1. Plant metabolism 2. Fate in soil (aerobic, anaerobic, water-sediment) 3. Fate in Water (Aqueous photolysis, hydrolysis) 4. Effect on aquatic organisms (Fish, daphnids, algae) 5. Effect on beneficials and non-target(Bee,

1) Japanese Ministry of Agriculture, Forestry and Fishery
2) Japanese Ministry of Health, Welfare, and Labor
3) Food Safety Committee in Cabinet office
4) JapaneseMinistry of Environment

8. Delayedneuro toxicity* 9. 90 days repeat oral 10. 21days repeatdemal toxicity* 11. 90 days repeat inhalation toxicity* 12. Sub-chronic neuro toxicity* 13. Sub-chronic delayed neuro toxicity* 14. 1 year-Chronic toxicity (oral) 15. Carcinogenicity 16. Reproductive toxicity 17. Teratogenicity 18. Mutagenicity (Ames, Chromosomal aberration, micronucleus) 19. General pharmacology 20. Animal metabolism (single& repeat/oral)	silk worm, natural enemy, avian) 6. Physical state, stability, degradability, color, odor, spectrum, melting points, vapor pressure, water solubility, organic solvent solubility, soil adsorption/ desorption, partition coefficients, density 7. Water pollution Potential 8. Biological concentration factor*
Residue	Efficacy and phyto-toxicity
1. Crop Residue 2. Residue in livestock* 3. Soil residue (laboratory and field) 4. Rotational crop Residue 5. Residual odor (tea) 6. Tobacco taste	1. Efficacy 2. Phyto toxicity to target crops 3. Phyto toxicity to srrounding crops 4. Phyto toxicity to rotational crops

*: Conditional requirements.

As shown in the Table 1 and Table 2, data requirementconcerning environmental toxicity and risk assessments are significantly different among EU, US and Japan. In the table shown below, comparison of risk assessment required is summarized. In any regions, consumer risk assessment is required in view of both long-term and shor- term exposure. One biggest difference in human risk assessmentsconcerning operator (sprayer) exposure is that Japan requires only sprayer risk assessment. This seems reasonable, since many farmer in Japan own rather small plot and specialization (different person conducts spray and other operation) is not common. On the other hand, regarding environmental risk assessment field, these three regions have significantly different requirement and assessment methods. Especially for the aquatic risk assessment, EU and U.S. have developed unique and complicatedcomputer model to predict environmental concentration. To run such model, specifically trained experts are sometimes necessary.

Table 2. Risk assessment required in EU, US and Japan for registration of agrochemicals

Category	Target	Required?		
		EU	US	Japan
Human	Consumer	Yes (Long & short term)	Yes (Long & short term)	Yes (Long & short term)
	Sprayer	Yes	Yes	Under planning
	Operator	Yes	Yes	No
	Resident	Yes	Yes	No
	By-stander	Yes	Yes	No
Environ-ment	Aquaticsa	Yes (Long & short term)	Yes (Long & short term)	Yes (short term only)
	Aq. macrophyte	Yes (Long & short term)	Yes (Long & short term)	No
	Benthics	Yes (Long & short term)	Yes (Long & short term)	Yes (short term only)
	Terr. plants	Yes (seedling vigor)	Yes (seedling vigor)	No
	Terr. mammal	Yes (Long & short term)	Yes (Long & short term)	No
	Avian	Yes (Long & short term)	Yes (Long & short term)	Yes (short term only)
	NTAb	Yes with many species (defined by ESCORTc)	Yes	Honey bee silk worm, and beneficial only
	Soil microbe	Yes	Yes	No

a: A set of fish, crustacean (daphnia), and algae.
b: Non-Target Arthropods.
C: European Standard Characteristics of Non-Target Arthropod Regulatory Testing.

4. The latest movement

As shown in Table 2, some change in regulatory requirementsand procedures in Japan

are planned or completed recently. Among these changes, avian risk assessment is now mandatory and the results are made open to public (http://www.jcpa.or.jp/labo/pdf/2016/index_birdrisk.pdf).Sprayer risk assessmentrequirement is not mandatory now but many basic studies to set up exposure model are in progress. It is notincluded in Table 1; however, a more drastic change, regulatory system to give approval to generic (off-patent) molecules in Japan, is in progress. Until April 2017, only five off-patented molecules (manzeb, propamocarb hydrochloride, acephate, glyphosate, and phenmedipham) have been registered and commercialized; however, all these off-patent molecules were developed by completing all data requirement (up to rat 2 years carcinogenicity study), which indicated that these are not the so called "generic(s)". From 1st of April, 2017, an amendment of notice12-nousan-8147 will be implemented to introduce TGAI specification, which will make it possible to evaluate the chemical identity among TGAIs (Technical Grade Active Ingredient) produced by different methods in different facilities. If two TGAIs are concluded as "chemically identical", many of data except product chemistry on second or later TGAI (i.e. genericTGAI) are not required. This drastic change made it possible to register a molecules with only about one million US$ investment (less than one-tenth of usual development cost in Japan), by which JMAFF intends to reduce cost of agricultural production.

5. Wrap up

Risk assessment is the core of regulatory (registration) system of agrochemicals, which secures and proves the "safety" of the molecules from many different aspects. Investing much cost ondata generation and risk assessment can NOT reduce actual risk in real world; therefore, it is necessary that authorities, industries and society discuss and define a more appropriate level of acceptable risk level based on the best and latest science, I hope. Too conservative risk assessments aiming completely zero-risk on agrochemicals will bring nothing to stable, efficient andsustainable agricultural production to feed hungry world.

END

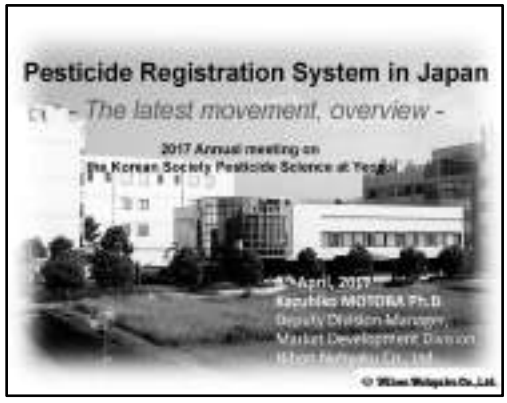
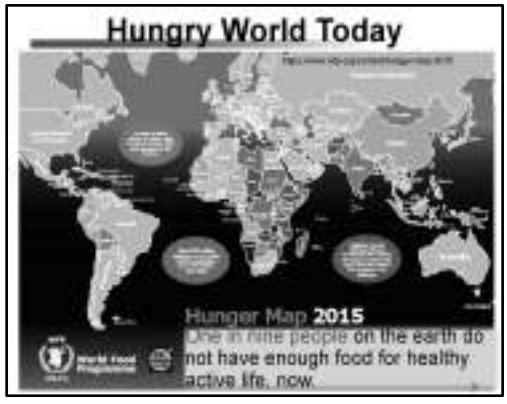
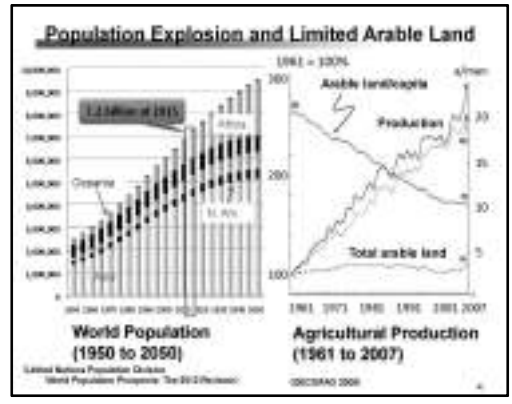
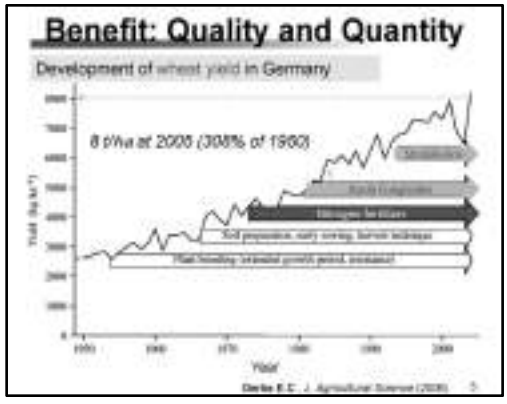

Pesticide Registration System in Japan
- The latest movement, overview -
2017 Annual meeting on
the Korean Society Pesticide Science at Yeosu
April, 2017
Kazuhiko MOTOBA Ph.D.
Deputy Division Manager,
Market Development Division

Table of Contents
✓ To feed hungry world
✓ Pesticide as a innovative tool for agriculture
✓ Regulatory system in Japan
✓ Comparison between other countries and region
✓ Wrap up message
RISK


Hungry World Today
Hunger Map 2015
One in nine people on the earth do not have enough food for healthy active life, now.


Population Explosion and Limited Arable Land
Arable land/capita
Production
Total arable land
World Population (1950 to 2050)
Agricultural Production (1961 to 2007)


Benefit: Quality and Quantity
Development of wheat yield in Germany
8 t/ha at 2000 (308% of 1960)
Year

Benefit: Labor Saving
Working Time (hr) for 10a (1/10 ha) paddy rice farming
300 kg/10a
600 kg/ha
If we don't have herbicide, more than 40% of employment must engage for weed control in Japan to maintain same production level of rice (actual population in farming = 4%).

Negative Aspects of Some Pesticides

✓ *Human health concern possible*

Parathion LD50: 5.56 mg/kg (Class 1a)
Methamidophos LD50: 16 mg/kg (Class 1a)
→ Withdrawn in many countries

✓ *Environmental impact possible*

Many non-selective insecticides: impact on natural enemy and beneficials
→Replaced by selective molecules

✓ *Persistence in environment possible*

DDT, BHC: highly persistent
→ Withdrawn in almost all countries

Balancing Benefit and Risk

Biological efficacy
- Low dose - high efficacy
- High selectivity
- Residual activity
- New MoA
- No cross resistance

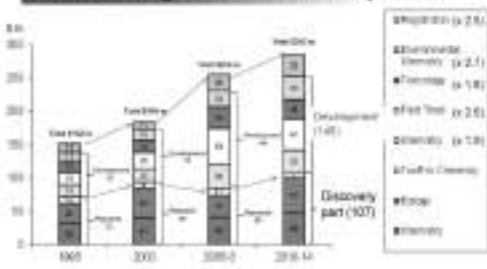
Safety
- To non-target organisms
- To crop (low phyto-toxicity)
- Low environmental impact (degradable, not mobile)

Contribution to sustainable Agriculture

User protection & convenience
- Low toxicity
- Easy use
- Easy application

Economy
- Cost benefit for farmer
- Applicability to IPM
- Patent, competitiveness

Increasing Cost for Development

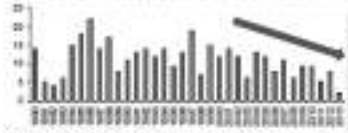


*Based on average value of 10 confidential company responses to a questionnaire

(Phillips McDougall-AgriService, April, 2016)

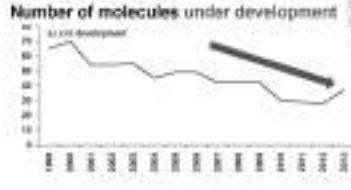
Higher Hurdle for Development

Number of molecules introduced to market



Development and Commercialization of new molecules become difficult year-by-year, which leads to decreased number of new molecules

Number of molecules under development



(Phillips McDougall, Apr.)

Basic law: Agricultural Chemicals Control Act.

✓ Agricultural Chemicals Control Act (Act No. 28)
- ✓ in preamble, states that the law intends to contribute;
  - ✓stable agricultural production
  - ✓health of nation
  - ✓Environmental protection
- ✓ defines data requirements
- ✓ defines evaluation procedure

Detailed rule is defined by a notice 12- nousan-8147

In EU, Regulation 1107/2009
In US, FIFRA (Federal Insecticide, Fungicide, Rodenticide act.

Data Requirement in Japan (for tox)

Study	Animal species	GLP/GLP
1. Acute-oral	Rat	TGAI, PPF
2. Acute dermal	Rat	TGAI, PPF
3. Acute inhalation	Rat	TGAI, PPF*
4. Dermal irritation	Rabbit	TGAI, PPF
5. Eye irritation	Rabbit	TGAI, PPF
6. Skin sensitization	Guinea pig	TGAI, PPF
7. Acute neuro toxicity*	Rat	TGAI
8. Delayed neuro toxicity*	Chicken	TGAI
9. 90 days repeat oral	Rat/Dog	TGAI
10. 21days repeat dermal toxicity*	Rat	TGAI
11. 90 days repeat inhalation toxicity*	Rat	TGAI
12. Sub-chronic neuro toxicity*	Rat	TGAI
13. Sub-chronic delayed neuro toxicity*	Chicken	TGAI
14. 1 year-Chronic toxicity	Rat/Dog	TGAI
15. Carcinogenicity	Rat/mouse	TGAI
16. Reproductive toxicity	Rat	TGAI
17. Teratogenicity	Rat/rabbit	TGAI
18. Mutagenicity	Bacteria, cells, mouse	TGAI
19. General pharmacology*	Rat, mouse, rabbit	TGAI
20. Animal metabolism	Rat	RM with ^{14}C

Submission Package (for Japan)

Risk assessment targets: comparison among three regions

Category	Targets	Japan	US and EU
Human	Consumer	Acute/Chronic	→
	Sprayer	Planned	Mandatory
	Operator	Unnecessary	Mandatory
	Resident/by-stander	Unnecessary	Mandatory
Environment	Aquatic	Acute	Acute/Chronic
	Aquatic Macrophyte	Unnecessary	Acute/Chronic
	Benthic	Conditional	Acute/Chronic
	Terrestrial mammals	Unnecessary	Acute/Chronic
	Avian	Acute (voluntary)	Acute/Chronic
	NTA (non target arthropods)	Beneficial	Mandatory
	Honey bee	Acute adult	Acute/Chronic-larvae/adults
	Soil organisms	Unnecessary	Mandatory
Crops	Primary Crops	Mandatory	→
	Rotational Crops	Conditional	Conditional

New Regulatory Movement in Japan

- ✓ Avian & aquatic risk assessment:
  Previously end-points (toxicity data) are required to submit but not utilized for risk assessment, since no exposure estimation model was available.
- ✓ Acute consumer exposure risk assessment:
  All active ingredient should be discussed in FSC to set up ARfD.
- ✓ Chironomid acute immobilization study:
  Daphnids are sometimes less sensitive to some specific class of chemicals.
- ✓ Setting TGAI specification:
  It makes "Generic" registration possible with significant data exemption.
- ✓ Sprayer (operator) risk assessment:
  To prevent incidents during application. Sprayer exposed to pesticides higher than consumer.
- ✓ NTA, additional species….

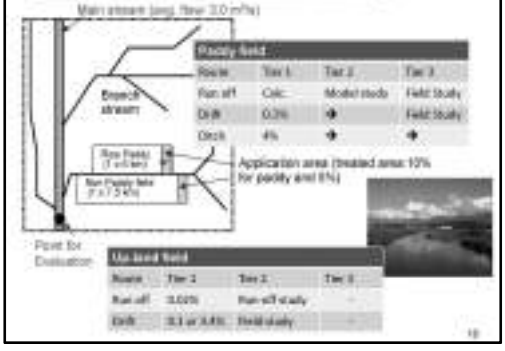
Environmental Risk Assessment

Assessment for Aquatics and Avian

- ✓ Previous regulation
  - ✓ Toxicity data for aquatics should be submitted. Classification (class A, B, Ba, and C) was given by only based on L(E)C50 value → Hazard assessment
- ✓ Present
  - ✓ Risk assessment will be done by comparison with exposure, then PEC calculation model is necessary.
  - ✓ Highly simplified PEC (river) model with tier system were given.

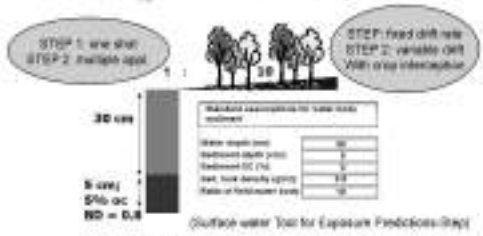
- ✓ Similar situation for avian.

PEC river Model in Japan



FORCUS model used in EU

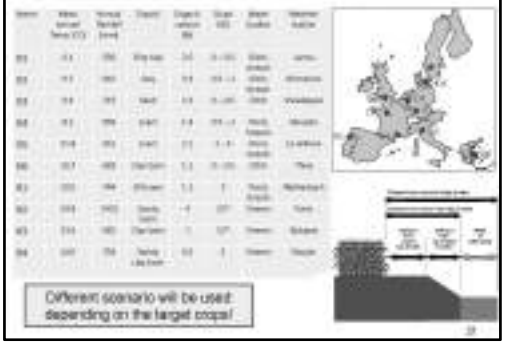
- FORCUS STEPS 1-2:
  - In-puts: molecular weight, water solubility, $T_{1/2}$ in soil, Koc, $T_{1/2}$ in sediment, n of spray, interval, dose



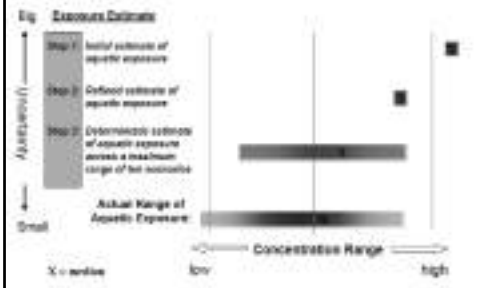
FORCUS STEP 3

- ✓ Drift: Drift Calculator
- ✓ Drainage: MACRO
- ✓ Run-off: PRZM
- ✓ Integration: TOXWA

Scenario Used in FORCUS STEP 3



Simple Model = Conservative



Avian Risk Assessment

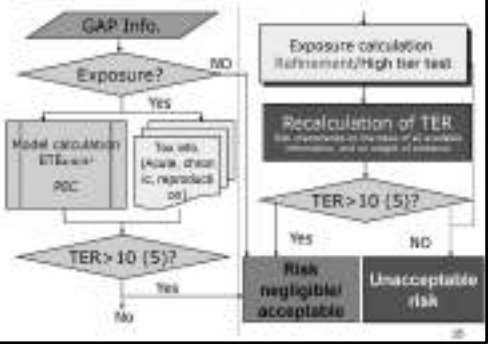
- ✓ Voluntarily conducted from 2012.
- ✓ Simple exposure calculation using GAP information (Tier 1) and crop residue data (Tier 2). Excel spread sheet available.
- ✓ Only for acute exposure

Model Formula for Avian Exposure in EU

- ✓ Avian eating much
- ✓ continuously eat
- ✓ only diet with maximum residue

➡ Extremely worst case!

Standard Procedure Employed in EU



Refinement & Mitigation

- ✓ If TER (Toxicity Exposure Ratio) can not satisfy pre-defined standard...
  - ✓ Parameter in exposure calculation formula will be changed.
    - Actual determination for residue in diet
    - Behavior observation of birds
    - Additional toxicology study to reduce UF (difficult now)
  - ✓ GAP change
    - Reduction of dose, number of spray
    - Deletion of target crops
    - Limitation of application timing
    - Limitation of use location (e.g. green house only)

Human Risk Assessment

ADI & ARfD

- ✓ In Japan, only chronic risk assessment using ADI (acceptable daily intake) was conducted until 2014.
- ✓ In case of detecting MRL violation (due to misuse, incident etc), risk assessment for short term in-take becomes necessary (it is impossible to eat only contaminated food for whole lifetime.)
- ✓ Short term exposure risk assessment using ARfD (acute reference dose) and short term exposure calculation (NESTI) were introduced.

Consumer Risk Assessment

- ✓ ADI/ARfD vs Oral Exposure (TMDI/EDI or NESTI) ratio.

Items	Japan	EU	US
Residue trial	4 to 2	8 to 4 (in NEU/SEU)	20 to 1
GLP	Yes (Except minor crops)	Yes	Yes
MRL	Table	OECD calculator	Specific calculator
Exposure Assessment	Excel spread sheet	Excel spread sheet	Toll Software
Processing study	Unnecessary	Necessary	Necessary

US OPPTS 860.1900 (part)

Residue data to exposure

MRL table

MRL	Maximum Residue (mg/kg)		
	2	4	8
0.10	0.025	0.0340	0.050
0.20	0.05	0.0707	0.100
1.0	0.5	0.701	0.707
10.0	0.00	8.82	7.70

Food Factor Table

Crops	Food Intake Amounts				
	Nationwide average (20.1 kg)	Male (20.1 kg)	Pregnant (20.0 kg)	Child (10.0 kg)	Child short term
Rice	154.2	180.2	170.0	80.7	170.2 (1.40)
Wheat	50.0	48.0	50.0	44.3	50.0 (1.40)
Soy bean	36.0	46.7	37.0	20.4	70.0 (1.40)
Sweet potato	8.0	8.0	8.0	7.0	130.0 (1.40)
Pear	8.4	7.0	9.1	9.4	400.0 (3.40)
Other veg	13.4	14.1	10.0	8.3	-

Risk for Worker (Incident & Suicide)

- ✓ Operator usually exposed to pesticides much higher than consumer. It means higher risk for sprayer.

Number of incidence recognized

Year / Categories		2010	2011	2012	2013	2014
Decease	Application	0(0)	0(0)	0(0)	0(0)	0(0)
	Misuse	3(3)	8(8)	2(2)	4(4)	6(6)
	Subtotal	3(3)	8(8)	2(2)	4(4)	6(6)
Intoxication	Application	11(31)	10(18)	18(36)	11(12)	11(22)
	Misuse	24(28)	30(32)	18(22)	13(18)	13(13)
	Subtotal	35(48)	38(48)	36(58)	24(30)	24(35)
Ground total		38(52)	36(48)	38(60)	28(34)	30(40)

(JMAFF HP: http://www.maff.go.jp/nouyaku/k_topics%27legal_review.html)

Sprayer Exposure Assessment

Items	Japan	United States	EU
Target	Sprayer only	Sprayer Operator Resident By-stander	Sprayer Operator Resident By-stander
Exposure estimation	Under development	PHED & ARETF Model	POEM, German BBA Model, EUROPOEM) → AOEM
Endpoints for evaluation	Not decided yet	Inhalation & dermal NOAEL	Systemic AOEL*
Scenarios	A few	Many	Many

* Systemic AOEL = (Oral NOAEL for sun scale study) x absorption rate (%) x 1/100

PHED: Pesticide Handler Exposure Database
ARETF: Agrochemicals Handler Exposure Task Force
POEM: Pesticide Operator Exposure Model
AOEM: Agrochemicals Operator Exposure Model

Generic Pesticide

Generics and TGAI Specification

- ✓ TGAI (Technical Grade Active Ingredient) contains not only active ingredient but also impurities, analogues as constituents. Toxicity data of TGAI reflects all constituents in TGAI.
- ✓ No article and rule are given in Pesticide Regulation law and Notice 12-Nohsan-8147 to define TGAI specification and to evaluate chemical identity and quality. No rule to alternate data between identical TGAIs is also given.
- ✓ By defining specification (new regulation from 1st April, 2017), chemical identity can be evaluated, which makes study exemption possible.

Generic becomes possible!

At the end of presentation

- ✓ In recent decades, more attention was paid to environment rather than human regarding pesticide risk.
- ✓ EU and US continue to introduce aggressive, conservative regulation and data requirement to pesticide regulation system (ED, Honey Bee, CMR, PECgw).
- ✓ Japanese regulation is following them about 10 years later. But the procedure and level seems slightly different and practical.

Personal Wrap Up Message

- ✓ Old, sometimes rather toxic or having high environmental impact molecules should be replaced by innovative low risk molecules. To facilitate this, strengthening regulation is inevitable.
- ✓ Complicated and precise risk assessment can not always give us correct answer.
- ✓ Investing much cost to pass through complicated risk assessment will not reduce actual risk in real world.
- ✓ Government authority, academia, society and industry should discuss about appropriate level of acceptable risk based on sound science.

[SPECIAL LECTURE 4]

Dr. **Kimberly Hodge-Bell**
Monsanto, USA

Integrated Agriculture Solutions - Biotechnology and Crop Protection

S-04

Kimberly Hodge-Bell, Ph.D.

• Associate Fellow and Senior Toxicologist at Monsanto Company
• St. Louis, MO, USA
• E-mail:

➢ Education

2002, Doctorate degree, Biomedical Science (Pharmacology and Toxicology), Meharry Medical College

2002 – 2004, Postdoctoral fellowship, University of California, Los Angeles

➢ Experience

a Diplomate of the American Board of Toxicology (DABT)

Committee member in the Society of Toxicology

Integrated Agriculture Solutions - Biotechnology & Crop Protection

Kimberly Hodge-Bell, PhD, DABT

Monsanto, USA

Monsanto is committed to bringing a broad range of solutions to help farmers get the most out of their crops while minimizing the amount of land, water and other resources that are required. The technologies that fuel these integrated solutions include biotechnology and crop protection.

Biotechnology has helped develop effective solutions for farmers over the past 30 years, strengthening plants' resistance to disease, insects and adverse weather conditions. The environmental benefits of biotechnology are notable. To ensure the quality and safety of biotech crops, a rigorous and integrated process is used to assess the safety. Specifically, researchers look for changes in allergenicity, toxicity, nutrient composition and level, unintended effects, and the safety of proteins included with the gene. The data generated from all of these studies is then submitted to regulatory agencies worldwide for review. Every major scientific body and international regulatory authority has deemed crop biotechnology and the foods currently available safe as other traditional crops.

Another integrated agricultural solution used by farmers is crop protection. Glyphosate, a crop protection product, is a versatile herbicide used by farmers, land managers and gardeners to control unwanted vegetation. Glyphosate-tolerant crop varieties transformed agriculture and greatly simplified weed control for corn, cotton and soybean farmers. Glyphosate works by inhibiting an enzyme present in plants that people and animals do not produce. Glyphosate safety is supported by one of the most comprehensive toxicology, crop residue, and environmental databases. Regulatory authorities establish ADIs as conservative acceptable (safe) human exposures. When measured exposure levels are taken into consideration, glyphosate exposures are orders of magnitude lower

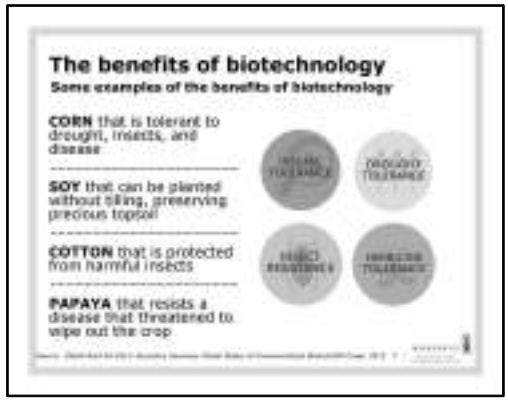
than the lowest established ADI for glyphosate (i.e., 0.5 mg/kg bw/day). Despite the favorable toxicology profile, specific mode of action, low human exposure and extensive regulatory reviews which have consistently concluded glyphosate does not cause cancer, the International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic to humans" in Category 2A (Guyton et al., 2015). IARC's classification is inconsistent with recent regulatory authorities and the Intertek Scientific Expert Panel conclusions that glyphosate is considered unlikely to pose a cancer hazard to humans. When used according to label directions, Glyphosate has a history of safe use for more than 40 years.

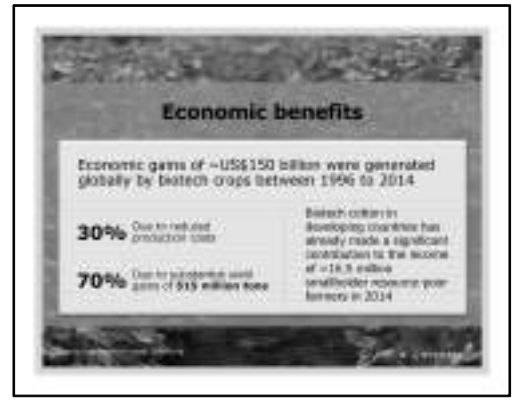
Integrated Agriculture Solutions
Biotechnology & Crop Protection
Kimberly Hodge-Bell, PhD, DABT
April 6, 2017

Who We Are
Monsanto at a glance

What do we do?
Working together for sustainable agriculture
Bringing a broad range of solutions to help nourish our growing world
Help agriculture to become more environmentally sustainable
Improve global food security

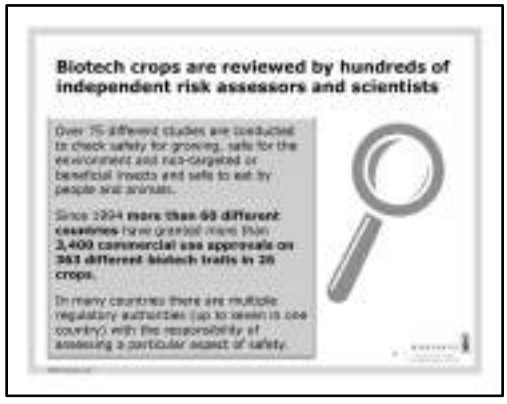
Integrated solutions drive improved Ag productivity, profitability for farmers
CROP PROTECTION
DATA SCIENCE
BIOTECHNOLOGY
BIOLOGICALS
SEEDING

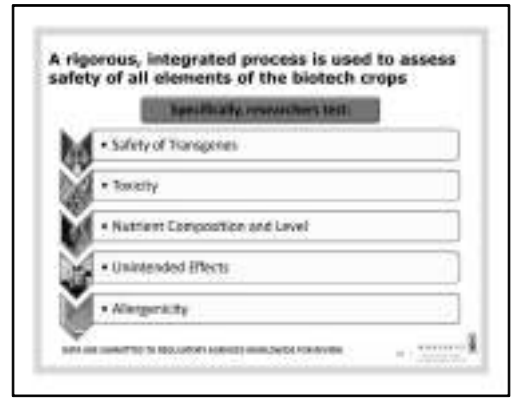

The benefits of biotechnology
Some examples of the benefits of biotechnology
CORN that is tolerant to drought, insects, and disease
SOY that can be planted without tilling, preserving precious topsoil
COTTON that is protected from harmful insects
PAPAYA that resists a disease that threatened to wipe out the crop


Economic benefits
Economic gains of ~US$150 billion were generated globally by biotech crops between 1996 to 2014
30%
70%

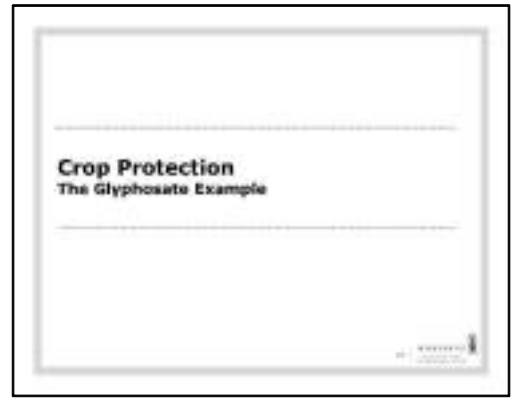
Environmental benefits

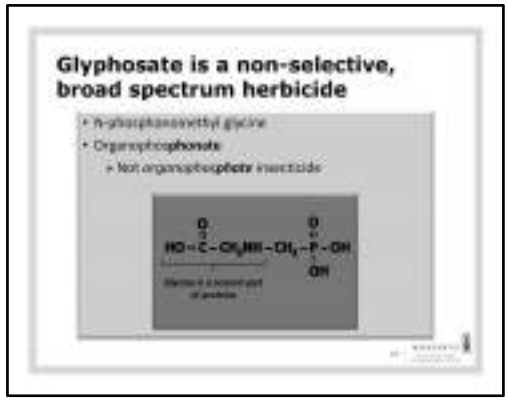
Biotech Crop Safety

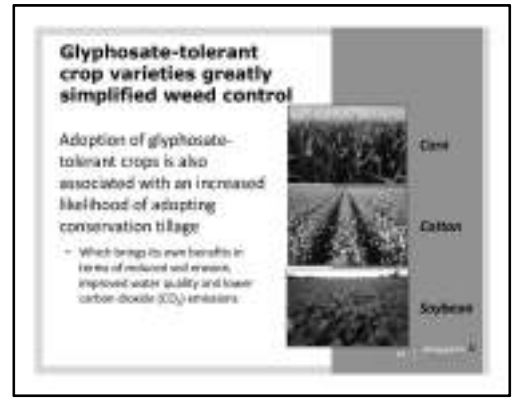

Biotech crops are reviewed by hundreds of independent risk assessors and scientists

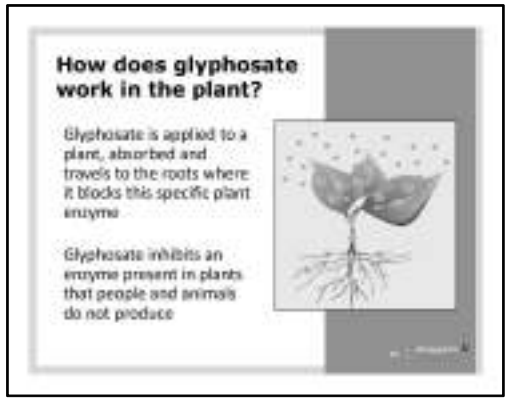

A rigorous, integrated process is used to assess safety of all elements of the biotech crops
• Safety of Transgenes
• Toxicity
• Nutrient Composition and Level
• Unintended Effects
• Allergenicity

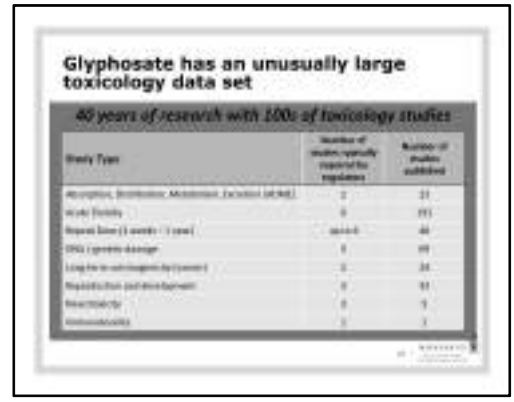
Consensus on safety by international science organizations

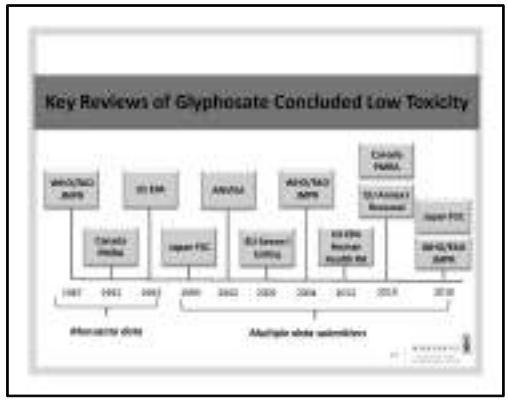

Crop Protection
The Glyphosate Example

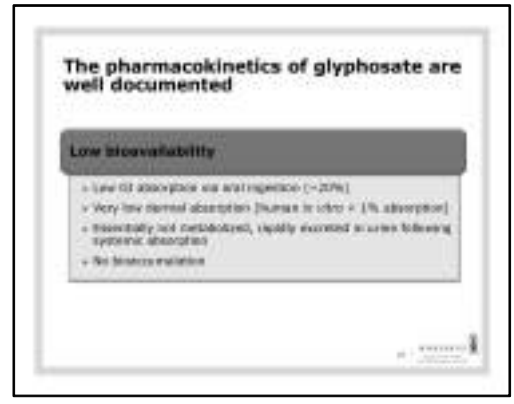

Glyphosate is a non-selective, broad spectrum herbicide
N-phosphonomethyl glycine
Organophosphonate
Not organophosphate insecticide


Glyphosate-tolerant crop varieties greatly simplified weed control
Adoption of glyphosate-tolerant crops is also associated with an increased likelihood of adopting conservation tillage
Corn
Cotton
Soybean

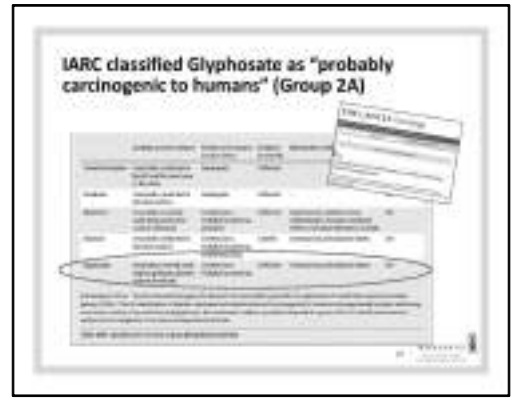

How does glyphosate work in the plant?
Glyphosate is applied to a plant, absorbed and travels to the roots where it blocks this specific plant enzyme
Glyphosate inhibits an enzyme present in plants that people and animals do not produce

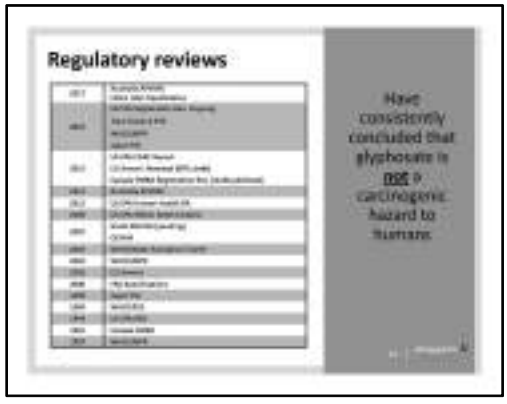

Glyphosate has an unusually large toxicology data set
40 years of research with 100s of toxicology studies


Key Reviews of Glyphosate Concluded Low Toxicity


The pharmacokinetics of glyphosate are well documented
Low bioavailability

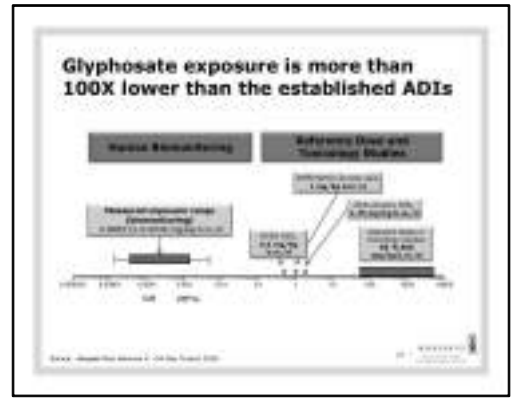
Regulatory reviews over 40 years
Not acutely toxic – oral, dermal, inhalation
Not irritating to skin
Not neurotoxic, not immunotoxic
Not a reproductive toxicant, not an endocrine disruptor, no birth defects
Not genotoxic, Not carcinogenic


IARC classified Glyphosate as "probably carcinogenic to humans" (Group 2A)

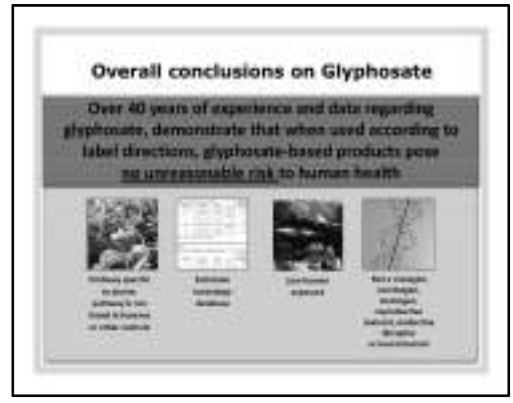

Regulatory reviews
Have consistently concluded that glyphosate is not a carcinogenic hazard to humans

Recent regulatory review conclusions

Expert panel convened to review IARC Monograph
Intertek Scientific & Regulatory Consultancy Services assembled an Expert Panel Review of IARC Monograph conclusions
"The Expert Panels conclude that glyphosate is unlikely to pose a carcinogenic risk to humans."


Glyphosate exposure is more than 100X lower than the established ADIs

World Health Organization herbicide ADIs determined through 2009 (mg/kg bw/day)


Overall conclusions on Glyphosate
Over 40 years of experience and data regarding glyphosate, demonstrate that when used according to label directions, glyphosate-based products pose no unreasonable risk to human health

Thank you

[SPECIAL LECTURE 5]

Dr. **Troy Seidle**
Humane Society International, Canada

3R Best Practices in Pesticide Regulation

S-05

Troy Seidle, Ph.D.

• Director, Research & Toxicology Department
• Humane Society International / Canada
• E-mail: tseidle@hsi.org

➢ Education

1998, UNIVERSITY OF WATERLOO, Applied Health Sciences, Waterloo, Canada

➢ Experience

1999 – 2000, INTERNATIONAL FUND FOR ANIMAL WELFARE / CANADA
2006, Scientific Consultant, EUROPEAN COMMISSION, JOINT RESEARCH CENTRE, Ispra, Italy
2007, Scientific Consultant, VIER PFOTEN, Vienna, Austria
2000 – 2006, Director of Science Policy, FOUNDATION TO SUPPORT ANIMAL PROTECTION, Virginia

➢ Publications

Seidle T, Qu T. "AXLR8 – Accelerating the Transition to a Human Pathway-Based Paradigm in Toxicology." In: Toxicity Testing Strategy in the 21st Century: Principles and Practices. Eds. S Peng & PL Carmichael. Beijing: Chinese Academy of Sciences (2016).

Seidle T, Robinson S, Holmes T, Creton S, Prieto P & Chlebus M. Cross-sector review of drivers and alternative approaches for acute systemic toxicity testing. *Toxicol Sci*. 2010; 116, 382-96.

Seidle T & Stephens M. Bringing toxicology into the 21st century: a global call to action. *Toxicol In Vitro*. 2009; 23, 1576-9.

3R best practices in pesticide regulation

Troy Seidle

Research & Toxicology Department, Humane Society International
휴메인소사이어티인터내셔널 Toronto, Canada

Decades of investment in novel testing approaches to replace, reduce or refine animal use in toxicology have yielded a substantial and ever-expanding toolbox of validated *in vitro* and computational models, waiver and other accepted non-testing strategies, and other innovative approaches that could be put to immediate use to improve the scientific relevance, efficiency and humaneness of regulatory testing and risk assessmentacross a number of product sectors. The European pesticide and chemical sectors will be used as case studies for examining progress toward uptake of available 3R approaches, using examples including new OECD in vitro guideline methods, deletion or waiver of redundant test requirements such as the 1-year dog and mouse carcinogenicity studies for pesticides, classification by calculation, and the first example of regulatory uptake of an adverse outcome pathway (AOP)-based test strategy as a model for ongoing movement toward '21st century non-animal toxicology' as articulated by the U.S. National Research Council, the European AXLR8 project and others.

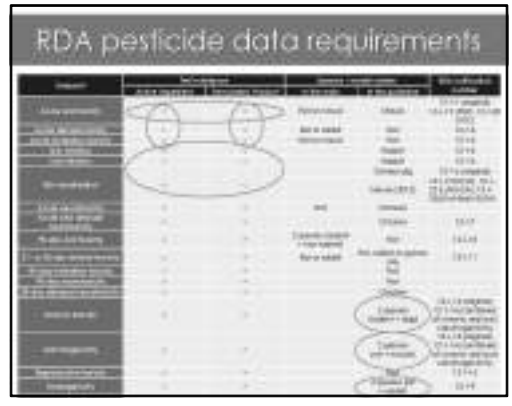
"3R best practices" in pesticide regulation: deletion of scientifically redundant animal test requirements
Korea Society of Pesticide Science | 6-7 April 2017

About
Expert team
Toxicology, ecotoxicology, pharmacology, regulatory science, endocrinology, biochemistry, neuroscience, law, etc.
Global presence
South Korea, Japan, Viet Nam, India, United States, Canada, Central America, Brazil, European Union, Australia and beyond
Approach
Working with policy makers, regulators, companies, scientists, and other stakeholders to build partnerships for progress
HSI is the leading international NGO working to advance non-animal testing & health research worldwide

HSI science team presence
OECD Test Guidelines, Chemicals & AOP programs
US EPA pesticide program dialogue committee
European Food Safety Authority accredited stakeholder
European Chemicals Agency accredited stakeholder
EU Competent Authorities for REACH and Classification and Labelling
European Medicines Agency interested party status
USTR Trade and Environment Policy Committee member
NTP Scientific Advisory Committee on Alternative Toxicological Methods
European Union Reference Laboratory for Alternatives
...and others

"3R best practices" concept
Strategies to Replace, Reduce or Refine animal use in toxicology, including:

"3R best practices" concept
Is it sufficient to reduce or refine animal use when full replacement is scientifically possible?


RDA pesticide data requirements

2017 toolbox of validated, globally recognized non-animal approaches

Safety concern	Name of test	OECD guideline
Skin corrosion / irritation	Reconstructed Human Epidermis (RHE), EPISKIN/EPIDERM, etc.	431, 435, 439
Eye corrosion / irritation	BCOP, ICE, IRE, Fluorescein Leakage, EpiOcular, etc.	437, 438, 460, 490, 491
Skin sensitisation	DPRA, Keratinocyte LuMI-neo (Keratinosens), h-CLAT, etc.	442C, 442D, 442E
Skin absorption	Human post-surgical skin; RHE models	428
Phototoxicity	3T3 Neutral Red Uptake (NRU)	432
Genetic toxicity	Ames, Chromosomal Aberrations, Micronucleus, etc.	471, 473, 476, 475, 487
Acute oral toxicity	3T3 NRU	OECD 420 + EURL ECVAM recommendation

OECD test guideline uptake in Korea

Endpoint	OECD TG #	Title	2008	2009	2014
Skin corrosion / 피부 부식성	430	In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test	5.20		
	431	In Vitro Skin Corrosion: Reconstructed Human Epidermis Test	5.24		
	435	In Vitro Membrane Barrier Test Method for Skin Corrosion			
	439	In Vitro Skin Irritation: Reconstructed Human Epidermis Test	5.28	V	
Eye irritation / 눈 자극성	437	Bovine Corneal Opacity and Permeability Test		8	
	438	Isolated Chicken Eye Test		11	
	460	Fluorescein Leakage Test			
	491	Short Time Exposure In Vitro Test			
	492	Reconstructed Human Cornea-like Epithelial Test		8	
Skin sensitisation / 피부 과민성	429	Local Lymph Node Assay: LLNA	5.17 (2.2)	1	5.1.1 (1)
	442A	Ames Test	5.1 (2.2)	4	5.1.2 (1)
	442B	Ames Test: LLNA	5.1 (2.2)	5	5.1.2 (2)
	442C	Direct Peptide Reactivity Assay	5.1 (2.2)	10	
	442D	ARE Nrf2 Luciferase Test Method	5.1 (2.2)		
	442E	Human Cell Line Activation Test (h-CLAT)			
Genotoxicity / 유전독성	471	In Vitro Mammalian Chromosomal Aberration Test	5.24		
	476	In Vitro Mammalian Cell Gene Mutation Tests	5.30		
High acute toxicity / 급성 독성	420	Acute Oral Toxicity Test			

Regulatory history & reviews

- Dogs traditionally required as a second species in repeated dosing studies of both 90-day and 1-year duration
- Retrospective data analyses beginning in 1990s began to critically examine the scientific added value of dog study data
  - TGD (Spielmann & Hess, 1996)
  - German BfR (Barbirath & Spielmann 1999, Spielmann & Gaertner 2001, Bue & Spielmann 2005)
  - US EPA (Bunton et al. 2005; Delaney et al. 2007)
  - Industry (Kolle et al. 2009; 2011)
  - Canada PMRA (Lino et al. 2011)

Question

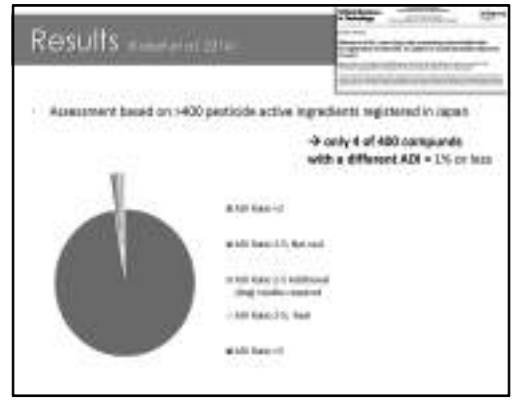
What if the 1-year dog study would not be available and an alternate ADI was derived based on the remaining toxicology database?

Method

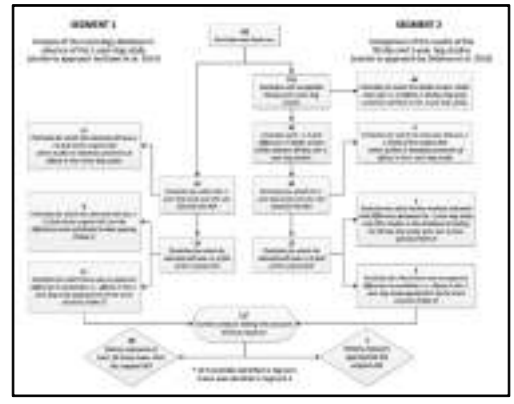
- Identification of registered compounds in Japan for which the ADI is based on the dog data (normally 1-year studies)
- 40 substances with data sets identified and analyzed for alternative ADI (without 1-year dog study)

	Dog	Data requested for risk assessment in MHLW				
		90-day dog study	1-year dog study	Gen. toxicity tests	2-generation reproduction	Rat developmental
Sweet potatoes	0.45	0.45, 1.37, 4.11, 12.3	0.45, 1.37, 4.11, 12.3	0, 10, 100, 1,000 mg/kg; 1,000 mg/kg; 0.1, 0.3, 1.0, 3.0; 0.1, 0.3, 1.0, 3.0; 0.1, 0.3, 1.0, 3.0

Method
Assessment criteria for alternative ADI
No difference
Potential difference
No real difference
Additional studies required
Real difference
Assessment


Results
Assessment based on >400 pesticide active ingredients registered in Japan
→ only 4 of 400 compounds
with a different ADI = 1% or less

Conclusions
More than 99% of ADIs unaffected; 1% of ADIs were 3-8x higher (within SF100)
Routine conduct of 1-year dog study is no longer scientifically justified
Recommendation to eliminate 1-year dog study from core battery of registration data requirements

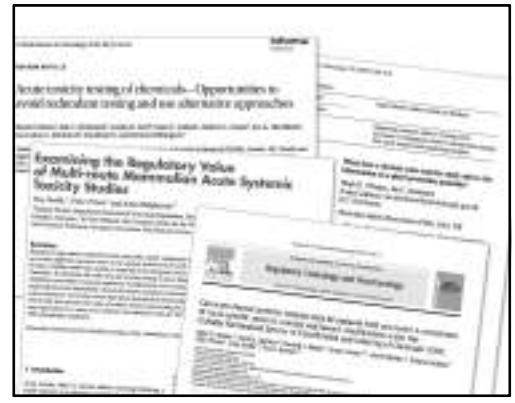


US
CA
JP
All but 2 major markets have removed routine 1-year dog study requirement

Acute dermal systemic toxicity

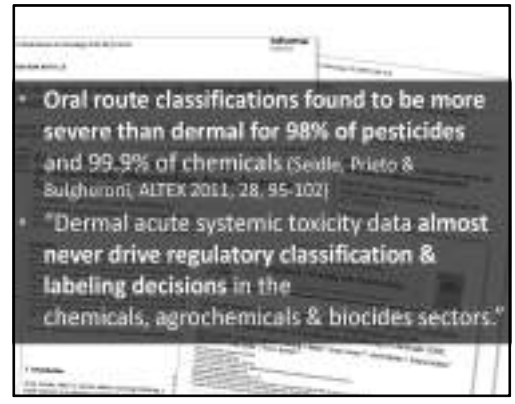
GHS' classification & labeling criteria for systemic acute toxicity

Classification	Category 1	Category 2	Category 3	Category 4
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Pictograms	[illegible]	[illegible]	[illegible]	[illegible]
Signal Word	Danger	Danger	Danger	Warning
Hazard statement	[illegible]	[illegible]	[illegible]	[illegible]
Precautionary statements	[illegible]			



Concordance among GHS oral-dermal classifications for 337 pesticide active ingredients

	ORAL				
DERMAL	NC	4	3	2	1
NC	154	96	12	1	1
4	0	6	4	1	0
3	1	1	8	6	2
2	0	0	1	4	1
1	0	0	0	1	11



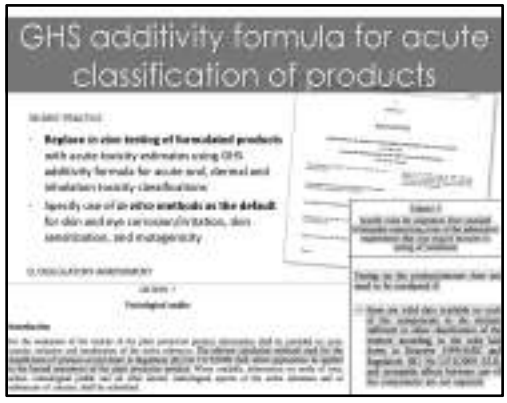
- **Oral route classifications found to be more severe than dermal for 98% of pesticides** and 99.9% of chemicals (Seidle, Prieto & Bulgheroni, ALTEX 2011, 28, 95-102)
- "Dermal acute systemic toxicity data **almost never drive regulatory classification & labeling decisions** in the chemicals, agrochemicals & biocides sectors."

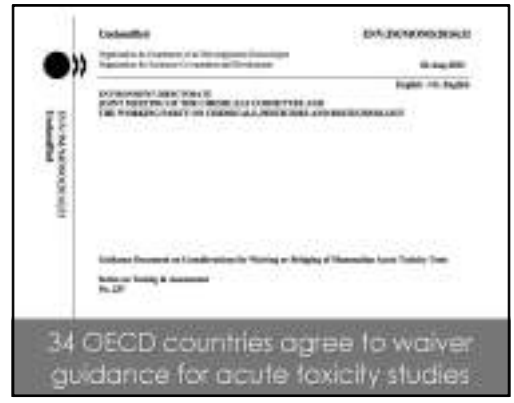
Data requirement	Objective	EU	US	JP	BR	CA	KR	AP
Acute dermal toxicity	Deferral/waiver of data requirement	✓	✓	?	?	?	?	?

Increasing acceptance of waivers for acute dermal toxicity

GHS additivity formula for acute classification of products

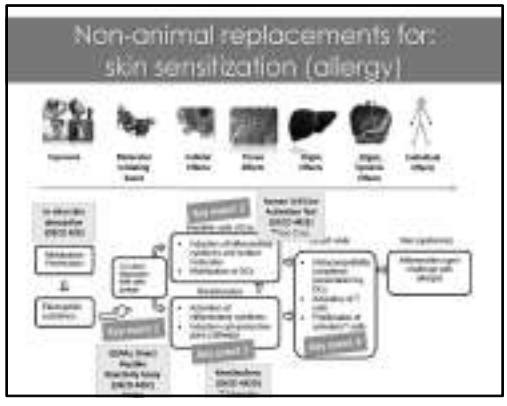
- Calculations based on GHS additivity formula provide a protective, non-testing alternative to redundant acute systemic testing *in vivo*
- Analysis of additivity formula predictions vs. 4-pack classifications of LD_{50}/LC_{50} for 200 agrochemical formulations (Canvers et al. 2013) found:
  - Oral LD_{50} prediction: >86% accuracy
  - Dermal LD_{50} prediction: >83-98% accuracy (no under-predictions)
  - Inhalation LC_{50} prediction: >86% accuracy

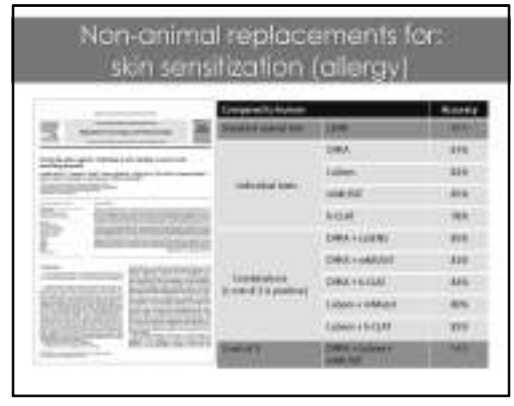

GHS additivity formula for acute classification of products


34 OECD countries agree to waiver guidance for acute toxicity studies

Tox21: human pathway-based approach to safety testing
2007: U.S. National Research Council "envisions a new toxicity testing system that evaluates biologically significant perturbations in key toxicity pathways using new methods in computational biology and a comprehensive array of in vitro tests based on human biology."
"Animal testing won't disappear overnight, but the agencies' work signals the beginning of the end."
Former NIH Director

PATHWAYS
to a Better Future
Brought to you by
HUMAN TOXICOLOGY PROJECT CONSORTIUM


Non-animal replacements for: skin sensitization (allergy)


Non-animal replacements for: skin sensitization (allergy)

"The difficulty lies, not in the new ideas, but in escaping from the old ones."
— John Maynard Keynes (1883-1946)

Thank You!

[SPECIAL LECTURE 6]

Dr. **Yonggyun Kim**
Andong National University, Korea

Eicosanoid Biosynthesis Inhibitors as Novel Synergists for Microbial Insecticides

S-06

Yong-Gyun Kim, Ph.D.

- Department of Pharmacology
- Faculty of Veterinary Medicine
- Cairo University, Egypt.

➢ Education

1984, Bacholar in Agriculture, Seoul National University, Republic of Korea.
1993, Doctorate degree, Iowa State University, USA
1994, Postdoctor Fellowship, University of California, Riverside, USA

➢ Experience

1994 – current, Professor, Andong National University
2016 – current, Chief-Editor, Archives of Insect Biochemistry and Physiology
2014 – current, Member, The Korean Academy of Science and Technology

➢ Publications

Identification and bacterial characteristics of Xenorhabdus hominickii ANU101 from an entomopathogenic nematode, Steinernema monticolum. *Journal of Invertebrate Pathology* (2017) 144: 74-87.

An endoparasitoid wasp influences host DNA methylation. *Sci Rep* (2017) 7: 1-16

A viral histone H4 supresses insect insulin signal and delays host development. *Dev Comp Immunol* (2016) 63: 66-77.

Eicosanoid Biosynthesis Inhibitors as Novel Synergists for Microbial Insecticides

Yonggyun Kim

Dept Bio-Sciences, Andong National University, Andong 36729, Korea

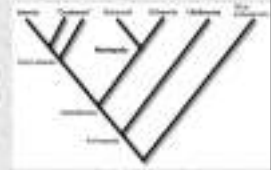
Eicosanoids are a chemical group of 20 carbon fatty acids that are polyunsaturated and oxygenated. They mediate various insect physiological processes including immune responses. Upon immune challenge, eicosanoids are biosynthesized from phospholipids by a catalytic activity of phospholipase A_2(PLA_2)at *sn-2* position to release arachidonic acid (AA). AA is then oxygenated by either cyclooxygenase or lipoxygenase to produce prostaglandin (PG) or leukotrien (LT), respectively. Both PG and LT mediate cellular and humoral immune responses of insects. Entomopathogenic bacteria included in two genera, *Xenorhabdus* and *photorhabdus*, induce significant immunosuppression of target insects by inhibiting catalytic activity of PLA_2 to shut-down eicosanoid biosynthesis. At least 8 different bacterial metabolites that directly inhibit PLA_2 have been identified in these two bacterial groups. However, they appear to be synthesized in different times after infection. In addition, they are different in inhibiting immune responses. These suggest that there are different types of PLA_2s in insects. These bacterial metabolites significantly enhance pathogenicity of other insect microbial pathogens including *Bacillus thuringiensis* (Bt) by suppressing host immune defense. Mixtures of Bt and the bacterial metabolites are collectively called “Bt-Plus”. Different Bt-Plus formulations are currently registered as crop protectants to control lepidopteran, coleopteran, and dipteraninsect pests.

Eicosanoid Biosynthesis Inhibitors as Novel Synergists for Microbial Insecticides

Yonggyun Kim
Andong National University
Korea

Introduction on insects

- Animalia
  - Invertebrata
    - Arthropoda
      - Chelicerata
      - Trilobitomorpha
      - Mandibulata
        - Myriapoda
        - Crustacea
        - Hexapoda



PLoS Biol (2014) 12: e1002008

Diversity of insects

- Hexapoda
  - Apterygota (Ametabola)
  - Pterygota
    - Exopterygota (=Hemimetabola)
    - Endopterygota (=Holometabola)

Science (2010) 340: 763-767

Insects as pest

Trend in insecticide development

Neurotoxins
- Organochlorine
- Organophosphate
- Carbamate
- Pyrethroid
- Neonicotinoid
- Diamide

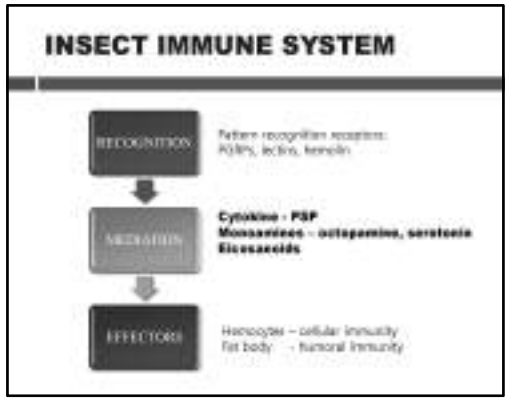
IGRs
- Chitin synthesis inhibitor
- Juvenile hormone analogs
- Ecdysone analogs

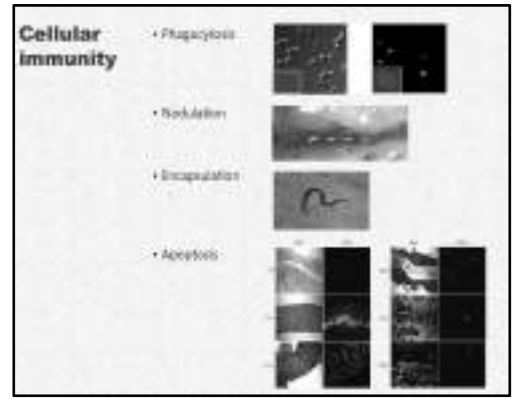
Biopesticide
- Bt
- Baculovirus
- *Beauveria*
- EPN
- dsRNA

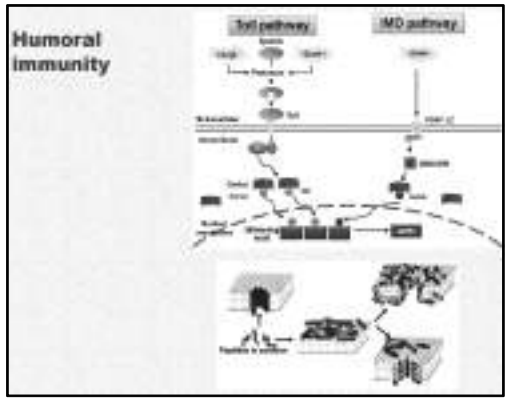
Novel targets to develop new insecticides

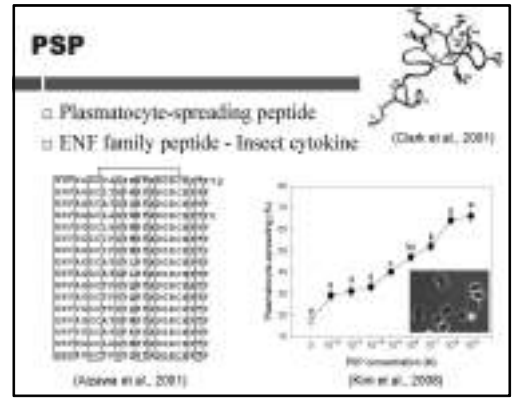
- Host-parasite molecular interactions

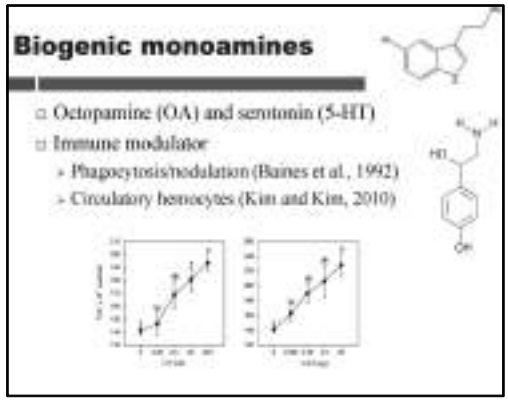
- Entomopathogenic microbes-derived insect immunosuppressants and their genes

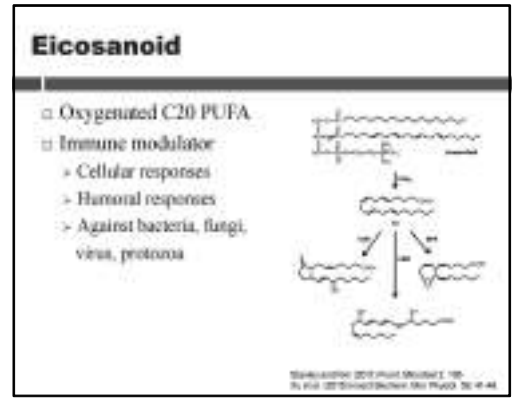

INSECT IMMUNE SYSTEM
RECOGNITION
Pattern recognition receptors:
PGRPs, lectins, keneidin
MEDIATION
Cytokine - PSP
Monoamines - octopamine, serotonin
Eicosanoids
EFFECTORS
Hemocytes – cellular immunity
Fat body - humoral immunity


Cellular immunity
• Phagocytosis
• Nodulation
• Encapsulation
• Apoptosis

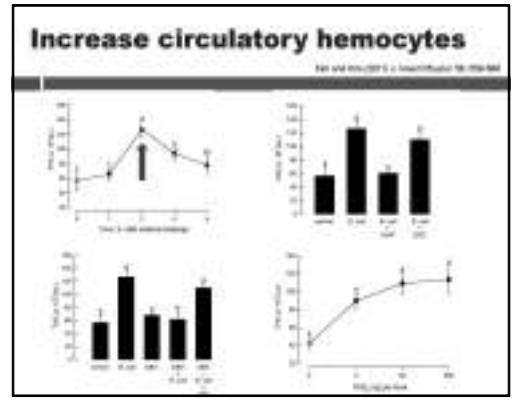

Humoral immunity
Toll pathway
IMD pathway

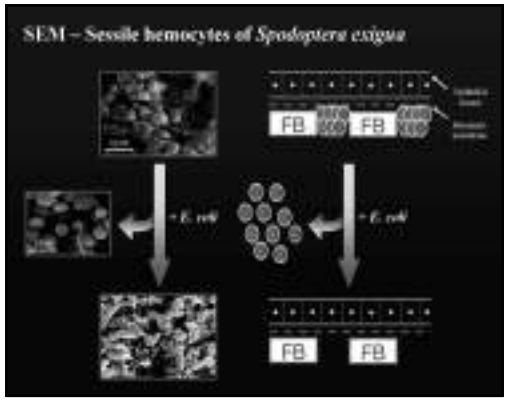

PSP
□ Plasmatocyte-spreading peptide
□ ENF family peptide - Insect cytokine
(Clark et al., 2001)
(Aspéve et al., 2001)
(Kim et al., 2008)

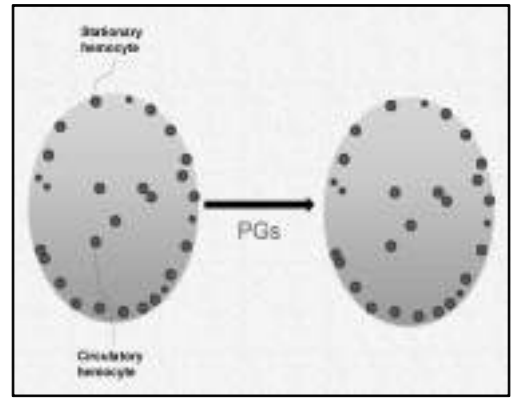

Biogenic monoamines
□ Octopamine (OA) and serotonin (5-HT)
□ Immune modulator
› Phagocytosis/nodulation (Baines et al., 1992)
› Circulatory hemocytes (Kim and Kim, 2010)


Eicosanoid
□ Oxygenated C20 PUFA
□ Immune modulator
› Cellular responses
› Humoral responses
› Against bacteria, fungi, virus, protozoa

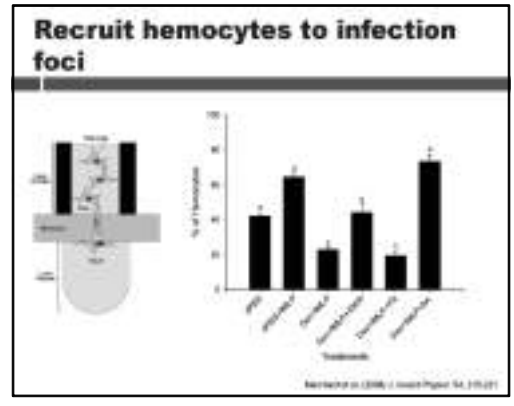
EICOSANOID MEDIATION ON HEMOCYTE MOBILIZATION



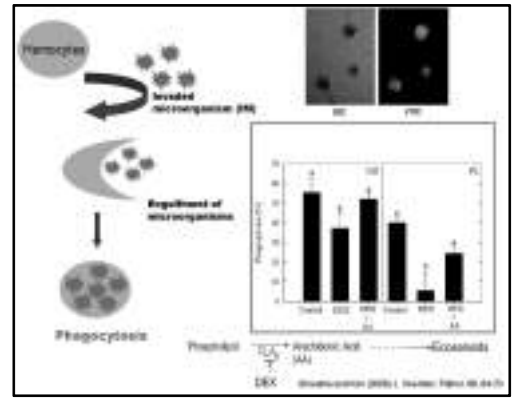




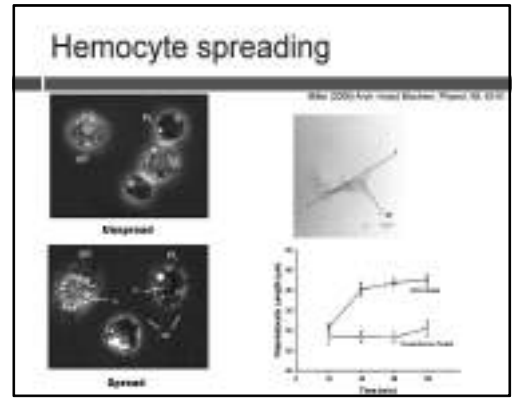
EICOSANOID MEDIATION ON HEMOCYTE MOBILIZATION TO INFECTION FOCI



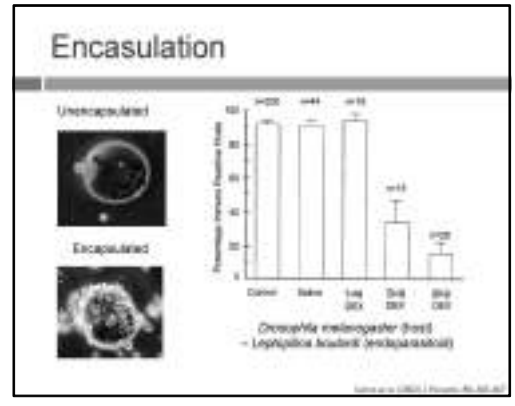
EICOSANOID MEDIATION ON HEMOCYTE PHAGOCYTOSIS



EICOSANOID MEDIATION ON HEMOCYTE SPREADING



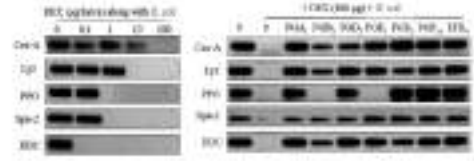
EICOSANOID MEDIATION ON HEMOCYTE ENCAPSULATION



EICOSANOID MEDIATION ON AMP BIOSYNTHESIS

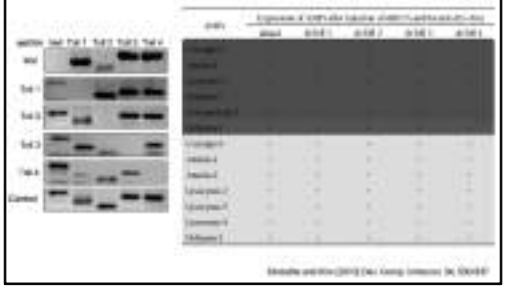
Eicosanoid - AMPs

- *Bombyx mori* (Morishima et al., 1997)
- *Drosophila melanogaster* – IMD pathway (Yajima et al., 2003)
- *Spodoptera exigua* (Shrestha and Kim, 2009)

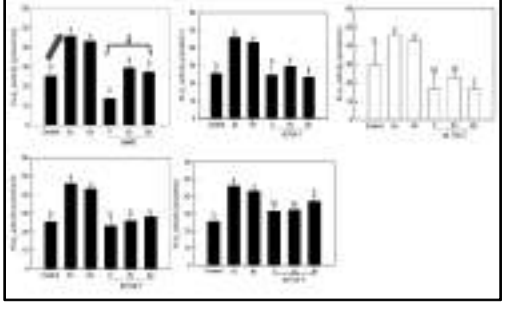


EICOSANOID PLAYS A MAIN ROUTE TO RELAY OTHER IMMUNE MEDIATORS (TOLL/IMD PATHWAYS)

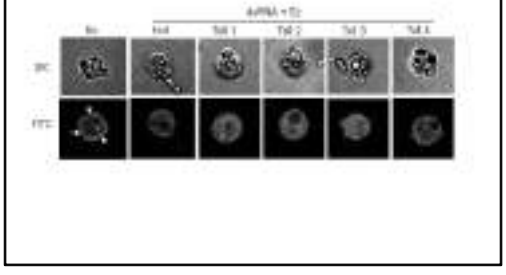
Toll/Imd pathways (*Tribolium*)

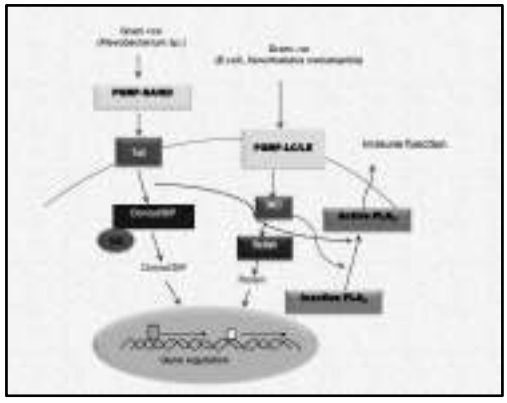


Toll/Imd signals activate PLA_2 activity

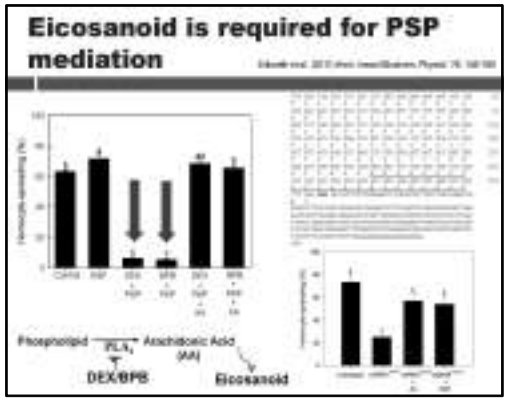


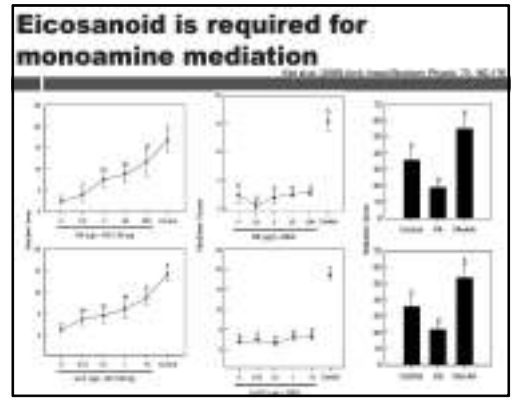
Toll/Imd signals induce localization of PLA_2 near to membrane

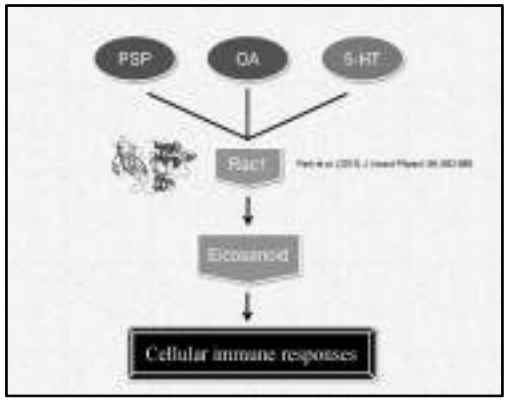




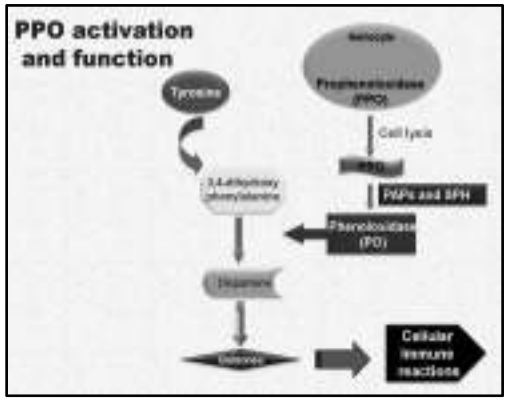
EICOSANOID PLAYS A MAIN ROUTE TO RELAY OTHER IMMUNE MEDIATORS (CYTOKINES)

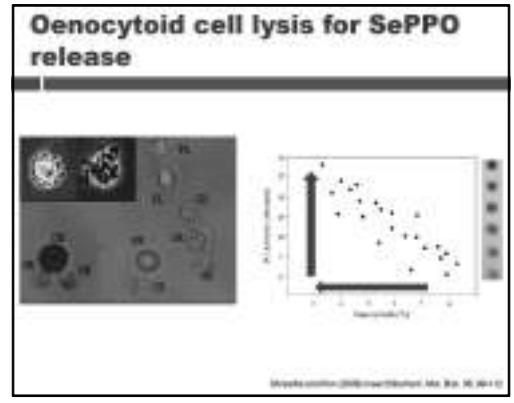


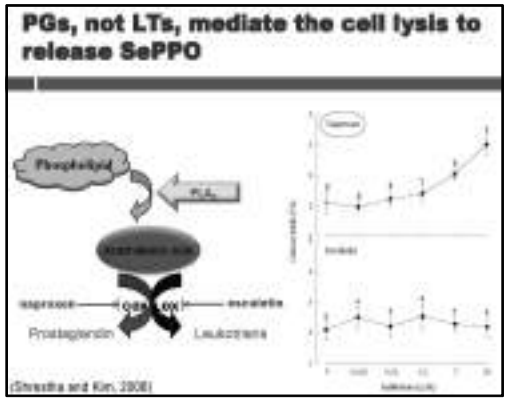


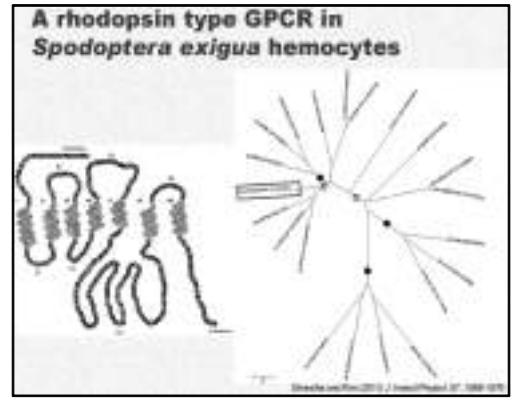


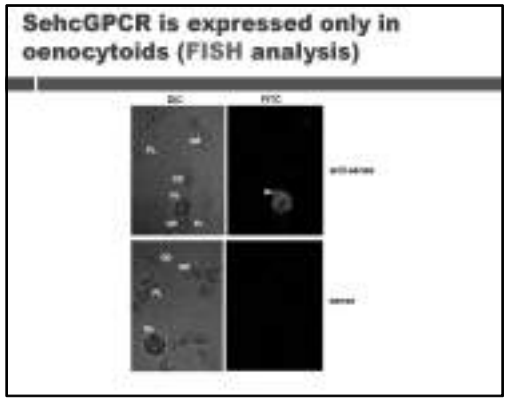
EICOSANOID ACTIVATES PPO AS A COMMON EFFECTOR FOR THE CROSSTALKS

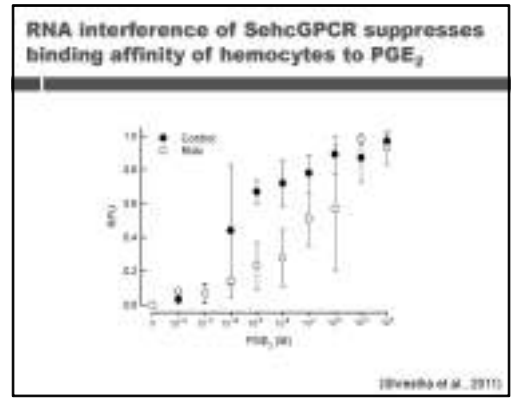

PPO activation and function
Tyrosine
Cell lysis
PAPs and SPH
Phenoloxidase (PO)
Cellular immune reactions

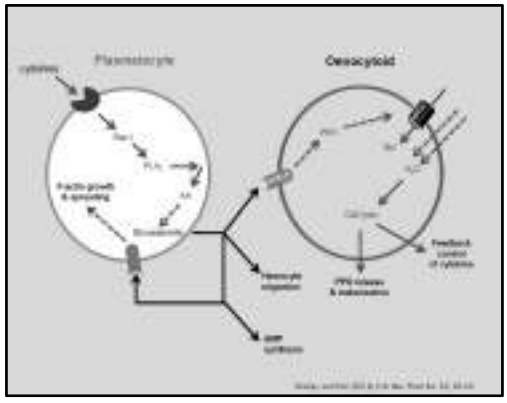

Oenocytoid cell lysis for SePPO release


PGs, not LTs, mediate the cell lysis to release SePPO
Phospholipid
Prostaglandin
Leukotriene
(Shrestha and Kim, 2008)

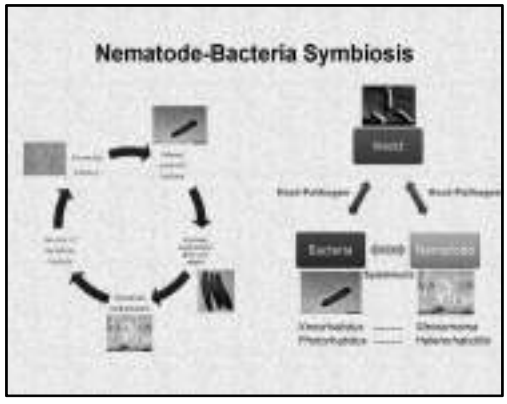

A rhodopsin type GPCR in Spodoptera exigua hemocytes

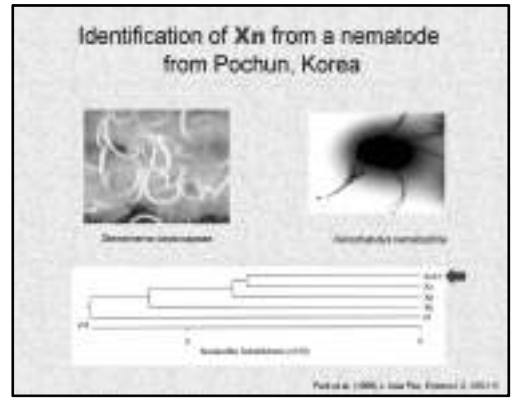

SehcGPCR is expressed only in oenocytoids (FISH analysis)
BF
FITC

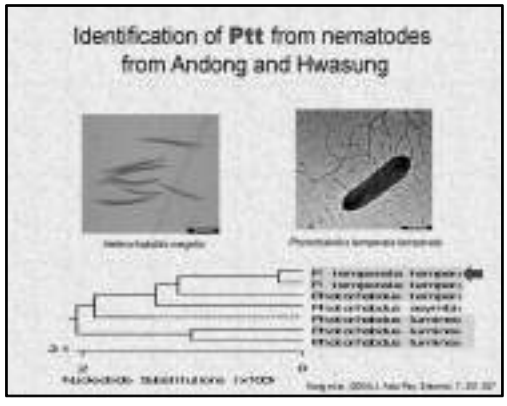

RNA interference of SehcGPCR suppresses binding affinity of hemocytes to PGE2
Control
RNAi

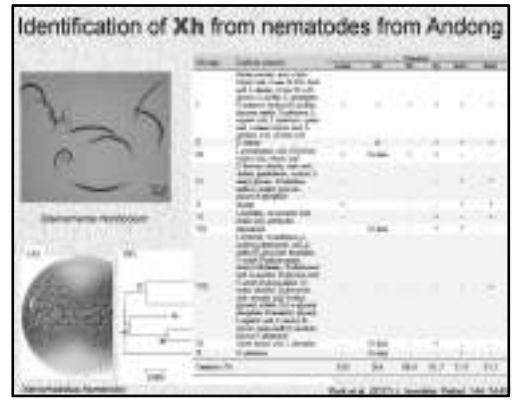


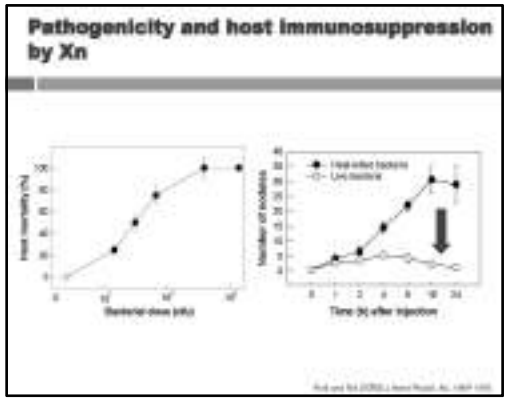
SIGNIFICANCE OF EICOSANOID SIGNALING IN INSECT IMMUNITY

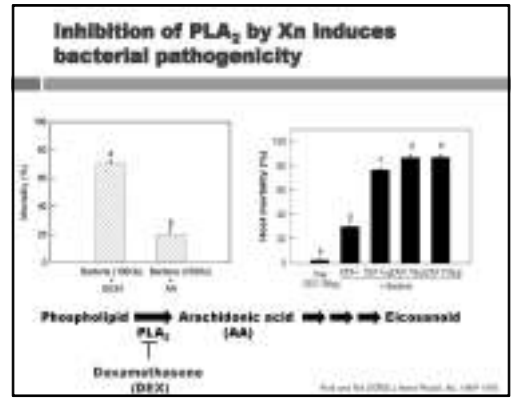


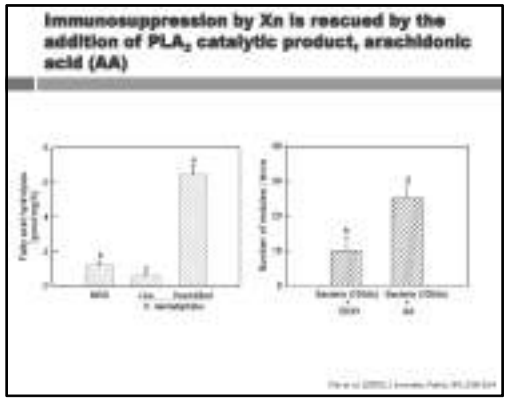


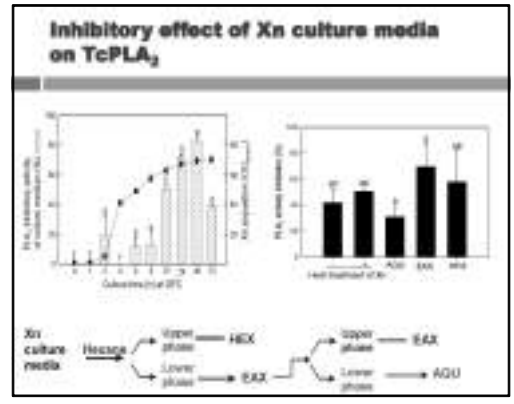


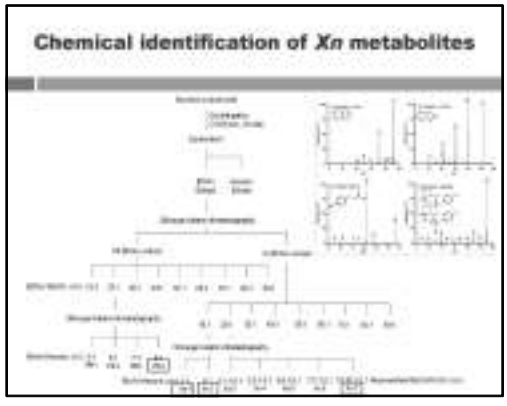


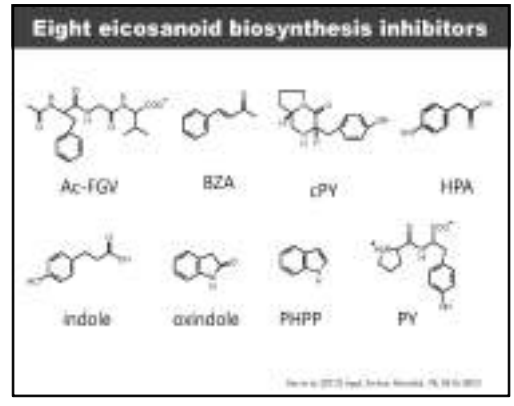

Pathogenicity and host immunosuppression by Xn


Inhibition of PLA2 by Xn induces bacterial pathogenicity
Phospholipid
PLA2
Arachidonic acid
(AA)
Eicosanoid
Dexamethasone
(DEX)

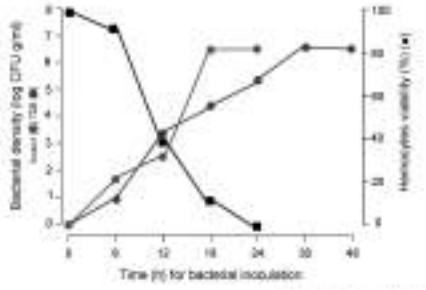

Immunosuppression by Xn is rescued by the addition of PLA2 catalytic product, arachidonic acid (AA)


Inhibitory effect of Xn culture media on TcPLA2
Xn culture media
Hexane
Upper phase
HEX
Lower phase
EAX
Upper phase
EAX
Lower phase
AQU

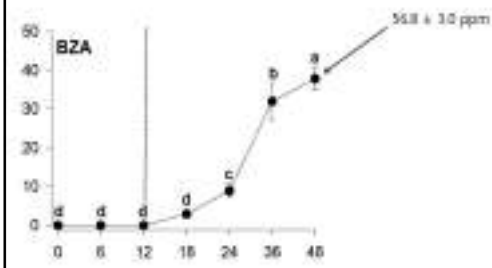

Chemical identification of Xn metabolites


Eight eicosanoid biosynthesis inhibitors
Ac-FGV
BZA
cPY
HPA
indole
oxindole
PHPP
PY

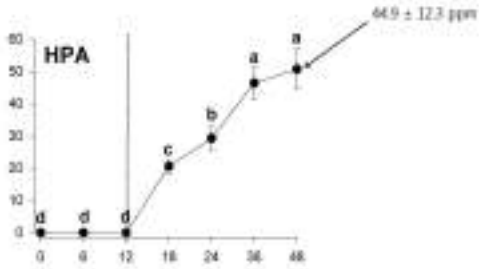
Bacterial growth and hemolytic activity



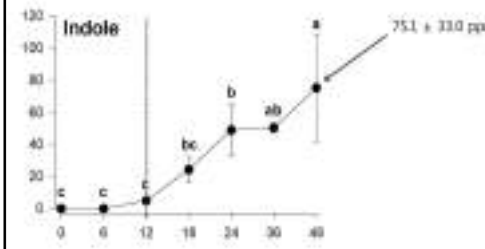
BZA



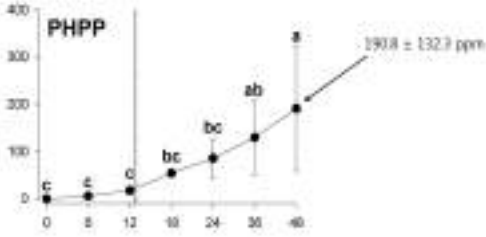
HPA



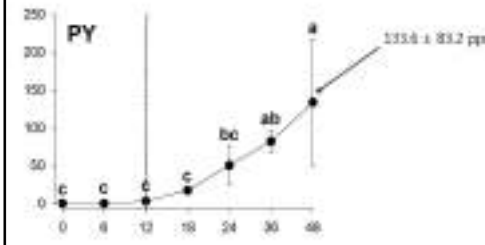
Indole



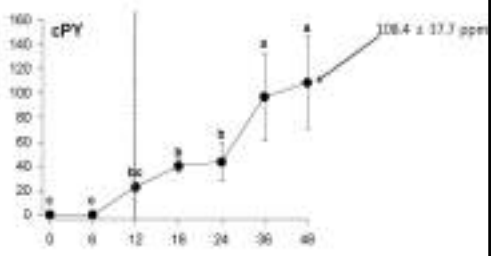
PHPP



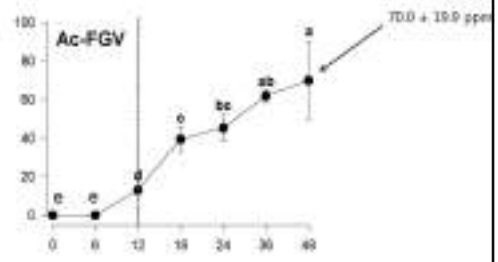
PY



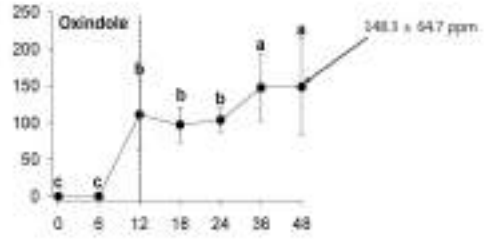
cPY



Ac-FGV



Oxindole



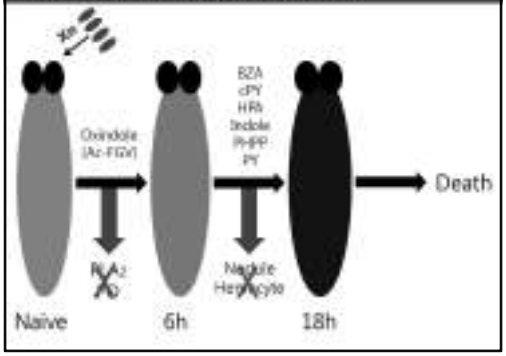
Time table of Xn metabolites

Growth phase	Metabolites
Initial (0 ~ 12 h)	(Ac-FGV)
	Oxindole
Late (12 ~ 24 h)	BZA
	cPY
	HPA
	Indole
	PHPP
	PY

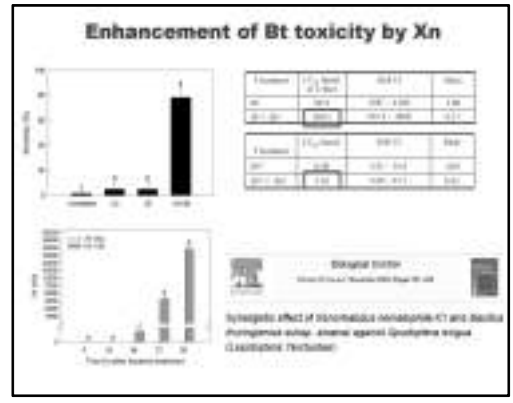
Biological functions of Xn metabolites

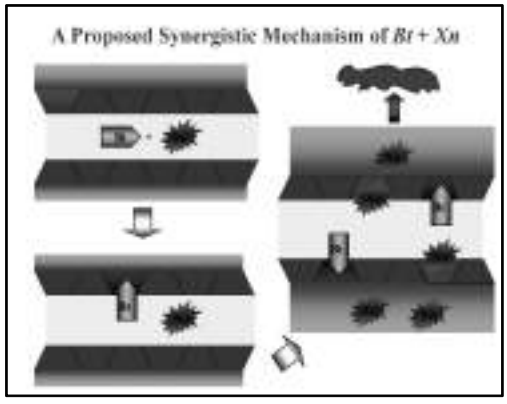
Immune target	Metabolite		Median inhibitor concentration (ppm)	
PAO	oxindole	BZA	1.07 (0.25 ~ 3.06)	1.88 (0.06 ~ 15.62)
PO	oxindole	P-HPP	0.85 (0.01 ~ 0.17)	0.09 (0.02 ~ 0.32)
Nodulation	BZA	HPA	1.47 (0.06-2.80)	23.82 (11.89 ~ 7.06)
Hemolysis	BZA	PY	2.28 (0.04 ~ 26.73)	1.75 (1.02 ~ 2.84)

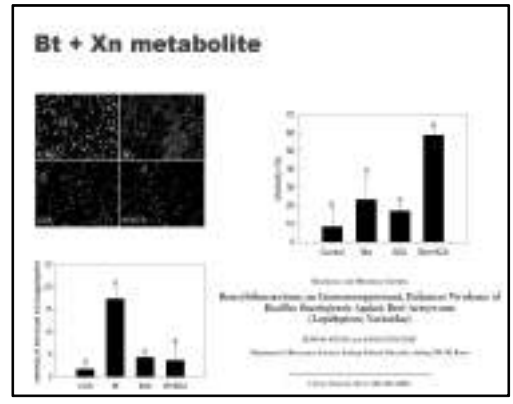
Time-course of pathogenicity

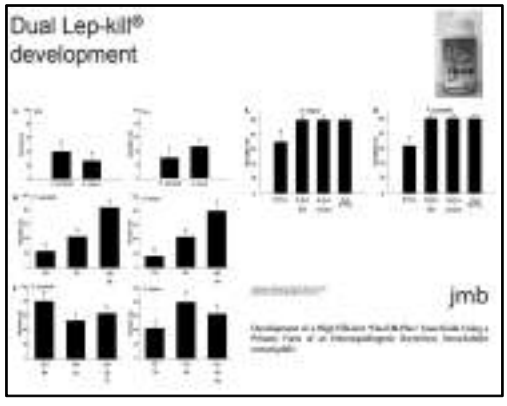


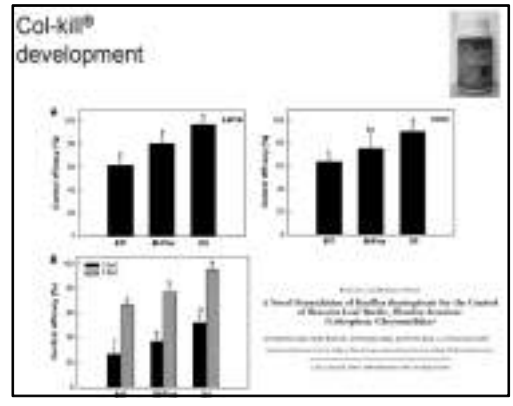
APPLICATION OF EBI TO CONTROL INSECT PESTS

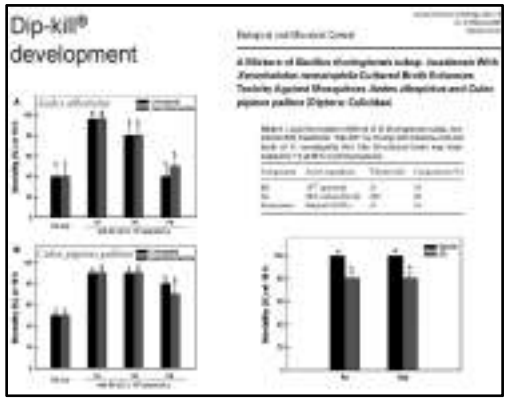












FUTURE DIRECTION:
IDENTIFICATION OF NOVEL EBI USING PLA_2 AS A MOLECULAR SCREENING SYSTEM

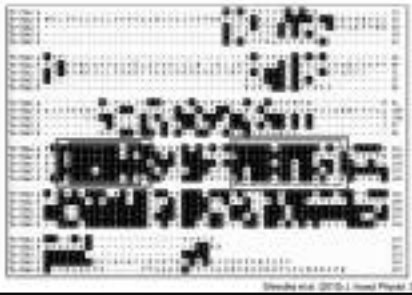
PLA_2

- A superfamily
- At least 15 different groups (Schaloske and Dennis, 2006)
- Three types
  - sPLA2 – Groups I, II, III, V, IX, X, XI, XII, XIII, XIV
  - iPLA2 – Group VI
  - cPLA2 - Group IV

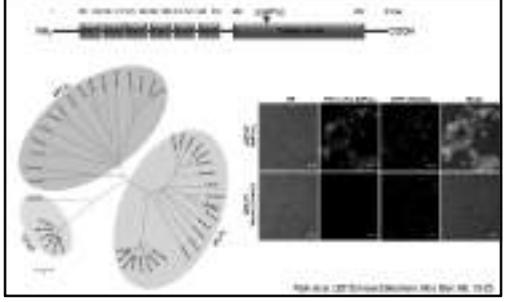
Insect PLA_2

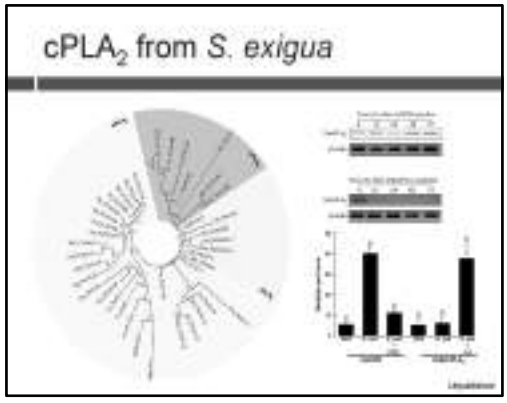
- Venom PLA_2s from hymenopteran insects
- Nonvenom and immune-associated PLA_2s
  - Biochemical evidences
  - Molecular identification
    - *Tribolium castaneum* – 4 $sPLA_2$ (Shrestha et al., 2010)
    - *Spodoptera exigua* – $iPLA_2$ (Park et al., 2015)
    - *Spodoptera exigua* – $cPLA_2$ (Unpublished)

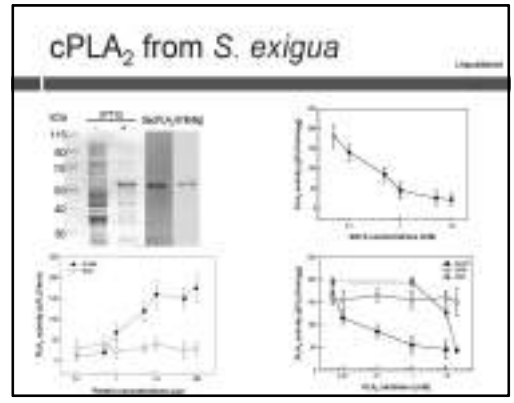
$sPLA_2$s of *T. castaneum*

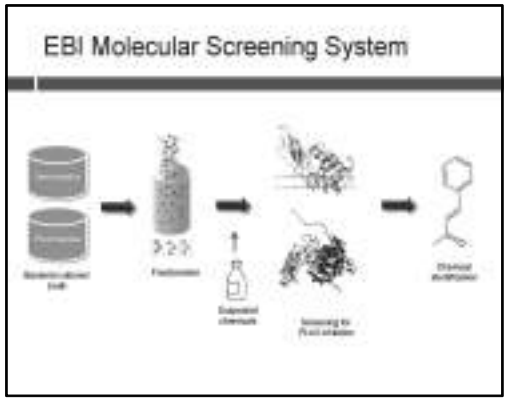


$iPLA_2$ from *S. exigua*




cPLA$_2$ from *S. exigua*


cPLA$_2$ from *S. exigua*


EBI Molecular Screening System

국립안동대학교
ANDONG NATIONAL UNIVERSITY

Oral presentation time table

[2017. April 7(Fri)]

	Symposia I (Grand ballroom I)	Symposia II (Grand ballroom II)	Symposia III (Notos)	Symposia IV (Zepiros)
	Chemistry I	Chemistry II	Toxicity	Biological Efficacy
Chairman	Prof. Lim (CNU)	Prof. Keum (KKU)	Dr. Sung (ESTech)	Prof. Kim (CBNU)
09:00~09:15	Seong-Hyun Hong (KNU)	Yi-Seul Jang (NIAS)	Chaw Ei Htwe Maung (Myanmar MOE)	Hee Nam Lim (KRICT)
09:15~09:30	Se-Yeon Kwak (KNU)	Md. Humayun Kabir (Univ. of Dhaka, Bangladesh)	Kwon BoWon (Kyung Nong)	Jae Woo Han (KRICT)
09:30~09:45	Abd Elaziz Sulieman Ahmed Ishag (Univ. of Khartoum, Sudan)	Md. Musfiqur Rahman (Univ. of Dhaka, Bangladesh)	Kwon BoWon (Kyung Nong)	Young Sook Kim (KRICT)
09:45~10:00	Seok Chang Su (Kyung Nong)	A. K. M. Mydul Islam (Univ. of Dhaka, Bangladesh)	Jin-Ho Kim (Hankook Samgong)	Dong Woon Lee (KNU)
10:00~10:15	Coffee break			
10:15~10:30	Jong-Soo Lim (Moghu Research)	DanBi Kim (NIAS)	Are-Sun You (NIAS)	Hyun-Seok, Koh (Kyung Nong)
10:30~10:45	Ahmed Zuhair Ismael (Iraq MOA)	Dong Yeol Lee (GNU)	Park Soojin (NIAS)	Dae-Hong Lee (Kyung Nong)
10:45~11:00	Sang-Hyeob Lee (KNU)	Min Woo Jung (SNU)	Park Soojin (NIAS)	Young-Su Lee (Gyeonggi-do Agricultural Research)
11:00~11:15	Hyo-Sub Lee (NIAS)	Minjun Kim (Farm Hannong)		Young-Bin Kwon (Kyung Nong)
11:15~11:30	Hansun Kwon (ThermoFisher)	Min Kyoung Paik (NIAS)		Kye-Hwan Lee (Kyung Nong)

Chemistry I

〈A-01〉 09:00~09:15

Comparison of Dissipation Ratio between Metconazole and Myclobutanil in Dropwort

Seong-Hyun Hong*, Jeong-In Hwang, Sang-Hyeob Lee, Se-Yeon Kwak, Min-Su Kang, Ja-Gun Kang, Jun-Sang Ryu, Kee-Sung Kyung[1], Jang-Eok Kim
School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea
[1]Environmental Sciences, Chungbuk National University, Cheongju 34134, Korea / 86

〈A-02〉 09:15~09:30

Degradation Patterns of Insecticide Dinotefuran in Soil

Se-Yeon Kwak*, Jeong-In Hwang, Sang-Hyeob Lee, Min-Su Kang, Jun-Sang Ryu, Seong-Hyeon Hong, Ja-Gun Kang, Jang-Eok Kim
School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea / 87

〈A-03〉 09:30~09:45

Establishment of Pre-Harvest Residue Limits of Picoxystrobin and Sulfoxaflor on Cucumber(*Cucumis sativus* L.)

Hee-Gon Kim[1], Ji-Yoon Kim[1], Kyung-Jin Hur[3], Abd Elaziz Sulieman Ahmed Ishag4*, Jang Hyun Hur[1,2,3]
[1]School of Natural Resources and Environmentral Science, Kangwon National University
[2]Environment Friendly Agricultural Products Safety Center, [3]Environment Friendly Agricultural Food Safety Center, 4Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Shambat, Sudan / 88

〈A-04〉 09:45~10:00

Optimization of pre-treatment method using dry ice of crop residue sample

Seok Chang Su*, Ji Kwang Yong, Lim Su Hwan, Jeon Sang Oh, Choi Jin Surk, Oh Ju Hwan, Lee Jae Yeong, Kim Joo Kyung
Safety Research Team, Central Research Institute, Kyung Nong Co., Ltd. / 89

〈A-05〉 10:15~10:30

Photodegradation of Methiozolin on Soil

Jong-Soo Lim*, Ki-Hwan Hwang, Sung-Hun Kim, Suk-Jin Koo, Jang-Hyun Hur[1]
Moghu Research Center Ltd., [1]School of Natural Resources and Environmental Science, Kangwon National University / 90

〈A-06〉 10:30~10:45

Residual Characteristics of Fungicide Fluquinconazole by Shapes of Crop

Ahmed Zuhair Ismael[1], Min-Su Kang, Jeong-In Hwang, Sang-Hyeob Lee, Se-Yeon Kwak, Jun-Sang Ryu, Seong-Hyeon Hong, Ja-Gun Kang, Kee-Sung Kyung[2], Jang-Eok Kim
School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea
[1]National Center for Pesticide Control, Ministry of Agriculture, Baghdad, Iraq
[2]Department of Environmental and Biological Chemistry, Chungbuk National University, Cheongju 28644, Korea / 91

〈A-07〉 10:45~11:00

Improvement of Extraction Efficiency for Multi-residue Analysis Methods of Pesticides in Agricultural Products with QuEChERS Method

Sang-Hyeob Lee*, Ahmed Zuhair Ismael[1], Jeong-In Hwang, Se-Yeon Kwak, Min-Su Kang, Jun-Sang Ryu, Ja-Gun Kang, Sung-Hyun Hong, Jung-Ah Do[2], Moon-Ick Jang[2], Gyu-Seek Rhee[2], Young-Deuk Lee[3], Jang-Eok Kim
School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea
[1]National Center for Pesticide Control, Ministry of Agriculture, Baghdad, Iraq
[2]National Institute of Food and Drug Safety Evaluation, Osong 28159, Korea
[3]Division of Life and Environment+al Science, Daegu University, Gyeongsan 38911, Korea / 92

〈A-08〉 11:00~11:15

Comparison of time sequential residual amount of systemic pesticides between different treatments in strawberries

Hyo-Sub Lee, Ji-Seon Shin, Su-Myeong Hong, Hye-Young Kwon, Dan-Bi Kim, Byeong-Chul Moon
National Institute of Agricultural Sciences, Chemical Safety Division / 93

〈A-09〉 11:15~11:30

Quantitative and Qualitative Confirmation of Pesticides in Food Extract Using a Hybrid Quadrupole-Orbitrap Mass Spectrometer

Hansun Kwon
Thermo Fisher Scientific Korea / 94

Chemistry II

〈B-01〉 09:00~09:15

A comparative study of pesticide residues in leafy lettuce and head lettuce

Yi-Seul Jang[1*], Geun-Hwan Gil[1], Kyeong-Aa Son[1], Yang-Bin Ihm[1], Chan-Sub Kim[1], Jae-Han Shim[2]
[1]Department of Agro-Food Safety / Agromaterial Assessment Division, NAS, RDA
[2]Department of Agricultural Chemistry, College of Agriculture and Life Science, Chonnam National University ,Gwangju, korea / 95

〈B-02〉 09:15~09:30

Residue analysis of picoxystrobin in oriental melon (*Cucumis melo* L. var. *makuwa*) using gas chromatography coupled with electron capture detector and mass confirmation- apply to dissipation kinetics, PHRL and risk assessment

Md. Humayun Kabir*, Md.MusfiqurRahman, A. M. Abd El-Aty[1,2], Hyung Suk Chung, Han Sol Lee, Jae-Han Shim
Natural Products Chemistry Laboratory, College of Agriculture and Life Sciences, Chonnam National University
[1]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University
[2]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University University of Dhaka ,Bangladesh / 96

〈B-03〉 09:30~09:45

Simultaneous quantification of methiocarb and its metabolites, methiocarb sulfoxide and methiocarb sulfone, in five food products of animal origin using tandem mass spectrometry

Md. Musfiqur Rahman*, A. M. Abd El-Aty[1,2], Hyung Suk Chung, Md. Humayun Kabir, Han Sol Lee, Jae-Han Shim
Natural Products Chemistry Laboratory, College of Agriculture and Life Sciences, Chonnam National University
[1]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea
[2]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University University of Dhaka ,Bangladesh / 97

〈B-04〉 09:45~10:00

Search of matrix components in spinach after QuChERS cleanup for pesticide multiresidue analysis by mass spectrometry analyses

A. K. M. Mydul Islam*, Hyeyoung Kwon, Su-Myeong Hong, Hyo-Sub Lee and Byeong-Chul Moon
Rural Development Administration, National Institute of Agriculture Sciences
*University of Dhaka, Bangladesh / 98

〈B-05〉 10:15~10:30

Volatilization of Sprayed Pesticides in Greenhouse Using a Lysimeter

DanBi Kim*, Taek-Kyum Kim[1], Yong-Duk Jin, Su-Myung Hong, Hye Young Kwon, Jin-ho Ro, Sung-Jin Lim, Byeong-Chul Moon
National Institute of Agriculture Sciences, RDA, Wanju, Korea
[1]Research Policy Bureau, RDA, Jeonju, Korea / 99

〈B-06〉 10:30~10:45

Residual Behaviour of Thiamethoxam and Its Metabolite in Pepper(*Capsicum annuum*) Plant under Various Conditions

Dong Yeol Lee[1,2*], Dong Kyu Jeong[1], Jin Hyo Kim[1]
[1]Divison of Applied Life Science, Gyeongsang National University, Jinju, Korea
[2]Gyeongnam Oriental Medicinal Herb Institute, Sancheong, Korea / 100

〈B-07〉 10:45~11:00

Dissipation characteristics of Cyantraniliprole and Its Metabolite in Minor Crop Millet during Cultivation

Min Woo Jung*, Jung Hak Lee, Hyejin Yu, Xiu Yuan, Jong Hwa Lee, Jiho Lee, Yongho Shin, Sujin Baek, Da Rae Jeon, Jeong-Han Kim
Department of Agricultural Biotechnology, Seoul national University / 101

〈B-08〉 11:00~11:15

Studies on Color Blending Trunk Injection Pesticides for Preventive of Pine Wilt Disease, Bursaphelenchus xylophilus on Pinus densifora

Minjun Kim*, Changseop Cho, Heegeun Ahn, Yongjun Kwon, Jaeyoung Song, Jongkeun Lee, Jisoo Lim, Jungkuk Eom, Yongoh Jang, Sang-Tae Seo[1]
FarmHannong Co.,Ltd.,
[1]Division of Forest Insect Pests & Diseases, Korea Forest Research Institute / 102

〈B-09〉 11:15~11:30

Development and application of Agricultural Products Risk Assessment System (APRAS)

Min Kyoung Paik*, Chinai Choi[1], Byung-Jun Park, Kyongmi Chon, Kyung-Hun Park, Byeong Chul Moon, Wook-Han Kim
Chemical Safety Division, National Institute of Agricultural Sciences, 166, Nongsaengmyeong-ro, Iseo-myeon, Wanju-gun, Jeollabuk-do, Korea
[1]KNA Consulting Co., 314, Gwanggyojungang- ro, Suji-gu, Yongin-si, Gyeonngi-do, Korea / 103

Toxicity

〈C-01〉 09:00~09:15

Oxidation Products, Acute Toxicity and Estrogenic Activity for Heterogeneous Fenton Oxidation of Bisphenol A

Chaw Ei Htwe Maung[1*], Il Kyu Cho[2], Byung-Jun Park[3], Kyu Hyuck Chung[4], Qing X. Li[5], Eunsung Kan[6]
[1]Biotechnology Research Department, Ministry of Education, Kyaukse, Mandalay Division, Myanmar
[2]Bio Control Research Center, Jeonnam Bioindustry Foundation, 495 Immyeon-ro, Gokseong, Jollanam-do 57510, Republic of Korea
[3]Department of Agro-Food Safety and Crop Protection, National Academy of Agricultural Science, Jeonju, Jollabuk-do 55365, Republic of Korea
[4]School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do 16419, Republic of Korea
[5]Department of Molecular Biosciences and Bioengineering, University of Hawaii, Honolulu HI 96822, USA 6Texas A&M AgriLife Research Center, Stephenville TX 76401, USA / 104

〈C-02〉 09:15~09:30

The Development of acute toxicity test method for *Macrobrachium rosenbergii*

Kwon BoWon*, Kim Jin, Ryu Seong Min, Lee Young-Jun, Kim Joo Kyung
Safety Research Dept - Kyung Nong Central Research Institute / 105

〈C-03〉 09:30~09:45

The fertilization action research of *Apis mellifera* depending on the plant secondary metabolite

Kwon Bowon*, Kim Jin, Ryu Seong Min, Lee Young-Jun, Kim Joo Kyung
Safety Research Dept - Kyung Nong Central Research Institute / 106

〈C-04〉 09:45~10:00

A study on the replaceability and the use of reference chemical in ecotoxicity test

Jin-Ho Kim*, Chul-Han Bae, Gwan-Seop Shin, Sung-Min Ryu[1], Jin Kim[1]
Hankooksamgong Agricultural Research Center [1]Kyungnong Central Research Institute / 107

〈C-05〉 10:15~10:30

Development of guidance for toxicological assessment in pesticide formulation change

Are-Sun You*, Jin a Oh, Sujin Park, Youmi Jo, Je Bong Lee, Yeon-Ki Park, Huenyoung Ha
National Institute of Agricultural Sciences / 108

〈C-06〉 10:30~10:45

Teratotoxicity study on Zebrafish Embryo of Cartop hydrochloride

Park Soojin*, Lee jin young, Mihye Jeong[1], Oh Jina, You Are-Sun, Youmi Jo, Je-bong Lee, Park Yeon-ki
Department of Agro-Food Safety & Crop Protection, National Institute of Agricultural Science, Rural Development Administration
[1]Research policy bureau, Rural Development Administration / 109

〈C-07〉 10:45~11:00

Toxicity evaluation and establishment of safety reference dose of fluthiacet-methyl

Park Soojin*, Baek Changhee[1], Lee jin young, You Are-Sun, Oh Jina, Youmi Jo, Je-bong Lee, Park Yeon-ki, Lee Keong il
Department of Agro-Food Safety & Crop Protection, NAS, RDA, [1]FMC Korea Ltd. / 110

Biological Efficacy

〈D-01〉 09:00~09:15

Exploring New Class of IGRs: Finding Hit K-222212 and Hit Optimization by Its Derivatives

Hee Nam Lim[1*], Seok Hee Lee[2], Seonghoon Kang[1], Yun Kyung Hwang[1], Jae Young Choi[2], Yeon Ho Je[2], Ill Young Lee[1], Young Kwan Ko[1]
[1]Center for Eco-Friendly New Materials, Korea Research Institute of Chemical Technology (KRICT)
[2]Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University / 111

〈D-02〉 09:15~09:30

Antifungal Activity of *Maesa japonica* Extracts and Isolated Compounds against Plant Pathogenic Fungi

Jae Woo Han
Center for Eco-Friendly New Materials, Korea Research Institute of Chemical Technology (KRICT), Daejeon 34114, Republic of Korea / 112

〈D-03〉 09:30~09:45

***Streptomyces* sp. KRA329 Producing Herbicidal Metabolites as Potential Biocontrol Agent**

Young Sook Kim*, Jae Deok Kim, Young Kwan Ko and Jung Sup Choi
Eco-friendly and New Materials Research Center, KRICT / 113

〈D-04〉 09:45~10:00

Change of responses of *Colletotrichum gloeosporioides* isolated from persimmon trees to MBC and dithiocarbamate fungicides

Hyeon Jeong An[1], Hee Been Na[1], Tae Heon Lim[1,2], Taehyun Chang[3], Janghoon Song[4], Dong Woon Lee[1*]
[1]Majorof Applied Biology, Kyungpook National University
[2]Nousbo Co., Ltd.,
[3]Major of Plant Resources and Environment, Kyungpook National University
[4]Pear Research Institute, National Institute of Horticultural and Herbal Science.
*Corresponding author / 114

〈D-05〉 10:15~10:30

Biological activity and characteristics of new fungicide KNF1036

Hyun-Seok, Koh*, Ho-Cheol Shin, Moo-il YEO, Cheol Jang, Ju-Kyung Kim
Central Research Institute, Kyung Nong Corporation / 115

〈D-06〉 10:30~10:45

Fumigation effects of Paladin® on trees after forest tending works

Dae-Hong Lee[1*], Jin-Won Seo[1], Won-Seok Choi[1], Tae-Ki Ha[1], Cheol Jang[1], Ju-Kyeong Kim[1], Dong-Geun Lee[2]
[1]Central Research Institute, Kyung Nong Corporation
[2]Yangsan National Forest Station, Southern Regional Office of Forest Service / 116

〈D-07〉 10:45~11:00

Insecticidal Susceptibility of Western Flower Thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) of Horticultural Crops in Gyeonggi Area

Young-Su Lee[1*], Hyun-Ju Lee, Soon-Sung Hong, Chang-Sung Kang
[1]Department of Agricultural Biology, Gyeonggi-do Agricultural Research and Extension Services / 117

〈D-08〉 11:00~11:15

Synthesis and Development of A New Selective Ryanodine Receptor Modulator Insecticide

Young-Bin Kwon*, Won-Hyung Lee, Joo-Kyung Kim, Hyeong-Min Kim
Central Research Institute, Kyung Nong Co., Kyungju 780-110, Korea / 118

〈D-09〉 11:15~11:30

Herbicidal Activity of Metazosulfuron against Sulfonylurea-Resistance Perennial Weed in Greenhouse

Kye-Hwan Lee*, Kyung-Hyun. Kim, Duk-Hwan Kim, Cheol Jang, Ju-Kyung Kim
Kyung Nong Corporation / 119

A-01

Comparison of Dissipation Ratio between Metconazole and Myclobutanil in Dropwort

Seong-Hyun Hong*, Jeong-In Hwang, Sang-Hyeob Lee, Se-Yeon Kwak, Min-Su Kang, Ja-Gun Kang, Jun-Sang Ryu, Kee-Sung Kyung[1], Jang-Eok Kim

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[1]Environmental Sciences, Chungbuk National University, Cheongju 34134, Korea

This study was to investigate the time dependent residual changes of systemic pesticides metconazole and myclobutanil in dropwort, and to compare the disspation rate and ratio with the initial residue based on the physicochemical characteristics of pesticides. Metconazole (20%, SC) and myclobutanil (6%, WP) were diluted 3,000 times and 1,500 times, respectively and sprayed 3 times at 7 days intervals on prior to harvest day. Residual amounts of metconazole and myclobutanil were analyzed by LC/UVD and GC/ECD, respectively. Recovery rates for residual analysis spiked with 0.4 and 2.0 mg/kg were 80.1-94.3%, and less than 10% of coefficient variation. Residual amounts of metconazole and myclobutanil were reduced from 2.15 to 0.87 and from 1.96 to 0.38 mg/kg, respectively. First order dissipation rate constants for metconazole was 0.008 and myclobutanil was 0.017. During the experimental period, the dissipation ratio versus initial residue amounts was found to be higher in myclobutanil (80.61%) than in metconazole (59.53%). Generally, pesticides with high solubility in water and low log P values are more likely to be lost due to high mobility in water or soil after spraying. The solubility of myclobutanil (132 mg/L at 20℃) was about 4 times higher than that of metconazole (30.4 mg/L at 20℃) and the log P value was 2.94 that is lower than metconazole (3.85). These results are consistent with above results, which suggests that the physicochemical properties of pesticides affect the residual characteristic.

Keyword : Dissipation ratio, Dropwort Metconazole, Myclobutanil, Physicochemical properties
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A-02

Degradation Patterns of Insecticide Dinotefuran in Soil

Se-Yeon Kwak*, Jeong-In Hwang, Sang-Hyeob Lee, Min-Su Kang, Jun-Sang Ryu, Seong-Hyeon Hong, Ja-Gun Kang, Jang-Eok Kim

School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea

To elucidate the degradation pattern of dinotefuran in soil, the residue amounts of dinotefuran (DIN) and its metabolites (DN, UF and MNG) were investigated. DIN was treated with concentration of 2 mg/kg on the radish cultivated and non-cultivated soil before seeding radish. The initial residue amounts of DIN was 2.01 mg/kg for radish cultivated soil and dissipation pattern shows a fast decrease during 21 and 30 days after treatement. Finally, residue amounts of DIN decreased to 100% of initial amount after 60 days with below limit of quantitaion (LOQ) and which result in the formation of UF upto 0.21 mg/kg. In case of non-cultivated soil, initial residue amounts of DIN was 2.00 mg/kg and it steadily decreased 84% with 0.32 mg/kg at 70 day. UF as well as DN were detected after 50 days in soil. Based on these different dissipation patterns of DIN in outdoor test, this study was conducted additional indoor test if these degradation patterns of DIN were due to microorganisms. The experiment was carried out by dividing into sterilized and unsterilized soil using the same soil as the outdoor test. DIN was spiked with 2 mg/kg in each of 20 g soil and analysed both of soil at 0, 1, 3, 7, 14, 21, 30, 45 and 60 day after treatment. The residue amounts of DIN in sterilized and unsterilized soil at 0 day were 1.97 and 1.99 mg/kg, and these were decreased with a similar decline curve by 45 and 54% at end of experiment day, respectively. The decrease rate of DIN in unsterilized soil was higher than that of DIN in sterilized soil DN and UF remained 0.01 and 0.03 mg/kg, respectively, in unsterile soil with microorganisms, and MNG was not detected in the same manner as the results of outdoor experiments respectively. From above results, the residue amounts of DIN tended to decrease rapidly in the soil, and degradation by soil microorganism was one of the factors that affected the decrease tendency.

Keywords : Dinotefuran, Degradation pattern, Microorganism, Soil, Sterilize
Corresponding author E-mail : jekim@knu.ac.kr

A-03

Establishment of Pre-Harvest Residue Limits of Picoxystrobin and Sulfoxaflor on Cucumber(*Cucumis sativus* L.)

Hee-Gon Kim[1], Ji-Yoon Kim[1], Kyung-Jin Hur[3], Abd Elaziz Sulieman Ahmed Ishag[4*], Jang Hyun Hur[1,2,3]

[1]School of Natural Resources and Environmentral Science, Kangwon National University,
[2]Environment Friendly Agricultural Products Safety Center,
[3]Environment Friendly Agricultural Food Safety Center,
[4]Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Shambat, Sudan

In this study, the residue patterns of picoxystrobin and sulfoxaflor registered in the cucumber were investigated to predict pre-harvest residue limit (PHRL). The pesticides were sprayed on cucumber grown in greenhouses of two different regions. The samples were harvested at 0, 1, 2, 3, 5, 7, 10 days after the spray and the residues were analyzed by LC-MS/MS. The limit of quantification (LOQ) was 0.005 mg kg^{-1} for picoxystrobin and sulfoxaflor. The recovery levels were 94.7±0.8% and 85.6±0.2% for picoxystrobin, and 86.4±2.4% and 92.5±2.6% for sulfoxaflor with spiked levels of 0.05 and 0.25 mg kg^{-1}, respectively. The respective values of biological half-lives for field 1 and field 2 were 2.2 and 2.6 days for picoxystrobin, 5.2 and 4.4 days for sulfoxaflor. According to current results, we recommend that the levels of PHRL on a cucumber are 1.29 mg kg^{-1} for picoxystrobin and 9.52 mg kg^{-1} for sulfoxaflor at 10 days before harvest time, correspondingly. This study may contribute to the safety control management of agricultural products in Korea.

A-04

Optimization of pre-treatment method using dry ice of crop residue sample

Seok Chang Su*, Ji Kwang Yong, Lim Su Hwan, Jeon Sang Oh, Choi Jin Surk, Oh Ju Hwan, Lee Jae Yeong, Kim Joo Kyung

Safety Research Team, Central Research Institute, Kyung Nong Co., Ltd.

As a result of analyzing the results of GLP (Good Laboratory Practice) system for analysis of crop residue, it is required to prepare a method for ensuring reproducibility through homogenization of samples of crops with high moisture content. In this study, the optimum method of pre-treatment of the sample was established and the homogenization method using dry ice was compared with method of the cutting homogenization.

In order to establish a standard for the sample pre-treatment method, the test was carried out with as follows. First, dry ice amount required for pre-treatment of grape, plum, peach, and watermelon samples, and changes in weight and time-dependent properties during the storage of the pretreated samples in the freezer were investigated. Second, the residual amount was measured in apple and persimmon for comparing the results between the sample the cut homogenization method and the dry ice homogenization method.

As a result of the test, regardless of the water content of each crop, the amount of dry ice used was equal to or greater than the weight of the sample, so that powdering and homogenization were possible, and the pulverized sample was gradually changed to a liquefied state after 30 minutes at room temperature. It was also found that the weight of the sample decreased by 28 ~ 33% during 12 hours in the freezer. In the comparison of pre-treatment method using apple and persimmon, dry ice homogenization method was 1.4 ~ 8 times better than the cutting homogenization method and this method is introduced as a sample pre-treatment method.

A-05

Photodegradation of Methiozolin on Soil

Jong-Soo Lim*, Ki-Hwan Hwang, Sung-Hun Kim, Suk-Jin Koo, Jang-Hyun Hur[1]

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Methiozolin is a new turf herbicide controlling annual bluegrass in various cool- and warm-season turfgrasses. This study was conducted to investigate the photodegradation of methiozolin on soil under a simulated sunlight condition using two radiolabeled tracers, [Phenyl-^{14}C] and [Isoxazole-^{14}C]methiozolin. The overall mass balance was 94.3 - 105.7% and 93.4 - 105.3% of applied radioactivity under the light irradiation and the dark control throughout the study, respectively. Under the light condition, [Phenyl-^{14}C] and [Isoxazole-^{14}C]methiozolin degraded and accounted for 70.9 and 62.2% of the applied at 32 days after treatment (DAT), respectively. The calculated half-lives were 77 and 46 days for [Phenyl-^{14}C] and [Isoxazole-^{14}C]methiozolin, respectively. Nonextractable radioactivity increased up to 18.9 and 24.2% of the applied for [Phenyl-^{14}C] and [Isoxazole-^{14}C]methiozolin at 32DAT, respectively. A small amount (<1.3%) and no volatile compound were produced from both the treated throughout the study. Six to seven minor metabolites (5.0%) were produced from both the treated soils throughout the study. On the other hand, under dark condition, [^{14}C]methiozolin was degraded much slower than the light condition (84.9 - 91.7% remained at 32DAT). These results suggest that photodegradation may be a major route of elimination of methiozolin in the environment.

A-06

Residual Characteristics of Fungicide Fluquinconazole by Shapes of Crop

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As pesticides sprayed on crops for controlling insect and disease could be remained in crops, it is important to understand residual characteristics of pesticides for ensuring the safety of harvested crops. The shapes of crops may be one of major factors determining residual amounts of pesticides in them. In this study, residual characteristics of fungicide fluquinconazole were investigated in leafy vegetables, such as Korean cabbage and shallot, which have different in surface area. Commercial fluquinconazole (10%, SC) was diluted 1,000 times with water and sprayed 3 times at interval of 7 days prior to harvest. The crops sprayed with fluquinconazole were harvested collectively on the last day of spraying. The residual amounts of fluquinconazole in the samples were analyzed with the pesticide residue analysis method, established by Ministry of Food and Drug Safety. The recovery rates of fluquinconazole spiked with concentrations of 0.2 and 1.0 mg/kg in Korean cabbage and shallot were ranged from 82.5 to 97.4%. The residue amounts of fluquinconazole in Korean cabbage and shallot ranged from 3.45 to 9.30 mg/kg and 0.89 to 4.44 mg/kg, respectively. Because of Korean cabbage have large surface area than shallot, Korean cabbage may have high adhesive property for pesticides than shallot. So, residual amounts of fluquinconazole in Korean cabbage was higher than shallot. From this results, it was found that the shapes of the crops greatly affect the residueal amounts of sprayed pesticide.

Keywords : Adhesive property, Fluquinconazole, Korean cabbage, Shallot, Surface area
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A-07

Improvement of Extraction Efficiency for Multi-residue Analysis Methods of Pesticides in Agricultural Products with QuEChERS Method

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This study was conducted to compare with the AOAC QuEChERS method and the improved method for reduced a matrix effect in three agricultural products, such as orange, green pepper and brown rice. AOAC QuEChERS method involves single-phase extraction of 10 g sample with 10 mL acetonitrile. On the other hand, the improved method extracted 10 g of sample with 50 mL acetonitrile. Clean-up step used in the AOAC QuEChERS method was the d-SPE using PSA, but C18 of SPE cartridge was used for the improved method. As a result, both methods were similar in linearity and limit of quantification. The ratios that satisfy the Codex guideline were 85% of total pesticides for AOAC QuEChERS method and 88% for the improved method. Matrix effects showed different results, however, the improved method was found to produce least matrix effect. Average matrix effect of AOAC QuEChERS method was -38%, and those of the improved method was -10%. Comparison of matrix effects for different agricultural products showed high variability for some residues such as dimethylthiocarbamate. It was indicated that the amount of co-extracting compounds that cause ionization suppression of pesticides depends on the agricultural products as well as on the sample preparation method employed. As a conclusion, the improved method appears to be a more suitable method because it can reduce the matrix effect rather than the QuEChERS method.

Key Words : Agricultural products, LC-MS/MS, Matrix effect, QuEChERS, Residue analysis
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A-08

Comparison of time sequential residual amount of systemic pesticides between different treatments in strawberries

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Strawberries are one of the main commodities in Korea and every year over 30 million dollar is earned through its exportation. However, the safety has recently become an important issue in the agro-trade, so it is important to produce safety Agricultural products. To confirm its safe production, the routine analysis of pesticides should give highest priority. In this study, time sequential residual amount of pesticedes were compared between different treatments in strawberries. As strawberries can be cultivated using hydroponic cultivation, so it is possible to use the intake treatment. The use of intake treatment can be lower amount of residual than using foliar treatment. In this study, we carry out experiment which compare the residual amount of different pesticide treatment by using two pesticides (azoxystrobin, carbendazim). Pesticides were treated with the safety standard and 2 times and then samples were collected 0, 1, 3, 6, 9, 13, 16 and 21 day and stored at −18 ℃. Samples were analyzed by LC and LC-MS/MS after pretreatment of LLE (liquid-liquid extraction) and QuEChERS. The strawberries treated with intake were detected to be less than Korean MRL in all treatment days. The difference of residual amounts in strawberry leaf treated between foliar and intake showed 50 and 88 times lower in case of azoxystrobin and carbendazim.

A-09

Quantitative and Qualitative Confirmation of Pesticides in Food Extract Using a Hybrid Quadrupole-Orbitrap Mass Spectrometer

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As world agricultural trade has expanded and concerns over food safety have grown, the enforcement of stricter pesticide regulations has become of utmost importance. In 2006, Japan introduced the Positive List System that established maximum residue levels (MRLs) for hundreds of agricultural chemicals in food, including approximately 400 pesticides, and set a uniform limit of 10 ㎍/kg (ppb) for chemicals for which MRLs have not been determined.1 In 2008, the European Parliament implemented Regulation (EC) No. 396/2005, which harmonized all pesticide MRLs for European Union (EU) member states and set default limits of 10 ㎍/kg for all pesticide/commodity combinations for which no MRLs have been set.

A pesticide safety review of about 1,000 active substances on the market was mandated by EU Directive 91/414/EEC and, upon its completion in 2009,led to the approval of only about 250 substances and effectively set the permissible levels of over 700 de-listed pesticides to the default limit. The EU and Japanese regulations are among the most stringent in the world and have fueled the need for faster and more sensitive analytical methods for cost-efficient, high-throughput screening and quantitation of multi-class pesticide residue.

Here, a method and performance utilizing the Q Exactive™ hybrid quadrupole- Orbitrap™ mass spectrometer is described. It consists of a generic chromatographic method and a full-scan data-dependent MS/MS (FS-ddMS2) mass spectrometric method with library searching and fragment confirmation. The FS-ddMS2 approach was used to generate calibration curves and analyze samples for targeted known compounds. In the typical acquisition setup demonstrated here, a simple full-scan data-dependent MS/MS experiment was associated with new preset confirmation settings for easier and faster method development. This talk shows current status as the leading mass spectrometric technique for high-resolution, high mass accuracy quantitative analysis.

B-01

A comparative study of pesticide residues in leafy lettuce and head lettuce

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Leafy lettuce and head lettuce are biologically similar to each other, the occurrence of disease and insect pest is similar. Leafy lettuce and head lettuce are simultaneously registration of pesticides in Korea with only the efficacy test on leafy lettuce. In the case of pesticides residue study for registration of pesticides, extrapolation is allowed for tomato-cherry tomato, red pepper-sweet peppers and spring onion-green onion. Pesticide residues vary greatly depending on the initial coating weight of pesticides. Amount of pesticide residues in leafy lettuce are higher than head lettuce, because leafy lettuce is higher surface/wight than head lettuce. In this study, we conducted a comparative residue study for leafy lettuce and head lettuce to determine the extrapolatability between crops and to calculate the applicable coefficient for setting the pesticide residue MRLs. The test items are boscalid. triflumizole WP and bifenthrin. imidacloprid WP registered with leafy lettuce (including head lettuce) in Korea. Leafy lettuce and head lettuce were sprayed twice at intervals of 7 days, and samples were taken at 0, 1, 3, 5, and 7 days after the last treatment. When sprayed according to guidelines on safe use of pesticides (2 times 3 days before harvest), amount of residues in leafy lettuce was 37.6 times higher than head lettuce for boscalid, 43.2 times higher for triflumizole, 45.2 times higher for bifenthrin, and 39.2 times higher for imidacloprid. Regardless of the kinds of pesticides, the pesticide residual amount in leafy lettuce was about 40 times higher than in head lettuce. Therefore, amount of pesticide residues in head lettuce can be estimated with residue study in lettuce to registrate pesticide and set the MRLs.

B-02

Residue analysis of picoxystrobin in oriental melon (*Cucumis melo* L. var. *makuwa*) using gas chromatography coupled with electron capture detector and mass confirmation- apply to dissipation kinetics, PHRL and risk assessment

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This study was carried out to determine the residual amounts of picoxystrobin in oriental melon (*Cucumis melo* L.) grown under plastic house conditions at two different sites (site 1 and site 2, cultivated in April and September 2016, respectively). Samples collected over 10 days were extracted using acetonitrile and salting out (using solid sodium chloride) and purified using Florisil solid-phase extraction cartridges. The analyte was determined using gas chromatography-electron capture detection (GC-ECD) and field-incurred residues were verified using gas chromatography-mass spectrometry (GC-MS). The calibration curve was linear over the range 0.02-2.0 mg/L with a coefficient of determination of R^2= 0.9998. The limits of detection and quantification were 0.003 and 0.01 mg/kg, respectively. Recoveries, tested at three spiking levels, were satisfactory with rates in the range 87.7-101.5% and relative standard deviations $\leq$ 9.6. The dissipation half-lives were 3.4 and 3.7 days for sites 1 and 2, respectively. Hazard estimates obtained using hazard quotients (HQs) revealed no health risk from the suggested pesticide application dosage when considering an adult's body weight, oriental melon consumption, and the accepted daily intake of picoxystrobin.

B-03

Simultaneous quantification of methiocarb and its metabolites, methiocarb sulfoxide and methiocarb sulfone, in five food products of animal origin using tandem mass spectrometry

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A simultaneous analytical method was developed for determination of methiocarb and its metabolites, methiocarb sulfoxide and methiocarb sulfone; in five livestock products (chicken, pork, beef, table egg, and milk) using LC-MS/MS. Samples were prepared using acetonitrile and salts and purified with dispersive- solid phase extraction procedure (d-SPE). Seven point calibration curves were constructed separately in each matrix and good linearities were observed in each matrix-matched calibration curve with coefficient of determinants (R^2) $\geq$ 0.991. The limits of detection and quantification were 0.0016 and 0.005 mg/kg, respectively, for all tested analytes in various matrices. Method was validated in triplicate at three fortification level (equivalent to LOQ, 2×LOQ, and 10×LOQ) with recovery rate ranged between 76.35 - 118.03% and relative standard deviation (RSD) $\leq$ 9.98%. The developed method was successfully applied to the market sample and no residues of methiocarb and/or its metabolites were observed in the tested samples.

B-04

Search of matrix components in spinach after QuChERS cleanup for pesticide multiresidue analysis by mass spectrometry analyses

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QuChERS method has been popular in sample preparation for pesticide multiresidue analysis owing to that it is simple and fast method compared to other existing methods. However, there is only a little or no information of its clean up characteristics considering matrix components are available. This study was conducted to determine the remaining matrix components in spinach extract after QuChERS clean up. Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS/MS) operated in the both positive and negative mode was used to identify components in matrix. Fragmentation of components was further confirmed by liquid chromatography- electrospray time-of-flight mass spectrometry (LC-ESI-TOF MS) analysis in the positive mode. The strategies to identify components include accurate mass spectra of parent ion, retrieving corresponding formula by software analysis and mass spectral decomposition pattern. Many flavonoid aglycones as well as their glycosidic derivatives were identified. The components characteristics spectrum peaks was identified (M-H) - at m/z 315.20 (isorhamnetin), 299.10 (diosmetin), 345.30 (spinacetin), and 359.40 (jaceidin). Gas chromatography amiable components were identified using gas chromatography-mass spectrometry (GC-MS/MS) after compare mass spectrum peaks with National Institute of Standards and Technology (NIST, USA) database. The result indicates the presence of phytol, fatty acids and fatty acids ester in matrix using QuChERS method. It can be anticipated that the identification of matrix components will help in further development of improved QuChERS clean up method.

Keywords: spinach; flavonoid; matrix components; LC-ESI-MS/MS; LC-ESI-TOF MS

B-05

Volatilization of Sprayed Pesticides in Greenhouse Using a Lysimeter

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In cultivation environment, various pesticides are used and some of them could be volatilized into the air. This could affect farmer's health. This study was carried out to investigate the volatilization of pesticides. The experiment was conducted in a lysimeter. The pesticides treated in lysimeter soil were ethoprophos (5.0% GR), diazinon (34.0% EC), alachlor (43.7% EC), metolachlor (40.0% EC), chlorpyrifos (2.0% GR), pendimethalin (31.7% EC), carbaryl (50.0% WP), napropamide (50% WP), tebuconazole (25.0% WP) and imidacloprid (2.0% GR). The volatilized pesticides in air were collected by personal air sampler with PUF tube. The collected samples were extracted using acetone in a soxhlet apparatus for 8 hours. The extracted pesticides were resoluted with acetonitrile. It was analyzed with LC-MS/MS. For 720 hours experiment, the largest vaporization amount of each pesticide in air was ethoprophos 15.24 $\mu g/m^3$, diazinon 5.14 $\mu g/m^3$, pendimethalin 2.70 $\mu g/m^3$, chlorpyrifos 1.76 $\mu g/m^3$, alachlor 1.40 $\mu g/m^3$, metolachlor 1.12 $\mu g/m^3$, carbaryl 0.27 $\mu g/m^3$, napropamide 0.22 $\mu g/m^3$, tebuconazole 0.11 $\mu g/m^3$ and imidacloprid 0.05 $\mu g/m^3$. The R value (coefficient of correlation) between volatilization and vapor pressure of pesticides is higher than 0.99. Therefore, there is high correlation between volatilization and vapor pressure of pesticides.

B-06

Residual Behaviour of Thiamethoxam and Its Metabolite in Pepper(*Capsicum annuum*) Plant under Various Conditions

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Neonicotinoid insecticides are widely used controlling aphids and other sap-sucking insects. Since these insecticides produce metabolites with toxicological relevance after application to plants, it is necessary to study the residual characteristics of the crops under various conditions to devise safety measures. This study was to analyze residual changes of thiamethoxam and its metabolite clothianidin in pepper(*Capsicum annum*), and then to compare residual characteristics by season(summer or winter), edible portion(fruits or leaves), and number of spray programs(once or three times with 7-day intervals). Thiamethoxam(10%, WG) was sprayed on pepper under greenhouse conditions. Samples were collected randomly up to 21 days and 28 days post-application after application during summer and winter, respectively. Insecticides were analyzed QuEChERS extraction and LC-MS/MS detection. The biological half-life of parent compound thiamethoxam in summer ranged 10.0 to 10.3 days and 19.8 to 23.9 days in winter. The residue of the parent compound apparently decreased while the metabolite residues were slowly increased, but the total amount of residues did not change. The biological half-life of total thiamethoxam in summer ranged 11.7 to 12.6 days and 21.0 to 24.8 days in winter. It indicated that, dissipation behavior of thiamethoxam on pepper, the residues of metabolites did not significantly affect the total residues. The study was found residual characteristics of thiamethoxam and its metabolite were varied in the parent compounds and their metabolites, depending on several factors (season, edible portion, or number of sprays), and that studies of residues should be differently approached, from different perspectives.

B-07

Dissipation characteristics of Cyantraniliprole and Its Metabolite in Minor Crop Millet during Cultivation

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Cyantraniliprole is an widely used insecticide during cultivation of crops such as red pepper, green onion, cucumber, and tomato. This study investigated dissipation characteristics of cyantraniliprole and its metabolite IN-J9Z38 in grain and straw of millet after spraying cyantraniliprole 10.26% oil dispersion formulation. For field trial, the pesticide was treated on 4 plots respectively (40/30 days, 30/21 days, 21/14 days and 14/7 days before harvest) with three replicates. Residues of the pesticide was analyzed by LC-MS/MS (shimadzu LC-MS 8040) and method limit of quantitation (MLOQ) were 10 ng/g for both of compounds. The linearity of calibration curve (r2) were $\geq$0.99 at the calibration range of 2.5-250 ng/mL. For the method validation, 5 g of macerated grain or straw of millet was treated with standard solutions at 10MLOQ and 50MLOQ levels. The samples were soaked with 5 mL of distilled water for 30 min and extracted with 10 mL of acetonitrile by shaking (30 min). Then, 4 g of MgSO4, 1 g of NaCl, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added into extracts, shaken for 1 min and centrifuged at 3,500 rpm for 5 min. The supernatant (1 mL) were transferred to dispersive SPE tube containing 25 mg of PSA and 150 mg of MgSO4. The tubes were shaken for 1 min and centrifuged at 13,000 rpm for 5 min before analysis. Recovery rates were in the range of 70-120% (RSD$\leq$10%) at all spiked levels (10MLOQ and 50MLOQ) for cyantraniliprole and its metabolite. In field study, the residue levels of cyantraniliprole decreased in grain, while the metabolite increased over time. In the case of straw, the residue levels of both were decreased over time. As a conclusion, our results, it could be used as a useful data for establishing PHI (Pre-harvest interval) of cyantraniliprole during cultivation of millet.

B-08

Studies on Color Blending Trunk Injection Pesticides for Preventive of Pine Wilt Disease, Bursaphelenchus xylophilus on Pinus densifora

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The tree injection have been knowm as main method for pine wilt disease control. existing tree injection chemicals can not know whether treat or not. so cost have been wasted. therefore this study was conducted to develop a highly visible tree injection chemicals. Blue9 and blue8 pigment dye showed highest visibility at abacectin 1.8% EC and emamectin benzoate 2.15% EC until 31 days of treatment. there were no difference in the effect on *bursaphelenchus xylophilus* and phytotoxicity on *pinus densifora.* accordingly, in order to prevent the loss of the cost, it is necessary to strengthen the selection criterion of the chemicals so that the pigment mixture product can be selected first when selecting the tree injection.

B-09

Development and application of Agricultural Products Risk Assessment System (APRAS)

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As the agricultural products that are detected excessively of hazardous substances such pesticides or heavy metals occur annually, agricultural industry constantly suffered and consumer anxiety is intensifying. Many institutions including National Institute of Agricultural Sciences NIAS) in Korea have conducted extensive agricultural products monitoring in order to establish standards or identify the status. However, the application rate of monitoring data is low because a piecemeal approach to dealing with non-compliance or screening-level risk assessment of hazardous substances is practical in reality. Recently, NIAS has established Agricultural Products Risk Assessment System (APRAS), which quickly and accurately predict the human risk assessment of hazardous substance for Korean people who eat agricultural products. APRAS provide the database of hazardous substances in agricultural products, and the integrated exposure and risk assessment in real time. The system can reduce the time, manpower and costs, and increase efficiency compared to existing approach. Furthermore, it is possible to perform a more realistic and scientific assessment by applying the characteristics of Korean people, such as intake and body weight. In the near future, it is expected to improve the utilization of the system by supporting other institutions accurate and realistic risk assessment results, which help rapid inter-agency response in case of crisis.

C-01

Oxidation Products, Acute Toxicity and Estrogenic Activity for Heterogeneous Fenton Oxidation of Bisphenol A

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This study focused on oxidation products, acute toxicity and estrogenic activity for a heterogeneous Fenton oxidation of bisphenol A using Fe3O4/multi-walled carbon nanotube (MWCNT) at the selected conditions (70mgL^{-1}BPA, 50 mg Fe3O$_4$/MWCNT, mol H_2O_2/mol BPA of 4, 20oC, pH 4). The final effluents from the heterogeneous Fenton oxidation of BPA at the selected conditions showed no biological toxicity when it was analyzed using 24 h born Daphnia magna. It also resulted in negligible estrogenic activity when the in-vivo estrogenic activity of BPA oxidation products was evaluated. The major oxidation productions generated from heterogeneous Fenton oxidation of BPA were 4-hydroxyacetophenone, 4-hydroxybenzoic acid, catechol and hydroquinone. Therefore, the heterogeneous Fenton oxidation of BPA using the Fe3O$_4$/MWCNT with low dose of H_2O_2 led to high removal of BPA with negligible biotoxicity and estrogenic activity. It would be a potential solution for highly efficient treatment and reuse of emerging contaminant-containing wastewater and water.

C-02

The Development of acute toxicity test method for *Macrobrachium rosenbergii*

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Domestic fallow and unused land area is approximately 160 thousands hectare, paddy is occupied 34 percent in total fallow and unused land. We forecast that it will be expanded continuously in future(2009, National Institute of Agriculture Sciences). Nowadays, unused land management solution has been suggested to make the high profit for farmers through the growing of eco-farming species in order to decrease the increase of unused paddy. The target species are *Misgurnus anguillicaudatus, Macrobrachium rosenbergii, Eriocheir Japonicus etc.*, in case of *Macrobrachium rosenbergii*, it has introduced in domestic and carried out farming technology development since 2012. With the successful breed in bulk, it is known for making the 19 times more profit than prior profit in rice. By the way, even though domestic genuses are sorted in 4 types (1977, Korea Animal-Plant Book), *Macrobrachium rosenbergii* was introduced from South Asia because domestic genuses were too small. Eventually, it could not find the marketability. To sum up, in order to introduce the *Macrobrachium rosenbergii* as eco-farming species successfully, we should check if it is safe for the agrochemicals. Eventually, the development of acute toxicity test method should be developed first. We are planning to develop the test method and validity with the related standard such as OECD test guideline and EPA OPPTS 850. 1035, would like to introduce aquatic crustacea toxicity test method and ecological data of *Macrobrachium rosenbergii* to make test method for *Macrobrachium rosenbergii.*

C-03

The fertilization action research of *Apis mellifera* depending on the plant secondary metabolite

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Synomone is one of the plant secondary metabolite that it can make a profit to both plant and pollen vector. Synomone is one of the effective strategy to attract pollen vector also, make the better competitiveness among plants. In case of strawberry and watermelon, they attract *Apis mellifera* can attractive as pollen vector. There are paper that if more bees are in the green house, the malformed fruit rate will be decrease and, make higher yield(2014, Agricultural research institute). We'd like to introduce our research plan to analyze the effect to the yield and attraction of pollen vector depending on the Synomone as follow. First, Strawberry (*Apis mellifera* dependent rate - Above 50 percent). Second, Oriental melon (The highest Apis mellifera dependent rate in curcubit vegetables). Third, Establishment of test method in apple to certify the attraction effect of pollen vector. The number of visiting in strawberry and oriental melon, we are planning to inspect the number of visit per unit area through OECD guidance document No. 75 "Honeybee brood test under semi-field conditions" standard. Also for the apple, we will verify the attraction effect via inspecting the pollen vector who visits target area per hour. Moreover, we are planning to evaluate the safety of Synomone through inspecting chronically affected fecundity of queen, growth condition of larva also.

C-04

A study on the replaceability and the use of reference chemical in ecotoxicity test

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The use of reference chemicals is mandatory testing for quality control of the test procedure and the suitability of test organism in the safety assessment of agricultural pesticides and industrial chemicals with environmental organisms.

The reference chemicals is recommended by test guidelines from OECD, National Institute of Environmental Research, and Rural Development Administration. However, pentachlorophenol sodium salt(PCP-Na) and dimethoate as a reference chemical in acute toxicity tests in fish and honeybee classified as restricted and prohibited material. Therefore, safe handling facility is necessary to use these materials and the replacement of reference chemical is required to prevent the dangerous occurrence by the consistent use for toxicity testing.

This study was conducted to find a alternative chemical with the safety of experiment. Potassium dichromate, 3,5-dichlorophenol, Copper(II) sulfate, which are suggested as reference chemical in the test guideline. Acute toxicity testing of these chemicals was performed by its single application to a carp(*Cyprinus carpio*), a ricefish(*Oryzias latipes*), and a muddy loach(*Misgurnus anguillicaudatus*).

The result showed that average 48-h LC50 value for fish was 215.75 ~ 248.25 mg/L of potassium dichromate, 2.12 ~ 2.20 mg/L of 3,5-dichlorophenol and 0.12 ~ 1.13 mg/L of Copper(II) sulfate. These results suggest possibility of 3,5-dichlorophenol and Copper(II) sulfate to use the reference chemical of the fishes because of toxicity level and reproducibility. In addition, specific and various additional tests should be carried out in the future.

C-05

Development of guidance for toxicological assessment in pesticide formulation change

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Submission of formulation change is required for change of inerts in increasing efficacy and decreasing adverse of registered pesticides. When formulation change is requested, data of physico-chemistry and bioavailability must be submitted, but not toxicological data. After request of formulation change, toxicological assessment is conducted then toxicological data is required in case of increasing toxicity. It was difficult for pesticide companies to check whether additional toxicity data was necessary or not because assessment criteria of formulation change is not described in RDA directives. For resolution of these problems, guidance for assessment criteria of formulation change in toxicological part was established. Toxicity and containing percent of inerts before and after formulation change was checked in each inert then addition·removement·increase·decrease of containing percent was combined. If additional toxicity was found, additional toxicological data was required. This quidance was considered to contribute to shortened pesticide commercialization in formulation change.

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C-06

Teratotoxicity study on Zebrafish Embryo of Cartop hydrochloride

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The zebrafish (Danio rerio) is a small tropical bony fish (teleost) that has emerged as an excellent vertebrate model organism because of its high genetic homology with humans(85% similarity), easy manipulation, short testing times(3-5 days), fast organogenesis, transparent embryos and high number of small size embryos. This study investigated to detect the possibility of developmental toxicity using the zebrafish against cartop hydrochloride. Zebrafish embryo developmental toxicity were measured with the different concentration and exposure period. As a result, lethality before hatching was high in the concentration of 1 μM(2.74×10^{-2} mg/L) or more, and zebrafish embryos in 0-24, 0-48 hpf(hours post fertilization) group died all before hatching. The hatching and abnormality rate of the zebrafish embryo by cartop hydrochloride was high in 0-48 hpf group, and embryo exposed to the 0.1 μM(2.74×10^{-3} mg/L) observed general retardation(large yolk size) and notochord formation. The effects of cartop hydrochloride on the zebrafish embryos were proportional to the exposure concentrations and periods. NOEC(No observed effect concentration) have been estimated under 0.01 nM (2.74×10^{-4} mg/L).

C-07

Toxicity evaluation and establishment of safety reference dose of fluthiacet-methyl

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Toxicity evaluation and establishment of safety reference dose was conducted based on results from various studies of fluthiacet-methyl(CAS No. 117337-19-6), a herbicide. The data used in the assessment include acute toxicity(rats), subchronic (rats, mice and dog), subacute neurotoxicity(rats), chronic toxicity(rats and dogs), combind chronic toxicity/carcinogenicity(rats), carcinogenicity(mice), two-generation reproductive toxicity(rats), developmental toxicity (rats anf rabbits), genotoxicity and ADME study. In ADME study, oral absorption was 55.82-62.10% and main excretion route was feces. Major adverse effects of flutiacet-methly observed are decreased body weight gain, effects on blood such as anemia and degeneration/necrosis of the liver. Fluthiacet-methyl showed no neurotoxicity, reproductive toxicity, teratogenicity and genotoxicity. Increase in the incidence of hepatocellular adenomas in male mice, exocrine pancreas cell adenomas and islet cell adenomas in male rats were observed in carcinogenicity tests. However, a genotoxic mechaism was unlikely to be involved in the tumor induction. Mouse liver tumor formation include: PPARα activation, enhanced cell proliferation, selective clonal expansion and liver tumor formation, and the MOA for hepatocarcinogenesis via PPARα activation in rodents is not likely to occur in humans. PPARα activation, Increase plasma CCK levels and expression of receptors CCK4 was induced pancreatic tumors(exocrine pancreas cell adenomas and islet cell adenomas) and It was a secondary effect subsequent to liver induction via PPARα mode of action. But based on the qualitative and quantitative differences between rat and human PPARα and CCK receptors, rodent pancreatic tumors formed via a PPARα mode of action are not relevant to humans. The lowest no-observed-adverse-effect level(NOAEL) in all tests was 0.1 mg/kg bw/day in an 18-month carcinogenicity study in mice. By applying a safety factor of 100 to the NOAEL, an acceptable daily intake(ADI) was established as 0.001 mg/kg bw/day. The lowest NOAEL among subchronic(90 days and 6-weeks study), teratogenicity studies was 0.6 mg/kg bw/day in 90 days study in rats and AOEL was established as 0.0033 mg/kg bw/day.

D-01

Exploring New Class of IGRs: Finding Hit K-222212 and Hit Optimization by Its Derivatives

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The currently used insecticides have been classified into insect growth regulators (IGRs), neo-nicotinoids, pyrethroids, organophosphates, carbamates etc. The IGRs and neonicotinoids are two major market leaders; recent environmental concerns, health risks and/or increasing resistance limit application of the neonicontinoids. In particular, the neonicotinoids are known to disturb a nerve system of honey bees, which makes honey bees lose their moving path. Recently, IGRs get paid more attention due to their low toxicity. The currently used IGRs are majorly juvenile hormone agonists (JHAs), ecdysone agonists, and chitin synthase inhibitors. Among them, the JHAs are reported to be more efficient and safer as they are insect-specific hormone-based agents. While the juvenile hormone antagoists (JHANs) with a different mode of action are highly expected to be complementarily used to control insects that are resistant to JHAs, no practical JHAN agents have been reported. Our project was inspired by the preliminary studies and screening system to find JHAs or JHANs that are set up by Je and co-workers.[ref] We first screened ~10000cpds that are provided by Chemical Bank in KRICT. Consequently, a total of ten compounds that hold JHAs or JHANs activities were identified. As a preliminary study, a structurally novel K-222212 compound was selected and its derivatives were synthesized. With these compounds, further in-vivo experiments for mosquitos and harmful insects have been conducted. The experimental results will be shortly discussed. This preliminary results suggest a new direction for development of novel insecticides.

ref) “Identification of plant compounds that disrupt the insect juvenile hormone receptor complex”, Je, Y. H. et al. PNAS, 2015, 112, 1733-1738.

D-02

Antifungal Activity of *Maesa japonica* Extracts and Isolated Compounds against Plant Pathogenic Fungi

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Synthetic fungicides are currently used as the primary means for controlling plant diseases. However, the intensive use of synthetic fungicides has polluted the environment, harmed human beings, destroyed non-target organisms, and led to the development of fungal resistance. The problems have stimulated the development of environmentally friendly means such as biological control using plant extracts. In this study, 220 different plant extracts have been investigated for their activity to control seven plant diseases on rice, wheat, tomato, and pepper plants. The methanol extract (3,000 μg/mL) of *Maesa japonica* (Myrsinaceae) showed 90, 83, and 75% control values against rice blast, wheat leaf rust, and rice sheath blight, respectively. The process from the preparation of plant materials to extraction using organic solvents was optimized. M. japonica extract showed broad antifungal spectra against 8 plant pathogenic fungi. Particularly, the mycelial growth of *Magnaporthe oryzae* was completely inhibited by methanol extract at the concentration of 333 μg/mL. Under the activity-guided fractionation against *M. oryzae*, triterpenoid saponins (maejaposides and maesasaponins) were isolated and identified from *M. japonica* extracts. Maejaposide C and maesasapnin V3 at respective concentration of 250 and 500 μg/mL exhibited >90% control values against rice blast. The results suggest that *M. japonica* and saponin compounds may contribute to the development of eco-friendly alternatives to chemical fungicides.

D-03

Streptomyces sp. KRA329 Producing Herbicidal Metabolites as Potential Biocontrol Agent

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Bioherbicidal metabolites produced by *Streptomyces* sp. are being studied as possible herbicides or herbicidal adjuvants to develop biological agents that are easily degradable and environment friendly. Also, isolation and structural identification of natural herbicidal-active compounds from *Streptomyces* sp. has been proved to be an effective approach for novel lead discovery of the herbicide development.

In the course of our screening of bioherbicidal agent, about 600 different soil actinomycete isolates were tested for control of problematic weeds in Korea. One of the isolates, KRA329, showed strong herbicidal activity against grass species, *Digitaria sanguiinalis, Panicum dichotomiflorum, Sorghum bicolor, Echinochlia crus-galli, Agropyron smithii* and broad leaf species such as *Solanum nigrum, Aeschynomene indica*, *Xanthium strumarium*, *Calystegia japonica*. The culture broth of KRA329 by foliar application showed phytotoxic symptoms of wilting or burn-down of leaves and stunting and finally plant death. The isolate KRA329 was identified as *Streptomyces* sp. based on its 16S rRNA gene sequence and morphological characteristics. Two herbicidal compounds, 329-M1 and 329-M2, were purified by column chromatography and high performance liquid chromatography. By NMR and ESI-MS analysis, the two compounds were identified as cycloheximide derivatives including glutarimide moiety. These results suggest that *Streptomyces* sp. KRA329 producing bioherbicidal metabolites can be developed as a biocontrol agent (BCA) for weed control and/or may provide as a lead molecule for a more efficient herbicide.

D-04

Change of responses of *Colletotrichum gloeosporioides* isolated from persimmon trees to MBC and dithiocarbamate fungicides

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Various isolates of *Colletotrichum gloeosporioides* were collected from infected fruits, leaves, and twigs of persimmon (*Diospyros kaki*) orchards in Sangju (79 isolates at 2013 and 48 isolates at 2016) and Yeongdong (68 isolates at 2016) from Korea. MBC (Methyl, Benzimidazole, Carbamates)-fungicides (carbendazim and thiophanate-methyl) and dithiocarbamates and other relative fungicide (mancozeb and propineb) which are registered on persimmon, were evaluated to survey of *C. gloeosporioides* growth responses on fungicide-media. All isolates collected in Sangju showed inhibited mycelium growth over 91% on medium with carbendazim (415 ㎍/㎖) however, one isolate of Yeongdong showed 87.6% compared to untreated control. The sensitivity of *C. gloeosporioides* isolates to thiophanate-methyl (700 ㎍/㎖) was similar between 2013 and 2016; however, the sensitivity of isolates to mancozeb (1,500 ㎍/㎖) and propineb (1,500 ㎍/㎖) were increased than that of 2013.

D-05

Biological activity and characteristics of new fungicide KNF1036

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Various experiments were carried out to evaluate commercial value of the new fungicide KNF1036 24% SC developed by Kyung Nong, efficacy and the phytotoxicity against major crop diseases in Korea were evaluated. In order to confirm the characteristics of KNF 1036 24% SC, the optimal treatment time, duration time of curative effect and treatment effect were examined against the marssonina blotch in apple. we conducted the evaluation of the effect and the phytotoxicity against 19 crop diseases including persimmon. As a result, KNF 1036 24% SC showed comparable efficacy each crop disease to the competing fungicide used in the field, and no phytotoxicity. in the test res ults against marssonina blotch in apple KNF 1036 24% SC showed excellent effect from mid May to early June, and its effect lasted about 30 days when KNF1036 24% SC was treated in mid May. Additionally, in order to evaluate KNF1036 24% SC efficacy against resistant strains(C. acutatum 4 species, C. gloeosporioides 7 species), the mycelial growth inhibition effects on Colletotrichum acutatum(12 species), and Colletotrichum gloeosporioides(20 species) were examined. As a result, KNF 1036 24% SC effect on Strobilurin resistant strains showed the same inhibitory effect on mycelial growth with that of the Azoxystrobin, strobilurin fungicide.

D-06

Fumigation effects of Paladin® on trees after forest tending works

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Originally developed as a methyl bromide replacement, dimethyl disulfide (DMDS; Paladin®) has demonstrated consistent performance as a broad spectrum soil fumigant used for the control or suppression of nematodes, various soil-borne plant diseases and weeds. DMDS was registrated as a soil fumigant and forest fumigant in 2015. Korea Forest Service is recently focused on forest tending works. The purpose of forest tending works is not only maintaining ecologically stable forest stand structure but also developing commercial forest. This study was conducted to investigate the effects on pine wilt nematode(*Bursaphelenchus xylophilus*) and japanese pine sawyer (*Monochamus saltuarius*) in trees after forest tending work.

Fumigation took placed at over 20㎥ and investigated the effect of nematode, pine sawyer and VOC according to the type of covering materials(PE tarpaulin, PVC sol).

After fumigation during 14 days, Paladin showed 100.0% mortality to pine wilt nematode and pine sawyer. And there was no significant diffent effect between PE tarpaulin and PVC sol. VOC concentration was higest shortly after treatment and decreased after covering the PE tarpulin/PVC sol. And paladin gas was perfectly eliminated 7 days after removal of covering meterials and trees.

D-07

Insecticidal Susceptibility of Western Flower Thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) of Horticultural Crops in Gyeonggi Area

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This study was conducted to monitoring the insecticidal susceptibility of Western flower thrips, *Frankliniella occidentalis*, damaging the horticultural crops in Gyeonggi area. Bioassay was conducted under laboratory and greenhouse conditions using commercial insecticides being used for the control of thrips at their recommended concentrations.

Neonicotinoid insecticides, especially acetamiprid SP, clothianidin SC, imidacloprid WP and thiacloprid WG were not toxic to *F. occidentalis* collected from horticultural crops. However, *F. occidentalis* collected vegetable greenhouse was very susceptible to the acetamiprid + spinetoram SC, clothianidin + spinetoram SC and methoxyfenozide + spinetoram SC which showed the control value over 90%. In greenhouse test, spinetram (5%) WG that had showed 100% of mortality to *F. occidentalis* in laboratory test also showed 87.4, 88.0, 98.3% of control values on 3, 6 and 9 days after treatment, while imidacloprid (10%) WP showed below 44% of control value.

Excepting the insecticide of spinosin or pyrrole class, there was a little class of insecticides for the effective control of *F. occidentalis* in horticultural crop areas. Considering all the results in this study, spinosin insecticides including over 4 and 10% of spinetoram and spinosad, and pyrrole insecticide including over 5% of chlorfenapyr should be recommended for the effective control of *F. occidentalis*, damaging the horticultural crops in Gyeonggi area.

KEY WORDS : Frankliniella occidentalis, Insecticidal resistance, Control

D-08

Synthesis and Development of A New Selective Ryanodine Receptor Modulator Insecticide

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Ryanodine receptor modulator insecticides are recently developed by modifying the structure of the natural product ryanodine and these mode of action is known to inhibit ryanodine receptor, calcium-induced calcium release channel. Ryanodine combines with the non-aqueous portion of the receptor that is distributed in nerve and muscle cells, so that it cause muscle contraction and inhibition of feeding activity of insects by disturbing the interaction between nerves. Insecticides in this group specially have strong effect on larvae of lepidoptera and low toxicity on mammals.

The purpose of this study is to synthesize and develop new compounds for selective modulator of insect ryanodine receptors. A series of pyrazole amide derivatives possessing carbamate linker instead of anthralinic amide have been synthesized. Their biological activities against various harmful insects and safeties for beneficial insects including honeybee have been evaluated. A compound having the strongest activity is selected and its commercialization is in progress with other tests, including toxicity and formulation.

D-09

Herbicidal Activity of Metazosulfuron against Sulfonylurea-Resistance Perennial Weed in Greenhouse

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Kyung Nong Corporation

This study was conducted to investigate weed control of Metazosulfuron against resistant biotype on sulfonylurea(SU) herbicides such as *Scripus. joncoides*, *Sagittaria Trifolia*, *Scirpus planiculmis* at the greenhouse. Imazosulfuron and Metazosulfuron were tested. *Scripus. joncoides*(Gyeong-ju biotype, Pohang biotype), *Sagittaria Trifolia* (Geoncheon biotype, Pohang biotype) and *Scirpus planiculmis*(Sesan biotype, Gunsan biotype, Bosung biotype, Youngam biotype) were used as perennial weeds in this study. Imazosulfon showed not good herbicidal efficacy against *Scripus. joncoides*(Pohang biotype), *Sagittaria Trifolia*(Pohang biotype) and *Scirpus planiculmis*(Sesan biotype), but Metazosulfuron showed excellent herbicidal efficacy against all of perennial weeds.

포스터 발표
poster presentation

〈P-01〉

2017년 신규 기준설정 농약 Fenoxasulfone 시험법의 실험실간 검증

도정아*, 윤지혜, 박신민, 신혜선, 임승희, 박원민, 정형욱, 이규식
식품의약품안전처 식품의약품안전평가원 식품위해평가부 잔류물질과 / 141

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National Agricultural Products Quality Management Service, Jeju Provincial Office 1Faculty of Biotechnology, College of Applied Life Science, Jeju National University / 143

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Kyung-Geun Ahn[1*], Haeun Joo[2], Heeju Kwon[2], Juyoung Jeong[2], Gi-ppeum Kim[2], Young-Sun Hwang[3], Won-Kap Yun[1], Young Deuk Lee[4], Myoung-Gun Choung[2]
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Haeun Joo[1], Kyung-Geun Ahn[2], Heeju Kwon[1], Juyoung Jeong[1], Gi-ppeum Kim[1], Young-Sun Hwang[3], Won-Kap Yun[2], Young Deuk Lee[4], Myoung-Gun Choung[1*]
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강원대학교 환경융합학부, 강원대학교 친환경농산물안전성센터, ㈜친환경농식품안전성센터 / 161

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도정아*, 윤지혜, 박신민, 신혜선, 임승희, 박원민, 정형욱, 이규식

식품의약품안전처 식품의약품안전평가원 식품위해평가부 잔류물질과

Fenoxasulfone은 1998년에 일본의 ㈜구미아이화학공업, ㈜이하라케미칼공업, ㈜케이아이연구소의 공동연구로 개발된 제초제로 식물에 대해 뛰어난 제초활성을 가지는 이속사졸린계 화합물이다. Fenoxasulfone은 국내에서는 2016년 사용 등록이 처음 요청되어 2017년 쌀에 대하여 신규 기준 설정이 예정된 농약으로 기준 설정에 따른 안전관리를 위한 시험법이 개발되었다. 본 연구에서는 공정시험법 마련을 위하여 신규 개발된 시험법에 대한 실험실간 검증을 수행하였다. 이에 따라 식품공전에서 정하고 있는 식품분류 중 대표농산물 6종(현미, 사과, 감귤, 감자, 고추, 대두)을 선정하여 개발된 시험법으로 검증시험을 수행하였다. Fenoxasulfone을 아세톤으로 추출한 후 디클로로메탄으로 액액 분배하고 아미노프로필 카트리지를 사용하여 불순물을 정제한 후, 대두 및 현미와 같은 지방성 검체의 경우 아세토니트릴 포화 헥산 용액으로 지방을 제거하는 과정을 추가하여 HPLC-UVD를 이용하여 검증을 수행하였다. 시험법 검증 결과, 회수율 범위는 71.0~116.4%, 분석오차는 8.85% 이하, 정량한계는 0.025 mg/kg 이었다. 이 결과는 국제식품규격위원회(CODEX)의 농약 '시험법 가이드라인(CAC/GL 40, 2003)' 및 식품의약품안전평가원의 '식품등 시험법 마련 표준절차에 관한 가이드라인(2016)'에 부합되는 것으로 확인하였으므로, 향후 식품공전에 수록되어 Fenoxasulfone의 분석을 위한 공정시험법으로 활용될 예정이다.

P-02

Achieving Fast, Efficient Separations in Environmental Applications using Superficially Porous Particle Column Technology

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Restek Corporation

Superficially porous particles (SPP) have been proven to provide fast and efficient separations. These particles feature a solid, impermeable core enveloped by a thin, porous layer of silica that decreases the diffusion path and reduces peak dispersion. When combined with highly selective stationary phases, the result is significant improvements in efficiency and sensitivity over fully porous particles (FPP) of similar dimension. With Raptor™ LC columns, Restek chemists became the first to combine the speed of superficially porous particles (SPP) with the resolution of highly selective USLC® technology. This new breed of chromatographic column allows you to more easily achieve peak separation and faster analysis times without expensive UHPLC instrumentation. The versatility of these columns makes them ideal for a variety of environmental applications. Restek LC columns offer the speed of superficially porous particles with the resolution of the highly selective stationary phases, allowing peak separation and faster analysis times to be achieved without expensive UHPLC instrumentation.

P-03

Characteristics of Residual Pesticides Reducing by Processing Methods in Immature Citrus

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We tested pesticide residues in 112 samples of immature citrus products for using as the baseline data about the safety management system. Because the immature citrus fruits are known to be healthy foods, and recently being sold in Korea as a new agricultural product-putgyul. The 60 citrus fruits that was based on the harvesting time and regional distribution were collected in the field. The 40 citrus products and 12 kinds of citrus beverages were purchased in the market. So, a total of 112 samples were analyzed for residual pesticides. The 320 pesticides were determined by multi-residue method, especially Mancozeb and Propineb which are most commonly used in citrus fruits were analyzed by the improved method of the Dithiocarbamates Rapid Quantitation(patent).

As a result, 30 kinds of pesticide residues were detected in the immature citrus fruits. In some citrus beverages, Carbendazim was detected in trace amounts, but there were no products exceeding the MRL.

Moreover, to provide the basic data on the safety to consumers and processing companies, we analyzed the trend of residual pesticide reduction during processing of the immature citrus fruit products. The citrus samples with pesticides were analyzed after 3 washing and 2 juicing methods. As a result, 11 pesticides were showed decrease by 16.7~100% in the washing method and 16.0~94.0% in the juicing method(extract whole citrus) and especially, all the pesticide residues that were detected tended to decrease in the juicing method(extract after removing citrus peel).

Therefore, it is concluded that the pesticide residues in the immature citrus fruits, which were suspected of safety problems, were safe. Even so, it is the best to eat after sufficient washing. Overall, it could be possible to provide safety management data to producers and processing companies if the residual pesticides are constantly monitored.

Key Words: Immature Citrus(Putgyul), Multi-residue analysis, Dithiocarbamates

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P-04

Comparison on the Reducing Efficiency of Detergents for Chlorfenapyr Residue in Sweet Persimmon

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This study was carried out to investigate thechlorfenapyrreduce effectwith detergents (water, ethanol, acetic acid, ethylene glycol, polyethylene glycol 4000 and Tween® 20).Thechlorfenapyr residue analysis carried out gas-liquid chromatography - electron capture detector (GLC-ECD).Water, 1% ethanol, 1% acetic acid, 1% ethylene glycol, 0.2% poly ethyleneglycol 4000 and 0.2% Tween®20 for the comparison of the washing efficiency. They effectively reduced upto 60% of the residue in sweet persimmon. As a result,1% ethanol and 0.2% Tween® 20 werethat was identified effective agents for the residue in sweet persimmon.

P-05

Cyclaniliprole의 환경행적 평가와 수계 노출농도 산출

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Cyclaniliprole (IKI-3106, 3-bromo-N-[2-bromo-4-chloro-6-[[(1-cyclopropylethyl) amino]carbonyl]- 1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide)의 환경 분해대사와 토양 및 수계 이동성에 관련된 보고서를 검토하여 환경에서의 잔류특성과 수계로의 이동성을 결정짓는 매개변수를 확정하고, 작물재배조건을 고려하여 환경노출농도를 산출하였다. Cyclaniliprole의 호기토양대사 DT50은 299~1,118일(평균 740일, 중위수 648일, 7 토양(미국 3, 유럽 2, 국내 2))로 5% 이상 생성되는 대사산물은 없었고, 혐기조건에서의 분해양상도 호기조건과 비슷하였다. Cyclaniliprole은 토양 광분해에 의해 NK-1375로 전환되어 최고 시점에는 40% 수준까지 검출되었고, 북위 40도 여름조건에서 광분해 DT_{50}은 25일로 산출되었다. 차광조건의 실내시험에 비하여 포장조건에서는 cyclaniliprole의 분해가 촉진되어 DT_{50}은 80~196일(평균 99일 3 토양(프랑스 1, 국내 2))이었으나 광분해산물 NK-1375는 검출한계(0.01 mg/kg) 미만이었다. Cyclaniliprole의 유기탄소기준흡착계수(Koc)는 각각 351~1,570 L/kg로 UK SSLRC 이동성 구분은 'moderately mobile to slightely mobile' 등급에 해당하였다. 광분해산물 NK-1375의 HPLC 방법에 의한 log Koc는 4.4 (pH 4~9)로 나타나 이동성이 낮을 것으로 생각되었다. Cyclaniliprole은 pH 4~9 범위에서 가수분해에 안정하였으나, 광에 의해 분해가 촉진되어 일본 봄 조건에서의 반감기는 2.2~2.7일이었으며 주 분해산물로 NU-536, NK-1375, NSY-137, TJ-537가 생성되었고 광증감 효과는 없는 것으로 나타났다. Cyclaniliprole 4.5% 액제의 고추-담배나방 방제 적용에 대한 지하수와 지표수의 연평균 추정농도는 각각 0.058 μg/L와 0.96 μg/L로 산출되어 잠정 설정한 음용수기준 100 μg/L에 근접할 가능성은 매우 낮았고, EPA GENEEC에 의한 지표수 추정농도도 2.9~3.2 μg/L로 담수어류, 물벼룩 및 녹조류에 대한 반수영향농도 81~630 μg/L 비해 매우 낮은 수준이었다. Cyclaniliprole의 잔류분을 토양, 지표수 및 지하수 모두에 대하여 cyclaniliprole과 NK-1375로 정의하였다.

P-06

Determination of the QuEChERS method for residual 127 pesticides in perilla leaf, by the HPLC/MS/MS

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In this study, the QuEChERS method and HPLC/MS/MS was used to analysis the pesticide residues in perilla leaf. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) multi-residue method has been receiving a lot of attention for the clean up method and the fast extraction of residual pesticide analysis. Each sample was treated with AOAC 2007.01 QuEChERS method and analyzed HPLC/MS/MS. Also, matrix-matched standard calibration was used for quantitative analysis. Triphenylphosphate (TPP) as internal standard material was used to validate the detection ability of an instrument. In recovering experiment, the 127 pesticides were spiked at two levels of 0.01 and 0.1 mg/kg in samples. The 102 pesticides of the 127 pesticides were within the normal range in recovery (70% to 120%) and RSD value (below 20%). So, the most pesticides were within the normal range except for some pesticides. The results showed that using QuEChERS method and HPLC/MS/MS could be applied to multi-residue analysis of pesticides in perilla leaf.

P-07

Development of Analytical Method for Flusulfamide Residues as Benzenesulfonamide Fungicide in Major Agricultural Commodities

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An analytical method was developed using HPLC-UVD/MS to precisely determine the residue of flusulfamide, a benzenesulfonamide fungicide used to inhibit spore germination. Flusulfamide residue was extracted with acetone from representative samples of five raw products which comprised apple, green pepper, Kimchi cabbage, hulled rice and soybean. The extract was diluted with large volume of saline water and directly partitioned into dichloromethane to remove polar co-extractives in the aqueous phase. For the hulled rice and soybean samples, n-hexane/acetonitrile partition was additionally employed to remove non-polar lipids. The extract was finally purified by optimized Florisil column chromatography. On an octadecylsilyl column in HPLC, flusulfamide was successfully separated from co-extractives of sample, and sensitively quantitated by ultraviolet absorption at 280 nm with no interference. Accuracy and precision of the proposed method was validated by the recovery experiment on every crop samples fortified with flusulfamide at 3 concentration levels per crop in each triplication. Mean recoveries ranged from 82.3 to 98.2% in five representative agricultural commodities. The coefficients of variation were all less than 10%, irrespective of sample types and fortification levels. Limit of quantitation(LOQ) of flusulfamide was 0.02 mg/kg as verified by the recovery experiment. A confirmatory method using LC/MS with selected-ion monitoring technique was also provided to clearly identify the suspected residue.

P-08

Development of Forchlorfenuron Residues in Agricultural Commodities Using HPLC-UVD/MS

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Forchlorfenuron is a kind of plant growth hormone that is currently utilized to accelerate cell division, expand cell volume and promote organ formation and protein synthesis. This experiment was conducted to establish a determination method for forchlorfenuron residues in crops using HPLC-UVD/MS. Forchlorfenuron residue was extracted with acetone from representative samples of four raw products which comprised apple, mandarin, green pepper, and Kimchi cabbage. The extract was diluted with large volume of saline water and directly partitioned into dichloromethane to remove polar co-extractives in the aqueous phase. The extract was finally purified by optimized Florisil column chromatography. On an octadecylsilyl column in HPLC, forchlorfenuron was successfully separated from co-extractives of sample, and sensitively quantitated by ultraviolet absorption at 265 nm with no interference. Accuracy and precision of the proposed method was validated by the recovery experiment on every crop samples fortified with forchlorfenuron at 3 concentration levels per crop in each triplication. Mean recoveries ranged from 75.2 to 94.4% in four representative agricultural commodities. The coefficients of variation were all less than 10%, irrespective of sample types and fortification levels. Limit of quantitation(LOQ) of forchlorfenuron was 0.02 mg/kg as verified by the recovery experiment. A confirmatory method using LC/MS with selected-ion monitoring technique was also provided to clearly identify the suspected residue.

P-09

Development of Rapidly Detection Kit Poisoning to Organophosphate Pesticide

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Pesticide need the improvement of human life, but overexposure of pesticide would be harmful to health. Specially, organophosphate chemicals were used as nerve gas such as Sarin, Soman, and VX agent. Social chaos also can be induced by nerve agents as terrorism weapon. Thus, various diagnostic kit should be performed to development for detecting the pesticide poisoning rapidly. Thus, we're carrying out the development of Immuno-Sensor kit and Bio-tube kit for detecting the exposure of organophosphate in human. Our goals are to detect a below 10ppm of LOD (limit of detection) for organophosphate and estimate the exposure state within 10min.

Key Words : Organophosphate, Diagnostic kit

P-10

Dissipation of Triazole Fungicides during Kimchi Processing made in Korean Cabbage

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This study was carried out to calculate the processing factors of triazole fungicides in Kimchi made in Korean cabbage. Hexaconazole and myclobutanil were sprayed 3 times at interval of 7 days prior to harvest day on Korean cabbage. Harvested Korean cabbage was washed by tap water for 30 secs, brined with 10% salt solution for 30 mins, and fermented during 14 days under condition of room temperature and 21 days at Kimchi refrigerator, respectively. Residue amounts of the pesticides in raw and processed products of Korean cabbage were analyzed using a GC-ECD. Their initial residues in Korean cabbage were 1.51 mg/kg for hexaconazole and 3.44 mg/kg for myclobutanil. The residue amounts of hexaconazole and myclobutanil in Korean cabbages were dissipated by up to 65.4-70.5% and 40.2-54.6%, respectively, through washing and brining. During fermentation, dissipation rates of myclobutanil were 78.3 under room temperature and 69.3% at Kimchi refrigerator, and hexaconazole was 94.4% both of fermentations. Processing factors of pesticides in processed Korean cabbage were calculated 0.32-0.06 for hexaconazole and 0.54-0.23 for myclobutanil, respectively. When processing factor is low, the residue amounts of processed products has been more removed compared with the initial residues. This results indicated that dissipation rates of hexaconazole is higher than myclobutani during Kimchi processing, there are many environmental factors affecting residue amounts. Overall, process and fermentation processing of Kimchi can give a significant dissipation effect above 69.0% on tested triazole pesticides in Korean cabbage.

Keywords : Hexaconazole, Kimchi, Korean cabbage, Myclobutanil, Processing factor.

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P-11

Dissipation Pattern of Flubendiamide on Jujube Fruits for Establishment of Pre-Harvest Residue Limits

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This study was performed to investigate dissipation pattern of Flubendiamide in jujube fruits, and to suggest the pre-harvest residue limits (PHRLs) based on their dissipation patterns and biological half-lives. The Flubendiamide was sprayed on jujube trees in two different fields at standard dose. The samples for analysis were harvested at 0 (2hr), 1, 3, 5, 7, 10 and 14 days after the treatment of Flubendiamide, and analyzed by a HPLC equipped with DAD (UV 210 nm). The method limit of quantification (MLOQ) was 0.01 mg/kg for the analysis of Flubendiamide in jujube fruits. The recovery ranged from 75.1% to 101.8% on jujube fruits. Residual amounts of Flubendiamide in jujube fruits were 0.18 ~ 0.61 mg/kg in field 1 and 0.12 ~ 0.19 mg/kg in field 2. The biological half-lives of Flubendiamide were 7.5 days in field 1 and 23.5 days in field 2. Therefore, the PHRLs of Flubendiamide is recommended as 1.18 mg/kg on jujube fruits for 10 days before harvest.

Key Words : Flubendiamide, Jujube, Pre-Harvest Residue Limits(PHRLs), Dissipation pattern

P-12

Effect of Cuticular Waxes and Fungicide Systemicity on Penetration of Azoxystrobin and Chlorothalonil into Apples

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Penetrative behaviors of fungicides systemic azoxystrobin and non-systemic chlorothalonil into apples were assessed with impacts by cuticular waxes of apples and systemic properties of fungicides. Wax-intact and -removed apples were immersed in diluted solutions of the fungicides and divided into washed and unwashed groups with running tap water. Apple samples collected at 1, 24, and 48 h after incubation were separated into four parts before analysis of fungicides: peel, pulp-1, pulp-2, and pulp-3. Most azoxystrobin residues (70.7 – 86.4%) in wax-intact apples were present in peels and were higher in pulp parts closer to peels. After removal of cuticular waxes, azoxystrobin concentrations in pulps were 2 times greater than those for wax-intact apples. Although washing apples with running tap water reduced 52.3 – 69.2% of azoxystrobin residues, the residues in pulps were still determined at similar concentrations before and after washing. On the other hand, chlorothalonil residues for all apple samples were determined in peels only regardless of wax removal and/or washing treatment, and 84.5 – 91.1% of the residues were removed from peels by washing. These results indicate how physicochemical properties of both fungicides could be influence their penetration behaviors through cuticular waxes of apples.

Keywords : Azoxystrobin, Chlorothalonil, Fungicide Penetration, Cuticular wax
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P-13

Establishment of hpRNAi for analysis of entomopathogenic fungal virulence-related genes

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Entomopathogenic *Beauveria bassiana* has been used as an effective biological control agent for long times, however little information about how it works in the pathogenesis in terms of epizootiology is available in this research field. Herein in a previous our study, complex I intermediate-associated protein 30 (*CIA30*) and autophagy protein (*Atg22*) genes were predicted to be strongly involved in the virulence of *B. bassiana* JEF-007. The *CIA30* gene is a complex I (NADH-ubiquinone oxidoreductase) early assembly factor that is involved in the first step of electron transfer chain in inner mitochondrial membrane. The *Atg22* has an important self-degradation mechanism to balance energy source in response to nutrient stress and in development. RNA interference (RNAi) technique can be used as a tool for determining gene function in filamentous fungi. To determine a fungal virulence-related genes in entomopathogenic B. *bassiana* JEF-007, *CIP30* and *Atg22* genes were used to knockdown by hpRNAi. We used the hairpin RNA (hpRNA) with SOD1 intron in pCAMBIA1302 vector for the silencing of two genes in JEF-007. This work provides a strong platform for future functional genetic studies of bean bug-pathogenic *B. bassiana*; the genes putatively involved in fungal virulence should be experimentally validated by knock-down in future studies.

Keywords: Autophagy protein, Beauveria bassiana, complex I intermediate-associated protein 30, hairpin RNA, RNA interference

P-14

Establishment of Residue Analysis Method of Etoxazole in Mandarin with HPLC

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This study was carried out to establish a single residue analytical method for determining insecticide etoxazole residues in mandarin. Analytical procedure for residue analysis were optimized with extraction, partition and purification with analysis by HPLC-PDA. In optimized condition, samples were extracted with acetonitrile (100 mL) containing 1N NaOH and partitioned with hexane (70 mL × 2 times). Purification with florisil SPE cartridge was done with washing 8 mL of hexane, collecting 10 mL of acetone/hexane (10:90, v/v). LOD (Limit of Detection) of etoxazole was 1.0 ng, and excellent linearity (R^2=0.9999) was achieved. LOQ (Limit of Quantitation) was 0.02 mg/kg in mandarin. Recoveries were measured at two fortification levels (10 LOQ and 50 LOQ) on mandarin samples and were 93.0 ± 3.9 % and 83.0 ± 7.1 % and coefficients of variation (CV) were less than 10% which showed good recovery and reproducibility. As a results, a single component analysis method of etoxazole was established.

P-15

Establishment of Residue Analytical Method of Fludioxonil in Paprika

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A single analysis method of fludioxonil in paprika was developed. The analytical procedures were optimized using different extraction, partition solvent, and purfitication method by analyzing with HPLC-VWD. After optimizing procedures sample was extracted by acetone, partitioned with dichloromethane (DCM) (100 mL + 50 mL), and purified with SPE-NH2 cartridge by collecting 10 mL of ethyl acetate/hexane(50/50, v/v) elution after washing 10 mL of ethyl acetate/hexane(30/70, v/v). Good linearity was achieved from 0.1 to 10.0 mg/kg of fludioxonil standard solutions. (R^2 = 1.0000) The limit of detection was 1.0 ng(S/N> 3), and limit of quantitation(LOQ) was 0.02 mg/kg. Recovery rates from paprika fortified at the level of 10 LOQ and 50 LOQ were 98.7 ± 2.8% and 89.2 ± 0.9%, respectively. As a results, a single residue analysis method of fludioxonil was established

P-16

Establishment Residue analysis method of boscalid in paprika with HPLC-VWD

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A single residue analytical method for detecting boscalid residue in paprika with high performance liquid chromatography (HPLC) was developed. For residue analysis in paprika, the procedures involved extraction with acetone, partition with solvent, purification with SPE cartridge were optimized, and analysis were done by HPLC. The partition efficiency with dichloromethane/hexane (20:80, v/v) 100 mL was 100 ± 2.2%. Purification with SPE-FL cartridge was done with washing 10 mL of ethyl acetate/hexane (5:95, v/v), collecting 10 mL of ethyl acetate/hexane (30:70, v/v). The recovery rate for SPE was 99.5 ± 3.4%. The limit of detection (LOD) was 0.5 ng, and limit of quantitation (LOQ) were 0.02 mg/kg for paprika. Standard calibration curve shows linearity from 0.1 mg/kg to 10.0 mg/kg (R^2 = 0.9999). Analytical method was established with a recovery rate of 107.4 ± 2.1% (10 LOQ) and 97.8 ± 0.3% (50 LOQ) by combining procedure.

P-17

Evaluation of Sources of Irreproducibility of Retention Indices under Programmed Temperature Gas Chromatography Conditions

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Retention index under the programmed temperature gas chromatography (PTGC) conditions (IT) is widely accepted despite of its unsatisfactory reproducibility. Effects of heating rate (rT), stationary liquid film thickness (df), inlet pressure (pi), initial temperature (To) and polarity of stationary phase (sp) on IT reproducibility were studied in the present work. We found that excellent reproducibility and regularity of IT values are obtainable at conditions of relatively low To and relatively high rT and pi, as well as use of quality thick-film column under an operation in a single-retention behavior region (i.e., cubic equation model region). Most solutes were eluted in the dual-retention behavior region under the commonly used conditions described in the literature (i.e., relatively high To and relatively low rT for thin-film columns). The IT reproducibility under the optimum condition was approximately 37 times better than that under the commonly used conditions. The present work further validated the concept about dual- and single-retention behaviors of solutes in PTGC and proposed a principle to improve IT reproducibility.

P-18

Fluthiacet-methyl의 환경행적 평가와 수계 노출농도 산출

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Fluthiacet-methyl (CGA-248757, KIH-9201, [[2-chloro-4-fluoro-5[(tetrahydro-3-oxo-1H,3H-[1,3,4]-thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]thio]-acetic acid methyl ester)의 환경 분해대사와 토양 및 수계 이동성에 관련된 보고서를 검토하여 환경에서의 잔류특성과 수계로의 이동성을 결정짓는 매개변수를 확정하고, 작물재배조건을 고려하여 환경노출농도를 산출하였다. Fluthiacet-methyl은 호기토양대사에 의하여 fluthiacet (CGA300402), CGA300403, CGA327066, CGA327067 및 A-CFPSA로 변환되어 각각 53~55%, 19~21%, 9~12%, 12~13% 및 12%까지 검출되었다. 호기조건 fluthiacet-methyl의 DT_{50}은 1~3일(국내 2, 미국 1 토양), 전체 잔류분의 DT_{50}은 38~44일(국내 2 토양)이었고, 혐기조건에서도 호기조건과 분해양상이 비슷하였다. Fluthiacet- methyl의 순수 광분해에 의한 DT50은 1.4일이었고, 분해산물 CGA300404의 검출수준은 42%에 달하였다. 미국 포장조건에서 검출되지 않은 CGA300403, CGA327066 및 A-CFPSA를 제외한 fluthiacet-methyl의 대사산물 포함 국내 토양 포장조건 DT_{50}은 1~9일(평균 2일)이었다. Fluthiacet-methyl과 fluthiacet의 유기탄소기준흡착계수(Koc)는 각각 427~1,455 L/kg과 95~26 L/kg이었고, UK SSLRC 이동성 구분은 'slightly mobile' 등급과 'moderately mobile' 등급에 해당하였다. Fluthiacet-methyl은 산성조건(pH 5)에서 가수분해에 안정하였으나, pH 7과 9 조건에서는 빠르게 fluthiacet으로 분해되었고 그 반감기는 각각 18일과 0.2일이었다. Fluthiacet-methyl의 순수 직접광분해 반감기는 4.9일이었으며 분해산물로 CGA330057이 11% 수준까지 검출되었고, humic acid를 첨가한 모의 자연수에서는 광증감 효과를 보여 일본 봄 조건에서의 반감기는 4일이었다. Fluthiacet-methyl 0.2% 함유 미탁제의 비농경지-일년생 및 다년생 잡초 방제 적용에 대한 지하수와 지표수의 연평균 추정농도는 각각 0.0016 μg/L와 0.17 μg/L로 산출되어 잠정 설정한 음용수기준 2 μg/L에 근접할 가능성은 낮았고, EPA GENEEC에 의한 지표수 추정농도도 0.43~0.54 μg/L로 담수어류, 물벼룩 및 녹조류에 대한 반수영향농도 2.9~2,300 μg/L에 비해 낮은 수준이었다. Fluthiacet-methyl의 잔류분을 토양, 지표수 및 지하수 모두에 대하여 fluthiacet- methyl, fluthiacet, CGA300404, CGA327067로 정의하였다.

P-19

GC-MS/MS를 이용한 참다래에서 113종 농약의 다성분 동시분석

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참다래는 비타민 C와 엽산, 칼륨 등의 영양소가 풍부한 기능성 열대과일로 잘 알려져 있다. 이러한 참다래는 일본 등에 수출하는 우리나라의 주요 수출 효자 품목으로써 농가 소득 증대에 기여하고 있다. 따라서 본 연구에서는 GC-MS/MS를 이용하여 참다래에서 113종 농약에 대한 다성분 동시분석법을 확립하여 수출 참다래에 대한 안전 관리를 강화하고자 수행되었다. 시료의 추출 방법은 QuEChERS법을 사용하였고 GC-MS/MS로 기기분석 하였다. 분석을 위한 검출한계는 (0.6~5.8) ㎍/kg 수준이었고 분석 정량한계는 (1.8~17.4) ㎍/kg 범위로 나타났으며, 분석 대상 성분의 직선성은 (5~100) ㎍/L 범위에서 R^2〉0.99이었다. 분석회수율은 저농도(20 ㎍/kg)에서는 51.9 %에서 121.2 %, 고농도(100 ㎍/kg)에서는 69.1 %에서 111.4 %의 수준이었으며, 상대표준편차는 19.6 % 이내로 나타나 20 % 이내의 기준을 만족하였다. 본 연구를 통해 QuEChERS 전처리와 GC-MS/MS를 이용한 113종 농약의 다성분 동시분석은 참다래에서 효과적으로 적용될 것으로 기대된다.

P-20

HPLC를 이용한 Penflufen 분석법 확립

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본 연구는 감자, 옥수수, 벼 등에 사용되는 국내 신규도입 살균제인 Penflufen의 분석법 확립에 관한 것이다. Penflufen은 침투이행성 약제로서 pyrazole-carboxamide의 분류에 속하는 alkylamide 살균제이며, succinate dehydrogenase(SDH)를 저해하는 작용기작을 가지고 있다. 2011년 영국에서 처음으로 등록 사용되었고, 주로 벼 등에 적은 약량으로 담자균류(*rhizoctonia solani* 등) 및 자낭균류(*helminthosporium spp.* 등) 등의 방제에 매우 효과적이며, 식물의 활력을 높여주는 부수적인 특성도 지니고 있다. 현재 미국, 캐나다, 브라질, 아르헨티나, 유럽, 호주 및 일본 등 여러 국가에 등록되어 사용 중이다. Penflufen은 백색결정 분말로서 이화학 특성으로는 분자량 317.41 g/mol, 분자식 $C_{18}H_{24}FN_3O$, 증기압 4.1×10^{-9}hPa (20℃), 1.2×10^{-8}hPa (25℃), 1.7×10^{-6}hPa (50℃), Log P(Kow) = 3.3 (pH 4, 7, 9), 융점 111.1℃, 밀도 1.21 g/cm^3 등이 있다. Penflufen의 분자구조 등의 화학성과 용해도 등의 물리성을 고려하여 HPLC 기기분석법을 확립코자 하였다. 주성분 및 방해성분 등의 머무름 시간, 주성분의 분리도 등을 고려하여 내부표준물질로 Di-n-butyl phthalate(DNBP)를, 이동상으로는 아세토니트릴과 증류수 혼합액(65/35 v/v, 본 용액 1 L에 아세트산 0.5 mL 첨가)을, 컬럼으로 C_{18}(250 ㎜ × 4.6 ㎜, 내경 5 ㎛)을 사용하였다. 본 분석법의 적합성을 검증하기 위하여 5point의 농도를 조제하여 직선성을 확인한 결과 상관계수(r^2)가 0.9992로 양호하여 본 분석법은 확립된 것으로 판단되었다.

P-21

LC-MS/MS를 이용한 딸기(*Fragaria ananassa* Duch.) 중 Sulfoxaflor 및 Thiacloprid의 경시적 잔류특성 연구

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현재 유통·판매되고 있는 딸기의 생산량은 전체 과채류 생산량(2,405,993 ton)의 10%(194,513 ton) 이상으로 168종의 농약이 딸기에 등록되어 생산에 기여하고 있으며, 등록된 농약의 안전사용기준 및 잔류허용기준(Maximum Residue Limit, MRL)을 설정하여 안전성을 확보하고 있다. 본 연구는 딸기(*Fragaria ananassa* Duch.)의 안전성 강화 및 확대를 위하여 생산단계 중 sulfoxflor 및 thiacloprid의 잔류허용기준(Pre-Harvest Residue Limit, PHRL)을 설정하고자 수행하였다. 딸기에 안전사용기준에 준하여 희석한 sulfoxaflor 및 thiacloprid를 포장 1, 포장 2에 기준량 1회 처리하였으며, 약제처리 후 2시간을 0일차로 하여 0, 1, 2, 3, 5, 7, 10일차에 시료를 채취하였다. 채취한 딸기 시료는 LC-MS/MS를 사용하여 분석하였으며, 두 약제의 분석정량한계(Method Limit of Quantiation, MLOQ)는 0.005 mg/kg이었다. 분석정량한계의 적합성을 판단하기 위한 회수율 시험을 수행한 결과 sulfoxaflor의 경우 102.6~107.3%, thiaclopird의 경우 75.8~85.6%로 유효회수율 범위 및 변이계수 10% 이내를 만족하였다. 딸기 중 sulfoxaflor 및 thiacloprid의 잔류량을 분석하여 산출된 잔류감소 회귀식은 sulfoxflor의 경우 포장 1, 포장 2에서 각각 $y = 0.9612e^{-0.3477x}$ ($R^2 = 0.9612$), $y = 0.3244e^{-0.145x}$ ($R^2 = 0.9701$)이었으며, thiacloprid의 경우 각각 $y = 0.5056e^{-0.16x}$ (R2 = 0.9701), $y = 0.3986e^{-0.099x}$ ($R^2 = 0.9737$)이었다. 회귀식을 바탕으로 산출된 두 약제의 생산단계 잔류허용기준은 sulfoxaflor 1.60 mg/kg, thiacloprid 4.45 mg/kg로 제안하였으며, 딸기 수확 10일 전 잔류량이 생산단계 잔류허용기준안 이하로 나타난다면 수확 시 딸기의 잔류량은 MRL 이하로 나타날 것으로 사료된다. 본 연구결과 국내 딸기 생산농가의 유통 전 농산물의 안전성에 기여함에 따라 소비자에게 안전한 농산물을 제공할 수 있으며, 농산물 안전관리를 강화할 수 있을 것으로 판단된다.

P-22

LC-MS/MS를 이용한 살충제 Tolfenpyrad의 잔류시험법 개발

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식품의약품안전처 식품의약품안전평가원 식품위해평가부 잔류물질과

Tolfenpyrad는 1991년 일본 미츠비시화학 주식회사(Mitsubishi Chemical Corporation)에서 개발한 약제로 알, 유충, 애벌레 및 성충 단계의 해충에 직접 접촉 작용하여 살충효과를 나타내며, 채소 등 작물의 배추좀나방, 진딧물 등을 억제하는 pyrazole계 살충제이다. Tolfenpyrad는 식물체내 tolyloxy 고리의 산화성 분해 및 아미드 결합의 가수분해로 대사되어 주요 대사산물인 OH-PT가 생성되는 것으로 보고되어 있으나, 작물잔류성시험을 통해 농산물 중 잔류 가능성이 없는 것으로 확인되어 tolfenpyrad의 잔류물의 정의는 모화합물로 관리하고자 하였다. 2017년 현재 코덱스, 미국 및 일본에서도 잔류물의 정의를 모화합물로 정하여 관리하고 있으며, 잔류허용기준은 감자, 아몬드 등을 포함하여 64종에 대해 0.01-70.0 mg/kg 수준으로 설정하여 관리하고 있다. 본 연구에서는 국내에서 기준 신설 예정인 아몬드, 피스타치오, 호두에 대한 수입식품 안전관리와 향후 수입식품 종류의 확대 가능성을 고려하여 식품공전에서 정하고 있는 대표농산물 5종(감귤, 감자, 고추, 현미, 대두)을 포함하여 범용적인 잔류시험법을 확립하고자 하였다. 농산물 중 tolfenpyrad를 acetonitrile로 추출하고 dichloromethane으로 액-액 분배하여 추출과 액-액 분배법을 확립하였으며, 대두와 현미 등 지방성 시료의 경우 n-hexane/acetonitrile 분배법을 추가하였다. 추출 및 액-액 분배 후 Silica 카트리지를 이용하여 불순물(간섭물질)을 정제하기 위한 정제조건을 확립하였으며 분석기기는 LC-MS/MS를 이용하였다. LC-MS/MS는 간섭물질의 영향을 받지 않으며, 선택성과 분석 감도를 높여 잔류물의 정확성 및 신뢰성을 확보할 수 있다. 개발된 시험법을 검증한 결과, 평균 회수율은 72.67-100.17%, 분석오차는 9.63%, 정량한계는 0.005 mg/kg로 나타났다. 본 연구에서 개발한 시험법은 국제식품규격위원회의 농약 시험법 가이드라인(CAC/GL 40, 2003), 및 식품의약품안전평가원의 '식품등 시험법 마련 표준절차에 관한 가이드라인(2016)'에 부합한 것으로 확인되어 향후 농산물 중 tolfenpyrad의 잔류물 안전관리를 위한 공정시험법으로 활용할 수 있을 것으로 기대된다.

P-23

LC-MS/MS를 이용한 참다래에서 207종 농약의 다성분 동시분석

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2016년 12월 31일부터 우리나라에서는 농약 허용물질목록 관리제도(Positive List System)를 도입하여 1차로 견과종실류 및 열대과일류를 대상으로 우선 적용하여 시행하고 있다. 이에 따라 참다래에 등록되지 않은 농약이 검출되면 0.01 mg/kg의 기준을 적용받기 때문에 잔류농약 안전성조사 결과 부적합 농산물이 증가하여 우리나라 참다래 재배농가들이 상당한 타격을 받을 것으로 예상된다. 따라서 본 연구에서는 LC-MS/MS를 이용하여 참다래에서 207종 농약에 대한 다성분 동시분석법을 확립하여 참다래의 잔류허용기준 강화에 따른 정확한 분석 결과를 도출하고자 수행되었다. 시료의 추출 방법은 QuEChERS법을 사용하였고 LC-MS/MS로 기기분석 하였다. 분석을 위한 검출한계는 (0.8~9.5) μg/kg 수준이었고 분석 정량한계는 (2.4~28.4) μg/kg 범위로 나타났으며, 분석 대상 성분의 직선성은 (1~100) μg/L 범위에서 R^2>0.99이었다. 분석회수율은 저농도(20 μg/kg)에서는 51.3 %에서 132.1 %, 고농도(100 μg/kg)에서는 52.2 %에서 127.2 % 수준이었으며, 상대표준편차는 18.4 % 이내로 나타나 20 % 이내의 기준을 만족하였다. 본 연구를 통해 QuEChERS 전처리와 LC-MS/MS를 이용한 207종 농약의 다성분 동시분석은 참다래에서 효과적으로 적용될 것으로 기대된다.

P-24

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Quantification of Acetamiprid and Thiacloprid Residues in Butterbur Grown under Regulated Conditions

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An analytical method was developed to quantify the residual levels of the neonicotinoid insecticides acetamiprid and thiacloprid in field-incurred butterbur samples using liquid chromatography-tandem mass spectrometry. Samples were extracted with acetonitrile and partitioned with dichloromethane. After partitioning, purification was conducted using a Florisil®cartridge. Linearityofthetwocompoundsoveraconcentration rangeof0.004–0.4μg/mL was excellent, with determination coefficients (R^2) $\geq$ 0.9998. The limits of detection (LOD) and quantitation (LOQ) for both acetamiprid and thiacloprid were 0.0006 and 0.002 mg/kg, respectively. The average recoveries for acetamiprid and thiacloprid at two spiking levels (0.02 and 0.1 mg/kg, i.e., 10 × LOQ and 50 × LOQ) were between 78.23 and 82.15%, with relative standard deviations $\leq$ 7.22%. The method was successfully applied to field-incurred samples treated with a commercial pesticide product, either once (zero or 7 days before harvest) or twice (0 and 7, 7 and 14, or 14 and 21 days before harvest). The highest and lowest residues were obtained for the 0 and 7 days' treatment and the 14 and 21 days' treatment, respectively. The developed method is simple and accurate and can be extrapolated to other leafy vegetables.

P-25

Monitoring and Safety Assessment of Pesticide Residues in Online openmarket Distribution Agricultural Products

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Pesticide residues in distribution agricultural products via online openmarket was monitored and assessed their safety. A total of 112 samples were purchased online openmarket distribution agricultural products (leafy vegetable) from February to August 2016 at four major online openmarket. These samples were analysed 220 pesticides by Multi-class pesticide multi-residue methods using GE-ECD/NPD and LC-UPLC/FLD and confirmed by TOF-MS and LC-MS/MS. As a result of analysis, 21 kinds of pesticides were detected in 27 samples (24.1%) such as perilla leaves, gyeojachae, spinach, korean cabbage etc, of which 3 samples (2.7%) such as 2 samples of gyeojachae (uniconazole, lufenuron) and 1 sample of lettuce leaf(ethoprophos) were violated Maximum Residue limits (MRLs). Risk assessment evaluated human health exposure with the ratio of EDI (Estimated daily intake) to ADI (Acceptable daily intake) of pesticides detected. %ADI (the ratios of EDI to ADI) were 0.0028~27.3172%.

Key words : pesticide residues, online openmarket, agricultural products, MRLs, %ADI

P-26

Mornitoring of Pesticide Residues in Agricultural Products Marketed from the Northern Area of Seoul(2015-16)

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This study was carried out to protect consumer health by excluding illegal commercial agricutural products that contain pesticide residues from market. Concentrations of 285 different pesticide residues were measured by multi-residue methods on a GC-ECD/NPD and an HPLC-DAD/FLD, for 3,207(3,276) commercial agricultural products collected from the Northern area of Seoul in 2015(2016). Residues were detected in 11.3(18.8)% of the products, with 0.3(0.4)% exceeding the maximum allowable residue limits(MRLs) for Korea. Pesticide residues that detected were most frequently found in 5 vegetable types: in 19.0% of leek; 10.2% of welsh onion; 9.9% of spinach; 6.1% of pepper and 3.8 % of perilla leaves in 2015. The results showed pesticide residues that detected were most frequently found in 6 vegetable types: in 7.5% of perilla leaves; 5.0% of leek and pepper; 4.1% of welsh onions; 3.7% of lettuce and 3.2 % of spinach in 2016. The highest levels of violation were found in leek(5cases), welsh onion, korean cabbage(3 cases), chicory, gyeojachae(2 cases) and water dropwort, chamnamul, chinese vegetable, spinach, chicory, lettuce, mustard leaf, green garlic (1 case) in 2015-2016. The most frequently detected pesticides over MRLs were procymidone, chlorfenapyr, cypermethrin, diniconazole, bifethrin, azoxystrobin in 2015-2016. Residual pesticides that exceeded their MRLs and listed in order of frequency were procymidone, diazinon, iprodione, chlorpyrifos, diniconazole, fludioxonil, chlorfenapyr in 2015-2016. These results indicate that the residue levels of pesticides in agricultural products collected from markets in the Northern area of Seoul are low enough to be considered safe.

Key words : pesticide residues, agricultural products, MRLs

P-27

Multiresidue Method for 7 Pesticides in major agri-exports using LC-MS/MS

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We developed the method for 7 pesticides to vitalization of our agricultural products export. Korea's major exporting countries are U.S.A, Japan, Hongkong and so on. These countries enforce Positive list system or Zero tolerance by technical barriers to trade. A multiresidue method for 7 pesticides (Cyantraniliprole, Cyflumetofen, Cyenopyrafen, Emamectin benzoate, Fluazinam, Oxolinic acid, Spinosad) has been developed using the QuEChERS EN method with high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) in 5 agricultural commodities (Apple, Paprika. Pear, Strawberry, Tomato). It was demonstrated that a minimal concentration of 0.5-10 μg/kg of analytes in matrix is required for the consistent identification of targeted pesticides with two MRM transition. To evaluation of matrix effects on the quantitative analysis each agricultural products, we compared to two calibration standards. To reduce interfering compounds from matrix, samples were diluted with buffer and acetonitrile before LC-MS/MS analysis. The Method was validated by the precision and accuracy results. All commodities spiked with two concentration levels of 10 and 100 μg/kg. The recoveries were within 77 ~ 102.1% with relative standard deviations (RSD) of $\leq$30% and the limit of quantification (LOQ) of method were 5.8 μg/kg below in all agricultural commodities. Linear calibration functions with correlation coefficients were obtained $r^2 > 0.99$.

For 7 pesticides residues analysis, this method with LC-MS/MS was used to identify and quantitate pesticides residues with concentrations ranging from 10 to >100 μg/kg in a variety of agricultural samples, demonstrating fitness for screening applications. We'd like to support the safety by this method when Korean farmers export their agricultural products.

P-28

Optimization of Simultaneous Analysis for Tebupirimfos and Tolclofos-methyl Residues in Ginseng and Dried Ginseng Using GC-NPD

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Ginseng(Panax ginseng C.A.Mey.) is widely used medicinal herb and food material in east Asia including Korea. Insecticide tebupirimfos and fungicide tolclofos-methyl are frequently used as soil incorporation treatment when initial stage of ginseng cultivation. This study was optimized to simultaneously determine the residues of tebupirimfos and tolclofos-methyl in ginseng and dried ginseng using solid phase extraction via gas chromatography - nitrogen phosphorus detector. Acetonitrile was used for pesticide extraction and partitioning was carried out by sodium chloride and magnesium sulfate anhydrous. Then purification of extract was using amino-propyl cartridge. The limit detection and quantification were 0.005 and 0.01 mg/kg from both pesticides. The linearities as concentration range of 0.005-1.0 μg/mL were good with determination coefficient(R^2) over 0.9999 from both experimental pesticides. Mean Recoveries at two different fortification levels were 85.0 - 92.9% with relative standard deviation(RSD) $\leq$ 1.55, 101.1-112.0% with RSD $\leq$3.13 from ginseng and dried ginseng for tebupirimfos analysis. The average recoveries showed 99.9 - 105.9% with RSD $\leq$2.46, 111.6 - 117.0% with RSD $\leq$2.15 from ginseng and dried ginseng for tolclofos-methyl analysis. The optimized method is expected to be usefully applied to the analysis of both pesticides in ginseng, dried ginseng, extracts and any products.

P-29

Penflufen의 환경행적 평가와 수계 노출농도 산출

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Penflufen (BYF 14182, K-1683, 5-fluoro-1,3-dimethyl-N-[2-(1,3-dimethylbutyl) phenyl]-1H-pyrazole-4-carboxamide)의 환경 분해대사와 토양 및 수계 이동성에 관련된 보고서를 검토하여 환경에서의 잔류특성과 수계로의 이동성을 결정짓는 매개변수를 확정하고, 작물재배조건을 고려하여 환경노출농도를 산출하였다. Penflufen은 호기토양대사에 의하여 BYF 14182-3-hydroxybutyl (K-1684, BCS-AA-10006)와 Acetyl BYF 14182 (K-1757, AE 2300037, BYF 14182-pyrazolyl-AAP)로 전환되어 각각 9~17% 및 2~12%까지 검출되었고, DT_{50}은 249~432일(미국 2 토양)이었다. Penflufen의 호기담수토양대사에서 5% 이상 생성되는 대사산물은 없었고, DT_{50}은 218~1,000일 이상(평균 512일, 3 토양(이태리 1, 국내 2))이었다. Penflufen은 혐기조건과 광 조건에서도 거의 분해되지 않았다. 밭 포장조건에서 penflufen은 2년경과 후 5~12%로 감소하여 그 DT_{50}은 65~263일이었고(유럽 6 토양), 논 포장조건에서 penflufen의 국내 2 토양의 DT_{50}은 103~122일이었다. Penflufen의 유기탄소기준흡착계수(Koc)는 각각 210~410 L/kg으로 UK SSLRC 이동성 구분은 'moderately mobile' 등급에 해당하였고, 토주용탈에 의한 RMF (Relative Mobility Factor)도 0.69로 확인되어 Koc에 의한 이동성 구분과 같은 등급에 속하였다. Penflufen은 pH 4~9 범위에서 가수분해에 안정하였으나, 광증감에 의해 분해가 촉진되어 일본 봄 조건에서의 반감기는 33~41일이었으며 분해산물로 BYF 14182-pyrazole-4-carboxamide와 BYF 14182-fluoro acid가 생성되었다. Penflufen 1.5% 입제의 벼-잎도열병 방제 적용에 대한 지하수와 지표수의 연평균 추정농도는 각각 0.089 μg/L와 2.6~4.3 μg/L로 산출되어 잠정 설정한 음용수기준 100 μg/L에 근접할 가능성은 매우 낮았으며, 지표수 최고농도도 담수어류, 물벼룩 및 녹조류에 대한 반수영향농도 103~5,100 μg/L 비해 매우 낮은 수준이었다. Penflufen의 잔류분을 토양, 지표수 및 지하수 모두에 대하여 penflufen, BYF 14182-3-hydroxy-butyl, BYF 14182- pyrazolyl-AAP로 정의하였다.

P-30

Residues and dissipation Half-life of Veterinary Antibiotics, Chlortetracycline, Enrofloxacin, and Sulfathiazole in soil

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Veterinary antibiotics contaminate plants via the following ways: veterinary antibiotics are given to animals, animals excreta are used as soil fertilizer, and finally the plants obtaining the nutrients from the contaminated soil. The consumers, in turn are might be at natural rsik, because of residues. The present study was carried out to determine the residues and estimate the half-life of three commonly used veterinary antibiotics, chlortetracycline (CTC), enrofloxacin (ENR), and sulfathiazole (STZ), in soil. Samples were extracted using modified citrate-buffered version of quick, easy, cheap, effective, rugged, and safe "QuEChERS" method followed by liquid chromatography coupled with tandem mass spectrometric analysis in positive ion mode. The linearity was good for the three tested antibiotics with determination coefficients (R^2)$\geq$ 0.9922. The average recovery rates at 2 spiking levels with three replicates for each of the levels were ranged between 77.1 and 114.8% with relative standard deviation (RSD) $\leq$ 20.2%. In batch incubation experiment (*in vitro* study), the half-lives of CTC, ENR, and STZ were ranged from 2.0 - 6.1, 2.2 - 4.5, and 1.1 - 2.2 days, respectively. Under greenhouse conditions, the half-lives of three target antibiotics in soil with and without radish were in between 2.5-6.9 and 2.7-7.4; 4.7-16.7 and 10.3-14.6; and 4.4-4.9 and 2.5-2.8 days, respectively. The concentrations of the tested antibiotics were decreased with time and consequently lower residues were observed in radish. In case ofhalf-life of ENR and STZ, the half-life of *vivo* study was longer than the *vitro* study it might be due to the application of organic fertilizer that diluted in water to grow crops *in vivo* study, also organic matter content in soil sample was higher for *vivo* study than that of *vitro* study, which may cause higher absorption power of antibiotic.

P-31

Shoot-and-Dilute GC-ECD for Analysis of Problematic Pesticides

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Sensitive pesticides like Captan, Folpet, DDT, and Deltamethrin degrade during hot splitless injection GC. This effect is magnified when analyzing food samples prepared using the QuEChERS procedure. Compound degradation leads to lower response factors and quantitative inaccuracies. To restore performance, more frequent maintenance on the splitless inlet and GC column are necessary. Maintenance includes splitless inlet liner and inlet seal changes, and GC column trimming. A way to keep the GC system up longer with more consistent response factors is to instead use split injection. We call split injection GC - “Shoot-and-Dilute GC”. Shoot-and-Dilute GC (split injection GC) with a Sky Precision split liner with quartz wool is recommended to prevent sensitive pesticide degradation that occurs during splitless injection. In addition to keeping the GC inlet up longer, the GC column lasts longer because less “dirt” is placed on it during split injection.

P-32

Simultaneous Determination of Veterinary Pharmaceuticals in Brown Rice Using LC-QTOF-MS

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This study was conducted to develop the simultaneous analysis of veterinary pharmaceuticals (VPs) in brown rice. The method for VPs in rice was developed and validated by LC-QTOF-MS. The method was established using solid phase extraction (SPE) cartridges. Efficiency of cartridges was tested in view of recovery and relative standard deviation (RSD). Hydrophilic lipophilic balance (HLB) cartridge was showed the higher result than that of tested other SPE cartridges. Recovery and limit of quantitation (LOQ) of tested 20 VPs in water were 70.3-112.0% and 0.076-7.6 μg/L, respectively. The precision was reliable since RSD percentage (1.1-5.7%) was below 20, which was the normal percent value. The results showed that the developed method might be used the monitoring of VPs in brown rice.

P-33

Simultaneous Determination of Veterinary Pharmaceuticals in Water Using LC-QTOF-MS

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This study was conducted to develop the simultaneous analysis of veterinary pharmaceuticals (VPs) in water. Simultaneous determination method for VPs in water was developed and validated by LC-QTOF-MS. The method was established using solid phase extraction (SPE) cartridges. Efficiency of cartridges was tested in view of recovery and relative standard deviation (RSD). Hydrophilic lipophilic balance (HLB) cartridge was showed the higher result than that of tested other SPE cartridges. Recovery and limit of quantitation (LOQ) of tested 20 VPs in water were 70.2-102.4% and 0.003-0.15 μg/L, respectively. The precision was reliable since RSD percentage (1.3-5.1%) was below 20, which was the normal percent value. These results were showed that the developed method might be used the monitoring of VPs in water.

P-34

The Effect of Ascorbic Acid on Ethoxyquin Recoveries

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Low ethoxyquin recoveries were observed from 4 agricultural commodities (paprika, pear, tomato, apple) in the QuEChERS EN method. The low recoveries is because of oxidative losses. Commodities that have strong antioxidative potential, such as citrus fruit and berries show good recoveries without adding antioxidants. To improve recoveries, ascorbic acid was added as antioxidant to paprika, pear, tomato, apple.

Recovery testing was perfomed in oder to find out the effect of ascorbic acid. Through the matrix-matched calibration method, good reproducibility and robustness, linearity was confirmed. The spiking levels for the recovery experiments were 0.010 and 0.1 mg/kg for GC-MS/MS analysis. Mean recoveries mostly ranged between 70 and 120 %, and RSD were generally below 20%. Limit of detections (LOD) were 0.8 to 1.4 μg/kg and limit of quantitation (LOQ) were 2.4 to 4.3 μg/kg. Correlation coefficients of the calibration curves were >0.998. Based on these results, the method has been proven to be highly efficient and robust and suitable

P-35

Transformation Rate of Insecticide Spirotetramat to Its Metabolites in Perilla Leaves

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Insecticides sprayed on crops for controlling insects may cause safety problem in agriculture products. It is important to predict their residual patterns of applied pesticides in order to ensuring safety of agricultural products. Also, it is necessary to know the effect of crops caused by the metabolites of pesticides. In this study, we investigated residual patterns of spirotetramat to its metabolites in perilla and the transformation rate to metabolites. Spirotetramat (SC including 22% a.i.) diluted 2,000 times in water was sprayed 2 times at interval of 7 days prior to harvest, and perilla leaves were harvested at 0, 1, 3, 5 and 7 days after final application. Residual amounts of spirotetramat and its metabolites in perilla leaves were analyzed using LC-MS/MS. Spirotetramat and metabolites recoveries spiked with 0.1 and 0.5 mg/kg in control sample were ranged from 85.5 to 110.9%. Residual amounts of spirotetramat in perilla leaves at harvesting days decreased from 24.42 to 17.25 mg/kg. While, the residual amount of metabolites keto and enol form increased from 0.19 to 0.29 mg/kg, showing a similar tendency. Glucoside form increased from 0.02 to 0.04, and mono form was below the limit of quantitation. Calculated as a sum of metabolites from spirotetramat based on residuals were converted up to 0.92%. Therefore, it is very important to know the transformation rate of parent pesticide to metabolites in order to ensure the safety of the final harvested agricultural products.

Keyword : Metabolites, Residual pattern, Spirotetramat, Transformation rate

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Translocation of Residual Diniconazole from Soil to Korean cabbage

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Translocation of residual diniconazole from soil to Korean cabbage was investigated. The recovery ranges in soil and Korean cabbage at two fortification levels of 0.2 and 1.0 mg/kg were determined 96.1 ~ 111.1 and 89.9 ~ 109.3%, respectively. Korean cabbages were cultivated during 43 days after soil treatment of diniconazole as two different concentrations in greenhouse. The samples of soil and Korean cabbage were collected at 0, 7, 14, 25, 35, 39, 43 and 35, 37, 39, 41, 43 days after application, respectively. The initial residue amounts were 5.18 and 13.23 mg/kg and decreased to 1.26 and 1.24 mg/kg at 43 DAT (day after treatment) and the half-life of diniconazole was about 27.7 and 12.4 day for the low and high concentration of diniconazole on treated soils, respectively. Residue level of diniconazole on Korean cabbage was 0.9 ~ 3.8% of initial concentration on soil. Therefore, the safe management guidelines of diniconazole for Korean cabbage-cultivating soils can be suggest as 12.4 mg/kg.

P-37

감자 중 Bifenazate와 대사물 Bifenazate-diazene의 LC-MS/MS를 활용한 동시 · 신속 분석법 확립

백수진, 이지호*, 이종화, 이정학, 신용호, 정민우, 전다래, 원수, 유혜진, 김정한

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Bifenazate는 과수 및 채소류 재배 시 발생하는 응애류 방제에 이용되는 살응애제이다. Bifenazate은 농산물 내에서 산화되어 대사물인 bifenazate-diazene으로 전환되므로 대사물의 분석이 필수이다. 현재의 공식 분석법은 bifenazate-diazene을 환원시켜 모화합물인 bifenazate로 전환하여 단일 화합물 형태로 분석하도록 하고 있다. 본 연구에서는 bifenazate-diazene을 환원하지 않고 대사물 자체로 분석하면서 bifenazate와 함께 동시에 분석할 수 있는 분석법을 LC-MS/MS를 활용하여 확립하였다. LC-MS/MS는 Shimadzu LCMS-8040을 이용하였고, ESI positive mode 및 scheduled SRM (selected reaction monitoring) mode를 사용하여 분석하였다. Bifenazate 및 대사물의 표준용액은 matrix matched 방법으로 제조하였고 분석정량한계(method limit of quantitation; MLOQ)는 10 ng/mL이었고, 검량 범위 4-200 ng/mL에서 검량선 상관계수(r^2)는 ≥0.99이었다. 마쇄한 감자 시료 10 g에 acetonitrile 10 mL를 첨가하여 진탕추출한 후, 4 g $MgSO_4$, 1 g NaCl를 첨가하여 1분간 격렬히 진탕하여 3,500 rpm에서 5분 간 원심분리하였다. 상등액 1 mL을 취하여 dispersive-SPE (MgSO4 150 mg, PSA 25 mg)를 이용하여 정제 후, 이를 13,000 rpm에서 5분간 원심분리하고 상등액 2 μL를 분석하였다. 분석법 검증을 위하여 bifenazate 및 bifenazate-diazene의 표준용액을 무처리 시료에 MLOQ의 1배, 10배 및 50배가 되도록 처리하여 각각 3반복 회수율 시험을 진행한 결과, bifenazate 및 bifenazate-diazene의 회수율은 모든 처리 수준에서 70-120.0% (RSD ≤10%)를 만족하였다. 본 연구를 통해 확립한 분석법을 통하여 감자에 잔류되는 bifenazate 및 대사물 bifenazate-diazene을 동시에 신속하게 신뢰성 있는 분석할 수 있을 것으로 판단된다.

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경남지역에서 재배된 수출용 신선딸기의 농약 잔류실태

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신선농산물에 대한 수입국 통관검사에서 안전기준을 위반하여 클레임이 발생되는 경우, 해당 농산물의 수입금지로 인한 금전적 손실뿐만 아니라 우리 농산물의 안전성에 대한 이미지 손상, 수입국의 통관검사 강화 등으로 수출에 막대한 지장을 초래하게 된다. 이에 국립농산물품질관리원에서는 신선농산물의 수출 진흥 및 농가소득 증대를 위해 수입국의 안전기준에 적합하게 재배된 농산물이 수출될 수 있도록 잔류농약 등의 유해물질에 대하여 수입국 맞춤형 안전성검사를 지원하고 있다.

파프리카, 배와 더불어 대표적인 수출 신선농산물인 딸기는 홍콩, 싱가폴 등으로 4,100여 톤(34백만 불, '16년 기준)을 수출하였으며, 이중 진주, 김해, 합천 등 경남지역에서 재배된 딸기가 82.6%를 차지하고 있다. 경남지역 시설재배 딸기의 본격적인 수출을 앞둔 2016년 11~12월 두 달 동안, 홍콩, 미국 등 5개국으로 수출예정인 418농가에서 재배된 딸기의 잔류농약 검사결과 acetamiprid, kresoxim-methyl, azoxystrobin 등 62종의 성분이 1,412회 검출되었으며, 이중 수입국 기준에 부합되지 않은 flonicamid, imicyafos, spirotetramat 등 32종(185회)의 약제가 검출된 115점에 대하여 해당 국가로의 수출이 보류될 수 있도록 조치하였다.

고품질의 안전한 우리농산물의 수출 확대를 위해서는 수입국의 안전기준에 부합하는 농산물을 생산하기 위한 재배농가의 노력과 더불어, 안정적 수출선 확보를 위한 수입국 맞춤형 안전성 검사의 지속적인 지원이 필요할 것으로 판단된다.

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계란에서 LC-MS/MS를 사용한 농약 다성분 분석

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현재 국내에서 계란 및 알에 40여개의 MRL이 설정되어 있고 최근에 닭에 대한 농약살포 등이 이슈화 되어 국내 및 외국의 MRL을 고려하여 총 15종 농약에 대한 다성분 동시분석법을 LC-MS/MS를 사용하여 개발하고자 하였다. LC-MS/MS는 Shimadzu 8040을 사용하였고, 분석정량한계(method limit of quantification; MLOQ)는 5 ng/g, 검량 범위 5 - 200 ng/mL에서 검량선 상관계수(r^2) ≥0.99를 대다수 농약이 만족하였다. 회수율 실험은 마쇄한 시료에 2MLOQ, 10MLOQ 수준 농약을 3반복으로 처리하여 2가지 전처리법(전처리법1, 전처리법2)으로 수행하였다. 전처리법 1은 전통적인 SPE 정제를 기반하였고 전처리법 2는 QuEChERS를 활용하였다. 전처리법 1은 마쇄한 계란 10 g에 acetonitrile + formic acid 1% 용액 10 mL를 첨가하여 진탕한 후, MgSO4 4 g, NaCl 1 g을 더하여 1분간 진탕추출을 하였다. 원심분리 후, 상등액 5 mL를 취하여 건고농축 한 후, DCM : MeOH(99 : 1)로 재용해 한 후, NH_4 카드리지를 사용하여 정제하였다. 정제액을 모두 건고농축 후 acetonitrile 용액 1 mL로 재용해하여 LC-MS/MS(ESI positive mode)로 분석하였다(5 μL injection). 전처리법 2는 마쇄한 계란 10 g에 acetonitrile과 acetonitrile + 0.1% formic acid 용액을 각각 10 mL 씩 첨가하여 진탕한 후, MgSO4 4 g, NaCl 1 g을 첨가하여 1분간 진탕추출을 하였다. 상등액 1 mL를 취하여 original dSPE(MgSO4 150 mg, PSA 25mg), C18 dSPE($MgSO_4$ 150 mg, PSA 25mg, C18 25 mg)를 사용하여 1분간 진탕 후, 원심분리하여 상등액을 취하여 LC-MS/MS(ESI positive mode)로 분석하였다(5 μL injection). 전처리법 1의 경우 대다수의 농약이 회수율 70 - 120%과 RSD ≤20%를 만족하지 못하였다. 전처리법 2의 경우, 대다수의 농약이 회수율 70 - 120%과 RSD ≤20%를 만족하였다. 추출의 경우 formic acid 0.1%를 첨가한 것이 더 좋은 결과를 나타내었다. dSPE에서는 C18이 더 좋은 결과를 나타내었고 결론적으로 formic acid가 추가된 C18을 함유한 dSPE를 사용한 것이 가장 좋은 결과를 나타내었다.

P-40

대두 중 Bromobutide와 대사물 Bromobutide-debromo의 GC-NPD를 활용한 분석법 확립

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Bromobutide는 sulfonamides계 제초제로서 일년생잡초인 피와 sulfonylurea계 제초제에 저항성인 잡초에 대하여 강한 제초활성을 나타내는 특징이 있어 다른 제초제와 혼합제의 형태로 사용되는 선택성 제초제이다. Bromobutide는 식물체에서 bromobutide-debromo로 대사되기 때문에 본 연구에서는 Bromobutide와 그 대사물 bromobutide-debromo의 잔류 분석법을 GC-NPD를 이용하여 확립하였다. 분석기기는 Agilent 7890B GC-NPD를 사용하였고, column은 DB-5ms계열을 사용하였다. Bromobutide 및 대사물의 분석정량한계(method limit of quantitation; MLOQ)는 0.02 mg/kg이었다. 검량범위 0.05-10 mg/kg에서 검량선 상관계수(*r2*)는 >0.99이었다. 균질화된 대두시료 20 g에 증류수 20 mL 첨가 후 20분간 방치하고 acetone 100 mL로 30분 간 진탕 추출한 다음 흡인 여과하였다. 포화 NaCl 수용액 50 mL와 증류수 450 mL을 넣은 분액깔때기에 여액을 넣은 후 dichloromethane 50 mL로 두 번 액액분배하였다. 유기용매 층을 anhydrous sodium sulfate에 통과시킨 후 40℃ 이하에서 감압농축하여 acetonitrile로 포화된 hexane 30 mL에 재용해한 후, hexane으로 포화된 acetonitrile 30 mL로 두 번 액액분배한 다음 acetonitrile 층을 anhydrous sodium sulfate에 통과시켜 40℃ 이하에서 감압농축 후 잔류물을 hexane 10 mL에 재용해하였다. 정제를 위해 유리 칼럼관에 alumina 5 g, anhydrous sodium sulfate 약 2 g을 충진한 후 dichloromethane:hexane (20:80, v/v) 50 mL으로 활성화시키고 추출액 10 mL를 첨가한 다음 세척 용액으로 50 mL 넣어 세척하고 dichloromethane:acetonitrile:hexane (50:1.5:48.5, v/v/v) 70 mL로 용출시켜 감압농축하고 4 mL acetone으로 재용해하였다. 분석법 검증을 위하여 무처리 대두 시료에 bromobutide 및 대사물을 MLOQ의 1배, 10배 및 50배가 되도록 각각 3반복 처리하고 회수율 및 정밀성을 측정하였으며, bromobutide 및 대사물의 회수율은 70-120% (RSD <10%)를 만족하였다. 본 연구에서 확립한 분석법을 통하여 대두에 잔류한 bromobutide 및 bromobutide-debromo의 동시 분석을 효율적으로 수행할 수 있을 것으로 사료된다.

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대중국 쌀 수출지원을 위한 현미와 백미 중 살균제 Hexaconazole, Tricyclazole과 살충제 Etofenprox의 잔류량 비교

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수출농산물은 국내 기준은 물론, 수출대상국의 식품기준에 맞는 농산물 생산이 최대 관건이다. 수출 쌀 안전성 평가의 판단 근거가 되는 농약잔류허용기준(MRL) 설정에 있어서 중국은 벼, 현미, 백미 기준으로 혼재되어 있고, 한국은 현미기준으로 설정되어 있어 백미상태로 수출하는 대중국 수출용 쌀에 국내기준을 중국기준에 곧바로 적용하기에는 어려움이 있다. 본 시험은 현미와 백미 중 농약잔류량 비교를 통하여 대중국 수출용 쌀의 안전성을 확보하기 위해 수행하였다.

시험은 전북 완주의 시험포장에서 수행하였으며, 품종은 조평벼였다. 시험농약으로는 hexaconazole+tricyclazole(4.5+22.5)% 액상수화제와 etofenprox 10% 유탁제를 안전사용기준에 따라 각각 750배와 1,000배로 희석하여 배부식 동력분무기를 이용하여 살포하였다. Hexacozole+tricyclazole의 시험구는 수확전 37-30, 44-37-30일, etofenprox 시험구는 21-14, 30-21-14일 처리구로 나누어 처리하고, 수확 후 현미/백미로 도정하여 공시시료로 사용하였다. Hexaconazole 잔류분석은 GC/ECD, tricyclazole과 etofenprox의 잔류분석은 HPLC/UVD를 사용하였다.

현미 중 hexaconazole, tricyclazole, etofenprox의 회수율은 각각 84.7-90.1%, 83.4-88.0%, 86.6-96.6% 이었으며, 백미 중 회수율은 각각 83.0-99.4%, 72.7-84.4%, 72.8-79.7% 범위이었다. 시험농약의 검출한계는 hexaconazole은 0.005 mg/kg, tricyclazole과 etofenprox는 0.01 mg/kg 이었다. 현미 중 hexaconazole의 잔류량은 검출한계 미만이었고, tricyclazole의 잔류량은 0.33-0.41 mg/kg, etofenprox의 잔류량은 0.03-0.04 mg/kg이었다. 백미 중 hexaconazole과 etofenprox의 잔류량은 검출한계 미만이었으며, tricyclazole의 잔류량은 0.17-0.22 mg/kg이었다. 백미 중 시험농약의 잔류량은 3농약 모두 한·중 양국의 기준에 적합하였고, 현미 중 잔류량은 hexaconazole과 tricyclazole 2농약만이 양국 MRL에 적합하였다. Etofenprox의 경우 현미 중 잔류량은 중국의 MRL(0.01 mg/kg)를 초과하였으나, 백미 중 잔류량은 검출한계 미만이고 수년간 모니터링 결과에서도 검출된 적이 없음을 들어, 백미 상태의 대중국 수출에는 문제가 없을 것으로 평가하였다.

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들깻잎 중 fenhexamid와 imidacloprid의 잔류량 변화 및 안전성 평가

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소면적 재배 작물인 들깻잎 중 농약의 경시적 잔류특성을 구명하고 농약의 섭취량을 산출하여 안전성을 평가하기 위하여 이 연구를 수행하였다. 시험농약은 살균제 fenhexamid(42% 액상수화제, 바이엘크롭사이언스)와 살충제 imidacloprid(8% 액상수화제, 바이엘크롭사이언스)이었으며, 각각 1,000과 2,000배 희석하여 200 L/10a의 액량으로 7일 간격 3회 살포하고 최종 약제 살포 후 0, 1, 3, 5 및 7일차에 시료를 채취하여 잔류농약을 분석하였다. 들깻잎 중 fenhexamid와 imidacloprid의 검출한계는 각각 0.05와 0.01 mg/kg이었으며, 회수율은 각각 75.1-85.1과 80.6-86.7%로 유효한 회수율 범위를 보였다. 또한 저장 기간 중 시험 농약의 안전성을 조사하기 위하여 저장 안전성 시험을 수행하였으며, 모두 74.7-83.8%의 회수율로 들깻잎 중 fenhexamid와 imidacloprid는 저장 기간 동안 안전한 것으로 판단되었다. 들깻잎 중 fenhexamid의 잔류량은 최종 약제 살포 당일 33.21-34.38 mg/kg으로 잔류허용기준인 30 mg/kg을 초과하였지만 5일차부터 잔류허용기준 미만으로 검출되었으며, 7일차에는 22.28-23.61 mg/kg으로 경시적으로 감소하였다. Imidacloprid의 경우 최종 약제 살포 당일의 잔류량은 3.07-3.23 mg/kg이었으며, 7일차의 잔류량은 1.14-1.16 mg/kg이었다. 또한 들깻잎 중 imidacloprid의 잔류량은 최종 약제 살포 당일부터 잔류허용기준인 7.0 mg/kg미만으로 검출되었다. 일일섭취추정량과 일일섭취허용량을 비교한 안전성 평가 결과 두 시험농약의 섭취량은 모두 일일섭취허용량 대비 약 0.01% 미만인 것으로 나타났다.

주제어 : 들깻잎, fenhexmid, imidacloprid, 잔류농약, 안전성 평가

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맥문동 중 dimethoate와 prochloraz-manganese의 잔류 특성 및 가공계수 산출

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이 연구는 맥문동 중 농약의 잔류특성을 구명하고 가공계수를 산출하기 위하여 수행하였다. 시험농약은 dimethoate(46% 유제, 한국삼공(주))와 prochloraz-manganese(50% 수화제, 한국삼공(주))이었으며, 시험 포장은 충남 청양에서 수행하였다. Dimethoate는 1,000배 희석하여 1 L/m^{2}의 액량으로 약제 처리일을 달리하여 2회(50-40, 40-30, 30-20) 토양관주처리 하였으며, prochloraz-manganese의 경우 2,000배 희석액을 200 L/10a의 액량으로 약제 처리일을 달리하여 3회(50-40-30, 40-30-21, 30-21-14, 21-14-7) 살포한 후 시료를 채취하였다. 채취한 시료는 관행적인 방법에 따라 건조 맥문동으로 가공하였으며, 수확일에 인접하여 살포한 처리구(dimethoate 30-20, prochloraz-manganse 21-14-7)의 맥문동은 생체 시료 중 농약의 잔류량을 산출하여 가공계수를 산출하였다. 시험농약의 검출한계는 각각 0.002(dimethoate-건조 전), 0.004(dimethoate-건조 후) 및 0.02(prochlroraz-manganese) mg/kg이었으며, 분석법을 검증하기 위하여 수행한 회수율 시험은 각각 88.1-94.1 (dimethoate)과 73.9-89.0 (prochlroraz-manganese)%의 범위이었다. 건조 후 맥문동 중 dimethoate는 〈0.004-0.026 mg/kg의 범위로 검출되었으며, 건조 전 맥문동의 경우 0.241-0.252 mg/kg의 범위로 검출되었다. 또한 prochlroraz-manganese의 경우 건조 후 맥문동의 모든 처리구에서 검출한계 미만이었지만 건조 전 맥문동에서 0.06-0.07 mg/kg의 범위로 검출되었다. 가공 전 잔류량과 가공 후 잔류량을 이용하여 산출한 dimethoate의 가공계수는 0.10-0.11, prochlroraz-manganese의 경우 0.29-0.33의 범위이었다.

주 제 어 : 맥문동, dimethoate, prochloraz-manganese, 잔류농약, 가공계수
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배추 재배 중 살충제 diflubenzuron의 잔류특성

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Diflubenzuron은 benzoylurea계 살충제로 감귤, 사과, 배, 복숭아, 고추, 수박, 파 등의 나방류 및 파리의 방제에 사용되며 배추의 배추좀나방 방제에 사용된다. 본 연구는 배추 재배 중 살포되는 diflubenzuron의 생물학적 반감기와 잔류특성을 파악하기 위해 수행되었다. Diflubenzuron 수화제 (특명, 7%)를 1000배 희석하여 수확 14일 전 배추에 살포 후 0, 1, 2, 3, 5, 7, 10, 14일차에 배추를 수확하여 UPLC-MS/MS를 사용하여 해당 약제를 분석하였다. UPLC-MS/MS는 Shimadzu LCMS-8040을 이용하였고, ESI positive mode를 사용하여 분석하였다. 분석정량한계(method limit of quantitation: MLOQ)는 0.005 ng/g이었고, 검량선 상관계수(r^2)는 $\geq$0.99이었다. 마쇄한 배추 시료 10 g에 acetonitrile 10 mL를 첨가한 다음 10분간 진탕추출 한 후 4 g $MgSO_4$, 1 g NaCl, 1 g NaCitrate, 0.5 g $Na_2Citrate\cdot 5H_2O$를 첨가하여 10분간 진탕하였고, 5분간 3500 rpm에서 원심분리 하였다. 상등액 1 mL를 취하여 dispersive SPE (25 mg PSA, 150 mg $MgSO_4$)를 이용하여 정제 후, 13,000 rpm에서 3분간 원심 분리하여 상등액을 500 uL를 취하여 acetonitrile 500 uL와 혼합하여 분석하였다. 분석법 검증을 위해 무처리 배추 시료에 diflubenzuron을 10MLOQ와 50MLOQ가 되도록 각각 3반복 처리하여 회수율을 측정하였다. Diflubenzuron의 평균회수율은 10MLOQ에서 97.6%, 50MLOQ에서 93.8%로 회수율 70~120% (RSD $\leq$20%)를 만족하였다. 저장안정성평가는 50MLOQ에서 104.2%로 나타났다. 농약 살포 후 경과일수별 잔류량은 포장1의 경우 0일차 2.63 mg/kg에서 감소하여 14일차에는 1.32 mg/kg이었고, 포장2에서는 0일차 6.92 mg/kg로부터, 14일차 1.82 mg/kg로 감소하였다. 배추에서의 diflubenzuron의 생물학적 반감기는 포장1에서 16.3일, 포장2에서 9.9일이었다.

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블루베리 중 살충제 Chlorfenapyr 및 Spinetoram의 잔류특성

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본 연구는 소면적 재배작물인 블루베리를 대상으로 chlorfenapyr 및 spinetoram 의 경시적 잔류특성을 구명하여 농약 품목등록 자료 및 잔류농약의 안전성 평가를 위한 기초자료로 활용하고자 수행하였다. 본 연구의 블루베리 품종은 드래퍼이며, chlorfenapyr 5% EC 및 spinetoram 5% SC 농약을 7일 간격 2회 경엽살포한 후 0, 1, 3, 5, 7일차에 시료를 채취하여 분석하였다. 블루베리 중 두 농약 모두 dichloromethane으로 분배한 후 florisil, NH2 cartridge column을 이용하여 정제 후 GC/μ-ECD, LC-MS/MS를 이용하여 분석하였다. Chlorfenapyr의 회수율은 78.8~101.5% 이었으며, spinetoram (XDE-175-J, L)의 회수율은 78.7~93.9%, 대사물질인 XDE-175-N-Demethyl-J, L의 회수율은 76.5~91.6%, XDE-175-N-Formyl-J, L의 회수율은 74.8~103.5%이었으며, 두 농약에 대한 MLOQ(method limit of quantiation)는 모두 0.01 mg/kg이었다. Chlorfenapyr의 잔류량은 0일차에서 0.61~0.74 mg/kg, 7일차에서는 0.24~0.26 mg/kg 이었고, spinetoram의 총잔류량은 0일차에서 0.32~0.38 mg/kg, 7일차에서는 0.06~0.08 mg/kg 이었다. 이것으로 블루베리의 안전성 평가에 유용한 자료로 활용될 수 있을 것으로 사료된다.

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비름(*Amaranthus mangostanus* L.) 중 Dimethomorph 및 Pyraclostrobin의 잔류특성 및 위해성 평가

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본 연구는 소면적 재배작물인 비름(*Amaranthus mangostanus* L.) 중 살균제 dimethomorph 및 pyraclostrobin (16+9.5%, SC)의 잔류특성을 구명하고 안전성을 평가하기 위하여 수행하였다. 처리구는 7일 간격으로 2회, 3회 살포횟수로 구분하여 배치하였으며, 시험 약제는 2,000배 희석하여 처리하였고, 최종 약제 살포 후 0일차를 기준으로 당일 수확하였다. 비름 중 dimethomorph 및 pyraclostrobin의 검출한계는 모두 0.01 mg kg^{-1}이었으며, 정량한계의 10배 및 50배 수준으로 회수율 시험을 수행한 결과, dimethomorph 89.6~96.3%, pyraclostrobin 70.8~96.8%, pyraclostrobin의 대사체 BF500-3은 77.6%~103.2%으로 나타났다. 비름 중 dimethomorph 및 pyraclostrobin의 잔류량은 수확 당일 처리구에서 4.56 mg kg^{-1}, 3.94 mg kg^{-1}으로 확인하였으며, 수확 21일 전에는 0.11 mg kg^{-1}, 0.20 mg kg^{-1}으로 나타났다. 현재 비름에 대한 안전사용기준은 설정되어 있지 않아 두 약제에 대한 유사 농산물인 엇갈이배추의 MRL을 적용한 결과, dimethomorph의 경우 모든 처리구에서 잠정 MRL 5.0 mg kg^{-1}을 초과하지 않았으며, pyraclostrobin의 경우 수확 21일 전 처리구에서 잠정 MRL 0.5 mg kg^{-1}이내에 잔류량을 확인하였다. 따라서 비름 중 dimethomorph 및 pyraclostrobin의 잔류특성을 구명한 결과 잠정 MRL을 초과하지 않는 수확 14일 전 3회 경엽처리를 적합한 안전사용기준으로 제안하였다. 또한 비름에 대한 dimethomorph 및 pyraclostrobin에 대한 추천 MRL을 포함한 전체 작물의 ADI 대비 식이섭취율은 각각 5.37%, 11.22%로 ADI 대비 식이섭취율 80%를 초과하지 않아 식이를 통한 노출 위험도는 안전한 수준으로 판단되며, 국내 잔류허용기준설정을 위한 연구 결과로 적합할 것으로 사료된다.

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사료 관리대상 농약 121성분 분석법 확립 및 검증

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2015년에 농식품부는 사료의 잔류농약에 대한 기준을 국제기준에 부합되게 조정하기 위해 기존 27성분에서 121성분으로 관리대상을 확대하였고, 적용대상 사료도 배합사료와 섬유질사료에서 곡류 및 콩류로 까지 확대하였다. 따라서, 이 연구의 목적은 성분확대 농약에 대해 통합 분석할 수 있는 다성분동시분석을 확립하고, 동시분석이 적용되지 않는 성분에 대해서는 계열 및 단성분 분석법을 확립하여 사료검정업무의 효율성 제고하고 검정결과의 신뢰도를 확보하고자 하였다. 먼저, 잔류허용기준이 설정된 농약 중 105성분에 대해 한번의 전처리를 통해 기기별로 그룹화하여 분석하는 다성분동시분석법을 확립하였고, 다성분동시분석법 적용이 불가능한 16성분에 대해 유사계통 농약들을 그룹화하여 일상적 분석의 효율성을 극대화하는 계열별 분석법을 확립하였고, 동시분석과 계열분석이 적용되지 않는 농약에 대해 정밀정량용 단성분 분석법을 확립하였다. 계열분석법으로는 산성농약, 극성농약, 극성해리성 그룹으로 분류하였다. 산성농약 계열 분석법은 pKa가 2~3인 약산성농약 6성분(2,4-D, bentazone, cycloxydim, dicamba, haloxyfop, MCPA)의 동시분석을 위해 0.1% formic acid를 함유한 acetonitrile을 추출 용매로 하였으며, 극성농약 계열 분석법은 LogPow가 (-)값을 갖는 극성농약 4성분(paraquat, diquat, cartap, chlormequat)의 동시분석을 위해 1% formic acid를 함유한 methanol을 추출 용매로 하였다. 극성해리성농약 계열 분석법은 약산성의 해리상수를 갖는 극성이 매우 높은 산성의 수용성 화합물 3성분(glyphosate, glufosinate, ethephon)의 동시분석을 위해 대상성분을 분해시키지 않고 이온화를 최대한 억압하기 위해 0.1% formic acid를 추출용매로 하였다. 단성분 EDB, pyrethrins, dithiocarbamate에 대해서도 최적의 추출 및 분석조건을 확립하였다. 121성분 분석법 확립을 통해 분석시간 및 분석비용을 절감하는 효과가 있었고, 사료 중 잔류허용기준 설정 농약에 대한 안전관리를 위한 분석체계를 구축할 수 있었다. 또한, 불량 사료의 유통을 원천적으로 차단하고 가축의 폐사 등의 위해사고 발생을 사전 예방할 수 있을 것으로 기대한다.

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사료 중 산성계열 6종 농약의 동시분석법 확립 및 검증

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농식품의 선택에 있어 품질, 위생, 안전에 대한 소비자의 관심도가 높아짐에 따라 축산분야에서 사료에 대한 안전성이 부각되고 있고, 이에 따라 사료 품질 및 안전관리가 더욱 강화되고 있다. 국내 사료 중 농약잔류허용기준이 설정된 농약은 121성분이며, 이 중 다성분동시분석이 가능한 105성분을 제외한 나머지 16성분에 분석법의 확립이 필요하다. 따라서 본 연구에서는 다성분동시분석이 어려운 16성분을 계열별로 분류하였고, 그 중 pKa(산해리상수)가 낮은 6종 농약(2,4-D, Bentazone, Cycloxydim, Dicamba, Haloxyfop, MCPA)에 대한 분석법을 확립하고자 하였다. 6종 농약의 특성상 유기 용매만으로 추출할 경우 회수율이 낮아지는 단점이 있다. 이러한 단점을 해결하기 위해 Acetonitrile을 추출용매로 하여 NaCl, $MgSO_4$, citrate buffer를 가하는 퀘쳐스(QuEChERs) EN법과 0.1% formic acid가 함유된 acetonitrile을 추출용매로 하여 NaCl, $MgSO_4$를 가하는 산성 퀘쳐스법의 결과를 비교하였다. 비교 결과 EN 퀘쳐스의 회수율은 49.24~88.30%로 나타났고, 특히 dicamba와 cycloxydim의 회수율이 낮음을 확인하였다. 반면에 산성 퀘쳐스의 회수율은 80.16~110.50%로 비교적 높은 값을 나타내었다. 결과적으로 산성퀘쳐스법을 이용하여 6종 농약을 분석하는 것이 회수율면에서 더 나은 결과를 도출할 수 있었다. 산성 퀘쳐스법에 대한 분석법 검증은 선택성, 직선성, 정확성, 정밀성 등을 기준으로 하였으며, 검증 요소 모두 검증 가이드라인 기준에 부합되어 분석법의 신뢰성을 확보하였다. 따라서 본 연구결과는 사료의 관리대상 농약 중 6종 농약에 대한 분석의 신뢰성 있고, 정확해졌을 뿐 아니라, 이를 적용하여 사료 중 산성농약을 검정하는데 효율적이라고 판단된다.

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소면적 작물 조(粟) 재배 중 Dimethomorph의 잔류특성

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Dimethomorph는 배추, 상추, 감자 등의 노균병 방제에 널리 사용되는 살균제이다. 본 연구에서는 소면적 작물인 조에 살균제 dimethomorph 16% 액상수화제를 살포하고, LC-MS/MS를 활용하여 조의 알곡과 짚 중 dimethomorph를 분석한 후 그 잔류 특성을 확인하였다. 농약살포는 약제기준으로 2,000배 희석하여 실시하였고, 수확 전 기준으로 50/40/30일전 처리구, 40/30/21일전 처리구, 30/21/14일전 처리구, 21/14/7일전 처리구에서 각각 3반복 처리하였다. LC-MS/MS는 Shimazu LC-MS 8040 (ESI+mode)을 사용하였고 분석정량한계(method limit of quantitation; MLOQ)는 모든 시료에서 10 ng/g이었고, 검량 범위 5-250 ng/mL에서 검량선 상관계수(r^2)는 ≥0.99이었다. 시료의 전처리를 위해서 균질하게 마쇄한 조의 알곡 및 짚 20.0 g을 칭량하여 150 mL 용량의 PTFE bottle에 넣고 증류수 20 mL를 첨가하여 30분간 습윤화하고 acetonitrile 100 mL를 가한 후 30분간 격렬히 진탕 추출하였다. 이후 NaCl 6 g을 첨가하여 1분간 격렬히 진탕하고, 3,500 rpm의 속도로 5분간 원심분리하였다. 상등액 10 mL를 정확히 취하여 감압농축하고, methanol/dichloromethane(1/99, v/v) 용액 4 mL에 재용해한다. SPE NH_2 cartridge (1 g)에 dichloromethane 5 mL를 흘려주어 활성화시켜준 뒤, 추출물 4 mL를 첨가하여 methanol/dichloromethane (1/99, v/v) 용액 7 mL을 이용하여 용출하였다. 용출액을 완전히 건고하여 acetonitrile 2 mL에 재용해하고 0.2 mL를 2 mL autosampler vial에 옮긴 후 acetonitrile 0.2 mL를 넣고 잘 섞어준 뒤, 이 중 5 μL를 LC-MS/MS로 분석하였다. 분석법의 검증을 위하여 10MLOQ 및 50MLOQ 수준에서 3반복 회수율 시험을 실시한 결과, 알곡 중 dimethomorph의 평균 회수율은 93.8% 및 82.7%이었고, 짚에서는 72.7% 및 76.3%이었다. 확립된 분석법을 통한 포장시료 분석 결과, 알곡과 짚 모두 최종 약제 살포 후 시간이 경과함에 따라 dimethomorph가 감소하는 경향을 나타냈다. 이 결과를 바탕으로 소면적 재배작물인 조에 대하여 dimethomorph의 잔류허용기준 마련과 안전사용기준 설정에 유용한 자료로 활용될 수 있을 것으로 판단된다.

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소면적 재배작물 아스파라거스(*Asparagus officinalis*) 시설재배조건에서 Spinetoram의 잔류특성

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백합과에 속하는 아스파라거스(*Asparagus officinalis*)는 최근 국민 소득 수준의 향상에 따른 식생활 변화와 고급채소의 수요 증대로 재배면적이 2005년 10ha에서 2015년 80ha로 재배면적이 점차 증가하고 있는 추세이다. 재배기간이 10년~15년인 다년생 작물로 해충에 노출 될 수 있는 기간이 길기 때문에 방제에 대한 지속적인 노력이 필요하며, 입경기부터 생육기에 나방류 해충의 발생에 따른 방제가 필요하다. 본 연구는 파밤나방(*Spodoptera exigua*) 방제에 활용되는 spinetoram 5% 입상수화제의 시설재배 10년생 아스파라거스에 대한 잔류 특성을 규명하여 안전사용기준 및 잔류허용기준 설정을 위한 기초자료로 활용하고자 하였다. 포장시험은 시설재배조건에서 약제를 2,000배 희석하여 7일 간격 2회 충전식 자동 분무기로 처리하였으며, 시료채취는 2회 약제처리 후 0, 1, 3, 5 및 7일차에 수행하였다. 분석법의 정량한계는 spinetram(XDE-175-J, L), N-demethyl spinetoram(J, L) 및 N-formyl spinetoram(J, L) 각각 0.002, 0.0002, 0.0002, 0.0002, 0.002 및 0.0002 mg kg-1이었다. 회수율은 정량한계의 10배 및 50배 수준으로 각각 3반복 수행하였으며 spinetram(XDE-175-J, L)는 각각 104.5~117.5% 및 89.0~93.8%, N-demethyl spinetoram(J, L)는 각각 80.5~88.9% 및 90.8~106.3%, N-formyl spinetoram(J, L)는 각각 104.3~118.9% 및 80.6~89.3%였다. 잔류량 산출은 모화합물 잔류량과 모화합물로 환산한 대사체 잔류량의 합을 spinetoram으로 하였다. 시설재배 아스파라거스에 대하여 spinetoram 5% 입상수화제 살포 후 0, 1, 3, 5 및 7일차의 최대 잔류량은 0.120 mg kg^{-1}, 0.057 mg kg^{-1}, 0.015 mg kg^{-1}, 0.005 mg kg^{-1} 및 0.003 mg kg^{-1}이었다. 이에 spinetoram 5% 입상수화제의 아스파라거스에 대한 안전사용기준 및 잔류허용기준(안)을 제안하고, %ADI를 산출하여 식이섭취에 따른 안전성을 확인하였다.

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소면적 재배작물 우엉(*Arctium lappa* L.)중 Imicyafos 및 Fosthiazate의 잔류양상

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본 연구는 뿌리혹선충 방제를 위한 imicyafos 및 fosthiazate를 선정하여 우엉에 대한 잔류허용기준 및 안전사용기준을 제안하고, 이에 대한 안전성평가를 위하여 수행하였다. Imicyafos 5% 입제 및 fosthiazate 5% 입제 농약을 각각 기준량(6 kg/10 a), 배량(12 kg/10 a)으로 파종 전 토양혼화 처리하였으며, 90일 후 어린 우엉 잎을 2회 채취한 후 수확기에 우엉 뿌리 시료를 채취하여 분석하였다. 두 약제 모두 LC-MS/MS를 이용하여 분석하였으며, imicyafos 및 fosthiazate의 정량한계는 0.005 mg/kg이었다. 우엉 잎과 뿌리 중 imicyafos 및 fosthiazate의 회수율 및 저장안정성 시험 결과, 모두 70.0~120.0%로 유효회수율 범위를 만족하였으며, 변이계수(Coefficient of variation, C.V.) 또한 10% 이하의 기준을 만족하여 대상 농약 성분에 대한 분석방법의 적합성과 시료의 저장기간 중 안정성을 확인할 수 있었다. 본 연구결과, 우엉 잎 중 imicyafos의 잔류량은 〈0.005~2.284 mg/kg이었고, fosthiazate의 경우 0.090~2.644 mg/kg이었으며, 우엉 뿌리 중 imicyafos 및 fosthiazate의 잔류량은 모두 0.005 mg/kg 이하인 것을 확인하였다. 본 연구결과를 근거로 하여 imicyafos 5% 입제 및 fosthiazate 5% 입제의 안전사용기준은 정식 전 토양혼화 1회처리를 제안하였다. 이를 바탕으로 우엉에 대한 imicyafos 및 fosthiazate의 위해성을 평가한 결과, 우엉 잎과 뿌리에 대한 ADI 대비 식이섭취율(%ADI)이 imicyafos 16.45%, fosthiazate 7.36%로 잔류농약의 주 섭취경로농산물 기준 일일섭취허용량의 80%를 초과하지 않으므로 식이를 통한 노출 위험도는 낮은 것으로 판단된다. 본 연구결과 우엉의 병해충 방제을 위하여 imicyafos 5% 입제 및 fosthiazate 5% 입제를 안전사용기준을 준수하여 사용한다면 우리나라 농식품 중 잔류독성 및 먹거리 안전관리에 일조할 수 있을 것으로 사료된다.

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수입 농산물의 잔류농약 단성분 모니터링 (Monitoring of single pesticide residue in Imported agricultural products)

이현숙*, 이한진, 최은혜, 정형욱, 이규식

식품의약품안전처 식품의약품안전평가원 식품위해평가부 잔류물질과

본 연구는 국내 수입되는 농산물의 잔류농약 실태를 조사파악하고, 위해평가 등의 안전성 평가를 수행한 후 정부차원에서의 식품 위생/안전 정책 수립의 과학적 근거를 마련하고자 수행되었다. 시료 수거는 인구 백만 이상의 전국 9개 지역(서울, 부산, 인천, 대구, 대전, 광주, 울산, 창원, 수원)을 선정한 후, 수입량 및 수입빈도가 높은 농산물 중 허용물질목록관리제도(Positive List System, PLS)가 시행된 견과종실류 및 열대과일류 등을 포함한 10품목(아몬드, 커피원두, 들깨, 오렌지, 바나나, 파인애플, 체리, 포도, 쌀, 고추)을 선정하여 총 114건의 농산물을 수거하였다. 대상 농약은 최근 10년간 모니터링을 하지 않은 ADI가 낮고 단성분 분석이 가능한 살균제(트리포린, 오리사스트로빈, 에톡시퀸, 하이멕사졸) 4종을 선정하여 모니터링을 수행하였다. 분석 대상 농약들은 모니터링에 앞서 시험법을 확인, 개선하고 매트릭스를 대표할 수 있는 농산물(감귤, 감자, 고추, 현미, 대두)에 적용하였고, 트리포린, 오리사스트로빈의 정량한계(LOQ)는 각각 0.005, 0.02mg/kg이었으며, LOQ 및 LOQ 10배, 50배 수준으로 처리 하였다. 에톡시퀸, 하이멕사졸의 정량한계는 각각 0.05, 0.02mg/kg이었고, 잔류허용기준(MRL)은 각각 0.5, 3.0 mg/kg이었으며, LOQ 및 MRL, MRL 10배 수준으로 처리하였다. 시험법 검증 결과 평균 회수율은 63.5-117.2%, 분석오차는 18.1% 이하, 상관계수(R^2)는 0.9974 이상의 수준으로 모두 국제식품규격위원회(CODEX)의 농약 시험법 가이드라인(CAC/GL40, 2003) 및 식품의약품안전평가원의 식품 등 시험법 마련 표준 절차에 관한 가이드라인(2016)에 적합하였다. 개선 및 검증한 시험법으로 총 114건의 수입 농산물에 대한 잔류농약 단성분 모니터링을 수행한 결과, 대상농약이 검출되지 않았다.

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수출대상국별 식품기준 맞춤형 농약안전사용지침 및 해외 잔류기준 설정

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수출농산물은 수출대상국의 식품기준에 맞는 농산물 생산이 최대 관건이다. 최근 세계 각국은 자국의 농업보호와 유통농산물의 안전성 확보를 위하여 식품안전관리제도를 강화하고 이를 비관세 무역장벽으로 활용하고 있다. 실제 우리농산물의 주요 수출대상국인 일본과 대만의 Positive List System(PLS) 시행 이후, 한국산 수출농산물 중 잔류농약 초과검출로 통관이 금지된 사례는 179회(일본 78회, 대만 101회)로 통관규제에 따른 수출위축 등 큰 경제적 손실을 초래하였다. 이러한 국제여건에 효율적으로 대응하고 수출농산물에 대한 농약사용 문제점을 해결하기 위해 농촌진흥청은 2003년부터 수출대상국별 작물별 적용병해충별로 사용가능한 농약과 사용방법, 안전사용기준, 양국의 잔류기준 등을 수록한 맞춤형 「수출농산물 농약안전사용지침」을 설정 · 보급해오고 있으며, 2016년 지침 설정수는 일본, 대만 등 11국가 30작물 1,747병해충, 20,694 농약품목이다. 또한 보다 적극적인 방법으로 안전성 위반농약이나 농업인이 선호하는 국내 등록농약 중 상대국에 기준이 설정되지 않은 농약에 대하여 수출대상국의 잔류기준(Import Tolerance, IT)을 설정해 오고 있다. 국내 등록농약의 수입국 잔류기준(IT) 설정은 2017.3월 현재, 일본에 고추 등 15작물 63농약, 대만에 사과 등 3작물 23농약, 홍콩에 딸기 10농약에 대한 의견을 제출하여, 일본에 피망 등 12작물 48농약, 대만에 사과 등 3작물 22농약의 IT를 반영시킴으로써 농산물의 수출확대 및 국제경쟁력 강화에 크게 기여하였다.

최근 들어 IT 설정은 대상국에 따라 2~16포장의 GLP 잔류성적과 더불어 면역독성 등이 포함된 독성자료와 약효약해 성적까지 요구하는 경우가 늘고 있어 현재 non-GLP 단포장 잔류성적을 적용하고 있는 우리나라로서는 잔류성 시험의 GLP 조기 도입과 농약등록 규정의 국제적 조화를 위한 제도 개선 등 이에 대한 대책이 시급히 마련되어야 할 것으로 본다. 또한 IT설정 업무의 효과적 추진을 위한 예산과 전문인력 확보와 더불어 해당국의 관련기관과의 소통 및 상호 협력체계 구축에도 공을 들여야 할 것이다.

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수출딸기(매향) 중 Cyenopyrafen과 Cyflumetofen의 잔류소실 특성 및 잔류농약 모니터링

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딸기(*Fragaria ananassa Duch.*)는 2015년 기준으로 생산량이 약 194,513톤, 생산액은 12,840 억원의 고소득 작물로써 대부분 시설재배에서 생산 되고 있다. 국내에서 재배되는 딸기 중 수출에 특화된 품종인 〈매향〉은 수출전문단지와 수출 선도조직등의 시장개척으로 수출이 크게 증가하고 있지만, 세계 각국은 자국의 농업과 소비자 보호를 위해 농식품의 안전 및 위해요소 관리를 점점 강화하고 있다. 국내에서는 수출농산물에 대해 지속적인 관리와 교육을 진행하고 약 320성분 동시다성분 분석법으로 잔류농약 안전성 조사를 수행하고 있지만, 수출딸기 재배 중, 320종에 포함되지 않은 미설정 농약성분을 사용하고 있어 국제적으로 수출국에 대한 잔류농약 문제가 발생할 소지가 있다. 따라서, 본 시험에서는 홍콩 등으로 수출되고 있는 딸기에서 국내 검사 미설정농약이면서 농가에서 많이 사용하고 있는 cyenopyrafen(상품명: 쇼크)과 cyflumetofen(상품명: 파워샷)에 대한 잔류 소실 특성을 확인하고 홍콩 등지에 수출되는 20농가의 딸기시료를 확보하여 cyenopyrafen과 cyflumetofen의 잔류량 실태를 조사하였다.

Cyenopyrafen은 농약 살포 후 45일 동안 시료를 채취하여 분석한 결과, 시험기간 동안 PLS 0.01 mg/kg 이상 이었으며 반감기는 6.1일 이었다. Cyflumetofen은60일 동안 수행한 결과, 60일 이후부터 PLS 기준 이하였으며 반감기는 6.1일 이었다. 이 2 성분은 PLS 기준 이하의 잔류기간이 매우 길어 수출 시 각별한 주의가 필요할 것으로 보인다. 20 농가의 모니터링 결과, cyflumetofen은 0.0024 mg/kg에서 0.24 mg/kg 수준으로 검출되었으며 cyenopyrafen은 0.0026 mg/kg에서 0.44 mg/kg 수준으로 검출되었다. 결과에서도 볼 수 있듯이 수출 시 부적합사례가 될 수 있어 농약사용에 대한 농가교육과 동시에 안전성평가와 모니터링이 필요할 것으로 판단된다.

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시설재배 오이(*Cucumis sativus* L.) 중 살균제 Fluopicolide 및 Metrafenone의 생산단계 잔류허용기준 설정

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시설재배 오이(*Cucumis sativus* L.)에 대하여 유통 시 잔류허용기준(Maximum Residue Level; MRL)을 초과하는 농산물로 인한 피해를 방지하고자 오이에 사용되는 fluopicolide 및 metrafenone의 생물학적 반감기를 산출하고, 생산단계 잔류허용기준(Pre-Harvest Residue Limit; PHRL)을 설정하고자 수행하였다. 오이의 포장시험은 구미(포장 1)와 상주(포장 2)의 시설재배지에서 수행하였으며, 두 농약을 안전사용기준에 따라 1회 살포 후, 2시간 후를 0일차로 하여 0, 1, 2, 3, 5, 7, 10일차에 시료를 채취하였다. Fluopicolide 및 metrafenone 모두 dichloromethane을 사용하여 분배하였고, SPE-NH2 cartridge로 정제하여 LC-MS/MS로 최종 분석하였다. 두 약제의 회수율 시험수행 결과, fluopicolide의 회수율은 74.2~84.2%, metrafenone은 84.7~97.2%이었으며, 분석정량한계 (Method Limit of Quantitation; MLOQ)는 0.005 mg/kg이었다. 오이 중 fluopicolide 및 metrafenone에 잔류량은 시간이 경과함에 따라 비교적 빠르게 감소하였으며, 두 약제에 대한 반감기는 fluopicolide의 경우 3.0일(포장 1)과 3.3일(포장 2), metrafenone의 경우 2.1일(포장 1), 2.0일(포장 2)로 나타났다. 오이는 급격한 증체율과 매끄러운 표면 특성을 가져 잔류농약의 희석효과로 비교적 짧은 반감기를 나타난 것으로 사료된다. 일자별 잔류량 감소추이를 토대로 fluopicolide의 PHRL은 2.92 mg/kg, metrafenone은 9.73 mg/kg로 제안하였다. 본 연구결과를 바탕으로 농약이 살포된 후 수확 시까지 fluopicolide 및 metrafenone의 잔류량 감소추이에 대한 예측이 가능하게 되어 오이의 안전한 생산·관리에 일조할 것으로 판단된다.

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아스파라거스 시설재배에서 Pyrethroid계 살충제 Acrinathrin 및 Etofenprox에 대한 잔류특성

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아스파라거스는 높은 영양학적 가치로 인하여 국내 소비가 지속적으로 증가하고 수출작물로도 주목받고 있다. 아스파라거스에 대한 농약의 잔류허용기준(Maximum Residue Limits, MRL)은 44개로 유사작물(셀러리, 풋마늘 등)에 비해 기준 설정이 부족한 실정이다. Pyrethroid계 살충제는 아스파라거스의 상품성에 주된 영향을 미치는 파총체벌레의 방제효과가 탁월한 약제로 최근 사용이 증가하고 있다. 본 연구는 아스파라거스 시설재배를 위한 pyrethroid계 약제인 acrinathrin 및 etofenprox의 작물 잔류특성을 구명하여 잔류허용기준설정(안) 및 생산단계잔류허용기준(안)을 제안하고자 한다. 포장시험은 시설재배조건에서 시료채취시기를 4월로 하여 설계하였으며, acrinathrin 5.7% 액상수화제 및 etofenprox+phenthoate 37(7+30)% 수화제를 각각 2,000배 및 1,000배 희석하여 7일 간격으로 2회 경엽살포한 후, 최종살포일로부터 0일, 1일, 3일, 5일 및 7일 후에 연속 수확하였다. Acrinathrin 및 etofenprox의 분석법 정량한계는 0.02 mg/kg 및 0.002 mg/kg 이었고, 정량한계의 10배 및 50배 수준에서 회수율은 acrinathrin 104.1~111.3% 및 etofenprox 83.8~90.82%였다. 최종 약제 살포후 0일, 1일, 3일, 5일 및 7일차의 최대잔류량은 acrinathrin 0.21 mg/kg, 0.16 mg/kg, 0.093 mg/kg, 0.053 mg/kg 및 0.027 mg/kg이었고, etofenprox는 0.15 mg/kg, 0.11 mg/kg, 0.043 mg/kg, 0.027 mg/kg 및 0.006 mg/kg이었다. 아스파라거스 시설재배조건에서 acrinathrin 및 etofenprox의 잔류량 결과에 근거하여 통계적 방법을 적용하여 감소상수 산출을 통한 생물학적 반감기는 각각 2.5일 및 1.6일이었다. 추가적으로, 아스파라거스 시설재배조건에서 acrinahtirn 및 etofenprox의 잔류허용기준(안) 및 생산단계 잔류허용기준(안)은 도출하였다.

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왕겨숯을 이용한 토양 중 잔류농약 경감효과 구명

황인성, 이효섭, 천재인, 홍수명, 권혜영, 문병철

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최근 소비자의 농식품 안전에 대한 관심이 높아지면서 GAP 및 친환경 농산물 인증의 수요가 증가하고 있다. 인증 수요는 증가하고 있지만, 기존 관행으로 농사를 지은 농가는 토양 중 잔류농약 검출로 인해 인증의 어려움을 겪을 것으로 사료된다. 토양 중 농약을 분해하는 방법은 많이 존재하지만 현장에서 적용할 수 있는 방안에 대해서는 부족한 실정이다. 따라서, 현장에서 적용할 수 있는 관리방안이 필요할 것으로 판단된다. 본 연구에서는, 왕겨숯 흡착을 이용한 토양 중 잔류농약 경감 구명을 위한 시험을 진행하였다. 왕겨숯은 친환경 비료로 관심을 받고 있고, 중금속 및 다양한 화합물을 흡착할 수 있는 것으로 알려져 있다. 왕겨숯의 농약 흡착능력을 평가하기 위해서 침투이행성 농약 5종(azoxystrobin, chlorpyrifos, diazinon, imicyafos, procymidone)을 선택하여 토양 2종(사양토, 양토)에 1 mg/kg을 첨가한 후 왕겨숯 0, 1, 2, 5% 첨가 후 170 rpm에서 12시간 동안 쉐이킹 하였다. 왕겨숯 흡착능력 평가를 위해 토양수 샘플러를 이용하여 토양수 채취 후 QuEChERS 및 LC-MS/MS를 이용하여 분석하였다. 분석결과 토양수 중 chlorpyrifos는 불검출 되었고, 나머지 농약들을 왕겨숯 2%이상에서 흡착효과가 나타나는 것으로 확인되었다.

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인삼 가공 과정 중 cypermethrin의 잔류 특성

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인삼을 건삼, 홍삼 및 농축액으로 가공하는 과정 중 cypermethrin의 잔류 특성을 구명하기 위하여 인삼 주산지인 충북 청주, 충남 금산 및 경북 영주의 4년근 인삼 재배지에서 포장 잔류 시험을 수행하였다. 시험농약(성보피레스 유제, 성보화학주식회사)은 안전사용기준에 준하여 1,000배 희석액(0.01 kg a.i./10a)을 수확 45일전까지 10일 간격으로 3회 살포한 후 관행적인 방법으로 시료를 채취하였다. 채취한 시료는 세척한 후 건삼, 홍삼 및 농축액으로 조제하여 분석용 시료로 사용하였다. 수삼 및 가공품 중 cypermethrin의 검출한계와 정량한계는 각각 0.03와 0.06 mg/kg이었으며, 정량한계, 정량한계의 10배 및 잔류허용기준 농도 수준으로 처리하여 분석한 회수율은 76.3-124.6%로 유효한 회수율 범위를 보였다. 인삼 및 가공품 중 cypermethrin의 잔류량은 모두 정량한계 미만이었으며, 침투성이 없는 농약을 경엽처리하여 분석부위인 뿌리까지 이행되지 않은 결과인 것으로 판단되었다.

주 제 어 : 인삼, 가공품, cypermethrin, 잔류농약
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잔류농약분석 숙련도 평가(Proficiency Test)를 위한 표준물질(Reference Material)의 분석

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본 실험은 잔류농약 숙련도 평가를 위하여 제공된 표준물질을 이용하여 다종농약성분의 정성 및 정량분석을 실시하였다. 분석에 사용된 농약 성분은 사용량이 많고 검출 빈도가 높은 성분을 중심으로 30종의 농약성분을 선정하였으며 실험에 사용된 분석법은 QuEChERS 분석법을 적용하여 LC-MS/MS 및 GC-MS/MS로 분석하였다. 시료는 안전성평가연구소에서 토마토를 이용하여 제조한 것으로 투입된 농약의 잔류농약 균질성과 안정성을 확인 한 후 분양 받아 분석하였다. 30종의 농약성분 중 임의의 20종 농약성분을 일정 농도로 첨가하였고 첨가 된 농약의 정량분석 결과를 토대로 정도관리를 수행하였다.

제조기관으로부터 무처리 토마토 시료(30 g)와 임의의 20종 농약성분이 첨가된 토마토 시료(30 g)를 수령하여 분석을 실시하였다. 시료 분석은 QuEChERS EN 15662 방법을 적용하여 반복구당 각각 5 g의 검체에 증류수를 첨가한 후 Acetonitrile로 추출하여 MgSO4와 PSA(primary secondary amine)등으로 추출·정제 하였다. 시료 추출물을 LC-MS/MS 및 GC-MS/MS를 이용하여 분석하였으며 실험결과 Acetamiprid를 비롯한 20종 농약성분이 검출되었고 R^2 값은 0.99 이상으로 높은 직선성을 나타내었다. 20종 농약성분의 검출농도는 0.054 ~ 0.716 mg/kg 수준이었으며 3회 반복구간 표준편차(SD, standard deviation)는 0.002 ~ 0.019 mg/kg 수준으로 확인되었다.

분석결과는 높은 정확성 및 정밀성을 나타내었으며 전처리 방법이 비교적 쉽고 용이한 QuEChERS 시료전처리방법과 미량의 시료를 고감도로 분석하는 LC-MS/MS 및 GC-MS/MS를 이용하여 잔류농약분석 숙련도 평가에 적절히 활용할 수 있다고 판단하였다.

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장어 중 펜디메탈린 시험법 검증
Method Validation of Pendimethalin in Eel

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식품의약품안전처 식품의약품안전평가원 식품위해평가부 잔류물질과

본 연구는 중국산 활장어(뱀장어)에 펜디메탈린이 검출되어 반송 · 폐기되었다는 해외 위해정보와 관련하여, 2017년 식품안전관리지침(수산물 안전관리)에 따라 수입 활장어(뱀장어) 중 펜디메탈린의 안전관리를 위하여 검사 시험법을 마련하고자 하였다. 펜디메탈린은 디니트로아니린계 제초제로 잡초발생 전 토양처리 제초제로 사용되며, 잔류허용기준은 가지 등 64종의 농산물에서 0.05~2.0 mg/kg로 설정되어있으나, 장어에 대한 기준 규격은 설정되어있지 않다. 펜디메탈린의 시험법 검증은 현행 식품공전 중 다종농약다성분 분석법-제2법을 적용하여 검증하였다. 펜디메탈린을 아세토니트릴로 추출하여, 헥산-아세토니트릴 분배법에 의하여 유지 및 비극성 불순물을 제거하고, SPE-Florisil로 정제한 후 GC-MS/MS로 분석하였다. 시험법 검증은 첨가농도 0.01, 0.1, 0.5 mg/kg 수준을 적용하였으며, 회수율, 정량한계 및 분석오차를 국제식품규격위원회(CODEX) 가이드라인에 준하여 실시하였다. 그 결과 펜디메탈린 시험법의 직선성(R^2)은 0.9983, 정량한계는 0.005 mg/kg, 회수율은 99.8~116.3%, 분석오차는 11.6% 미만으로 시험법의 정확성 및 정밀성을 확인하였다. 본 연구에서 검증된 시험법은 수입 활장어(뱀장어) 대상으로 펜디메탈린의 잔류검사를 위한 시험법으로 활용될 예정이다.

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타액 중 농약 다성분의 GC-MS/MS를 이용한 동시 분석법 확립

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통계청 사망원인 자료에 의하면 2006년부터 2012년까지 농약으로 인한 국내 사망자수는 21,353명으로 매년 3,000명 이상이 농약중독으로 사망하고 있고, 근래에는 타살사건(농약 사이다) 등이 빈번히 발생하고 있어 중독원인 농약 수사의 신속정확한 확인이 절대적으로 필요하다. 본 연구는 연구대상 농약 55성분에 대해 GC-MS/MS를 활용하여 생체시료 타액 중 농약의 동시 다성분 분석법을 확립하였다. GC-MS/MS는 Shimadzu GCMS-TQ8040을 사용하였고, column은 Rxi-5Sil MS를 이용하였다. GC-MS/MS 기기분석조건의 확립을 위해서, 개별 성분의 표준용액을 주입하여 SCAN 및 product ion 분석을 통하여 성분당 2개 이상의 SRM (Selected reaction monitoring)조건을 확립하였다. 농약 55 성분 중 53성분의 분석정량한계(MLOQ, Method limit of quantitation)는 5 ng/mL이었다. 정량을 위한 검량선은 5-100 ng/mL 범위에서 matrix matched calibration 법을 사용하였으며, 55 성분 중 52성분에서 검량선 상관 계수(r^2)는 ≥0.99 이었다. 회수율 실험을 위해 타액 100 μL를 취하여 MLOQ, 5MLOQ, 15MLOQ 수준으로 농약 처리 후 0.1% formic acid가 함유된 acetonitrile 200 μL를 첨가하고 1,200 rpm에서 1분간 진탕 추출하였고 40 mg MgSO4, 10 mg NaCl을 가하여 1,200 rpm에서 1분간 진탕 추출하였다. 진탕액을 13,000 rpm에서 5분간 원심분리하여 상등액 120 μL를 취하여 4 : 1 비율로 matrix matching한 후 GC-MS/MS에 1 μL 주입하여 분석하였다. 회수율 실험 결과, 전체 농약 55성분 중 회수율 70-120% (RSD ≤20%)를 만족하는 성분은 MLOQ, 5MLOQ 및 15MLOQ에서 각각 전체의 89.1%, 96.4% 및 98.2%이었으며, 해당 농약들은 동시 정량분석이 가능하여 법과학 분야에 효과적으로 활용할 수 있을 것으로 사료된다.

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팥(*Vigna angularis* Willd.) 중 살균제 Chlorpyrifos 및 Tefluthrin의 잔류특성 및 안전성평가 연구

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소면적 재배작물은 잔류농약 모니터링 결과 부적합 발생비율이 높고, 농약의 품목수가 다른 작물에 비해 상대적으로 적어 병해충의 방제에 어려움을 겪고 있다. 본 연구에서는 소면적 재배작물인 팥(*Vigna angularis* Willd.)을 선정하여 대상 작물에 발생하고 있는 검거세미밤나방 방제에 효과적인 chlorpyrifos 2.0% 입제와 tefluthrin 0.5% 입제를 선정하여 잔류성 시험을 수행하였다. 본 연구의 시험약제인 chlorpyrifos 2.0% 입제 및 tefluthrin 0.5% 입제를 각각 기준량(6 kg/ 10 a), 배량(12 kg/ 10 a)으로 파종 전 1회 토양전면혼화처리 하였으며, 수확시기에 맞추어 팥(알곡) 시료를 채취하였다. 채취한 팥 시료 중 chlorpyrifos 및 tefluthrin의 잔류분석을 위하여 dichloromethane으로 2회 분배하였고, SPE-NH2 catridge를 이용하여 정제한 후 GC/ECD에 주입하여 최종 분석하였다. 두 약제의 회수율시험 결과 chlorpyrifos는 82.9%~88.9%, tefluthrin은 78.1%~92.2%로 유효회수율 범위인 70%~120%를 만족하였고, MLOQ (Method Limit of Quantitation)는 모두 0.005 mg/kg이었다. 팥 중 chlorpyrifos 및 tefluthrin의 잔류량은 기준량과 배량 처리구에서 모두 MLOQ 미만으로 검출되었으며, 각각 약제의 총 잔류량으로부터 산출한 ADI 대비 식이섭취율(%ADI)은 63.5655%, 7.7773%로 팥 중 ADI 대비 식이섭취율이 80%를 초과하지 않아 안전관리에 문제가 없을 것으로 사료된다. 이에 팥 중 chlorpyrifos 및 tefluthrin의 안전사용기준은 파종 전 1회 토양전면혼화처리로 제안하였으며, 이러한 결과가 소면적 재배작물 팥 재배 중 피해를 주는 검거세미밤나방 방제에 효과적으로 활용될 뿐 아니라 소면적 재배작물의 잔류농약 안전관리에 도움이 될 수 있을 것으로 판단된다.

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Chemical and Biological Indicators of Soil Quality in Organic and Conventional Farming Systems

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Organic farming has been popular worldwide and has expanded in recent years due to environmental, economic, and social concerns. In Egypt, up to 2.23 % of the agricultural area is managed organically. The transition from conventional to organic farming is accompanied by changes in an array of soil properties and processes that affect soil properties. These changes in soil properties under Egyptian condition are not well documented. Therefore, the present study was carried out to investigate comparative effects of organic and conventional farming systems on various soil chemical and biological indicators under Egyptian condition. Surface soil samples (0-30 cm) were collected from eight field pairs (organic field and its conventional counterpart) to represent organic farms in three locations (Fayoum (Tubhar village), Belbeis city (SEKEM Organic Farm) and Salhyia city). The organic fields have been in organic practice for 4, 6, and 10 years (Fayoum samples), 20 and 30 years (SEKEM samples), 8, 10 and 12 years (Salhyia samples) since organic farming certification. The soil chemical and fertility indicators included soil organic carbon, T-N, pH, EC, CEC, exchangeablecations, and available N, P, K, Fe, Mn, Cu and Zn. The biological indicators included population count of total bacteria, actinomycetes, fungi and azotobacter as well as the activity of dehydrogenase, alkaline phosphatase and urease enzymes.

Soil organic carbon, T-N, CEC, and available N, P, K, Fe, and Mn were significantly higher in soils of organic system than in the conventional system. These soil quality indicators further increased with increasing the period of organic farming practices. On the other hand, soil pH, EC, available Cu and Zn were higher in the conventional system than in the organic system. Findings indicate also that soil under organic farming system exhibits higher microbial population and activity.

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광범위 신규살균제 KNF1036의 이화학특성 및 제형연구
Research of physico-chemical properties and formulations for broad spectrum fungicide, KNF1036

허원, 이원형, 김형민, 김주경

주식회사 경농 중앙연구소

최근 요구되고 있는 혁신 작물보호제는 인류 생존에 필수 요소인 식량 및 에너지 확보를 위한 식물 바이오매스의 생산과 관리 기술의 핵심 요소로 필요 불가결한 상태이며, 또한 유전공학의 발달과 더불어 소비자들의 요구를 반영하는 즉, 식물 병원균에 대하여 환경 내의 저투입으로 높은 활성을 나타내며 다양한 병원균을 동시 방제할 수 있는 고효율 광범위 살균제의 개발이 지속적으로 요구되고 있다.

본 연구에 사용된 KNF1036은 strobilurin계 살균제로 최근 가장 활발한 연구가 진행되고 있는 호흡저해제 분야로 이는, 세포의 에너지 대사에 직접적으로 관여하는 미토콘드리아 내의 전자전달계를 차단함으로서 효과적인 식물 병원균 제어기술로 우수한 살균활성을 기대할 수 있다.

KNF1036의 이화학적 특성검토를 위하여 성상, 녹는점, 가비중, 증기압, 폭발성, 수용해도, logP, 유기용매 용해도 등을 분석하였다.

이러한 이화학적 특성을 바탕으로 제형을 검토한 결과, WP, EC, DC, SC, WG 등 다양한 제형이 개발 가능하였으며, 상업화 제형으로는 SC를 선발하여 최종처방을 확립하였다.

KNF1036 SC의 제형적인 특성 검토를 위하여 경시안전성 및 광안전성 시험을 실시한 결과, 유효기간은 4년 이상이 가능하였고 광안정성은 대조약제보다 안정적인 것으로 분석되었다. KNF1036 원제의 독성검토결과, 인축에 안전하였고 수서생물에 대해서는 strobilurin계 살균제와 유사한 독성을 보였다.

KNF1036 SC의 경우, 국내 대면적 포장시험 및 해외 여러 국가의 포장시험에서 여러 작물의 다양한 병해에 대해 우수한 살균 효과를 입증하였으며, 다양한 제형을 개발함으로서 글로벌 진출 시 각국의 상황에 맞는 여러 제형의 제품에 적용이 가능할 것으로 기대된다.

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양명아주 추출물을 함유한 유기농자재 중 유효성분 ascaridole의 보관 온도에 따른 함량 변화

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전 세계에 고루 분포하는 양명아주는 남미가 원산지이며 구충작용을 하여 병해충 방제에 많이 사용되고 있다. 양명아주 추출물을 원료로 한 유기농자재는 유효성분의 함량에 따라 작물의 병해충 방제 효능이 달라질 수 있고 제품의 조성과 보관 상태에 따라 유효성분의 지속기간도 달라질 수 있다. 이러한 유기농자재가 시중에 품질관리를 받지 않고 목록공시에 등록되어 생산, 유통되고 있어 제품별로 효능을 중심으로 한 유통기한 설정 평가가 필요한 실정이다. 이에 본 연구에서는 양명아주 추출물을 함유한 액상 유기농자재의 보관 온도에 따른 함량 변화를 조사하여 유통기한 관리를 위한 기초자료로 활용하고자 하였다. 시험 방법은 유기농자재 3종을 6가지 온도(0℃, 23℃, 30℃, 40℃, 45℃, 54℃)에 보관한 후 유효성분인 ascaridole 함량의 변화를 조사하였으며 그 결과, ascaridole의 반감기는 30℃ 조건에서 3.9일~27.7일로 나타났으며 다른 온도 조건에서도 비슷한 결과가 나타났다. 특히 유효성분의 반감기가 낮게 나타난 제품은 목초액이 포함된 제품으로 pH가 4 이하로 낮게 나타났다. 이를 통해 유기농자재 제품 중 양명아주 추출물의 함량과 부자재의 조성, 제품의 pH 조건에 따른 유효성분의 지속기간이 차이를 보이는 것을 알 수 있었고, 양명아주 추출물 내 유효성분의 열에 대한 안정성은 보관 온도에 따른 차이는 보이지 않았다는 것을 확인할 수 있었다.

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왕겨숯의 농약 흡착능력 평가 시험

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농약은 농업의 생산성을 증대하여 식량문제를 해결하는데 크게 기여하였고, 농산물의 질을 향상시키는데 큰 도움이 되었다. 그러나 농약이 작물에 살포되면서, 작물에 부착되지 않은 농약은 토양에 잔류하여 작물로 흡수·이행되어 안전성 문제를 발생시킬 수 있지만 토양 중 잔류농약 관리방안에 대한 연구는 부족한 실정이다. 토양 중 잔류농약 관리를 위해 왕겨숯을 이용한 연구를 진행하였다. 숯은 다공성의 특징으로 유기물이 잘 흡착되도록 하는 성질을 가지고 있고, 친환경 비료로 많은 관심을 받고 있기 때문에 현장 적용기술로 적절할 것으로 판단된다. 실험은 boscalid, dimethomorph, tricyclazole 3종을 이용하였고, 실험방법은 왕겨숯을 토양무게 및 증류수량의 0, 1, 2, 5% 첨가한 후, 180 rpm으로 12시간동안 진탕한 다음 pH미터기로 pH를 측정하였다. 토양과 토양수 sampler를 이용해 추출한 토양수를 QuEChERS 방법 및 LC-MS/MS를 이용하여 분석하였다. 왕겨숯의 순수 흡착능력 결과에서는 왕겨숯을 첨가할수록 농약 흡착능력은 높게 나타났고, 5% 이상 처리시 무처리구 보다 90% 이상 시험농약이 흡착이 되는 것을 확인할 수 있었다. 토양 중 왕겨숯을 처리하였을 때 무처리구 보다 왕겨숯 5% 처리하였을 때 최대 43% 토양수로 농약이 적게 이동되는 것으로 나타났다.

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정향 정유를 함유한 유기농자재의 지표성분 설정 및 분석법 개발

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정향은 인도네시아가 원산지인 도금양과 식물로서, 이의 꽃봉오리는 향신료, 의약으로 이용되어 왔다. 현재 꽃봉오리에서 얻은 정유는 항균효과가 있어 유기농자재의 원료로 이용되고 있다. 이에 정향 정유의 지표성분을 선정하고 유기농자재 중 지표성분의 정량분석법을 확립하고자 하였다. 정향 정유 중 항균성이 있고 다량 함유된 것으로 알려진 eugenol, β -caryophyllene, α -humulene을 지표성분으로 선정하였으며, GC-FID를 이용하여 기기분석 조건을 설정하였다. 그리고 HLB SPE 카트리지를 이용하여 전처리법을 확립하였다. 설정된 기기분석 조건에서 3 성분의 표준품을 이용하여 검량선을 작성한 결과 1~50 ppm 범위에서 상관계수 0.999 이상의 직선성을 보였으며, 정량한계 (limit of quantitation, LOQ)는 1 μg/mL 이었다. 분석법의 정밀성, 재현성 등의 평가를 위해 50 LOQ, 5 LOQ, LOQ 수준으로 회수율을 시험한 결과 평균 회수율은 88.8~107.6%이었고 상대표준편차는 2.6~7.8%로 나타났다. 시중에 유통되는 정향 정유 또는 추출물을 함유한 유기농자재 중 지표성분을 분석한 결과, 정유에서는 추출 부위에 따라 32.08-95.17%가 함유되어 있었으며 유기농자재에서는 0.67-3.09%가 함유되어 있었다. 본 연구를 통해 선정된 지표성분과 정량분석법은 정향 정유를 함유한 유기농자재의 분석에 활용될 수 있을 것으로 판단된다.

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피마자 추출물을 이용한 유기농자재 중 Ricinine의 보관온도에 따른 안정성 평가

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식물추출물을 이용하는 유기농자재는 추출물 내 포함된 유효성분의 함량에 따라 작물의 병해충 방제 효능이 달라질 수 있다. 현재 유기농자재는 꾸준한 품질관리를 받지 않고, 목록공시에 등록되어 생산, 유통되고 있어 효능을 중심으로 한 유통기한 설정 평가가 부족한 실정이다. 본 연구를 통해서 시중에 유통 되고 있는 피마자 추출물을 함유한 유기농자재 2종을 6가지 온도(실온, 0℃, 30℃, 40℃, 45℃, 54℃)에 보관한 후 유효성분 ricinine 함량의 변화를 조사하여 유기농자재 유통기한 관리를 위한 기초자료로 활용하고자 하였다. 그 결과, ricinine의 반감기는 제품 A 10.8 ~31.5일, 제품 B 6.3~10.5일로 나타났다. 이를 통해 유기농자재 제품 중 피마자 추출물의 함량과 부자재의 조성에 따라 지표성분의 지속기간이 차이를 확인 할 수 있었다.

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피마자유를 이용한 유기농자재 중 Ricinoleic acid의 보관온도에 따른 안정성 평가

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식물추출물을 이용하는 유기농자재는 추출물 내 포함된 유효성분의 함량에 따라 작물의 병해충 방제 효능이 달라질 수 있다. 현재 유기농자재는 꾸준한 품질관리를 받지 않고, 목록공시에 등록되어 생산, 유통되고 있어 효능을 중심으로 한 유통기한 설정평가가 부족한 실정이다. 이에 본 연구에서는 시중에 유통되고 있는 피마자유(2종) 및 이를 활용한 유기농자재(2종)를 각 보관온도(실온, 30℃, 35℃, 45℃, 54℃)에 따라 ricinoleic acid의 반감기를 산출하여 유통기한을 위한 기초자료로 활용하고자 하였다. 그 결과, 피마자유의 반감기는 약 21.7~46.2일, 유기농자재의 반감기는 28.9~57.8일을 확인하였다. 이를 통해 유기농자재 중 피마자유 반감기는 보관온도에 따라 차이를 보이지 않았지만, 함량과 부자재의 조성에 따라 유효성분의 지속기간이 차이가 날 수 있을 것으로 확인되었다.

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Combined Toxicities of Endosulfan with Phenanthrene against Zebrafish Adults (*Danio rerio*)

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Many studies on toxicity of an individual pollutant were carried out but not many studies on toxicity of combined pollutants were performed. However, in the nature, pollutants do not exist individually but simultaneously and have toxic effects on various ecological organisms. Endosulfan and phenanthrene are known to be frequently found in the agricultural environment. Therefore, in this study, individual and combined toxicity tests of endosulfan and phenanthrene on zebrafish (Danio rerio) adults were carried out based on OECD Guideline 203. The acute toxicities of endosulfan against the fish after 96 h of exposure were 1.0 μg L^{-1} for LC_{10} and 4.6 μg L^{-1} for LC_{50}. The acute toxicities of phenanthrene were 710μg L^{-1} for LC_{10} and 920 μg L^{-1} for LC_{50}. To evaluate mixture toxicity of two compounds that have adissimilar mode of action, independent action (IA) model was used. Based on LC_{10} and LC_{50} values for 96 h obtained from individual acute toxicity tests, the acute toxicity tests with four different combinations of compound concentration levels were performed. The results showed that the combination of LC_{50} value of endosulfan and LC10 of phenanthrene produced synergistic effect, suggested by the fact that experimental value is larger than the value calculated using IA model. To observe enzyme activity, head and body samples were separately prepared from the dead or living fish. The results of acetylcholinesterase (AChE) activity showed lower activity in the bodies of zebrafish treated with endosulfan alone and combined, compared with the control. In case of glutathione S-transferase (GST), a combined treatment gave higher activity than an individual. Carboxylesterase (CE) expressed higher activities by endosulfan than phenanthrene in both head and body. For evaluation of lipid changes after exposure, whole fishes were prepared. When the lipid changes in zebrafish after exposure were compared, an increase in lipids with an m/z ratio of 769.52, 795.53, 841.55 and a decrease in lipid with an m/z ratio of 885.64 were observed as the concentration of both compounds were increased. Those four lipids may be considered as a lipid biomarker to identify the toxicity of phenanthrene and endosulfan on organisms in the water system.

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Establishment of ADI and AOEL for pyflubumide through evaluation of Toxicological data

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Toxicity evaluation and establishment of safety reference dose for pyflubumide was conducted with acute toxicity, short term, long term study(rat, dog), carcinogenicity(rat, mouse), reproduction, teratogenicity, genetic toxicity, ADME study. In ADME test, oral absorption was 52% and main excretion route was feces. Residue level in tissue was very low. The major metabolites were NNI-0711-aniline-isobutyryl 및 NNI-0711-acid. Category of Acute toxicity was 5. In short term studies, there were anemia, change of heart, and hepatocelluar hypertrophy then the lowest NOAEL was 1.2 mg/kg bw/day. In 1-year dog and rat study, anemia, change of kidney, adrenal gland and bile duct then the lower NOAEL was 0.85 mg/kg bw/day. In carcinogenicity study for rats, there were effects of liver, heart, adrenal gland then NOAEL was 0.735 mg/kg bw/day. In carcinogenicitiy test for mice, spleen weight increased then NOAEL was 4.00 mg/kg bw/day. In reproductive study, there were effects of heart, liver, ovary, thyroid gland, and lung then NOAEL was 0.781 mg/kg bw/day. In teratogenicity test, body weight gain and food consumption decreased then the lowest NOAEL was 20 mg/kg bw/day. .There was no reproductive and teratogenic effect. In genotoxicity test, the result of all the tests was negative. As a result, the lowest NOAEL was 0.735 mg/kg bw/day in rat carcinogenicity study and safety factor was 100. ADI was established as 0.0074 mg/kg bw/day. The proper NOAEL among short term and teratogenicity studies was 1.20 mg/kg bw/day and correction factor was 0.52, then AOEL was established as 0.0062 mg/kg bw/day.

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Fluensulfone 및 함유품목의 환경생물에 대한 위해성평가

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fluensulfone은 식물기생선충에 특이적으로 작용하는 heterocyclic fluoro alkenyl sulfones의 살선충제로 특히 뿌리혹선충, 시스트선충 등에 효과적이다. 주 효과는 직접접촉에 의한 섭식중지, 운동능력 감소, 마비 등으로 선충의 교살 및 산란 · 부화 억제이나 정확한 작용기작은 밝혀져 있지 않다. fluensulfone의 환경생물에 대한 독성과 fluensulfone을 함유한 품목의 환경생물에 미치는 영향을 알기위해 담수어 2종(냉수어, 온수어), 물벼룩, 녹조류의 수서생물과 조류, 지렁이, 꿀벌과 같은 육상생물, 천적 등 유용곤충에 대한 독성 및 영향시험성적서를 검토하였다. fluensulfone 원제의 환경생물에 대한 독성 검토 결과 담수어류에 대한 급성독성에서 무지개송어(냉수어종)와 블루길(온수어종)의 LC50은 38 mg/L, 13 mg/L 이었고 물벼룩의 EC50은 29 mg/L 이었으며 녹조류의 EC50은 0.242 mg/L이었다. 육상생물 중 조류(북미산 메추라기)의 LD50은 1506 mg/kg, 지렁이의 LC50은 153 mg/kg 으로 영향이 낮았고 꿀벌에 대한 급성독성 중 접촉과 섭식 독성의 LD50은 각각 〉170 ㎍/bee, 〉197 ㎍/bee로 영향이 낮은 것으로 판단하였다. 포식성 응애(*Typhlodromus pyri*)에 대한 LR50은 2083 ml/ha, 포식성 응애(*Hypoaspis aculeifer*)에 대한 EC_{50}은 205.9 mg/kg로 천적에 대한 영향이 낮았다. 담수어, 물벼룩, 녹조류의 독성시험 결과로 fluensulfone 원제는 GHS 분류기준에 따라 급성수서환경유해성 구분1로 분류하였다. fluensulfone을 함유한 플루엔설폰 40% 액상수화제 품목의 경우 잉어에 대한 급성독성 LC50은 2.8 mg/L로 어독성 Ⅲ급으로 구분하였고 물벼룩의 EC50은 0.8 mg/L이었다. 어류, 물벼룩의 품목 독성 성적과 녹조류, 조류, 지렁이, 꿀벌에 대한 원제의 독성 성적을 근거로 위해성평가를 수행한 결과 모두 위해성이 낮은 것으로 판단하였다.

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Metazachlor 및 함유품목의 환경생물에 대한 위해성평가

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국내에 신규 등록된 metazachlor는 chloracetamide 계통의 발아 전 처리제로 겨울·봄 유채, 양배추, 꽃양배추, 녹색꽃양배추, 순무, 무, 관상수, 과수 묘목, 산림 등에 사용된다. 주 작용기작은 condensing elongase 효소시스템과의 상호작용으로 지질생합성 시 very-long-chain fatty acid 형성을 억제시켜 제초작용을 나타내는 것으로 세포 분열 및 조직의 분화를 억제시킴으로 결과적으로 어린잡초의 생육을 억제하거나 기형을 유발시키는 것이다. metazachlor의 환경생물에 대한 독성과 metazachlor을 함유한 품목의 환경생물에 미치는 영향을 알기위해 담수어 2종(냉수어, 온수어), 물벼룩, 녹조류 등의 수서생물과 조류, 지렁이, 꿀벌과 같은 육상생물, 천적 등 유용곤충에 대한 독성 및 영향시험성적서를 검토하였다. metazachlor 원제의 환경생물에 대한 독성 검토 결과 담수어류에 대한 급성독성에서 무지개송어(냉수어종)와 블루길(온수어종)의 LC_{50}은 7.8 mg/L, 10.9 mg/L 이었고 물벼룩의 EC_{50}은 33.7 mg/L 이었으며 녹조류의 EC_{50}은 0.0318 mg/L이었다. 육상생물 중 조류(북미산 메추라기)의 LD50은 〉2000 mg/kg, 지렁이의 LC50은 〉1000 mg/kg으로 영향이 낮았고 꿀벌에 대한 급성독성 중 접촉과 섭식 독성의 LD50은 각각 〉100 ㎍/bee, 〉72.19 ㎍/bee로 영향이 낮은 것으로 판단하였다. 기생봉(*Aphidius rhopalosiphi*)과 포식성 응애(*Typhlodromus pyri)*에 대한 LR_{50}은 모두 〉7.5 L./ha로 영향이 매우 낮았다. 담수어, 물벼룩, 녹조류의 독성시험 결과로 metazachlor 원제는 GHS 분류기준에 따라 급성수서환경유해성 구분2로 분류하였다. metazachlor을 함유한 메타자클로르 1.5% 입제 품목의 경우 잉어에 대한 급성독성 LC_{50}은 17.8 mg/L로 어독성 Ⅲ급으로 구분하였고 물벼룩의 EC50은 〉10 mg/L이었다. 어류, 물벼룩의 품목 독성 성적과 녹조류, 조류, 지렁이, 꿀벌에 대한 원제의 독성 성적을 근거로 위해성평가를 수행한 결과 모두 위해성이 낮은 것으로 판단하였다.

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Toxicity category and classification of penflufen in GHS system

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Toxicological data was assessed for toxicity category and classification of penflufen, There are no effect related to treatment in acute toxicity. Category of acute oral, dermal and inhalation toxicity was 5, 5, 4 respectively. Category of skin and eye irritation, and skin sensitization was 'not classified'. In rat and dog 90 day studies, there are liver weight increase and hepatocellular hypertrophy and the lowest NOAEL was 5.6 mg/kg bw/day. In l year dog study, there was liver toxicity. In rat chronic/carcinogenicity study, there was liver toxicity and tubulostromal tumor that was not related to human. In mouse carcinogenicity study, there was liver toxicity. In reproduction and teratogenicity study, there are decrease of body weight and food consumption, and liver toxicity. There are no effect of reproductive toxicity and terotogenicity. The results of genotoxicity studies were negative. Therefore penflufen was classified category 5 of acute oral an dermal toxicity and category 4 of acute inhalation toxicity but not classified the other toxicity.

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WBD법을 이용한 벼 재배 농작업자의 Orysastrobin 노출량 평가

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벼 재배 중 농작업자가 농약을 조제 및 살포할 때 살포액에 의한 호흡 및 전신 노출량 측정 및 평가를 위하여 전남 장성지역 내 10개소의 벼 재배 포장에서 살균제 Orysastrobin 액상수화제를 power sprayer (PS)기로 살포하여 호흡 및 전신노출량을 측정하였다. 시료의 분석은 LC/MS/MS를 이용하였으며, Orysastrobinn의 정량한계(LOQ)는 0.005 ㎍/mL이었다. 포장회수율은 100LOQ (2.5 ㎍/mL)수준으로 처리하여 분석한 결과 92.8~106.4%이였고 LOQ, 10LOQ, 100LOQ 수준으로 처리하여 분석한 시료의 회수율은 80.1~111.5%이였다. 10개 벼 시험포장에서 Orysastrobin 12% 액상수화제 조세시 및 살포시 농작업자의 피부노출 총량은 각각 30.60~402.90 ㎍과 4.25~27.02 mg 수준이였고, 호흡노출량은 0.265 ㎍이였다. 약액 조제 시 병을 헹구는 과정에서 주로 하박과 장갑에 노출이 집중되었고, 살포자의 경우에는 하체(엉덩이, 허벅지, 정강이)에 많이 노출되는 경향을 보였다.

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농약의 수생생물에 대한 위해성 평가

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논이나 밭, 과수원 같은 농경지에 살포된 농약은 비산이나 유출 등을 통해 비표적 환경생물(육상 및 수생)에 나쁜 영향을 미칠 수 있다. 따라서 농약 등록을 위해서는 환경생물에 대한 위해성 평가는 필수적이다. 특히, 논에 농약사용은 농약의 유효성분이 논물에 녹아 관개수와 직접 연결된 하천이나 호수에 유입될 수 있으므로 우리나라와 같이 벼 재배용 농약 사용량이 많은 경우에는 수생생물에 대한 위해성 평가는 매우 중요하다. 일반적으로 수생생물에 대한 위해성 평가는 단계별로 이루어지는데 1단계에서는 녹조류, 물벼룩, 어류에 대한 급성독성(EC_{50} 또는 LC_{50})과 환경추정 농도(PEC)를 비교하여 위해성이 없으면 다음단계 평가는 생략한다. 그러나 위해성이 있으면 2단계 만성독성(NOEC)과 PEC를 비교하게 되고 안전성이 인정되면 추가평가는 생략한다. 만약 2단계 평가에서도 위해성이 있으면 3단계 평가에 필요한 자료를 요구하게 된다. 그러나, 수생생물에 대한 위해성 평가방법과 기준은 국가별로 다소 차이가 있다. 이에 유럽과 미국, 우리나라의 수생생물에 대한 위해성 평가방법과 기준을 비교하였다. 또한 우리나라의 수생생물에 대한 위해성 평가방법과 기준에 따라 원예용과 벼재배용 농약을 예를 들어 수생생물에 대한 위해성 평가를 실시하였다.

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머귀나무를 이용한 Isopimpinellin의 추출 및 환경생태독성

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최근 농약 안전성에 대한 관심이 높아지면서 잔류농약에 대한 소비자의 우려가 국내 농업에 있어 항상 이슈가 되고 있는 실정이다. 따라서 최근 연구에서는 약효가 뛰어나고 안전성이 높은 친환경추출물 등의 대체제에 대한 연구가 활발히 진행되고 있는 상황이다.

머귀나무 종자의 주성분 중 하나인 Isopimpinellin은 살충 및 살균 성분을 함유하고 있어서, 천연농약으로 사용할수 있고, 물이나 알코올 등으로 손쉽게 추출이 가능해 화학농약의 대체제로 활용할 수 있다고 보고되고 있고, 선행 연구를 통해 약효를 검증하였다.

따라서 본 연구에서는 머귀나무 종자에서 추출 가능한 Isopimpinellin 함유 종자유의 추출효율 및 Isopimpinellin 함유량을 확인하고, 종자유를 활용한 수생태 독성 시험을 통해 안전성을 파악하고자 하였다.

머귀나무 종자유의 추출효율은 증류수 추출 시 최대 9.53 %, 에탄올 및 메탄올 추출 시 각각 10.87 % 및 11.20 %의 최대 종자유 추출 수율을 보였으며, 추출 용매를 사용하지 않고 압착 추출 시 18.5 %의 가장 높은 수율을 보였다. 압착 추출물 중 Isopimpinellin의 함량은 약 2.8 %로 확인되었다. 머귀나무 종자유의 어류 급성독성시험 결과 잉어 급성 96h-LC_{50} 값은 >10 mg/L로 나타났으며, 물벼룩 급성독성시험을 실시한 결과 48h-EC_{50} 값은 >10 mg/L 나타났다.

keywords: Isopimpinellin, 머귀나무, 잔류농약, 친환경

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물벼룩을 이용한 벼재배용 농약 제품 및 원제의 혼합독성평가

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대부분 농가에서는 노동력 절감 및 병해충 방제효율을 증대시키기 위해 농약을 혼합 살포하고 있다. 국내에서 농약을 혼합 살포할 때 인체에 미치는 독성영향을 평가한 선행연구는 있지만 환경생물에 미치는 독성영향에 대한 연구는 거의 없는 실정이다. 2015년 벼재배 농가 128가구를 대상으로 농약 혼용 사용 조사 결과 살균제와 살충제의 혼용이 전체 혼용 조합 중 70% 이상을 차지하여 제일 빈도가 높았다. 따라서 6-8월 사이 많이 사용된 살충제 2종과 살균제 2종을 선정하여 농약 혼용에 따른 물벼룩 급성 독성을 평가하고자 하였다. 농약 제품은 tricyclazole 75% wettable powder(WP), phenthoate 47.5% emulsifiable concentrate(EC), fenobucarb 50% EC, haxaconazole 10% EC, 농약 원제는 tricyclazole 96.5%, phenthoate 91.5%, fenobucarb 96%, haxaconazole 92.2% 을 선정하여 시험을 진행하였다. 시험은 단일 농약 제품과 원제의 물벼룩 급성독성시험 후 시험농약을 2종 6조합, 3종 4조합, 4종 1조합으로 물벼룩에 노출시켜 물벼룩 급성독성시험을 진행하였다. 농약 혼합물에 의한 독성은 toxicity unit(TU)으로 표현하였고 mixture 농도처리는 $\sum 0.25$, $\sum 0.5$, $\sum 0.75$, $\sum 1.0$, $\sum 1.5$ TU로 하여 계산하였다. 농약제품인 경우 fenobucarb EC+ phenthoate EC 와 hexaconazole EC + tricyclazole WP 은 TU 값이 1보다 크게 나와 독성이 감소하는 상쇄(antagonistic) 효과가 나타났고 나머지 9개 조합에서는 TU값이 1보다 작게 나와 독성이 상승하는 상승(synergistic) 효과를 나타냈다. 반면 농약 원제 혼합 시험에서는 fenobucarb + hexaconazole, hexaconazole + phenthoate, fenobucarb + hexaconazole + tricyclazole 조합은 TU 값이 1보다 크게 나와 독성이 감소하는 상쇄효과가 나타났고 fenobucarb + tricyclazole, phenthoate + tricyclazole 조합은 TU 값이 1로서 첨가효과가 나타났다. 나머지 6개 조합에서는 TU 값이 1보다 작게 나와 독성이 상승하는 상승효과를 나타냈다. 본 시험결과 2종 혼합에서는 제품과 원제의 TU 값의 경향이 다르게 나타났고 3종과 4종 혼합에서는 fenobucarb + hexaconazole + tricyclazole 조합을 제외하고는 제품과 원제의 TU 값이 1보다 작아 독성이 상승하는 상승효과를 나타냈다. 본 시험결과 원제와 제품에서 나타나는 혼합 독성경향이 다름을 확인 할 수 있었고 실제 농약의 혼합독성을 평가할 때 농약의 원제 뿐 만 아니라 제품에 대한 혼합독성 평가가 필요할 것으로 생각된다.

P-79

살포시스템에 따른 감귤 잎 중 Dithianon의 부착량 비교

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본 연구에서는 감귤의 병해충방제에 사용되는 디티아논 수화제를 이용하여 약제 살포시 살포시스템에 따른 감귤 잎의 부착량을 조사하여 효율적인 약제 처리시스템을 개발하고자 하였다. 이를 위해 관행적으로 사용되고 있는 engine type spray를 대조구로 하여 감귤과원에 적합하게 개량된 speed spray 및 sprinkler의 약제 살포시 감귤나무의 높이에 따른 부착량 및 감귤 잎의 앞·뒷면의 부착량을 비교 평가하였다. 시험은 제주도에 위치한 감귤시험장 감귤과원에서 진행하였다. 높이에 따른 부착량의 경우 감귤나무의 전체에 골고루 부착되는지 알아보기 위해 상·중·하로 채취하여 분포도를 알아보았으며, 감귤 앞·뒷면의 부착량의 경우 약제 살포시 감귤 잎의 안쪽까지 부착되는지 알아보기 위해 감귤 잎의 앞·뒷면에 filter paper를 부착하여 실험에 사용하였다.

분석은 UPLC-UVD를 이용하여 분석하였으며 감귤 잎, filter paper를 분석 하였다. Dithianon의 정량한계는 0.02 mg/kg수준이며, 회수율 시험결과 90.7~116.8% 수준의 회수율을 나타내었다. Engine type spray의 높이에 따른 부착량은 상부에서 2.82 $\mu g/cm^2$로 가장 높은 부착량이 나타났으며, speed spray의 경우 중앙부에서 3.42 $\mu g/cm^2$, sprinkler의 경우 상부에서 4.67 $\mu g/cm^2$로 나타났다. 감귤 잎의 앞 · 뒷면 부착량은 engine type spray의 경우 중앙부 앞면에서 0.23 $\mu g/cm^2$, speed spray의 경우 중앙부 뒷면에서 0.36 $\mu g/cm^2$, sprinkler의 경우 중앙부 앞면에서 0.44 $\mu g/cm^2$로 가장 높은 부착량이 나타났다.

P-80

이미다클로프리드 처리 방법에 따른 벼멸구의 독성반응과 최적 사충율 관찰 시기 설정

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해충의 살충제에 대한 독성 반응은 처리 방법에 따라 다를 수 있다. 본 연구에서는 침투이행성 살충제 이미다클로프리드를 이용하여 벼멸구의 세 가지 다른 처리 방법에 대한 독성 반응을 평가하고 특성을 규명하여 보았다. 처리 방법으로는 종자침지법, 유묘침지법 그리고 미량국소침지처리법을 선정하였으며, 벼멸구 접종 후 6일 동안 다양한 농도에 대한 사충율을 평가하였다. 그 결과 세 종류 처리 방법에 대한 반수치사농도(LC_{50})와 반수치사시간(LT_{50})은 시간과 농도가 증가함에 따라 각각 감소하는 경향을 나타내었다. 최적 반수치사농도 산출을 위한 관찰 시기는 6일 동안 관찰 시간 별 각 농도의 치사율에 대한 표준 편차가 가장 높은 구간으로 결정하였는데, 종자침지법과 유묘침지법은 4일차에서, 그리고 미량국소침지처리법은 1일차에서 사충율 관찰이 적합한 것으로 나타났다. 이러한 결과는, 해충에 대한 살충제 독성 수준은 처리 방법에 따라 다르게 나타나며, 실험을 통해 최적 관찰 시기를 사전 설정 후 독성 평가를 수행하는 것이 필요함을 의미한다.

P-81

잔디사용 주요 살균제의 살포자 및 출입자 위해성평가

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농작물 재배를 위해서 반드시 작물보호제를 사용해야 하듯이 비 농경지인 잔디밭, 골프장, 정원, 가로수 등에도 병해충을 방제하기 위해서는 농약을 사용해야 한다. 농경지에 사용되는 작물보호제의 경우는 위해성평가 등이 체계화되어 비교적 잘 관리되고 있으나 비농경지의 경우는 그렇지 못한 것이 현실이다. 우리나라에 등록되어 있는 농약은 약 1900여종이 되며 이 중에 400여종이 수목이나 잔디 에 등록되어 있다. 잔디만 등록된 농약도 280여종이다. 본 연구에서는 잔디에 등록되어 있는 주요 살균제의 살포작업자 및 잔디밭에서 하는 활동 중 대표적인 골퍼(golfer)에 대한 위해성을 평가하여 앞으로 비농업용 농약의 안전관리체계구축에 기여하고자 하였다. 국내 잔디밭에 사용하도록 등록된 30종 살균에 대하여 급성경구, 경피에 대한 독성을 조사하고, 캐나다에서 사용하고 있는 독성정도를 기준으로 한 재출입 허용기간을 설정하였다. 또한 현재 국내 농작업자 평가모델과 미국에서 개발한 주거지 위해성평가 표준지침 등을 이용하여 살포자 및 출입자(골퍼)에 대한 위해성을 평가하였다. 잔디사용 살균제의 급성경구독성은 30품목 중 1품목만이 보통독성이었고 나머지는 저독성으로 구분되어있으며 재출입허용기간도 모두 24시간으로 설정되었다. 24시간으로 설정된 살균제는 살포 후 4시간까지는 출입이 불가하지만 4~12시간에는 보호장비를 착용한 전문가가 1시간 동안 출입이 가능하며, 12~24시간에는 농약이 살포된 작물에 직접 접촉하지 않는 작업이 가능하다. 국내사용 농작업자위해성평가 모델을 6시간 8ha 살포하는 시나리오를 이용하여 평가한 결과 7품목이 추가의 노출시험이 필요한 것으로 조사되었다. 또한 미국에서 사용하는 거주자 위해성평가모델을 이용할 경우, 호스연결살포기(Hose-end sprayer)를 사용하고, 1일 4ha를 살포하는 시나리오에서 살포작업자 및 골퍼 모두 1차 평가에서 11품목이 상위 단계의 평가가 요구되었다. 그러나 이와 같은 결과는 최악의 시나리오를 이용한 평가이므로 정밀한 추가평가가 요구되는 것으로 판단되었다.

P-82

피펫법과 분무법에 의한 국내 농약방제복의 침투성 평가

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농작업자의 노출을 감소시킬 수 있는 가장 효과적인 방법은 농약방제복을 착용하여 농약의 침투를 물리적으로 차단하는 것이지만, 아직 국내에서는 농약방제복에 대한 침투성을 측정하고 평가하는 방법이 미흡한 실정이다. 본 연구에서는 미국 및 유럽 등에서 적용하고 있는 농약 방제복 성능기준 표준시험법을 적용하여 국내 시판되고 있는 8종 방제복과 일반 shirt 1종을 선정하여 침투성을 확인하고 평가하였다. 연구방법으로 농약 방제복 성능기준 표준시험 방법 중 EN/ISO 27065에서 제시하는 ISO-22608(피펫법)과 EN 14786(분무법)지침서를 적용하였으며, pendimethalin 유제(제품명:스톰프)와 tebuconazole 액상수화제(제품명:실바코)를 이용하여 농약방제복의 침투성을 확인하였다. GC/MS분석을 이용하여 분석법을 확립하였으며 분석법 검증을 위한 시험편과 흡습지에 대한 평균회수율은 pendimethalin유제는 96.2~107.2%였고, tebuconazole액상수화제는 96.1~111.5%으로 적합하였다. 피펫법에서 방제복에 대한 pendimethalin 유제의 침투성은0.0~43.1%, 발수성은 0.9~93.5%, 체류성은 4.9~95.3%였다. 그리고 tebuconazole액상수화제의 침투성은 0.3~32.8%, 발수성은 0.4~113.7%, 체류성은 1.2~62.9%였다. Shirt에서는 pendimethalin 유제(62.0%) 및 tebuconazole액상수화제(21.4%)모두 높은 침투성을 확인하였고, 방제복 1종에서 두 농약에 대해 높은 침투성을 확인하였다. 농약 성상을 비교 했을 때 유제의 경우가 더 높은 침투성을 나타내었다. 분무법에 의한 pendimethalin 유제의 침투성은 0.1~51.9%였고, tebuconazole 액상수화제는 0.01~7.3%였으며 shirt의 침투성은 각각 73.9%, 48.1%였다. 피펫법과 분무법의 침투성 결과를 비교 하였을 때 pendimethalin 유제는 유사하거나 분무법이 다소 높았고, tebuconazole액상수화제는 유사하거나 분무법에서 다소 낮은 결과를 확인하였다. 이 결과로 8종의 방제복 중 1종의 제품과 shirt에서 피펫법과 분무법 두가지 모두 높은 침투성을 확인하였고, 7종의 방제복은 높은 침투저항성을 확인 하였다. 이로써 농작업자는 농약 노출에 대한 안전을 위해 작업 시 반드시 농약방제복을 착용하여야 하며 국내에서 판매되고 있는 방제복에 대한 침투저항성 허용기준 및 관련 표준시험법 구축의 제도마련이 필요할 것으로 판단된다.

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Control Effect of Chemicals against Bacterial fruit rot on JuJube by *Xanthomonas arboricola*

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Recently, jujube has become popular as a fresh fruit. Therefore, the diseases such as anthracnose and bacterial fruit rot that the symptom appears on the fruit surface may have a large impact on the merchantability. The symptoms of jujube fruit by bacterial fruit rot by *Xanthomonas arboricola* include initially small water-socked, reddish spots and gradually become brown and enlarged and the surface eventually became sunken, while internal tissues turned deep brown. And in leaf, a small water-socked spot appear and turn to a polygonal spot with halo. Considering recent hot weather conditions, outbreak of bacterial leaf rot is expected, but there are no registered control chemicals to date. In this study, control effect of several chemicals against bacterial fruit rot was evaluated both *in vitro* and field test. In paper disk assay, bacteriocidal effect was best in oxytetracycline which of inhibitory zone was 28.7㎜ followed by oxolinic acid 19.5㎜ and streptomycin 15.7㎜ at a concentration of 200ppm. In field tests at Boen and Jincheon region of Chungbuk province, control effect was good at 1,000 times diluted concentration of streptomycin(A.I. 20%, WP), oxytetracycline(A.I. 17%, WP), oxolinic acid(A.I. 20%, WG) and validamycin A(A.I. 20%, WG) whose control value was average 84.4%, 83.7%, 79.1% and 77.6% relatively. At 500 times diluted concentration of streptomycin treatment in the early stage of growth, phytotoxicity with yellowing of young leaf edge was appeared.

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Control Effect of Chili Pepper Bacterial Wilt by *Bacillus amyloliquefaciens* M27, Antagonistic to A Wide Range of Pathogenic Bacteria and Fungi

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Bacterial wilt is caused by the soilborne plant pathogen *Ralstonia solanacearum* attacks over 11 plant species in *Solanaceae* in worldwide, and approximately 9 plant species are recorded as hosts in Korea. *B. amyloliquefaciens* M27 was strongly inhibited the growth of *Ralstonia solanacearum*. *Pectobacterium cartovora* subsp. *cartovororum, Acidovorax citrulli*, *Agrobacteerium tumefacienes* and *Burkholderia glumae*. Incidence of Chili papper bacterial wilt by treatment of 20 fold, 50 fold, 100 fold and 200 fold of LB broth cultural liquid of *B.amyloliquefaciens* M27 was showed 7.4%, 16.7%, 22.2% and 31.5%, respectively, whereas that of control was 47.2%. on October. Also, on October incidence of Chili papper bacterial wilt by treatment of 20 fold, 50 fold, 100 fold and 200 fold of LB broth cultural liquid of *B.amyloliquefaciens* M27 was showed 6.1%, 6.1%, 9.1% and 11.4%, respectively, whereas that of control was 39.4%. These results indicate that *B.amyloliquefaciens* M27 could be a prospective eco-friendly antibacterial agent for the control of chili pepper bacterial wilt by *R. solanacearum*.

P-85

Control Efficacy of Several Fungicides against Downy Mildew of Onion at Nursery Seedling Stage

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During the nursery seedlings stage at Muan in 2013, moisture and temperature conditions were favorable for the occurrence of onion downy mildew. Several fungicides were evaluated for controlling onion downy mildew at the stage of seedling. The disease incidence of the untreated control was 51.1%, which was sufficient to evaluate the fungicide efficacy for the control of onion downy mildew. Incidence at the mancozeb treatment (MAN treatment) was 4.4% and it was the highest control value of 91.4%. Incidence at the various fungicides treatment (MIX treatment) consisted of amectotradin plus dimethomorph, floupicolide plus propamocarb HCL, or dimethomorph plus pyraclostrobin was 10.0%, and the control value of the MIX treatment was 80.4%. Among the tested fungicides, amectotradin plus dimethomorph (AME plus DIM treatment) was selected and treated for three times to the nursery seedlings. Incidence of AME plus DIM treatment was 12.2%. There was significant difference among the three different treatments and the untreated control. These results suggest that the application of fungicides on onion nursery seedlings was effective to control the onion downy mildew.

P-86

Deletion in Cytochrome b Gene of *Colletotrichum acutatum* Resistant to Pyraclostrobin

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Pepper fruits infected in anthracnose were collected from Dalsung, Goryung, Chilgok and Gunwi area and pathogen were isolated. No. of isolates was 35 from Goryung, 23 from Dalsung, 62 from Chilgok and 52 from Gunwi. Each isolates were cultured on potato dextrose agar(PDA) media for 7 days at 25℃ and mycelial disk was taken by punching with 5mm puncher. The mycelia disk were moved to PDA media amended with 100 ppm of pyclostrobin and cultured 5 days at 25℃. Most of them could not grow on pyclostrobin amended media but few isolates grow slowly This means few isolates are resistant to pyraclostrobin. Effective concentration of resistant isolates on pyraclsotrobin was measured using microplate. Spore suspension was prepared from 10 days' culture media of each isolates. Pyclostrobin was serially diluted by 3 times from 2000ppm using 2 times potato dextrose broth. Each cell of microplate received 100 ul of spore suspension and diluted pyclostrobin solution and measured absorbance at 595nm using microplate reader after incubation for 3 days at 25℃. EC50 of resistant isolates was around 20ppm but sensitive isolates was around 2 ppm. To compare gene of resistant and sensitive isolates, the full sequence of mitochondrion gene was acquired from NCBI(accession No. KR349346) and primes were designed with primer 3. based on aquired sequence. DNA fragment was amplified with cytF1/cytR1 primers and sequence of cytochrome b gene was analyzed. Deletion of G at position 1023 was observed in resistant isolates. It is not certain that this 1023 G deletion confers resistance to pyraclostobin but considered to be related to resistance

P-87

Field Evaluation of Systemic Acquired Resistance Activator against Pear Scab caused by *Venturia nashicola*

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Pear scab caused by the fungus *Venturia nashicola* is the most devastating disease on Asian pear (*Pyrus pyrifolia*). Control of this disease is conducted mainly by spray of various kinds of fungicides, but there is concern about the resistance strains caused by excessive and repeated use of fungicides. In this study, the control efficacy of a commercially available systemic acquired resistance (SAR) activator, Acibenzolar-S-methyl, was tested in the Naju area. A conventional synthetic fungicide program with 10 time-spray schedule was included for comparison. The SAR activator was substituted 4 times in the middle of synthetic fungicide program. It showed sufficient condition to evaluate the efficacy of SAR activator for the control of pear scab since disease incidence of the untreated control plot was 33.2% or 83.7% on leaves or fruits, respectively. When the 10 time-spray fungicide program was tested, pear scab was occurred by 4.4% or 12.4% on leaves or fruits, respectively. However, pear scab on leaves was occurred by 4.6% at treated plot with fungicide program and SAR activator, resulting in the 86.2% of control value. Incidence of the fruit disease was 11.5% at treated plot with the fungicide program and SAR activator, showing 86.4% of control value. These results suggest that the SAR activator can control effectively the pear scab and be used to establish the program for controlling the pear scab.

P-88

Genomic characterization of newly found virus in *Riptortus pedestris* (Hemiptera: Alydidae)

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Picorna-like viruses have been isolated in various insects as having biological characteristics similar to picornaviruses. Here, a novel viral genome was founded in the *Riptortus pedestris* (bean bug) infected with an insect pathogenic fungus *Beauveria bassiana* (*Bb*) from transcriptome analysis. The viral genome consists of 10,554 nucleotides except the poly A tail and this genome contains helicase, protease, RNA-dependent RNA polymerase (RdRp), VP3 and VP1. The virus was obtained in all stages (three of small, medium, large nymphs and adults) of reared R. pedestris in the laboratory and was detected in the thorax, midgut, abdomen, productive organ, muscle and fat body, but not in the head. This work can provide an understanding the rearing system of *R. pedestris* or relationship between host and microorganism.

Keywords: picorna-like virus, RdRp, *Riptortus pedestris*, transcriptome

P-89

*Myrothecium roridum*에 의한 아욱잎마름병 발생

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아욱(*Malva verticillata* L.)은 아욱과의 두해살이풀로 동규라고도 불린다. 2016년 9월경, 아욱 재배 포장에서 잎이 불규칙한 모양으로 황화되고 마르는 증상이 발생하였다. 병든 잎에서 원인균을 분리하고, 균학적 특성 조사와 internal transcribed spacer(ITS) rDNA 영역의 염기서열 분석으로 병원균을 동정하고, 병원성을 검정하였다. 병든 아욱잎에서 *Stemphylium* sp., *Cercospora* sp., *Myrothecium* sp.가 분리되었고, 6개 균주를 단포자 분리하였다. 각 균주의 병원성을 확인하기 위하여 건전한 아욱 유묘의 잎에 포자현탁액을 분무 접종한 결과, *Myrothecium* 균주 접종구에서만 병이 발생하였다. 병원균의 동정을 위해 감자한천배지(PDA)에서 균학적 특성을 조사한 결과, 포자퇴(sporodochia)는 검정색으로 배지 표면에 다수 형성되었고, 포자는 투명하고, 격막이 없으며 원통형내지 타원형으로 크기는 5.3~6.7× 1.9~2.5μm로 조사되어 *M. roridum*으로 동정되었다. 이와 같은 동정 결과의 확인을 위하여 ITS-rDNA 영역의 염기서열을 분석한 결과, NCBI GenBank에 등록된 *M. roridum* 99~100%의 상동성을 나타내었다. 따라서 이 병을 *M. roridum*에 의한 아욱잎마름병으로 명명하고, 병의 발생을 국내에서 처음으로 보고한다.

P-90

Responses of *Colletotrichum gloeosporioides* isolated from persimmon trees in Sangju and Yeongdong to Qoi and DMI-fungicides

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68 and 48 isolates of *Colletotrichum gloeosporioides* were collected from persimmon (*Diospyros kaki*) trees in Sangju and Yeongdong, respectively in 2016. DMI (DeMethylation Inhibitors)-fungicides (prochloraz, difenoconazole, fluquinconazole, metconazole, myclobutanil and tebuconazole) and Qoi (Quinone outside Inhibitors)-fungicide (trifolxystrobin) registered on persimmon were evaluated to survey of *C. gloeosporioides* growth responses on fungicide-media. All DMI-fungicides showed over 91% inhibition rate against all isolated *C. gloeosporioides* on medium. However, only 13 and 6.3% of *C. gloeosporioides* isolates were showed over 91% inhibition rate on trifolxystrobin media. Therefore, we summarize, Qoi-fungicides cannot use for control of *C. gloeosporioides* in persimmon orchard.

P-91

Survey on Wintering and Sore Scattering of Ginseng Gray Mold using Modified *Botrytis* Selective Media

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Gray mold caused by *Botrytis cinerea* occurs during the entire growth period of ginseng, and it occurs in all parts such as leaves, stems, and fruits. The pathogens penetrate through the head and stalk, infect the roots and cause great damage to the ginseng production. However, studies on the occurrence and occurrence of ginseng gray mold pathogens are still insufficient. Therefore, this study investigated the wintering and infection rate of the pathogens using modified *Botrytis* selective medium on the ginseng field in Chungbuk province, and the spore scattering was investigated after wintering. In order to investigate the scattering rate of ginseng gray mold spores, a selective medium was prepared by modifying the method of Edwards and Sedden (2001). In the ginseng field, dead stem and rice straw on the ground were collected from January to investigate the contamination of gray mold. The spores scattering were investigated at the end of December and in the middle of February. The contamination rate of ginseng gray mold was high in Chungju area. In 2 year old ginseng, contamination rate of dead stem and rice straw was 70%, and that of 5 year old ginseng was 69% in dead stem and rice straw. The pathogens are wintering in the dead stem and rice straw on the ginseng field in winter. Spores were found to be scattered in February in all the areas surveyed, the amount of spores collected was high in 5 year old ginseng in the Chungju area. The results of this experiment show that spraying a fungicide at the time of spore scattering can prevent the infection of pathogen.

Key Word : Ginseng, Gray mold, Spore Scattering, Wintering

P-92

Tricyclazole 함유 입제의 파종동시처리 적용을 위한 용출제어 처방설계

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육묘상처리제 시장은 파종동시처리기의 보급 증가로 이앙동시처리에서 파종동시처리 시장으로 확대되고 있으며 파종과 동시에 손쉽게 약제를 처리할 수 있어 작업자 편의성을 증대시키는 장점이 있다. 반면 파종동시처리 시, 출아 약해 없이 안전하게 처리할 수 있는 원제는 한정되어있어 용출제어를 위한 지속적인 소재탐색 및 제조 기술의 개발이 요구되어 지고 작물에 대한 안전성, 속효성과 잔효력을 균형적으로 발현할 수 있는 유효성분의 용출제어가 중요한 핵심품질특성이다.

본 연구에서는 수용해도(596ppm)가 높아 약효 지속성이 낮은 살균제인 tricyclazole이 함유된 입제를 용출제어 기술을 적용하여 국내 최초 파종동시처리제로 개발하였다.

Tricyclazole 함유 입제의 용출속도를 제어하고 속효성과 잔효력을 최적화하기 위하여 다양한 소재를 이용하여 입제 처방을 검토한 결과, 수중용출도는 poly vinyl alcohol(PVA)계 결합제, Propenoic acid polymer계 코팅소재를 사용한 처방에서 96.2%였던 것이 파라핀왁스와 폴리머수지를 적정 비율로 사용한 처방에서 16.5~26.6%로 크게 낮아진 수중용출도를 나타내었다. 파라핀왁스와 폴리머수지 처방에 대한 온실시험 및 포장시험에서 벼에 대한 안전성을 확인하였다. 또한 포장에서 벼 잎집무늬마름병, 벼 흰잎마름병을 대상을 방제효과를 확인한 결과, 각각 87.5%와 79.1%로 대조와 비등이상의 방제효과를 나타내었다.

본 연구를 통하여 다양한 용출제어 입제처방 스크리닝과 처방 별 이화학 수중용출도 분석 및 생물활성검정을 통하여 벼에 안전하고 속효성과 잔효력을 균형적으로 발현할 수 있는 최적의 입제 처방을 확립할 수 있었다.

P-93

국내 benomyl 저항성 벼키다리병균의 β-tubulin 유전자 변이

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최근 벼키다리병균에 대한 몇 가지 종자소독약제의 저항성 모니터링 결과, 약제저항성 균의 비율이 증가하고 있는 추세이다. 이 중 benzimidazole계에 속하는 benomyl 저항성 균의 분포비율이 2006년 비해 2014년에 약 3배 증가한 것으로 조사되었다. 국내 분포하는 benomyl 저항성 키다리병균의 유전자 변이를 조사하기 위하여 2006~2007년과 2013~2014년에 분리한 균주 중 감수성 13개, 중도저항성 2개, 저항성 16개 균주를 선발하여 β-tubulin 유전자를 분석하였다. 그 결과, 중도저항성 균주는 200번 codon에서 [TTC(Phe)→TAC(Tyr)] 변이와 235번 codon에서 [GGC(Gly)→GGT(Gly)] 변이가 나타났다. 반면 저항성 균주의 경우, 167번 codon에서 [TTC(Phe)→TAC(Tyr)] 변이와 235번 codon의 [GGC(Gly)→GGT(Gly)] 변이가 나타났다. EC_{50}값과 MIC값 기준에 의해 저항성균주로 분류되었던 16개 균주 중 2개 균주(CF322, CF379)는 유전자 분석에 의해 중도저항성인 것으로 조사되어, 추후 저항성 정도가 다른 균주를 대상으로 유전자 분석을 실시하고, 저항성 기준을 재설정할 필요가 있다. 본 결과는 benomyl 저항성 벼키다리병균의 β-tubulin 유전자의 변이를 국내에서 최초로 확인한 것이다.

P-94

국내 벼키다리병균의 Ipconazole에 대한 감수성 기준 설정

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2006년부터 2007년까지 국내에서 분리한 벼키다리병균 139 균주와 2013년부터 2014년에 분리한 451 균주에 대해 균학적 특성 조사와 translation elongation factor 1 alpha(TEF) 유전자 염기서열 분석을 통해 동정하였고, 기내 유묘검정을 통해 병원성을 확인하였다. 그 결과, 2006~2007년과 2013~2014년 분리균주 중 병원성이 있는 균주는 각각 124 균주, 375 균주로 나타났다. 이 균주를 대상으로 ipconazole에 대한 감수성 기준을 설정하기 위하여 한천희석법을 이용하여 균사생장 억제정도를 조사하고, 각 연도별 EC_{50} 값과 최소억제농도(MIC)를 계산하였다. 2006~2007년 균주의 평균 EC_{50} 값은 0.484 μg/mL이었고, 범위는 0.0586~2.4973μg/mL로 조사되었고, 2013년 균주의 평균 EC_{50} 값은 0.2563μg/mL, 범위는 0.0491~1.3504μg/mL이었다. 2014년 균주의 경우 평균 EC_{50} 값은 0.4749μg/mL, 범위는 0.0831~2.984μg/mL로 조사되었다. MIC값의 경우, 기존에 보고된 일본 균주는 0.1~6.25μg/mL 범위였으나 국내 균주의 경우 6.25μg/mL 이상인 균주가 연도별로 66.3%(2006~2007년), 44.9%(2013년), 58.9%(2014년)의 빈도로 나타났다. 이와 같이 일본 균주의 ipconazole에 대한 MIC값에 비해 국내 키다리병균의 MIC값이 높은 경향이 있는 것으로 나타나 ipconazole에 대한 저항성 모니터링이 지속적으로 필요하다고 할 수 있다.

P-95

미토콘드리아 호흡 저해하는 pyraclostrobin과 penthiopyrad에 대한 인삼 탄저병균(*Colletotrichum panacicola*)의 저항성 발현

명경선, 연은솔, 김신화, 김기훈, 윤세나, 최다희, 도지원, 민지영, 김흥태

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인삼 탄저병이 발생한 잎에서 분리한 *Colletotrichum panacicola* 균주를 가지고 미토콘드리아 호흡을 저해하는 strobilurin계 살균제인 pyraclostrobin과 숙신산 탈수효소 저해(SDHI) 살균제인 penthiopyrad에 대한 저항성 모니터링을 실시하였다. 실험에 사용한 균주는 2005년과 2006년에 강원도와 제주도를 제외한 7개 도의 11포장에서 분리한 12균주와, 2015년과 2016년도에 전북, 전남, 충남, 충북의 14포장에서 수집된 40균주를 사용하였다. 살균제에 대한 병원균의 감수성 정도의 조사는 한천희석법으로 실험하였으며, 엑셀 프로그램에서 EC_{50}값을 구하여 비교하였다. Pyraclostrobin에 대한 평균 EC_{50}값은 2005년과 2006년 분리 균주는 0.015 $\mu g\ mL^{-1}$이었으나, 2015년과 2016년 분리 균주는 100 $\mu g\ mL^{-1}$ 이상으로 살균제에 대한 저항성이 크게 증가하였다. Pyraclostrobin에 대한 C. panacicola의 저항성 기준은 병원균의 EC_{50}값이 0.5 $\mu g\ mL^{-1}$ 이상일 경우 저항성 균주로 판단하였다. 그 결과 2005년과 2006년에 분리한 12균주는 모두 감수성 균주이었지만, 2015년과 2016년도에 분리한 38균주는 2균주만 감수성이었을 뿐, 나머지 모든 균주가 저항성으로 나타났다. Pyraclostrobin은 2004년에 등록되어 식물병 방제에 사용되어 오는데, 2005년과 2006년에 분리한 병원균에서는 전혀 저항성 발현을 확인하지 못하였지만, 10여년 동안 사용하면서 저항성이 증가해서 분리한 균주 중 95%에 해당하는 균주가 저항성 균주로 밝혀졌다. 하지만 SDHI 살균제인 penthiopyrad에 대해서는 2004년과 2005년에 분리한 균주 중에서 EC50값이 100 $\mu g\ mL^{-1}$인 4균주를 제외하면 평균 EC_{50}값이 8.534 $\mu g\ mL^{-1}$이었으며, 2015년과 2016년에 분리한 40균주의 평균 EC_{50}값도 4.394 $\mu g\ mL^{-1}$이었다. 이상의 결과를 보면 미토콘드리아의 호흡을 저해하는 strobilurin계 살균제와 SDHI 살균제에 대해서 인삼 탄저병의 반응은 매우 달라서, strobilurin계 살균제인 pyraclostrobin에 대한 저항성은 매우 심각하였으나, SDHI 살균제인 penthiopyrad에 대해서는 저항성 발현이 거의 없는 것으로 판단되었다.

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3-Carvomenthenone and Its Structural Analogues Show Insecticidal Efficacy against *Plodia interpunctella* and *Tribolium castaneum*

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The insecticidal toxicities of active constituent of the *Eucalyptus dives* leaves and its analogues against *Plodia interpunctella* and *Tribolium castaneum* were determined. 3-Carvomenthenone was isolated by chromatographic techniques and determined by EI-MS, ^{13}CNMR, ^{1}HNMR, DEPTNMR, ^{1}H–^{1}HCOSY, and HMQC. In the fumigant bioassay against *P. interpunctella* larvae and adults, cyclohexenone exhibited the strongest insecticidal toxicity (LD_{50} against larvae and adults, 2.45 and 3.63 $\mu g/cm^3$), followed by methylcyclohexenone (2.95 and 4.24 $\mu g/cm^3$), seudenone (3.02 and 4.44 $\mu g/cm^3$), and 3-carvomenthenone (52.4 and 68.7 $\mu g/cm^3$). In the contact bioassay, cyclohexenone (LD_{50} against larvae and adults, 17.25 and 19.35 $\mu g/cm^2$) exhibited the most potent toxicities against T. castaneum larvae and adults, followed by methylcyclohexenone (28.32 and 37.24 $\mu g/cm^2$), seudenone (31.24 and 36.26 $\mu g/cm^2$), and 3-carvomenthenone (92.12 and 96.32 $\mu g/cm^2$). The results show that no functional radical on the backbone (2-cyclohexen-1-one) was more toxic than other chemicals. In the structure-activity relationships between 3-carvomenthenone analogues and insecticidal toxicity, the mode of the insecticidal action of 3-carvomenthenone, cyclohexenone, methylcyclohexenone, and seudenone was through the dermal organs of *T. castaneum* and *P. interpunctella.* This study indicates that the *E. dives* oil, 3-carvomenthenone, cyclohexenone, methylcyclohexenone, and seudenone have potential capacity for the development as safety natural agents to control the stored food/grain insects.

P-97

Chlorantraniliprole 저항성 초파리모델 확립과 분자생물학 및 생화학적 차이점 규명

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작물보호제는 농업의 생산성을 위해 절대적으로 필요하기 때문에 작물보호제 내성, 저항성 해충의 증가는 농업에 있어서 매우 중요한 문제이다. 그러나 우리는 여전히 작물보호제에 대한 내성과 저항성 발전의 기초가 되는 분자 및 세포 기작을 명확히 이해하지 못하고 있다. 노랑초파리(*Drosophila melanogaster*)는 연구가 많이 되어 있는 동물모델로 다양한 연구에 활용되어 왔지만, 작물보호제 관련 약제 저항성 모델로는 활용이 되어 있지 않다. 우리는 노랑초파리의 수컷에 Ethylene methane sulfonate를 처리하여 전체 염색체에 돌연변이를 유도하고 Chlorantraniliprole을 두 가지 농도로 처리하여 저농도와 고농도로 도태된 두 종류의 저항성 초파리 계통을 확립하였다. 최근에 개발된 Chlorantraniliprole은 곤충 근육의 Ca^{2+} 농도를 조절하는 Ryanodine 수용기(RyRs)에 작용하는 diamide계 작물보호제이다. 접촉독성과 섭식독성 평가법을 활용하여 두 종류의 저항성 초파리 계통의 저항성 지수를 산출하였다. 접촉독성 평가법에서 두 종류의 저항성 초파리 계통은 저항성 지수에 차이가 없었지만, 섭식 독성 평가법을 이용한 결과는 통계적으로 유의한 저항성 지수 증가를 확인 할 수 있었다. RyR 발현량 비교 결과에서는 두 가지 저항성 계통에서 RyR의 발현이 유의성 있게 감소되었고, 주 약제 효소인 Acetylcholinesterase와 Glutathione-S-Transferase 활성은 증가되었다. 이 결과들은 확립된 두 종류의 저항성 초파리 계통이 해독 관련 효소의 활성에 관여할 것임을 의미한다. 두 종류의 저항성 초파리 계통을 이용하면 아직 밝혀지지 않은 약제 내성 및 저항성 기작을 밝혀내는데 도움이 될 것이라고 생각된다.

검색어 : 초파리, Chlorantraniliprole, 저항성, Ryanodine, Acetylcholinesterase, Glutathione-S transferase

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Differential Transcription Profiles of Diamondback Moth Larvae Following SublethalTreatment of Five Different Insecticides

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To identify genes that commonly respond to the treatment of different insecticides, 3rd instar larvae of the diamondback moth, *Plutella xylostella,* were treated with sublethal doses (<LC_{10}) of chlorantraniliprole, cypermethrin, dinotefuran, indoxacarb and spinosad via leaf dipping. Then, transcriptomic profiles of treated larvae were compared with that of untreated control. A total of 117,181 transcripts in average with a mean length of 662 bp were generated by de novo assembly, of which 35,329 transcripts were annotated. Among them, 207, 153, 336, 360, and 262 transcripts were determined to be up-regulated whereas 117, 47, 92, 115, and 81 genes were down-regulated following treatments with chlorantraniliprole, cypermethrin, dinotefuran, indoxacarb and spinosad, respectively. Finally, with the criteria of >10 X fold change (FC) and $p < 0.05$ or >4 X FC, $p < 0.05$ and $q < 0.2$, the genes commonly over-transcribed in all treated insects were selected and their over-transcription levels were confirmed by quantitative PCR. These commonly responding genes included three cytochrome P450 genes (Cyp303a1, Cyp6a20 and CYP9E2), three cuticle protein genes (LM-8, LM-19 and TM-A3A), lavesin-1, acyl-CoA Δ11 desaturase, glucose dehydrogenase, nose resistant to fluxetine protein 6, chorion peroxidase and protein yellow. As the five test insecticides have distinct structure and mode of action, the genes identified in this study were suggested to be involved in general chemical defense at the initial stage of intoxication. Their possible roles in tolerance and resistance development were further discussed.

P-99

Evaluation of 3,7-Dimethyl-2,6-octadienal and Its Derivatives as Mite Indicator and Acaricidal Agents against *Haemaphysalis longicornis* and *Dermatophagoides* spp.

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Severe fever with thrombocytopenia syndrome (SFTS) caused by SFTS virus is a serious threat to human health and leads to economic instability. 3,7-Dimethyl-2,6-octadienal derived from *Melissa officinalis* leaves and its derivatives have shown an acaricidal function against *Haemaphysalis longicornis*, known to harbor the SFTS virus, and *Dermatophagoides* spp., and an indicative function (color change) on *Dermatophagoides* spp. In a fumigant bioassay, 2,4-octadienal (LD_{50}, 0.93, 0.86, and 0.86 $\mu g/cm^3$) was 29.4-, 29.5-, and 58.2-fold more toxic than DEET (LD_{50}, 27.23, 25.47, and 49.72 $\mu g/cm^3$) against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*, respectively, followed by 3,7-dimethyl-6-octenal (LD50, 1.21, 1.42, and 0.49 $\mu g/cm^3$), 2-octenal (2.84, 2.02, and 0.25 $\mu g/cm^3$), and *M. officinalis* oil (2.06, 1.42, and 1.97 $\mu g/cm^3$). Moreover, the color of *D. farinae* and *D. pteronyssinus* was altered from colorless to golden brown by treatment with *M. officinalis* oil, 3,7-dimethyl-2, 6-octadienal, 3,7-dimethyl-6-octenal, and 2,4-octadienal. These finding indicated that *M. officinalis* oil, 3,7-dimethyl-2,6-octadienal, 3,7-dimethyl-6-octenal, and 2,4-octadienal could be developed as functional agent associated with SFTS virus and allergen.

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Food Protective Effects of 3-Methylbenzaldehyde Derived from *Myosotis arvensis* and Its Analogues Associated with Color Change against Stored Food Mites

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Systemic anaphylaxis can occur after consumption or contact with foods and grains contaminated by stored food mites (*Tyrophagus putrescentiae*). Spread of systemic anaphylaxis might be prevented by control of the infestation of *T. putrescentiae* by natural acaricides. In this regard, the potential abilities of 3-methylbenzaldehyde derived from *Myosotis arvensis* oil and its structural analogues to act as new acaricide and mite kit (mite color change) against *T. putrescentiae* were evaluated. LD_{50} values demonstrated that 2,4,5-trimethylbenzaldehyde (0.78 $\mu g/cm^3$) had the highest vapor action against *T. putrescentiae*, followed by 2,4-methylbenzaldehyde (1.14 $\mu g/cm^3$), 2,5-dimethylbenzaldehyde (1.29 $\mu g/cm^3$), 2-methylbenzaldehyde (1.32 $\mu g/cm^3$), 2,3-dimethylbenzaldehyde (1.55 $\mu g/cm^3$), 3-methylbenzaldehyde (1.97 $\mu g/cm^3$), and 4-methylbenzaldehyde (2.34 $\mu g/cm^3$). Color change of 7 methylbenzaldehyde analogues mixed with 2,3-dihydroxybenzaldehyde against *T. putrescentiae* showed mite color change from colorless to reddish brown, which was evident to the naked eye. There were no antagonistic interactions between 2,3-dihydroxybenzaldehyde and the methylbenzaldehyde analogues. These findings suggest that the methylbenzaldehyde analogues could be developed as dual functional agents to protect from allergic skin reactions to stored food products.

P-101

Insecticidal activities of essential oils derived from *Cinnamomum* sp. barks and their components against *Ricania* sp. (Homoptera: Ricaniidae)

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The insecticidal activities of major components derived from *Cinnamomum cassia* and *Cinnamomum zeylanicum* oils against *Ricania* sp. nymphs and adults were examined by leaf dipping and spray bioassays. LC50 value of *C. cassia* and *C. zeylanicum* oils was 37.66 and 72.62 mg/L, respectively, in the leaf dipping bioassays against *Ricania* sp. nymphs, and 77.38 and 134.86 mg/L in the spray bioassays against *Ricania* sp. adults. The insecticidal activity of C. cassia oil was 1.74-1.93 times greater than that of *C. zeylanicum* oil. In the components of *C. cassia* and *C. zeylanicum* oils examined by GC-MS, the content of cinnamaldehyde derived from *C. cassia* and *C. zeylanicum* oil was 80.20% and 46.23%, respectively. Based on LC50valuesof cinnamaldehyde, hydrocinnamaldehyde and 4-hydroxy-3-methoxycinnamaldehyde against *Ricania* sp. nymphs and adults, cinnamaldehyde (LC50, 31.25 and 62.43 mg/L, respectively) demonstrated the most potent insecticidal activities, followed by 4-hydroxy-3-methoxycinnamaldehyde (172.19 and 212.77 mg/L) and hydrocinnamaldehyde (275.05 and 318.63 mg/L). The findings showed that *C. cassia* oil, *C. zeylanicum* oil and cinnamaldehyde have potential value in management of *Ricania* sp. and could be valuable as effective natural insecticides.

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Insecticidal toxicities of carvacrol and thymol derived from *Thymus vulgaris* Lin. against *Pochazia shantungensis* Chou & Lu.

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The insecticidal toxicities of five essential oils against *Pochazia shantungensis* adults and nymphs, newly recorded pests, were evaluated. The LC_{50} values of *Thymus vulgaris, Ruta graveolens, Citrus aurantium, Leptospermum petersonii* and *Achillea millefolium* oils were recorded as 57.48, 84.44, 92.58, 113.26 and 125.78 mg/L, respectively, against *P. shantungensis* nymphs using the leaf dipping bioassay, and 75.80, 109.86, 113.26, 145.06 and 153.74 mg/L, respectively, against *P. shantungensis* adults using the spray bioassay method. Regarding volatile components identified in *T. vulgaris* oil, the LC_{50} values of carvacrol and thymol using the leaf dipping bioassay against *P. shantungensis* nymphs were 56.74 and 28.52 mg/L, respectively. The insecticidal action of *T. vulgaris* oil against *P. shantungensis* could be attributed to carvacrol and thymol. Based on the structure-toxicity relationship between thymol analogs and the insecticidal toxicities against *P. shantungensis* nymphs similar to the LC_{50} values against *P. shantungensis* adults, the LC_{50} values of thymol, carvacrol, citral, 2-isopropylphenol, 3-isopropylphenol, and 4-isopropylphenol were 28.52, 56.74 and 89.12, 71.41, 82.49, and 111.28 mg/L, respectively. These results indicate that the insecticidal mode of action of thymol analogs may be largely attributed to the methyl functional group. Thymol analogues have promising potential as first-choice insecticides against *P. shantungensis* adults and nymphs.

P-103

Isolation and Identification of a 2,4-D butyl ester-degrading Enterobacter sp. M11-3

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In order to renovate 2, 4-D butyl ester contaminated soil through biodegradation, a high-effective 2, 4-D butyl ester degrading bacterium was isolated from pesticides industry soil in Yantai of Shandong, China using enrichment culture and HPLC method. The strain identification was studied, the best cultivated conditions and pesticides degrading substrate range of the strain was also determined. The 2, 4-D butyl ester degradation rate of the screened strain M11-3 was 99.98% after 120 h cultivation. Based on 16S rRNA gene sequence analysis, the strain was identified as Enterobacter sp. The best cultivated temperature and pH of the strain were 25℃and 7.0. After 96 h cultivation, the degradation rate of the strain to chlorpyrifos and 2, 4-D butyl ester was 37.25 ~ 54.34%, the degradation rate to atrazine,imidacloprid, bupirimate, hexaflumuron, acetamiprid and cypermethrin was 15.53 ~ 28.77%.

P-104

New Available Quarantine Technology for Application of Ethyl Formate

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Methyl bromide (MeBr) has been widely used for quarantine and pre-shipment (QPS) in Republic of Korea. It has resulted unreasoned use of MB occupational health issues such as acute and chronic inhalation to fumigators and related workers as well as it is an ozone deplete substance. The cylinderized ethyl formate (EF) formulated with carbon dioxide (CO2), as an chemical alternative for MeBr, was successfully adapted in different types of imported fruits in terms of efficacy to against target pest with relatively short exposure time and less damage of the commodities compared with current MeBr treatment. However, there were some limitations to extend use of cylinderized EF formulation due to its commercial use are cost-ineffective in comparison with MeBr and restrict emission of CO2. Here, we proudly present the new technology of EF vaporizer which are specially designed to be use liquid EF (Fumate, registered product in Korea) with nitrogen, an inert carrier gas. This new EF application technology is expected to be cost-effectiveness and provide safer fumigation and reduce CO2 emission.

P-105

New Quarantine Trials for Using Liquid Ethyl Formate with Nitrogen Application on Imported Citrus Fruits_Cost Effectiveness and Worker Safety

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Methyl bromide (MeBr) had being planned to phase-out and recommended to reduce by the International Plant Protection Convention (IPPC) because it was listed as ozone depleting substance (ODS) and chronic inhalation toxicity to fumigators and related workers. Ethyl formate (EF) is the one of the potential valuable alternatives to MeBr in terms of its safety for consumers(food additive and naturally occurred) and worker with higher Threshold Level Limit (TLV=100 ppm) in comparison with current commercially available alternative fumigant. The cost-effective use of liquid EF applications with nitrogen gas was developed by cooperative research between Murdoch University in Australia and Animal and Plant Quarantine Agency (QIA) in Republic of Korea aimed to replace the cylinderized EF formulated with carbon dioxide, which is considered as a cost-ineffective method for quarantine practice. Our research demonstrated that new liquid applications technology in 10 m^3 scale-up fumigation in quarantine practice in Korea. The results shown that completely control all stage of citrus mealybug (*Planoccous citri*) have been achieved at dose 70 gm^{-3} of liquid EF and 5°C for 4hr fumigation. No phytotoxic damages were examined on imported citrus (oranges, grapefruits and lemons) and unlike the current unacceptable MeBr applications. its applications fully met the quarantine guideline and protocol for worker safety.

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나무주사제인 아바멕틴 유제 및 에마멕틴벤조에이트 유제의 소나무 수체 내 잔류 동태 연구

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(주)팜한농 작물보호연구센터 완제연구팀, [1]국립산림과학원 산림병해충연구과

소나무재선충병의 방제 기법으로 활용되는 나무주사제는 항공방제에 비해 저렴하고 효율적으로 방제가 가능하다는 장점을 갖지만, 약제 처리 수목을 확인할 때 식별이 어렵다는 단점이 있다. 본 연구는 약제 주입 후 시인성 개선을 위해 색소를 첨가했을 때의 소나무 내 잔류 동태를 평가하고자 하였다.

본 연구는 기존약제인 아바멕틴 1.8% 유제와 에마멕틴벤조에이트 2.15% 유제, 그리고 아바멕틴 1.8% 유제에 Blue9 색소, 에마멕틴벤조에이트 2.15% 유제에 Blue8 색소를 첨가한 4가지 약제를 사용하였으며, 각 약제별 소나무 10주에 흉고직경 1cm 당 1ml로 수간에 천공 처리한 후 경과 일수 별 색소무첨가 및 색소첨가 약제처리 소나무 주가지의 상, 중, 하에서 잔류경향을 비교하였다.

아바멕틴은 약제 주입 후 13일차에 색소무첨가는 0.004~0.017 mg/kg, 색소첨가는 0.006~0.009 mg/kg이 검출되었고 31일차에 색소무첨가는 0.014~0.024 mg/kg, 색소첨가는 0.016~0.027 mg/kg이 검출되었다. 그리고 약제 주입 후 70일차에 색소무첨가는 0.014~0.024 mg/kg, 색소첨가는 0.023~0.027 mg/kg이 검출되었고 123일차에 색소무첨가는 0.013~0.022 mg/kg, 색소첨가는 0.016~0.042 mg/kg이 검출되었다. 또한 색소무첨가와 색소첨가 간에는 잔류량에 차이가 없었으며, 가지 상중하 간에도 차이가 없었다.

에마멕틴벤조에이트는 약제 주입 후 13일차에 색소무첨가는 0.072~0.220 mg/kg, 색소첨가는 0.162~0.722 mg/kg이 검출되었고 31일차에 색소무첨가는 0.085~0.137 mg/kg, 색소첨가는 0.112~0.529 mg/kg이 검출되었다. 그리고 약제 주입 후 70일차에 색소무첨가는 0.148~0.203 mg/kg, 색소첨가는 0.163~0.526 mg/kg이 검출되었고 123일차에 색소무첨가는 0.131~0.192 mg/kg, 색소첨가는 0.225~0.455 mg/kg이 검출되었다. 또한 색소무첨가 약제에 비해 색소첨가 약제의 잔류량이 높았으며, 가지 상중하 간에는 차이가 없었다.

따라서 시인성 개선을 위해 색소를 첨가한 약제의 처리 후 소나무 수체 내 약제의 전달력은 기존약제(색소무첨가) 대비 비등 이상이었다.

※본 연구는 국립산림과학원 위탁과제 '소나무재선충병 선제적·맞춤형 방제 전략 및 기술 연구'의 지원에 의해 수행되었음.

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농작물 주요 해충 3종에 대한 식물추출물의 살충 효과

박부용, 이상구, 장홍섭, 전윤호, 전성욱, 정인홍, 박세근, 이상범*

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기능성 식물인 고수(*Coriandrum sativum)*, 계피(*Cinnamomum cassia*)의 추출물로 농작물 주요해충인 복숭아혹진딧물(*Myzus persicae*), 점박이응애(*Tetranychus urticae*), 배추좀나방(*Plutella xylostella*)에 대한 살충 효과를 검정하였다. 식물체 추출 방법은 수증기 증류추출, 용매 추출, 초임계 추출 3가지 방식을 사용하였다. 실내 검정은 해충별 기주식물의 잎 절편을, 3,000배로 희석한 각 추출물에 침지한 후 건조시킨 다음 각 해충을 접종하여 24시간 뒤 사충수를 조사하였다. 추출방식 별로 각 추출물들의 살충 효과가 상이하였는데 고수 추출물의 경우 수증기 증류법으로 추출한 물질이 배추좀나방 유충에 대한 살충 효과가 가장 우수하였다. 계피 추출물의 경우 수증기 증류법으로 추출한 물질은 배추좀나방 유충, 점박이응애, 복숭아혹진딧물에 대한 살충 효과가 우수하였으며 용매 추출물은 점박이응애에 살충 효과가 있는 것으로 조사되었다.

P-108

대추과원 해충 2종의 약제 방제 효과 시험

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대추나무에서 대추나무잎혹파리(가칭)와 복숭아심식나방이 발생하여 농가에 피해를 주고 있다. 대추나무잎혹파리의 경우 어린잎을 안쪽으로 말아 내부에서 흡즙을 하며, 복숭아심식나방의 경우 대추 과실 속으로 유충이 파고 들어가 피해를 준다. 따라서 대추에 발생하고 있는 해충 두 종에 대한 약제 선발 시험을 수행하여, 농가에서 방제시 효율적인 약제를 선정 할 수 있도록 하였다. 시험 수행은 대추나무잎혹파리는 보은과 옥천 포장에서 스피네토람 입상수화제 등 5종, 복숭아심식나방은 보은과 원주에서 페니트로티온 수화제 등 5종에 대해서 선발시험을 수행하였으며, 그 결과 대추나무잎혹파리에서는 보은과 옥천에서 각각 스피네토람 입상수화제 94.2%, 91%, 에마멕틴벤조에이트 유제 93.3%, 92.7%, 아세타미프리드 수화제 91%, 93.1%, 사이안트라닐리프롤 분산성액제 93.7%, 93.9%, 복숭아심식나방의 경우 보은과 원주에서 각각 페니트로티온 수화제 89.6%, 90.8%, 아세타미프리드 수화제 85.4%, 85.8%, 델타메트린 유제 94%, 93.2%, 클로란트라닐리프롤 수화제 92.9%, 93.2%, 플루벤디아마이드 액상수화제 97.6, 94.8%의 방제가를 나타내었다. 두 종 해충 모두 약제 5종에 대해 선발 시험 결과 80% 이상의 방제가를 나타냈으며 그중 4종은 90% 이상의 방제가를 나타내었다. 따라서 농가에서 두 종에 대한 해충 방제를 위한 약제 처리시 본 시험에 사용한 약제를 이용하면 효율적으로 방제가 가능할 것으로 판단된다.

P-109

버섯파리(Lycoriella sp.)에 살충활성을 나타내는 *Bacillus thuringiensis* 균주의 분리 및 특성 조사

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양송이버섯 재배지에서 발생하는 버섯파리(*Lycoriella* sp.: Sciaridae)의 유충은 버섯균사를 직접 섭식하고 가해함으로서 버섯의 생육 및 수확량을 감소시켜 상품성을 크게 떨어뜨리는 피해를 나타내고 있는 해충이다. 버섯파리 유충을 방제하기 위하여 대부분 살충제를 사용하고 있지만, 농약잔류와 저항성의 문제로 인해 방제가 어려운 실정이다. 그렇기 때문에 안정적으로 사용할 수 있고 효과도 우수한 생물적방제의 개발 및 연구가 필요한 실정이다. 따라서 본 연구에서는 양송이버섯 재배시 발생하는 버섯파리 유충을 생물학적 방법으로 방제하기 위하여 곤충병원성세균인 *B. thuringiensis* 균주를 분리하여 특성을 조사하였다. 파리목 유충에 대하여 살충활성을 나타내는 10개의 *B. thuringiensis* 균주를 선발하였다. 버섯파리(*Lycoriella*), 작은뿌리파리(*Bradysia agrestis*), 흰줄숲모기(*Aedes albopictus*), 토고숲모기(*Aedes togoi*), 빨간집모기(*Culex pipiens pallens*) 유충에 대하여 생물검정을 실시하였으며, 버섯파리와 작은뿌리파리에 높은 살충활성을 나타내는 3개의 *B. thuringiensis* 균주를 선발하였다. 선발한 균주를 농도별로 생물활성 검정을 한 결과는 버섯파리 유충에서 B균주의 LC_{95}값은 $1.5X10^7$(cfu/ml)로 가장 높은 살충 활성을 나타냈으며, A균주와 C균주의 LC_{95}값은 $2.6X10^7$(cfu/ml), $6.7X10^7$(cfu/ml)으로 기준균주 D보다 높은 살충활성을 나타내었다. 파리목 해충에 대하여 높은 독성을 나타내는 *B. thuringiensis* 균주의 내독소단백질의 활성화 여부를 확인하기 위하여 SDS-PAGE로 단백질 패턴을 조사하였으며, cry 유전자를 확인하기 위하여 plasmid DNA 패턴을 확인하였다.

P-110

시설 원예작물 재배지에서 작은뿌리파리의 약제 처리방법에 따른 방제효과 비교

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시설 원예작물 재배지에서 피해가 많은 작은뿌리파리(*Bradysia difformis*)의 방제를 위하여 2017년 현재 약 30여 품목이 등록되어 있을 만큼 매년 등록 약제 종류가 늘어나는 추세에 있다. 작은뿌리파리는 파프리카, 오이, 토마토, 딸기, 백합 등 시설 채소류와 화훼류 재배지에서 연중 지속적으로 피해가 발생하고 있어 이들 작물을 재배하고 있는 농가에서는 한 작기가 5~9개월이 소요되는 동안 작은뿌리파리를 방제하기 위하여 3~9회 정도 약제를 살포하는 것으로 조사되었다. 그러나 대부분의 농가들에서 약제 살포 후에도 만족스럽지 못한 효과로 인하여 재배작물의 피해가 발생하고 있어 그 원인을 구명하기 위하여 본 연구를 수행하였다. 약제 처리방법은 크게 두 가지로 나누어 시험하였으며, 첫 번째는 지제부 관주처리였으며, 두 번째는 점적관수였다. 대부분의 작은뿌리파리 등록 약제는 지침서에 지제부 관주처리로 기술되어 있지만, 최근 노동력 부족, 재배농가의 고령화 등으로 점적관을 통하여 살충제나 살균제를 처리하는 농가가 많아 두 가지 방법을 구별하여 약제를 처리한 후 약효를 비교하였다. 그 결과 점적관을 통하여 약제를 처리한 시험구에서 작은뿌리파리 성충의 황색 끈끈이트랩 유인수가 지제부 관주 처리한 시험구보다 2배 이상 많았으며, 무처리구와 차이가 없을 정도로 살충효과가 현저히 낮았다.

P-111

양송이버섯 친환경 방제를 위한 에센셜 오일의 포식성 천적 스키미투스응애 (*Stratiolaelaps scimitus*) 치사율 검정

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국내 양송이버섯 재배지에서 가장 피해를 심하게 주는 해충은 버섯파리와 버섯응애이다. 버섯파리 유충은 균사와 자실체를 파먹으며 절단시키고 유충, 성충 모두 이동 중에 각종 세균과 푸른곰팡이 등의 병원균을 몸에 묻혀 매개함으로써 복합 피해를 유발하기도 한다. 버섯응애 또한 배지의 균사를 먹으며 절단시키기 때문에 밀도가 심할 경우 핀발이가 전혀 이루어지지 않게 되어 수확이 불가능해진다. 그러나 버섯파리 방제용으로 등록된 약제는 diflubenzuron, teflubenzuron, cyromazine 3종뿐이며 등록된 약제마저도 복토 후에는 농약잔류 문제로 약제사용에 제약이 많아 방제가 어려운 실정이다. 버섯응애 방제용 약제는 아직까지 등록되어 있지 않아 방제법이 시급하다. 본 연구는 버섯파리 유충과 버섯응애의 살충, 살비력이 우수한 에센셜 오일을 선별하는 과정에서 버섯파리와 버섯응애의 포식성 천적과 병행하여 사용할 수 있도록, 필터페이퍼가 1장 깔린 직경 5 mm의 페트리디쉬에 스키미투스응애 성충 10개체씩(3반복) 옮겨 넣어 500, 1000, 2000배로 희석한 127종의 에센셜 오일을 살포 후 스키미투스응애 치사율을 조사하였다. 그 결과 500배 희석 오일은 약 50종에서 50%이상의 치사율이 관찰되었으며 2000배 희석 오일의 경우 시나몬바크 66.6%, 타임 56.6%, 윈터그린 53.3%, 아버비테 43.3%의 치사율이 관찰되었다. 이러한 에센셜 오일들은 버섯파리와 버섯응애 방제에 효과가 우수하더라도 스키미투스응애에게는 높은 치사율을 보였기 때문에 병행해서 사용하기에는 어렵다. 향후 양송이 수확에 영향이 없으며 버섯파리와 버섯응애의 살충, 살비효과가 높은 오일 선별, 적정 오일 농도 그리고 효율적 이용에 관한 추가 시험이 필요하다.

P-112

작은뿌리파리(*Bradysia agrestis*)에 살충활성을 나타내는 새로운 *Bacillus thuringiensis* 균주의 특성 분석

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작은뿌리파리(*Bradysia agrestis*) 유충은 다양한 작물의 뿌리를 직접 가해하여 생육을 억제하고 고사시키는 유충의 방제는 대부분 화학적 살충제에 의존하고 있다. 화학 농약의 사용은 생산물의 안전성, 저항성 개체의 출현, 인 · 축에 대한 피해 및 환경오염 등을 유발하는 문제점이 있다. 따라서 작은뿌리파리(*B. agrestis*)를 환경에 안전하면서 효과적으로 방제할 수 있는 생물적 방제에 대한 연구가 필요한 실정이다.

본 연구에서는 국내토양으로부터 분리 및 보관된 균주들 중 파리목 유충에 활성이 높다고 알려진 spherical type의 내독소결정성단백질을 형성하는 균주를 선발하였다. 작은뿌리파리(*B. agrestis*)는 충남대학교 생물적해충제어 실험실에서 사육하였고, 부화한지 5-6일 되는 작은뿌리파리(*B. agrestis*) 유충과 이집트숲모기(*A.aegypti*) 유충에 대하여 생물검정을 수행하였다. B 균주는 두 종 모두 100% 살충활성을 나타내었다. 기준균주인 K 균주는 이집트숲모기(*A. aegypti*)에 대하여 100%의 높은 살충활성을 나타내었고 작은뿌리파리(*B. agrestis*) 유충에 대한 살충활성은 70%의 방제효과를 나타내었다. B, K 균주의 작은뿌리파리(*B. agrestis*) 유충에 대한 LC_{95}값은 각각 1.4×10^8, $>10^8$ 이었다. B, K 균주의 단백질 패턴을 분석하기 위하여 SDS-PAGE를 실시하였다. 추후 연구에서 살충활성이 있는 cry gene을 확인하기 위하여 PCR 실험을 수행할 예정이다.

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조명나방 (*Ostrinia furnacalis*) 발생 패턴과 피해 해석

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해충이 언제, 어디서, 어떻게 발생하는가? 또 언제 어디서 어떻게 작물에 피해를 주는가? 를 아는 것은 해충의 피해를 예측하고, 밀도를 효율적으로 조절하기 위한 선결 조건이다. 조명나방의 성충은 5월 초순 발생을 시작하여 5월 말 1화기, 7월 중순 2화기, 9월 초순 3화기의 발생 피크를 이루며 년 3회 발생하였다. 유충은 5월 말경 나타나 옥수수 잎에 피해를 주기 시작하며, 피해가 이후 급격히 증가하다 6월 17일을 전 후로 더 이상 증가하지 않았다. 발생 초기에는 1, 2, 3령 충이 혼재하고 있으며, 줄기 피해는 6월 10일 경에 나타나기 시작하여 6월 17일을 전후로 급격히 증가하였다. 이는 3령 충의 발생 패턴과 일치하여, 줄기 피해는 3령 충으로부터 시작되는 것으로 추정된다. 조명나방의 최적 약제 방제 시기는 줄기 피해를 주는 3령 충이 줄기 속으로 들어가기 2-3일 전이다.

검색어: 조명나방, 발생, 잎피해, 줄기피해, 방제시기

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플룩사메타마이드(Fluxametamide)의 생물활성 및 약효 · 약해 평가

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Fluxametamide는 2007년 일본의 닛산화학공업(주)이 개발한 이소옥사졸린 골격의 신규 살충. 살비제이다. 이 화합물은 나비목, 총채벌레목, 파리목, 딱정벌레목, 노린재목, 응애목 등 광범위한 해충종에 대해 높은 살충효과를 보이며, Fluxametamide 유제 10%로 2013년부터 일본식물방역협회를 통하여 약효.약해시험을 실시했다. 이 약제는 50~25 ppm의 농도에서 채소류, 차등을 가해하는 광범위한 해충 및 응애류에 대해 높은 실용성을 보였으며, 대상작물 및 주변작물에 안전성도 확인되었다. Fluxametamide 9% 유제의 살충제로서의 생물활성 및 약효 · 약해를 알아보고자 에 대한 약효 · 약해 특성을 평가하였다.

Fluxametamide의 작용기작은 신경전달물질에서 억제적 작용을 하는 GABA (Gamma - aminobutyric acid)의 전달을 경합적으로 막아서, 신경을 교란시켜 해충을 치사에 이르게 하는 것이 확인되었다. 또한 Fluxametamide는 페닐피라졸계 살충제와 동일한 작용을 하지만, Fluxametamide는 다른 부위에 결합하는 것이 확인되어 페닐피라졸계 살충제에 저항성을 갖는 해충에도 높은 효과를 보여주는 신규 작용성이 있는 물질이다. 그러므로, 다른 작용기작의 혼재약제에 저항성이 있는 해충에 대해서도 높은 살충효과를 나타낸다.

Fluxametamide 9% 유제를 고추(단고추류 포함) 꽃노랑총채벌레에 발생초기 7일간격 2회 경엽처리 하였을 경우 92.1~96.2%의 방제가로 대조약제(에토펜프록스.펜토에이트 37% 수화제, 스피네토람 5% 입상수화제)와 비등의 효과를 보였다. 또한, 고추(부촌, 바이킹, 슈퍼마니따) 및 단고추(피에스타, 슈페리어)에 대한 약해시험 결과 기준량, 배량에서 모두 약해가 나타나지 않았다.

또한, Fluxametamide 9% 유제를 파(쪽파포함) 파밤나방에 발생초기 10일간격 3회 경엽처리 하였을 경우 94.3~99.3%의 방제가로 대조약제(플루페녹수론 5% 분산성액제)와 비등 이상의 효과를 보였다. 또한, 대파(금장외대파) 및 쪽파(재래종)에 대한 약해시험 결과 기준량, 배량에서 모두 약해가 나타나지 않았다.

Fluxametamide는 채소류에 생기는 나비목 및 총채벌레목 등에 우수한 방제효과를 보였으며, 약해에 안전하여 실용성이 높을 것으로 판단된다.

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Benzobicyclon 제초제 처리에 따른 IR-8 벼의 유전자 전사체 분석

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과거에 Sulfonylurea계 제초제가 주류를 이루고 있었으나 저항성 잡초의 출현에 따라 현재 수도용 제초제는 Benzobicyclon, Mesotrione 및 Tefuryltrione과 같은 HPPD계통의 약제들로 대체되고 있다. 이들 약제는 Carotenoid 생합성에 관여하는 효소인 HPPD(4-hydroxyphenylpyruvate dioxygenase)를 억제하여 잡초에 백화현상을 일으켜 고사시키는 제초제로서 일반적으로 찰벼, 흑미계통 및 장립형인 통일형(Indica) 품종 등에서 약해가 발생하는 것으로 알려져 있어 제품 포장지에 주의사항을 표시할 뿐만 아니라 농민들 또한 이에 대하여 인지하고 있다. 그러나 최근 일부 일반형 품종에 대한 Mesotrione 함유 제초제처리 시 약해발생 연구가 보고되어, 이에 대한 원인 분석 및 대책이 필요한 실정이다. 본 연구에서는 Benzobicyclon을 처리한 것과 처리하지 않은 개체의 Transcriptome Sequencing을 통해 유전자 발현값을 얻어 차별 발현 유전자를 분석하였다. 분석결과 처리한 것과 하지 않은 것의 유의성 있는 유전자는 3875개의 유전자가 감소 또는 증가하는 유의성 있는 결과를 나타냈으며, 그 중 1936개의 유전자는 처리한 benzobicyclone을 처리한 것에서 감소를 나타냈으며, 이 중 Os01g0600900(NSimilar to Chlorophyll a-b binding protein 2, chloroplastic.), Os11g0115350(Similar to Non-specific lipid-transfer protein 2.) 및 Os12g0115050 (Non-protein coding transcript.)의유전자가 10이상의 발현량 감소를 타냈다. 이와는 반대로 유전자 발현 증가한 유전자는 1939개의 유전자로 Os12g0274750 (Hypothetical gene), Os11g 0707000(Ribulose-bisphosphate carboxylase activase (EC 6.3.4.-) (Fragments)), Os11g0707000(Non-protein coding transcript), Os11g0707000(Similar to Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic) 및 Os11g0660500 (Similar to Translationally controlled tumor protein (Fragment))의 유전자가 10배 이상의 발현 증가를 나타냈다.

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벼 이앙동시 제초제 처리 후 관수시기별 약효 및 약해 변동

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한국에서 벼농사 작업별 노동투입시간은 매우 빠르게 감소하고 있다. 기계화 및 규모화에 의하여 육묘, 이앙, 시비 등의 노동투입시간은 2005년도부터 연평균 2~8% 감소하였다. 그러나 제초제 저항성잡초 발생 및 확산으로 제초제 사용량과 처리횟수는 내년증가하고 있는 실정이다. 벼 재배면적 대비 제초제 사용횟수는 1995년에 약 1.17회였으나 2013년에는 2.09회로 빠르게 증가하고 있다. 그리고 전국 벼농사 농가수 중에서 65세 이상 농가는 매년 평균 12.9%로씩 증가하여 2013년에는 57.1%로 나타났다. 고령화에 따른 가구당 벼 재배면적도 빠르게 증가하고 있는데, 특히 10ha 이상 농가수는 매년 2% 이상 증가하고 있다. 따라서 본 연구는 고령화 및 규모화로 급속하게 변화하고 있는 벼농사에서 제초제 저항성 강피, 물달개비, 올챙이고랭이 등이 확산할 경우 제초제 사용횟수가 증가하고 있어 노동력이 증가하고 있기 때문에 제초에서 노동력을 줄일 수 있는 이앙동시제초제의 약효를 증진시키고 약해를 경감시킬 수 있는 방법을 연구하였다. 본 연구는 전북 익산에 위치한 국립식량과학원 시험포장에서 실시하였다. 시험에 사용된 약제들인 펜트라자마이드+벤조비싸이크론+이마조설푸론 입제와 브로모브타이드+피라크로닐+피라조설푸론-에칠 입제를 이앙과 동시에 제초기(일본 Yamato 농기계)를 이용하여 살포하였으며, 이앙은 6월9일에 하였다. 제초제 처리 후 관수시기별 약효 및 약해정도를 알아보기 위하여 관수 시기는 제초제 처리 직후(0일), 그리고 3, 5, 7일에 하였다. 약해는 이앙 후 10, 20, 30일에 달관으로 조사하였으며, 약효는 이앙 후 30일 및 45일에 조사하였다. 관수시기별 구당면적은 592㎡로 하였다. 이앙동시 제초제 처리 후 관수시기가 빠를수록 약해는 낮고, 잡초방제효과 높게 나타났다. 두 제초제 처리 후 5, 7일 관수에서 약해는 제초제 처리 후 30일까지 지속되었다. 처리된 2종류의 제초제간 약효는 뚜렷한 차이는 없었으나 약해는 이앙 후 10일에는 제초제 처리 후 5, 7일 관수에서 브로모브타이드+피라크로닐+피라조설푸론-에칠 입제가 펜트라자마이드+ 벤조비싸이크론+이마조설푸론 입제 보다 다소 높게 나타났으나 이앙 후 20일 이후 부터는 펜트라자마이드+벤조비싸이크론+이마조설푸론 입제가 브로모브타이드+피라크로닐+피라조설푸론-에칠 입제 보다 다소 높게 나타났다. 약효는 2종류 제초제 모두 제초제 처리 후 3일 이내 관수할 경우는 이앙 후 45일까지 97% 이상 나타났으나 제초제 처리 후 5일, 7일 관수할 경우는 이앙 후 30일 및 45일 모두 강피와 올방개 방제효과가 낮게 나타나 전체방제효과는 상대적으로 낮게 나타났다.

주제어 : 이앙동시, 제초제, 관수시기, 약효, 약해

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Effects of Paclobutrazole on the Composition of Phytosterols and Selected Terpenoids in *Perilla frutesecens* Leaves

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Paclobutrazol (azole type compound) is a synthetic plant growth regulator. Its mode of action is related to the inhibition of the biosynthesis of plant hormone (gibberellic acids/brassinosteroids). The compound and related azole fungicides are well-known inhibitors of cytochrome P450s in steroids and several natural product biosynthesis. *Perilla frutescens* (Perilla) is an important crop plant as a source of vegetable oil (seed) and fresh vegetable (leaves). In comparison with seed oils, the phytochemical compositions of Perilla leaves are not well documented. In this study, the phytosterol compositions in Perilla leaves were determined with gas chromatography-mass spectrometry. In addition, some important terpenoid alcohols (e.g., phytol) were also measured. Azolyl compounds open gives inhibitory effects over terpenoid biosynthesis since the oxidase of terpenoid biosynthesis are affected by azoles. According to instrumental analysis, the major phytosterol in Perilla leaves was b-sitosterol. Phytol was found as a major terpenoid alcohol. Quantitative analysis of the composition of steroids and phytol in Perilla plants, treated with paclobutrazol was determined and the results were evaluated in consideration of pesticide application rates and the duration of application. The results will give valuable information, related to the effects of plant growth regulators on the primary/secondary metabolite profiles of crop plants.

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The Plant Growth Stimulation Effect of Geraniol on Spinach and Preparation of Its Emulsified Solution with Cyclodextrin

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This study was carried out theinvestigation of environmentally friendly emulsifier for preparation ofgeraniol emulsionandthe effect of geraniol on spinach development. Cyclodextrin is a well-known emulsifier composed of six to twelve glucose. Emulsion efficiency of α-or β-cyclodextrin was tested for geraniol. β-cyclodextrin can beeffectivelysuspended 1.17 mL geraniol/g in aqueous solution. For the field experiments, the emulsified solution was treated on the spinach at Namhea in winter. This crop was classifiedusing leaf number to four groups such as <11, 11-15, 16-20 and >20. Next, we analyzed the distribution ratio of each group after harvest. The harvest yield of third group(16-20) was significantly increased 12%p sincetreating 25 mg/Lgeraniol solution.

폐 회

연구상 시상

○ 우수 논문 발표상

1. 화학분야 수상자 : 회원 ( )
   논문제목 :

2. 독성분야 수상자 : 회원 ( )
   논문제목 :

3. 해충분야 수상자 : 회원 ( )
   논문제목 :

4. 병해·제초분야 수상자 : 회원 ( )
   논문제목 :

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   논문제목 :

2. 수상자 : 회원 ( )
   논문제목 :

3. 수상자 : 회원 ( )
   논문제목 :

4. 수상자 : 회원 ( )
   논문제목 :

경품 추천

2017년 임시총회 및 추계 학술발표회 개최지 소개

학회장 인사