Asia Pesticide Residue Mitigation through the Promotion of Biopesticides and for Enhancement of Trade Opportunities

Synthesis of the Field Training Workshop, 25-26 August 2020

The Field Training on was conducted virtually on 25th and 26th of August 2020 through BlueJeans virtual platform. It was organized through the Asia Pesticide Residue Mitigation Project (APRMP) by APAARI and IR4 project (Rutgers University). The project is supported by the Standards and Trade Development Facility (STDF)/World Trade Organization (WTO), the United States Department of Agriculture (USDA), and the Food and Agriculture Organization of the United Nations (FAO) and German Development Agency (GIZ) as knowledge partners. A total of sixty-two participants attended the training from the partner countries. The training aimed to improve participants' understanding of field training practices, such as general field GLP practices, Standard Operating Procedure, understand a protocol and use of field equipment for pesticide mitigation studies.

Dr. Michael Braverman, IR4 Project, Rutgers University, USA and Dr. Ngan Chai Keong, Principal Research Officer, Soil Science, Water and Fertilizer Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) were the resource persons for the field training. Participants actively interacted during the training by asking their questions and clarifications in the operations related to the project.

The field training aimed to cover the major aspects in two phases. In the first phase, combination of chemicals and active ingredients that are applied to the crops simultaneously in the field and study the residue decline study. In the second phase, selective active ingredients on residue mitigation are selected. The decline in those active ingredients is monitored by application of invasion chemicals followed by the application of biopesticide specific to the pest.

First day of the field training started with the introduction about the field training by Dr. Michael and acknowledgement to the project sponsors. He welcomed the representatives from different partnering countries including Pakistan, Nepal, Indonesia, Vietnam, Malaysia, Sri Lanka, Thailand, Bangladesh, Cambodia, Laos, and Philippines. Good laboratory practices overview, protocols, standard operation procedures (SOP) pertaining to the field operations, quality assurance auditing, sprayer calibration and walking speed, and equipment needs.

The technical session began with the overview on Good Laboratories Practices (GLP). GLP is a quality system concerned with the organizational process and conditions under which studies are planned, performed, monitored, recorded, archived and reported. It ensures the quality, integrity, and reliability of data. GLP is different from the certification by the facts that GLPs are designed by United States Environmental Protection Agency or Organisation for Economic Co-operation and Development (OECD) member countries that are designed for single study. It is a description of the quality system in SOP. GLPs are a documentation and auditing procedure on how well you actually performed a research activity. On the other hand, Certifications are established by the Certification group or ISO Members that are established for repetitive studies and contains the description of quality system in quality

manual. Certification is a measure on your abilities to perform an analysis or procedure in order to conduct research in the future.

Overview and definitions related to GLP: GLP Provides a framework for responsibilities of key study personnel, General requirements for Testing Facilities, Requirements for handling and distributing test, control and reference materials, Study Planning and Conductance Generation of records, and report preparation and their retention. Important definitions related to the GLP are described below:

"sponsor" is the entity that pays the bills and holds registration
"study director" is the individual responsible for the overall conduct of a study
"Quality Assurance Unit" is a person except the study director, management or other individuals involved in the study, designated by testing facility management to perform the duties relating to quality assurance of the studies
"Test Facility Management" is the management where the study director resides
"Testing Facility" is the person who actually conducts a study, i.e., actually uses the test substance in a test system
"Study" is the experiment at one or more test sites, in which a test substance is studied in a test system under laboratory conditions or in the environment to determine or help predict its effects, metabolism, Product performance, environmental and chemical fate, persistence and residue, or other characteristics in humans, other living organisms, or media. It does not include basic exploratory studies.
"Person" is an individual, partnership, corporation, association or any legal entity
"Standard operating procedure" is the Written, approved procedures that describe in detail standard and repetitive procedures
"Protocol" is the approved, written document that clearly indicates objectives, study design and all methods to be used in conducting the study
"Test System" is the entity that the test substance is tested in or added to the system
"Test Substance/Article" is the material or device administered to a test system
"Reference substance" is the chemical substance or mixture, analytical standard, or material other than a test substance, feed, or water, administered to or used in analyzing the test system to establish basis for comparison
"Control Substance/Article" is the material other than a test substance, feed, or water, administered to the test system during the study
"Raw Data" is the laboratory worksheets, records, memoranda, notes, or exact copies, that are the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of the report of that study. All changes to raw data must be made without obscuring the original entry. All changes must be initialled and dated by the person making the change, accompanied by an explanation for the change
"Computer Data" could be defined as either magnetic media or first hard-copy printout - data cannot be overwritten or erased
"Archives" is the area used for the orderly storage and expedient removal of all raw data, documentation, protocols, specimens, and interim and final reports

The study must be conducted according to an approved Protocol. The Protocol specifies how the study is to be carried out. It has enough detail so that those actually conducting the study can envision how the study is to be conducted and so at a later time one can envision how it was done. For each routine activity there needs to be a standard operating procedure

that describes how the activity is to be performed. These serve as a "cookbook" to outline the activity and specify the general procedure and its documentation.

Maintaining Good Documentation: Documentation should permit the complete reconstruction of a study. Key points to be considered in maintaining good documentation includes:

- Recording data directly, promptly and legibly in indelible ink (never pencil)
- Making the note of initial and date all observations and any resulting changes, but do not obscure original data
- Writing down the initial and date only work you have performed
- Do not document selectively or in advance of performing the activity
- Do not use white-out correction fluid or tape
- Do not use ditto marks as raw data
- Copy all heat sensitive paper and stamp "exact copy"
- Explain why any raw data not used was not used
- Verify critical calculations using a second person and document this
- Properly head all pages, tables, columns; identify units
- Describe Statistical & Calculation Procedures used
- Sign, Date, and File automated printouts (e.g., QC forms)
- Retain all Raw Data (original records) in the Study File
- Documentation must allow another person to be able to accurately reconstruct what you have done
- Keep all *original* observations including those observations recorded directly into a computer
- Sign and date all computer printouts
- Never back-date anything

Documentation of Amendments and Deviations: Amendment is a planned change to the protocol in advance of the time the event or action occurs in the study. Deviation is something that is done during the course of a study that is different from the protocol. All amendments and deviations should be approved by the study director, sponsor representative, QA unit, Laboratory Research Director, and Field Research Director.

There are top ten important parts of a GLP program that should be addressed that includes:

1. Appoint a study director and quality assurance
2. Participants must be competent, as a result of education, training and experience
3. Follow the protocol and SOPs
4. Conduct studies in adequate and clean facilities
5. Identify test and control articles and document their use
6. The study director is the single point of control for the conduct of a study.
7. Document and correct all deviations
8. Do not commit fraud; all information must be signed and dated
9. Everything related to a study must be archived controlled by an archivist.
10. Do not allow any pencils or white out to be kept at field or laboratory facilities. Everything must be written in ink and any corrections must allow the view of the original entry.

Some questions were put forward that included what test systems will be used in the present study, "what are the reference substances that will be used in the study in the laboratory and not in field?", "if there are different instructions in protocol and SOP which procedure should be followed?", "What type of paper work that the company uses to send the samples from field to the laboratory?", "Who provides the authorization for amendment?" which was rightly answered by the participants from Pakistan and Indonesia. One of the participants had a clarification on who should the copy of translated document. It was clarified that all the documents should be in English for this study and any internal documents could be signed and approved by the study director.

Followed by this general overview and definitions pertaining to the GLP, the training was provided on using and working with protocols and changes. The protocols contained the series of examples related to calibration, using the reference standards, calibrating the field equipment, and test study design.

Justification and objectives of the protocol should include all the purpose and objectives of the study clearly that are approved by the study director. Test system design and statistical method was briefed with an example on usage of sprayer for the field studies. All the equipment should be calibrated with the permissible level of deviation from the mean values. This was illustrated by putting forward a practical question to the participants by Dr. Michael. While calibrating the equipment, it should be noted that the result output from the equipment should attain three consecutive values of the approved mean values. Participants were asked with the yes or no question to assess the methodology that has been followed. Most of the participants considered the equipment as calibrated if there are three correct outputs but not consecutive, which was clarified in the discussion. Complete details on experimental times, date, sample details, data regarding calibration, data and procedure should be addressed well in details throughout the project.

Following aspects or record for the protocol should be maintained at the minimum:

01- Names of all personnel conducting specific research functions
02- Amendments and deviations from protocol and standard operating procedures (including copies of signed protocol changes received prior to submission of the Field Data Book to the Regional Field Coordinator).
03- Test site information
04- Plot maps
05- Test substance receipt, use and container/substance disposition records
06- Test substance storage conditions (including temperatures)
07- Data regarding calibration and use of application equipment
08- Treatment application data
09- Crop maintenance pesticides and cultural practices, test plot history, and soil information. (Reporting soil information from typical farm service soil analysis labs, or past history for the farm, or from official documents, such as the SCS Soil Survey for the test plot area is adequate for this study. The nature of this study is such that soil characteristics do not need to be determined under GLP standards.)
10- Residue sample identification, collection, storage conditions and handling (Weight measurements are considered estimates for the samples collected from field or processing

trials, and the scales/balances used for this purpose do not need to be maintained in strict adherence to GLP.) Residue sample identification, collection, storage conditions and handling (Weight measurements are considered estimates for the samples collected from field or processing trials, and the scales/balances used for this purpose do not need to be maintained in strict adherence to GLP.)

11. Residue sample shipping information

12- Description of crop destruction, or explanation for lack of destruction

13- Meteorological/Irrigation records (temperature/humidity records for greenhouse trials)--required from planting of annual crops or for a minimum of one month prior to the first application onto perennial crops, until last residue sample collection. These records do not need to be determined under GLP standards.

14- Pass times (if applicable) and other data to confirm amount of material applied to plots

15- Equipment maintenance records with indication of routine vs. non-routine nature of maintenance

16- Other applicable data requested in the IR-4 Field Data Book necessary for confirmation that the study was conducted in accordance with the protocol. Compliance with GLP's is not required for the collection of data associated with crop phytotoxicity.

Usage of SOP based on the example of document used by Dr. Michael in his past studies were discussed. Standard Operating Procedures (SOP'S), Personnel Records, Recording Raw Data Determining Significant Figures and Rounding Numbers, Transferring 1R-4 Field Data Books and Raw Data to 1R-4, Site Selection, Plot Layout Requirements, Crop Establishment, Maintenance and Destruction, Collecting Phytotoxicity Data, Receipt and Storage of Test Substance and Adjuvants, Determining Spray Volume, Determining Test Substance and Adjuvant Amounts for Spray Mix, Measuring Test Substances, Transferring a Test Substance or Adjuvant from the Original Container to Secondary Containers, General Procedures for Mixing a Test Substance and application of test substances, Cleaning Test Substance Application Equipment were some of the key points highlighted in the SOP example.

GLP Field Residue Studies

Following standard practices are recommended for the pesticide residue analysis:

- 4 plots- 1 Control and 3 Treated at least 20 meters apart.
- Calibration- 3 consecutive times 5%
- Apply at 400, 500, 600 ml formulated test substance per hectare in 800-2000L/Ha spray volume
- 2 applications at 14 days apart
- Target PHI 3 days
- From each plot collect 2 untreated, 2 treated samples 24 fruit/sample at 3 days after last application.

Receipt, storage and disposition of test substance should be maintained as per the SOP. Harvested samples should not be cut in the field should be taken to lab and cut/analysed.

Field Data Notebook Instructions:

- All entries should be clear, understandable, legible, and made with a ballpoint pen in indelible blue or black ink.
- Changes to the raw data can only be made by drawing a single line through the original
- entry so as not to obscure it. The date, signature (or initials) and reasons for change (brief description
- or Error Code) must accompany any change. Acceptable Error Codes include:
- ME=Measurement Error CE=Calculation Error NR=Not Recorded SP=Spelling Error EE=Entry Error NA=Not Applicable UE=Unnecessary Entry IC=Incorrect Comment LE=Late Entry IE=Illegible Entry IW=Inappropriate Word WE=Wrong Entry TE=Transcription Error AW=Accidental Writeover PE=Pagination Error
- Other error codes can be used, however, the codes must be outlined in an approved SOP or noted in the notebook

Details on using the sprayer and application of pesticide to the plants in the field was provided with the pictures taken from the field trials. Calculations for plot size, spray volume per plot, Volume test substance per plot, Liters/plot, and Seconds/pass were explained. Importance of pass time during the application of the pesticide was highlighted. The participants were introduced to the software called "Metronome" that could be used to calculate the walk speed during the application of pesticide and use of sprayer. This would help in maintaining the uniformity on the application of the pesticide in the field.

The following are the General Items and Equipment Needed for the Conduct of GLP Residue Field Trials:

Field Items:

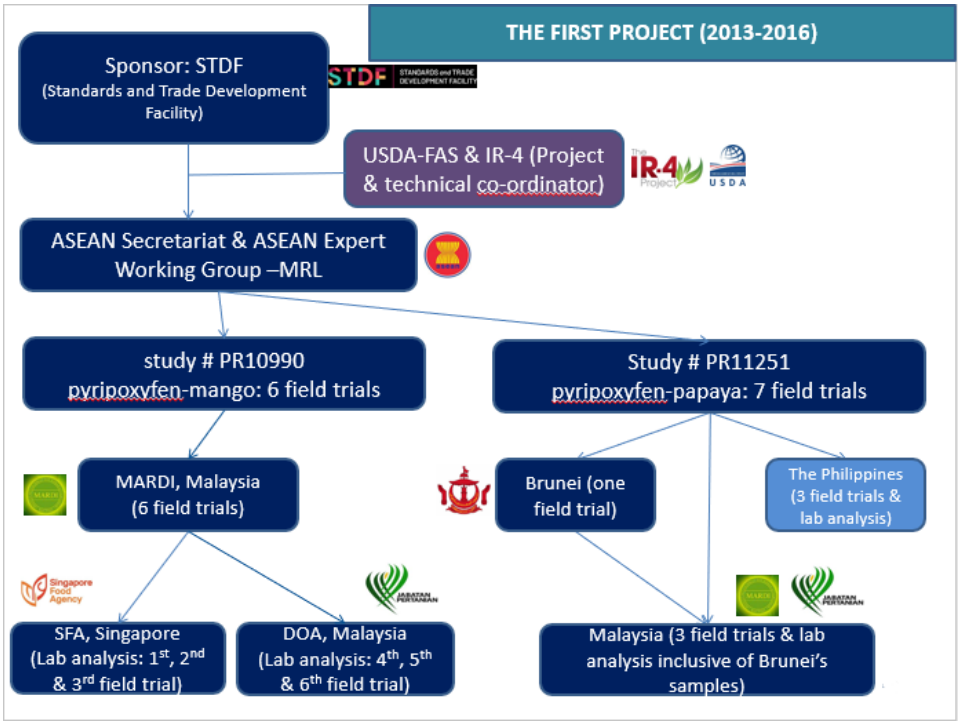
- Mist blower, Backpack /Knapsack sprayer/sprayer for treatment applications
- Metronome (or phone app) to help keep pace when making application.
- Environmental monitor for wind speed and direction, air temperature, %Relative Humidity, at application
- Soil Thermometer with 4" probe
- pH test strips to test water used to mix test substance
- Graduated cylinders, beakers, pipettes, etc., for measuring test substance and water carrier
- Water jugs for carrying water to field, if needed.
- Flagging, "no entrar/no cosechar" tape, plot markers, etc., to identify and delineate field plots
- Ladder for harvest, if needed.
- Bags for samples
- Safety equipment (gloves, spray suit, rubber boots, respirator, goggles, etc.)
- Ice chest and wet ice or blue ice packs for transporting samples back to the freezers; one for treated samples, one for untreated samples
- Data logger for test substance transport to maintain integrity while in transit
- Data loggers for transport of samples from field to freezers (one for each cooler)
- Possible Data loggers for shipping containers with samples and dry ice (one for each container)

Office or Laboratory:

- Small refrigerator for storage of test substance (to keep Test substances from excessive heat or cold; verify recommended storage temp and conditions)
- Data logger to record temperature of test substance while in storage
- Freezers for field crop samples (store samples at -18°C until sent to analytical lab); ideally, one freezer for treated samples, one for untreated samples
- Data loggers for freezers, to monitor temp of samples while in storage
- Back-up generator for sample freezers (12-hour run time) and, ideally, an alarm system to alert personnel of a freezer failure. Plan to transfer samples in case of emergency.

The first day session ended with the discussion and clarification on the discussed topics on use of sprayer, taking the appropriate volumes of spray liquid, sampling, sample size, and deviations for the application.

The second day of the field training mostly covered the experiences from the past projects, and examples of the field record books from Dr. Michael. On the second day of the field training conducted on 26 August 2020, Dr. Ngan Chai Keong, Principal Research Officer, Malaysian Agricultural Research and Development Institute (MARDI) presented on the Field Training Experiences and Chemical Compatibility. He was involved with the STDF sponsored pesticide management in papaya and mango. A brief outline on the project details are given below in the flow chart indicating the commodidites and organisations involved in the field training of the project.



Dr. Ngan participated in the field training organised for ASEAN countries that was organised by Dr. Michael. Hands-on field training and walking speed calibrations were demonstrated for the participants. Major takeaway points from the training included:

- Field measurement and recording.
- Calibration of discharge rate of spraying equipment.
- Calibration of sprayer walking speed.
- Pesticide application.
- Sampling protocol.
- Shipment of samples to local laboratory and Singapore.
- Quality assurance of the field phase of supervised residue trials.

Dr. Ngan shared that himself and the partnering countries were benefited on exposure to conducting supervised residue field trial according to Good Laboratory Practice standard. Recording of field measurement, data and information. It also increased the networking and continuous collaboration with international partners.

It is important that the solution mixtures of pesticide products are to be applied on selected crops followed by crop samplings (multi-residue decline studies). Compatibility test for selected pesticides before multi-residue decline studies began after the field training. The MARDI formulated a Procedure to conduct pesticide compatibility test that included the following steps:

1. Wear gloves, mask, goggles and whatever safety equipment is listed on the labels.
2. Get 2 clean containers that can hold over a liter, preferably one with a lid that you can close and shake.
3. Get a strainer that you can use to filter out any precipitate. Something like the photo below.
4. Put the test substances in order with all the EC formulations first , then SL, then SC formulation. Within each formulation type, create an order of the smallest volume to largest volume(Acetamiprid 0.25 ml/L... up to Malathion 2.5ml/L).
5. Pour 1 liter of water into one container.
6. Add 0.25 ml Acetamiprid to the water.
7. Shake well, observe the color, write it down and take a picture.
8. Add the second product to the same first liter of solution you started with.
9. Shake well, observe the color, write it down and take a picture.
10. Wait a few minutes and see if there is any odd color change, heat given off or any precipitate formed.
11. If the mixture is to cloudy or you can't tell if a precipitate has formed, pour from one container to the other container through the strainer.
12. Keep repeating the process with each new product you add,
13. If there is some type of adverse reaction (Lets say after adding product number 5), go back and prepare products 1-4. Skip 5 and add number 6 instead and keep going until you reach a problem.
14. If any are left out, prepare a second liter of water an combine only the ones that were left out. Hopefully, they will all get along ok.
15. After you have determined the maximum number that can all be within one liter mix we want to know if the mixture is phytotoxic.
16. Place the mixture in a sprayer and see if the mixture is spraying out ok without clogging.
17. Shake well and spray 4 separate areas (Replications) of areas (Maybe about 1 square meter/plot) with a dense population of small weeds. Observe sprayed plots at

3, 7 and 14 days after application for any leaf burn, stunting or odd appearance. Set up a control plot too. Record and photograph.

18. After spraying look at the nozzle and the bottom of the sprayer for any unusual amount of clumps/clogging.

Followed by Dr. Ngan's sharing of experience, Dr. Michael briefed the components of field training notebook with Dos and Don'ts. The major aspects covered included documentation and Corrections , Personnel, Notes and communication, Amendments and deviations, Test substance receipt and storage, test site maps, plot plan and details, equipment, Sprayer calibration, walking speed, calculations, Application records, study differentiation, Harvesting and sampling, Sample Inventory, Freezer temperature logs, Sample shipping/transfer, and Weather records. There was active discussion from the participants on the field training aspects and maintaining the field training notebook.