Standard Operating Procedures for the Application of Pesticides in Agricultural Research at the NJAES Research Greenhouse Facilities

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General Guidelines for Standard Operating Procedures

SOP #1.0 General requirements for the development and use of Standard Operating Procedures (SOP’s) for use in greenhouse/growth chamber research.

SOP #2.0 Responsibilities of the Study Director

SOP #3.0 Scope or the Quality Assurance Unit

Experimental Design and Layout

SOP #4.0 Design and site selection for greenhouse/growth chamber studies

SOP #5.0 Commodity establishment and maintenance

SOP #6.0 Soil Testing Procedures

Calibration of Spray Equipment

SOP #7.0 Calibration of instruments and gauges
<table>
<thead>
<tr>
<th>SOP #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>#8.0</td>
<td>Calibration of a liquid sprayer</td>
</tr>
<tr>
<td>#9.0</td>
<td>Calibration of Granulator Applicators (for high tunnel use only)</td>
</tr>
<tr>
<td></td>
<td><strong>Procedures for Pesticide Applications</strong></td>
</tr>
<tr>
<td>#10.0</td>
<td>Procedures in the application of pesticides</td>
</tr>
<tr>
<td>#11.0</td>
<td>Measuring a pesticide formulation</td>
</tr>
<tr>
<td>#12.0</td>
<td>Herbicide application with CO2 backpack sprayer</td>
</tr>
<tr>
<td>#13.0</td>
<td>Cleanup of application equipment</td>
</tr>
</tbody>
</table>
**SOP #1.0:** General requirement for the development and use of Standard Operating Procedures (SOP’s) for use in greenhouse research.

**PURPOSE:** To provide guidance in conducting greenhouse and controlled environment research.

**PROCEDURES:**

1. Studies which are conducted in the support of the registration of pesticides will have SOP’s for all phases of the research.

2. Each SOP will be revised when needed. All earlier revisions will be retained.

3. Any deviations from the SOP’s that would significantly affect the results of the study must be documented in writing.

4. The following is the format to be used for each Standard Operating Procedure:

   **SOP #:** (SOP number in numerical order [1 to n]). **Title:** (SOP title). **Revision #:** (serially beginning with 1.0 after the initial draft).

   **REVISED OR WRITTEN BY:** (Name of person revising the original SOP or developing a SOP).

   **PURPOSE:** (Brief description of the purpose of the SOP).

   **PROCEDURES:** (Describe the operating procedures in numerical order from beginning to end so that a reasonably intelligent person with some knowledge of the situation can carry out the procedures without any verbal input from other sources).
SOP #2.0: Responsibilities of the Study Director

PURPOSE: To provide information on the responsibilities of the Study Director.

PROCEDURES:

1. The Study Director has the responsibility for the following:
   
a. Assure that the study is carried out according to an approved protocol.

b. Assume that personnel, resources, facilities, equipment, materials and methods are available as scheduled for the conduct of the project.

c. Make sure that all personnel conducting the study understand the protocol and SOP’s for the project.

d. All raw data, summaries and other items connected with the study that need to be retained are stored in archives.

e. Maintain a master timetable for all field research projects under the study director’s control.

f. Maintain on file a current summary of experience and a job description for all key people engaged in the study.
**SOP #3.0:** Scope of the Quality Assurance Unit.

**PURPOSE:** To assure that herbicides and other pesticides are applied accurately and safely while conducting research in greenhouse and environmental chambers at Rutgers, SEBS.

1. Personnel will self inspect procedures annually, and additionally, whenever necessary, until and unless a university Quality Assurance Unit is able to inspect the procedures.

**SOP #4.0:** Design selection for greenhouse and environmental chambers studies.

**PURPOSE:** To assure samples are large enough to obtain the required data or samples with sufficient uniformity.

**PROCEDURES:**

1. Environmental climate settings will be made in accordance with acceptable horticultural practices for the commodity.

2. The greenhouse or environmental chamber will be large enough to accommodate the required number of replications, buffer zones and treatments in accordance with an approved protocol and for the commodity to be grown under commercial conditions yielding samples of sufficient size for analysis where required. Verification of temperature and humidity settings should be made at this time. Light intensity and accumulation should be verified throughout the greenhouse or growth chamber before placement of plants.

3. Locate plant materials with sufficient isolation to minimize contamination from external sources such as commercial operations or other research studies.

4. Cultural practices (potting, pruning, planting & etc.) should be performed prior to final plot layout and marking when these practices may affect plot location.

5. The experimental design as specified by the protocol should be used. If there is none, then use a commonly accepted experimental design such as a complete randomized block design.
The experimental design used should be documented. A minimum of 3 replications should be used.

6. Prepare a plot map showing the location of each plot on the site and the North azimuth. The plot map should contain permanent reference points, if possible, so that the plots can be relocated after the study is terminated. Retain the plot map in the study folder.

7. Assign each plot a number representing the replicate and treatment (i.e. plot # 105 is the first replicate of treatment #5). The plot map should indicate the location of each plot by plot # and provide sufficient information to identify the replicate and treatment assigned to each plot.

8. Lay out each plot in the greenhouse or chamber using a steel tape or other suitable measuring device to accurately locate the plots on the site.

9. Identify both ends of each plot with a marker of sufficient visibility to be seen easily throughout the duration of the study. Identify border or guard rows and alley ways with markers.

10. Number each stake with a permanent number corresponding to the plot number on the plot map. Include other numbers, letters, or abbreviations below the plot number on the stake that may be needed to describe additional treatments or conditions.

**SOP #5.0:** Commodity establishment and maintenance.

**PURPOSE:** Assure that commodities are grown under good agricultural practices and provide a uniform crop for study.

**PROCEDURES:**

1. Have on hand a reasonably up-to-date publication on the production of the commodity
under study (i.e. Commercial Vegetable Production Recommendations published by Rutgers Cooperative Extension). If no publication exists for the production of the commodity, consult with an agricultural specialist familiar with the production practices for the commodity and document the practices required to produce the commodity under simulated commercial conditions.

2. Determine pH and soil fertility requirements of the commodity. Obtain random samples of soil for testing from the supplied soil mix. Have the mix tested to determine how well it will meet the requirements of the commodity. Refer to articles on growing commodity in soilless mix and greenhouse/growth chamber conditions. When field soil is needed for greenhouse or growth chamber studies, it should be air dried before bringing into the facility.

3. Condition the soil mix as necessary to bring the soil reasonably within the requirements of the commodity.

4. Determine the correct species and variety to use as specified by the study protocol. If the variety is not specified, determine the variety most commonly used in the area by commercial producers and use it for the study. If a commercial producer is providing the plants, try to select plants as uniform in growth and color as possible.

5. Determine within and between row spacing(s) and seed depth as specified. Plant the seed or transplant in reasonably straight lines or rows with fairly accurate measurements to assure the commodity is planted according to specifications.

6. Identify each treatment row as a plot # in such a manner so that it will be visual throughout the life of the study.

7. Irrigate or perform other agricultural practices as necessary.

8. Maintain the commodity in a healthy state and good growing condition throughout the life of the study as directed using good agricultural practices.

9. If pesticides are applied to the commodity to prevent losses due to pests not under study, they should be applied according to the relevant SOP’s in this document. If this is a residue study, no pesticide should be applied that would interfere with the chemical analysis of the pesticide under study. If in doubt, call the analytical chemist or analytical laboratory identified in the protocol to receive the residue samples.
10. Maintain a written record of all practices performed.

**SOP #6.0: Soil Testing Procedures (if applicable to greenhouse/growth chamber studies)**

**PURPOSE:** To collect soil samples which are representative of the areas being tested for variations in nutrient status. To assure that good soil management practices are performed according to soil test recommendations.

**PROCEDURES:**

1. Walk the field area to be sampled looking for variations in soil color, texture, and drainage. If variations in the soil exist from one end of the field to the other, collect samples that are representative of each region where there is variation in the soil. If variations in soil exist between replications of an experiment, sample each replication.

2. The number of soil samples collected and tested for nutrient status will vary depending upon the extent of variation in the field and the objectives of the researcher. The researcher may choose to test each region of variation in the field and adjust soil management practices for each region according to soil test recommendations. If the researcher does not want to adjust soil management practices for each region, samples from each region can be combined to form a composite sample.

3. Soil samples can be collected with either a shovel or soil-testing probe. Samples should consist of the upper 8” of topsoil. Do not touch soil with hands while collecting samples. Place soil samples in boxes or bags that are free from contamination.

4. Air-dry soil samples and send to a soil testing laboratory. Include with sample information required by the laboratory to make needed fertility recommendations.
**SOP #7.0:** Calibration of instruments and gauges.

**PURPOSE:** To assure that all instruments and gauges used in the research studies are reasonably accurate and in good working order.

**PROCEDURES:**

1. Each gauge or instrument used in research study (i.e. sprayer pressure gauge, temperature and humidity gauges, balances, photometers etc.) should be periodically tested to determine that it is reasonably accurate. If the item is used continuously, it should be tested frequently enough to assure its continued accuracy (i.e. monthly, after every 10 hrs. use etc.). If the item is used infrequently, it should be tested before it is first used in the research study and as often thereafter as necessary to assure its accuracy. Those gauges or instruments that give inconsistent results or are not accurate to within desired tolerances should be repaired or replaced.

2. The CO₂ cylinders should be inspected and tested regularly in accordance with all pertinent regulations.

3. Refer to pages in the manual for the calibration method. If no method is available then cite how to proceed.

**SOP #8.0:** Calibration of a liquid sprayer

**PURPOSE:** To determine the delivery rate sprayer and make adjustments as necessary to ensure an accurate application of the pesticide.

**PROCEDURES:**

1. Visually inspect pumps, hoses, pipes, fittings, regulators, gauges, o-rings, screens, filters, and tanks for obvious wear or potential leaks and repair or replace as necessary.
2. Select an appropriate nozzle tip for the desired spray rate (gal/acre) with regard to spray volume, pressure, and ground speed.

3. Determine whether all nozzles are discharging uniformly by spraying water at a uniform pressure and catching the discharge from each nozzle in a separate container for a given length of time. Begin timing the discharge after the system is operating. If the discharge varies widely, replace all nozzle tips that give a much larger or much smaller discharge. Variation among nozzle tips should be less than 10%. Repeat the above procedure until all nozzles are discharging uniformly.

4. Determine the acreage of a plot (i.e. a 5’ x 20’ plot is 0.0023 acres). Multiply the acreage of a plot by the number of replications. This will be the total area receiving a spray treatment. Determine how much water should be sprayed on this area to give the desired spray rate (gal/acre).

5. Fill the sprayer with a measured volume of water, pressurize the system, and remove air bubbles from lines.

6. Select an area from the border rows that is equivalent in size to that of a treated area in the study. Spray this area at the speed normally used in application or in accordance to guidelines of the protocol.

7. Carefully measure the amount of water remaining and determine the amount needed to spray the treated area. Repeat steps 4 to 7 a minimum of 2 additional times to confirm consistent operation.

8. If the amount of water sprayed out is with 2% of the amount that should be applied, then the sprayer is calibrated and the same settings should be used in actual application. If it is not within this range then repeat steps 2 to 8 after adjusting the pressure, ground speed, or nozzle tips until you are reasonably sure you are applying the correct volume of spray/acre.

**SOP #9.0:** Calibration of Granulator Applicators (for applications in high tunnel greenhouses)

**PURPOSE:** To determine the delivery rate of the granular applicator and make adjustments as necessary to ensure an accurate application of the pesticide.
**PROCEDURES:**

1. Determine that the spreader is in good working order and good mechanical condition. Make sure that the openings to release the granular material are not clogged and free of debris.

2. Refer to the manual for the calibration methods. If no method is available then proceed as follows.

3. Wear protective clothing as necessary and fill the spreader at least half full of the material to be applied. Attach a pan under the spreader to catch the material as it is released.

4. Measure an area of 0.01 acre or 435.6 square feet in close proximity to the area to be treated.

5. Determine the approximate setting of the openings and the approximate speed to operate the applicator for the desired amount of active ingredient/acre.

6. Operate the applicator over the measured distance and collect the output in the pan attached to the spreader.

7. Weigh the material from the pan and multiply by 100 to give the amount applied per acre.

8. Continue with steps 5 to 7 until the desired rate is achieved with 5% of the total/acre.

**SOP #10.0:** Procedures in the applications of pesticides.

**PURPOSE:** To describe the procedures used in pesticide application.
PROCEDURES:

1. All personnel involved in the mixing, application, storage and cleanup of pesticides should be properly trained and licensed.

2. Equipment used in the application of the pesticides should be inspected and calibrated as indicated under SOP #8 and 9.

3. Personnel mixing and applying the pesticide should wear appropriate protective clothing as stated on the pesticide label.

4. The pesticide concentrate should be measured out as indicated under SOP #11.

5. Add water to the mixing container to be about 25% of the final spray volume, add the pesticide(s), triple rinse the graduated cylinder (liquids) or plastic bag (dry formulations) into the container. Add water by pouring against the side of the container opposite the volume marks to reach the correct volume. Use a defoamer if needed. Place used syringes in containment beakers until they are triple rinsed.

6. Agitate by pouring back and forth against the side of a second mixing container a minimum of 5 times, or until the pesticide is well mixed.

7. Pour the spray solution into the spray tank. Add additional water if needed to reach the final spray volume needed.

8. Agitate the spray mix before and during application to insure an even mix of the pesticide and water.

9. Make sure all settings of pressure, speed, boom height, granular flow, etc. are set according to specification from the calibration as previously performed.

10. Apply the treatments beginning with the lowest concentration and work up to the highest concentration.

11. Just before entering each plot make sure you are traveling at the correct speed and turn on the sprayer or release the granules. Maintain the speed through the plot.
12. Turn off the sprayer to stop granular flow at the end of the plot.

13. Correct calibration will result in the use of all the spray solution at the end of the last plot.

14. If the pesticide application is for maintenance of the plots, then apply the pesticide to all the plots in the study according to the directions on the pesticide label.

15. When the application of all the treatments is complete, triple rinse all disposable syringes, containment beakers, mixing containers, and any other pesticide contaminated articles into the spray tank. Pull all nozzles and screens and rinse separately, and reinstall in boom. Dilute the triple rinse water sufficiently to be sure that the labeled rate is not exceeded, and dispose of the rinse water by spraying on the border rows around the study. Triple rinse the spray tank, hoses and boom using the same procedure. Use a commercial spray tank cleaner according to the label instructions when experience indicates an advantage to the use of the product.

16. Certain emulsifiable concentrate (EC) formulations cannot be completely cleaned with water. Use acetone or a mixture of acetone and water to clean these residues. Follow with a triple rinse with water. **Note: Acetone must be stored in a fireproof containment closet and the acetone rinse must be collected and stored in a fireproof containment closet for approved disposal. Do not spray acetone onto the soil.**

**SOP #11.0:** Measuring a pesticide formulation

**PURPOSE:** To assure an accurate dosage in the application of pesticides in field research.

**PROCEDURES:**

I. Liquid

1. All measuring and weighing of pesticides must be done in the fume hood of the pesticide storage room. Proper personal protective equipment must be used while handling pesticides. Use the smallest clean graduated cylinder or disposable syringe large enough to hold the
volume of pesticide needed. It should be graduated in increments small enough to read to an accuracy with +/- 1% of the total volume required (i.e. if 100 ml is needed the smallest division on the cylinder should be 1 ml or less).

2. If the opening of the cylinder is too restricted to allow pouring of the pesticide from the original container without danger of spillage, then do one of the following:

   a. Use a clean beaker with a pour lip as an intermediate and fill the cylinder from it or

   b. Use a clean funnel that is large enough to allow filling the cylinder with a minimum of spillage.

3. Wear or use appropriate safety equipment while handling pesticide concentrate.

4. When measuring a liquid in a graduated cylinder, take the reading of the liquid in the cylinder at the bottom of the meniscus. Pour the liquid directly from the graduated cylinder into the mixing container.

5. When measuring a liquid in a disposable syringe, insert the syringe into the liquid the minimum depth needed to insure filling without drawing air. Fill the syringe, and deliver the required amount, return the remainder to the container, and place the syringe in a containment beaker to prevent contamination of the syringe or mixing area.

II. Dry

1. The balance should be calibrated prior to weighing the quantity for use in the study by following the directions of the manufacturer in the manual.

2. Select a clean jar with a tight fitting lid, a plastic bag, or other container suitable to hold the desired amount of pesticide. Tare it on the scale following the manufacturer’s directions.

3. Select and wear or use appropriate safety equipment while handling pesticide container. All weighing and measuring of pesticides must be done inside the fume hood of the pesticide storage room.

4. Label the container to identify it as to the appropriate treatment or plot number.
5. Weigh the concentrate in a tared container. Return excess to original pesticide container. Seal the empty scoop into the last container to be triple rinsed in the pesticide storage room.

6. Twist and fold plastic bags 180 degrees, and secure with a number 8 rubber band looped over the bag 3 times. (Note: additional loops abrade the bag upon opening that interferes with triple rinsing.)

7. To mix wettable powder (WP) formulations, pull on the folded end of the bag to remove the rubber band, fill the bag ¼ full with water, twist the bag closed with some air captured inside, and shake to mix. Pour the slurry into the mixing container and triple rinse the bag and the empty measuring scoop, if present, into the mixing container.

8. To mix dry flowable (DF) and water dispersible granular (WDG) formulations, pull on the folded end of the bag to remove the rubber band, invert the bag in the mixing container above the water and pour the dry granular into the container. Triple rinse the empty plastic bag and the empty measuring scoop, if present, into the mixing container.

9. Dispose of the triple rinsed plastic bags and disposable measuring scoops in the trash. Do not litter the research site. Return reusable triple rinsed scoop(s) and jars to the pesticide storage room for cleaning with soap and water before reuse. Use water sparingly in the sink when washing scoops and measuring equipment.

**SOP #12.0:** Herbicide application with CO2 backpack-sprayer.

**PURPOSE:** To describe methods used for calibration and spraying with backpack-sprayer.

**PROCEDURES:**

1. Inspect backpack-sprayer (i.e. CO2 tank, pressure gauges, regulators, hoses, fittings, nozzles, screens, filters, o-rings, sprayer tank) for obvious wear or potential leaks test with clear water, and repair or replace as necessary. Be sure the CO2 tank(s) have been inspected and tested within the period of time specified in all pertinent regulations.
2. Applications are normally made using #8004 spray nozzle tips and 30 psi pressure to give an approximate 25 gal/acre spray rate at walking speed. Since walking strides differ for each person, the applicator should choose a comfortable walking pace that can easily be maintained during spray application of large studies. It is important that the applicator avoids slowing his/her speed with fatigue.

3. Calibrate sprayer as described in SOP #6. Adjust either the spray volume, the spray pressure, or nozzle tips to accommodate the small variations in applicators walking speed.

4. The spray volume can easily be adjusted to accommodate the applicators walking speed to prevent any herbicide from being left in the sprayer tank after spraying. Determine the spray solution volume needed to spray the treatment area. Select an area of the field equivalent in size to that of the treatment area to be sprayed with water. Walk at a comfortable pace while spraying this area. Adjust the spray solution volume up or down until the exact amount, +/- 2%, needed to spray the treated area is determined. The boom should “blow air” at the back end of the last plot. Repeat the calibration a minimum of 3 times with the volume of spray solution determined to be correct, before switching from clean water to pesticide treatments.

5. Calculate the amount of herbicide concentrate (liquid or dry formulation) to be applied to the treatment area. Prepare tank mix as described in SOP #10 and 11.

6. Wear appropriate protective clothing and gloves during spray applications and while handling herbicides, preparing tank mixes, and cleaning up.

7. Rinse the mixing containers, spray tank and boom before applying different herbicides, or before spraying a lower rate of an herbicide after spraying a higher rate. Dispose of the rinse water by applying it to the border rows in the front, rear, or along the sides of the experiment, following the same procedures as if it were a treatment. Use reasonable care not to apply a dose higher than the labeled rate to any border area.

8. Follow procedures outlined in SOP #10 for cleaning up and disposal of excess spray solution.

**SOP #13.0:** Cleanup of application equipment

**PURPOSE:** To assure that the pesticide application equipment is decontaminated without adversely affecting personnel or the environment.

**PROCEDURES:**
1. All personnel involved in the mixing, application, storage, and cleanup of pesticides should be properly trained.

2. When the application of all the sprayable liquid treatments is complete, triple rinse all disposable syringes, containment beakers, mixing containers, and any other pesticide contaminated article into the spray tank. Pull all nozzles and screens and rinse separately, and reinstall in boom. Dilute the triple rinse water sufficiently to be sure that the labeled rate is not exceeded, and dispose of the rinse water by spraying on the border rows around the study. Triple rinse the spray tank, hoses and boom using the same procedure. Use a commercial spray tank cleaner according to the label instructions when experience indicates an advantage to the use of such a product.

3. Certain emulsifiable concentrate (EC) formulations cannot be completely cleaned with water. Use acetone or a mixture of acetone and water to clean these residues. Follow with a triple rinse with water. **Note: Acetone must be stored in a fireproof containment closet and the acetone rinse must be collected and stored in a fireproof containment closet for approved disposal. Do not spray acetone onto the soil.**

4. Dispose of the triple rinsed plastic bags and disposable measuring scoops in the trash. Do not litter the research site. Return reusable triple rinsed scoop(s) and jars to the lab for cleaning with soap and water before reuse.

5. Triple rinse disposable protective clothing and discard in the trash. Triple rinse reusable protective clothing before leaving the greenhouse.

6. Triple rinse empty granular applicators in the pesticide storage room after use and dispose of the rinse water as outlined in number #2 above.