IRAC Susceptibility Test Methods Series
Version: 1.0

Method No: 031

Details:

Method:	No: 031
Status:	Approved
Species:	Rape stem weevil *(Ceutorhynchus napi)* Cabbage seedpod weevil *(Ceutorhynchus obstrictus)* Cabbage stem weevil *(Ceutorhynchus pallidactylus)* Cabbage stem flea beetle *(Psylliodes chrysocephala)* Flea beetles *(Phyllotreta spp.)* (For the pod midge *Dasyneura brassicae* a different approach should be used)
Species Stage	Adults
Product Class:	Synthetic pyrethroids (IRAC MoA 3A)

Photograph: Courtesy of Udo Heimback

Comments: The method was developed by Julius Kühn-Institut (JKI). It is currently being widely used in Germany for monitoring sensitivity of flea beetle and weevil species in oilseed rape to synthetic pyrethroids. This method is suitable for Resistance Monitoring and also for Susceptibility Baseline provided that additional rates/replicates are tested to obtain LD50 values.

Objectives:

Susceptibility Baseline: ☒ Resistance Monitoring: ☒

Description:

Materials:
Insect-proof containers, fine pointed brush, glass beakers for test liquids, syringes/pipettes for liquids or weighing balance for solids, acetone, 20ml glass vials with lids, vial roller (or hotdog roller), small funnel to transfer beetles to vials, paper towels, ventilated holding cage, maximum/minimum thermometer.

Method:

(a) Collect approximately 100 - 150 adult beetles at different locations across the infested field. Some beetles can be attracted by yellow colour and then collected. Store beetles in a perforated plastic bag. Place some dry paper towel at the bottom of the bag, and add some oil seed rape leaves as food source. The insects should not be subjected to excessive temperature, humidity or starvation stress after collection.

(b) Use the attached recording sheet for sampling details and other information that may be useful for tracking samples and interpreting susceptibility results later on.

(c) Ship the bag as quickly as possible to the test laboratory, transportation method should avoid excessive temperature, humidity or starvation stress

(d) It is recommended that on arrival to the laboratory, the beetles be released into a ventilated holding cage with the possibility to pick up water and left to recover overnight.

(e) The standard test synthetic pyrethroid is lambda-cyhalothrin (technical available from Fluka). Other synthetic pyrethroids can be used, but the vial application concentrations may need to be adjusted to take account of differences in inherent potency between different pyrethroids. It is advisable to initially run a comparison study with lambda-cyhalothrin if an alternative pyrethroid is chosen for your study.

(f) The test containers are glass vials with an internal surface area of 20-80 cm^2. Newly purchased vials

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should be cleaned of potential residues from their manufacture by soaking overnight in soapy water, rinsing with acetone and air drying for at least 4 hours before use.

(g) Determine the surface area of the glass vials by:

(h is the height of the vial, r is the radius of the bottom)
Surface Area = Area of bottom + Area of the side
Surface Area = $\pi r^2 + (2 \pi r)* h$

(h) Prepare accurate dilutions of the technical grade compound in acetone. For lambda-cyhalothrin suitable test concentrations in µg per cm^2 glass surface have been determined as follows:

0.0375 µg/cm² (50% of the European field application rate of 7.5 g ai/ha),
0.015 µg/cm² (20% rate),
0.003 µg/cm² (4% rate)
Acetone only Control

(i) Glass vials should be filled with 500-1500 µl (depending on vial size, solution should cover base of vial when placed horizontally) of solution and rotated at room temperature until the acetone is completely evaporated. The acetone should be left to evaporate at least 4 hours.

(j) A minimum of two replicates of each concentration and control are required (i.e. 8 vials per test). Additional rates and replicates may be utilised if a full dose response is required for LD50 values generation.

(k) Place a minimum of ten adult beetles per vial (a funnel can be helpful in transferring the beetles to the vial), cap and store horizontally at 20 ± 2ºC and avoiding exposure to direct sunlight.

(l) During the test it is necessary to shake the vials carefully from time to time to separate beetles which are tangled together or to unmatch copulating pairs (the upper beetles of a copulating pair otherwise does not come in contact with the glass surface)

(m) After 24 hours assessments are carried out. Shake the vials carefully to stimulate the beetles and wait 30-60 seconds until the beetles start moving again. It should be noted, that weevils and flea beetles are able to simulate death. So sometimes it is necessary to wait longer to assess the fitness of the beetles. Count dead (including moribund) and alive beetles. Beetles which cannot make co-ordinated movements as compared to the control should be considered dead.

(n) Express results as percentage mortalities. Where control mortality is greater than 20% the study should be considered as invalid for the purposes of susceptibility monitoring.

(o) Using the following 'susceptibility rating scheme' evaluate the test population as being in one of the following categories: (from this evaluation no final conclusion on resistance status can be drawn yet. For P. *chrysocephala* and *Ceutorhynchus obstrictus* resistance has been shown using the scaling provided here.

1) **Susceptible:** mortality at 0,015 µg/cm² =100%

2) **Decreased susceptibility:** mortality at 0,015 µg/cm² between 90 and 100%

3) **Resistance suspected:** mortality at 0,015 µg/cm² < 90%, or at 0,0375 µg/cm² < 100%

Sample Information Recording Sheet:

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Sampling details	
Susceptibility problem previously apparent:	Yes/No
Date of beetle collection:	
Address:	
Name of collector	
Street	
Postal code	
City	
Region	
Geographical position (GPS), if available:	
Crop:	
Average number of insecticide applications in the region:	
Recent insecticide applications in the sampled field:	
Product	
Application date	
Number of applications	

24 hour Assessment Sheet:

	Replicate 1			Replicate 2		
Application rate	**Dead**	**Alive**	**% Mortality**	**Dead**	**Alive**	**% Mortality**
50%						
20%						
4%						
Control						

Precautions & Notes:

1. Where glass equipment is used it must be adequately cleaned with an appropriate organic solvent before re-use to prevent cross-contamination.
2. Different batches of technical grade insecticide may vary in concentration of active ingredient (usually between 85-99% a.i.). Purity needs to be taken into account when preparing the test solutions.

For further information please contact the IRAC International Coordinator:
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