Kobe organic and Kobe non-organic: 10% RHEUM OFFICINALE BAILL PLANT EXTRACT: Efficacy on the tomato powdery mildew *Oidium lycopersici* - Semi-field Test in 2011

*Test Guideline(s)*
Based on EPPO guideline 57 (2) for powdery mildew on cucurbits and other vegetables, semi-field trials

*Author(s)*
Guido MKM Sterk, Master in Zoology

*Study Completion Date*: 01/03/2012

*Test Facility*
Test Facility Name: IPM Impact
Test Facility Address: Gierkensstraat 21 3511 Hasselt Belgium

*Sponsor*
Onze Livre BV
Wim Duijenbergplantsoen 29 4/F, Office 04, 6221 SE Maastricht The Netherlands.

*Study Number*: OIDILY11-01A
PAGE RESERVED

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Applicant/Sponsor: Onze Livre BV
Wim Duisenbergplantsoen 29 4/F,
Office 04, 6221 SE Maastricht The Netherlands

Study Director:

Study Director’s Name, degree Lic. Guido Sterk  
Test Facility Name: IPM Impact  
Date 01/03/2012

Test Facility Management:

Manager’s Name, degree Lic. Guido Sterk  
Manager’s Title  
Test Facility Name: IPM Impact  
Date 01/03/2012

Applicant/Sponsor

Onze Livre BV:
Wim Duisenbergplantsoen 29 4/F,  
Office 04, 6221 SE Maastricht The Netherlands

Onze Livre BV Representative  
Date
QUALITY ASSURANCE STATEMENT

Not applicable

Study Number
OIDILY11-01A

Study Title
Kobe Organic 1.2 SL and Kobe Non-Organic 1.2 SL: Efficacy on the tomato powdery mildew Oidium lycopersici - Semi-field Test in 2011

__________________________________________  ________________________________
First, Middle Initial, Last Name                        Date
Quality Assurance Unit Auditor’s Title
QAU Auditor's Company Affiliation
CERTIFICATION OF AUTHENTICITY
Indicate Full Study Title Here
Kobe Organic 1.2 SL and Kobe Non-Organic 1.2 SL: Effects on the tomato powdery mildew *Oidium lycopersici* - Semi-field Test in 2011

We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report provides an accurate record of the procedures and results.

*Report by: Lic. Guido Sterk*

---

Study Director’s Name Guido Sterk
Study Director’s Title Lic.

Date 01/03/2012

*Approved by: Lic. Guido Sterk*

---

Manager’s Name Guido Sterk
Manager’s Title Lic.

Date 01/03/2012

---

Study Monitor’s Name

Date

*Study Initiation Date:*
01 11 2011

*Date Study Completed:*
31 12 2011

*Sponsor:*
Onze Livre BV
Wim Duisenbergplantsoen 29 4/F,
Office 04, 6221 SE Maastricht The Netherlands
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Kobe Organic 1.2 SL and Kobe Non-Organic 1.2 SL: Efficacy on the tomato powdery mildew *Oidium lycopersici* in a non-GLP Semi-field Test in 2011

Guido MKM Sterk

1.0 SUMMARY

Materials and Methods:

Test Item, Control and Reference Item

**Test Item**
The test item and the information concerning the test item were provided by the sponsor:

Name: Kobe 1.2 SL Organic
Kobe 1.2 SL Non-organic

Active Ingredient Content: 10% RHEUM OFFICINALE BAILL PLANT EXTRACT

Type of Formulation: SL

Water Amount in this Study: 20 mL/plant (Spraying just before beginning of run-off)

Target Amount in this Study: Two dose levels for each formulation of Kobe 1.2 SL.
The dose rates are documented in the raw data and reported in the final report

Storage: Stored at low humidity, out of direct sunlight at a temperature less than 40 °C, in special agro-cabinets, Asecos, developed for the storage of plant protection compounds

Safety Precautions: Routine safety and hygienic procedures were applied
Control
Control: Tap water
Water Amount in this Study: 20 mL/replicate (plant) = 160 mL/object (Spraying just before beginning of run-off)

Reference Item
The information on the reference item according to the test item container label and data sheet:
Name: AgriTrap / Agri 50
Manufacturer: CAI
Active Ingredient Content: alginate
Type of Formulation:
Type: Insecticide /Fungicide
Water Amount in this Study: 20 mL/plant (Spraying just before beginning of run-off)
Target Amount in this Study: One dose level
Storage: Store at low humidity, out of direct sunlight at a temperature less than 40 °C
Safety Precautions: Routine safety and hygienic procedures

Test System
Taxonomic Group: Tomato powdery mildew (Deuteromycetes)
Species: Oidium (neo)lycopersici (=Oidium lycopersicum)
Origin: IPM Impact
Stage at Delivery: Infested plants
Stage at Test Start: Infested plants

Test Units
Type and Size: Test units:
The trial were made on tomato (Solanum lycopersicum, var. Moneymaker).
- 1 plant/replicate
- 8 replicates
Identification: Each test unit was uniquely identified with study number, treatment and replicate number.
<table>
<thead>
<tr>
<th>Test Conditions</th>
<th>Test Environment:</th>
<th>Greenhouse compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature:</td>
<td>25 °C ± 5 °C</td>
<td></td>
</tr>
<tr>
<td>Relative Humidity:</td>
<td>Ca. 70 ± 10 %</td>
<td></td>
</tr>
</tbody>
</table>

**Cropping considerations**

1. Tomato plants are potted into separate pots (pot surface size = 81 cm², 1 plant per pot. They were infested with infested tomato leaves.
2. BBCH Stage: 55 (first signs of individual flowers)
3. Each replicate was placed on separate tables. Treatments were separated by plastic holding large foam shields to avoid spray drift or are kept in a separate places.
4. Trial treatments were prepared according to protocol.
Food
Food: Not relevant. Fungus disease.

Application of the Test Item, the Control and the Reference Item
Application: Single application with a “Birchmeier Super Star 1.25 L” hand-held sprayer
Reason for this Route of Administration: Worst case scenario
Application rate of the Test Item: Kobe 1.2 SL Organic was be sprayed with a defined concentration of 0.15 and 0.2 % formulated compound.
Kobe 1.2 SL Non-Organic was be sprayed with a defined concentration of 0.15 and 0.2 % formulated compound.
The spray volume will be 20 mL per test plant achieving spray coverage just before beginning of run-off.

Concentration of the Test Item Spraying Dilution: 0.15 and 0.2 % formulated compound
Concentration of the Reference Item Spraying Dilution: 0.3% formulated compound AgriTrap / Agri 50
Spraying Scheme: 1. control, 2. Two dose rates of each test item, 3. Standard Application Rate: 8x20 ml spraying solution was applied on 8x1 plant.
Documentation: A technical report with all the details of the trial in an Excell file is stored at IPM Impact.

Course of the Test
Individuals: High infestation
Introduction Procedure: Infested leaves
Exposure Time: 15 days

Test Parameters
Population density Degree of infestation according to official EPPO guideline
Phytotoxicity Any observations on phytotoxicity would be recorded, but no phytotoxicity was observed.

DATA TO COLLECT:
Assess plant growth in treated and untreated plants by direct measurement of height or foliage density and by digital photographs.
Record phytotoxicity as % of total leaf area affected by chloroosis and necros
Record any other symptom or plot differences observed using a scale appropr the symptom.
Record changes in vegetative behavior when present.

Result Evaluation: degree of infestation
Corrected % = (1 - n in T after treatment n in Co after treatment ) * 100
n=degree of infestation T = test compound Co = control
Degree of infestation was determined 15 days after exposure to the test item and the reference item, respectively. It was corrected according to the corresponding results of the control group by the following formula (Abbott, 1925)

Statistical Analysis: Not performed.

Validity Criteria of the Study
Control Mortality: High infestation of powdery mildew (>4 on the EPPO scale)
Standard: >90 % control in the standard, compared to the control
Conclusion:

- Kobe 1.2 SL Non-Organic and Kobe 1.2 SL Organic at all dose rates had a high efficacy against powdery mildew.
- AgriTrap / Agri 50 had a high efficacy, erasing the powdery mildew from the leaves. The trial is valid.
- The mean degree of infestation of powdery mildew in the control was more than 4. The trial is valid.
2.0 GENERAL STUDY INFORMATION

2.1 Study Objectives
The purpose of this study was to measure the effects of a spraying treatment of Kobe 1.2 SL Organic and Kobe 1.2 SL Non:Organic on powdery mildew on tomatoes.

2.2 Test System Justification
Based on EPPO guideline PP1/57(2) Powdery mildew on cucurbits and other vegetables.

2.3 Study Personnel

<table>
<thead>
<tr>
<th>Test Facility Name Study Personnel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Management: Guido Sterk</td>
</tr>
<tr>
<td>Study Director: Guido Sterk</td>
</tr>
<tr>
<td>Gierkensstraat 21</td>
</tr>
<tr>
<td>3511 Hasselt</td>
</tr>
<tr>
<td>Belgium</td>
</tr>
<tr>
<td>Principal Investigator: idem</td>
</tr>
<tr>
<td>Technical Personnel: idem</td>
</tr>
</tbody>
</table>

2.4 Proposed Study Execution Dates
Experimental Start Date: 12 2011
Experimental Completion Date: 12 2011

3.0 MATERIALS AND METHODS

Summary
The trial was carried out at IPM IMPACT trial site in Linter Belgium. Tomatoes of the variety Moneymaker were placed on tables in the greenhouse at a spacing of 100 cm between the crop and 50 cm between the rows. Plots existing of 1 plant and measuring 1 m by 1 m replicated 8 times were used in a randomized complete block design. Irrigation on the table was given during the first 8 weeks after planting thereafter drip irrigation continued throughout the growing season. Assessment of the disease levels were done on a weekly basis a day before the treatments application starting from November 2011. All leaves were checked once after treatment. The treatments were applied using handheld sprayer to deliver an equivalent spray volume of 1000 litres per hectare. There was only one treatment.

Phytotoxicity was checked for 7 days from each treatment after the application according to the official EPPO guideline. The rest of the cultural practices were done as usual. Data was analyzed using the Abbott formula and according to the official EPPO guideline. The trial was completed on 31th of December 2011.
3.1 **Test Guidelines**
This study was designed to comply with the following methods: EPPO guideline : PP1/57(2) Powdery mildew of cucurbits and other vegetables.

3.2 **Test System**

3.2.1 **Chemical System**

3.2.1.1 **Test Item**
- **Name:** Kobe 1.2 SL Organic and Kobe 1.2 SL Non:Organic
- **Formulation:** 1.2 SL
- **Active substance(s):** RHEUM OFFICINALE BAILL PLANT EXTRACT
- **Product Use:** Fungicide

3.2.1.2 **Reference Items**
- **Control**
  - **Name:** water

- **Toxic Standard**
  - **Name:** AgriTrap / Agri 50
  - **Formulation:** alginate
  - **Product Use:** Insecticide/ Fungicide

3.2.1.3 **Test Vehicle**
- Tap water

3.2.1.4 **Application Information**
Test item application rates were based on the results of non-GLP range finding study(ies) and consultation with the Sponsor’s Study Monitor.
Spray Solution Table

<table>
<thead>
<tr>
<th>Nominal Product</th>
<th>Concentration (mg/mL)</th>
<th>Total Spray Solution Made (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kobe 1.2 SL</td>
<td>5 x 20 ml = 100 ml</td>
<td>8x20 ml = 160 ml</td>
</tr>
<tr>
<td>Organic</td>
<td>1,5 and 2 mL/L</td>
<td></td>
</tr>
<tr>
<td>Kobe 1.2 SL</td>
<td>5 x 20 ml = 100 ml</td>
<td>8x20 ml = 160 ml</td>
</tr>
<tr>
<td>Non-Organic</td>
<td>1,5 and 2 mL/L</td>
<td></td>
</tr>
<tr>
<td>Agri 50</td>
<td>3 mL/L</td>
<td>8x20 ml = 160 ml</td>
</tr>
<tr>
<td>Water</td>
<td>8x20 ml = 160 ml</td>
<td></td>
</tr>
</tbody>
</table>

Application Details

Application Order:
1. Control
2. Kobe Organic 1.2 SL (test item): 2 dose rates
3. Kobe 1.2 SL Non-organic (test item): 2 dose rates
4. Agri 50 (standard)

Method of Application: Spraying.
Application Equipment: Birchmeier Super Star
Number of Nozzles: 1
Spray Nozzle Type: Swivel
Spray Pressure: 2 bar
Application Speed: +/- 50 ml/min.
Height above Target: 10 cm
Calibration Procedure: Spray Equipment Clean-up: Rinsing with tap water – every active ingredient, water included, has its own Birchmeier Super Star sprayer

Documentation: All application data are documented in the study records.

3.2.2 Biological System

3.2.2.1 Test Organism

Taxonomic Group: Deutomycetes
Species: *Oidium lycopersici*
Sex: Not applicable
Source: *IPM Impact*
Stage at Delivery: Heavy infestation
Life Stage at Treatment: Heavy infestation
Age at Treatment: Not applicable
3.2.3 **Physical System**

3.2.3.1 **Acclimatisation (Pre-Test)**

- **Pre-Test Location:** Not applicable
- **Temperature:** 
- **Relative Humidity:** 
- **Light Intensity:** 
- **Light Regime:**

3.2.3.2 **Test Units**

- **Exposure Units:** 1 tomato /replicate was placed in a plastic recipient (30x20x12)
- **Identification of Test Units:** Each test unit was uniquely identified with study number, treatment and replicate number

3.2.3.3 **Test Conditions**

- **Test Location:** Controlled-environment room
- **Exposure period:**
  - Temperature: 25 ± 5°C
  - Relative Humidity: 70 – 10% Light
  - Intensity: 5000 – 10000 lux
- **Post-exposure period:**
  - Temperature: 25 ± 5°C
  - Relative Humidity: 70 – 10% Light
  - Intensity: 5000 – 10000 lux
- **Light Regime:** 16 light, 8 hr dark (see climatic conditions during the trial in appendix)
- **Instrumentation:** Hobo U12 data logger Temperature/Relative humidity/Light
- **Documentation:** Test conditions were recorded with suitable instruments and documented in the raw data. The source, preparation date, and storage conditions of the food are documented in the raw data.
3.3 Test Design

Treatment Groups: Water Control, test items, standard

Exposure Period
Length of Exposure Period: 15 days
Sample Size: All leaves

3.4 Test Conduct

3.4.1 Exposure Period
15 days

3.5 Parameters Observed

Degree of infestation
The number and degree of infested leaves were counted directly on the leaves.

Fytotoxicity measurement
Assess plant growth in treated and untreated plants by direct measurement of plant height or foliage density.
Record phytotoxicity as % of total leaf area affected by chlorosis and necrosis.
Record any other symptom or plot differences observed using a scale appropriate to the symptom.
Record changes in vegetative behavior when present.

3.6 Result Analysis

3.6.1 Parameter 1
Degree of infestation formula
Evaluation in the population density by using the Abbott formula

Corrected mortality = (1 – (N in T after treatment/N in Co after treatment)) * 100
N = Degree of infestation T = test compound Co = control
3.6.2 **Parameter 2**
Phytotoxicity: No Phytotoxicity was observed in this trial

3.6.3 **Documentation**
Statistical procedures and computer program used to analyze the study data are referenced in the study records.

3.7 **Validity Criteria of the Study**
Control Mortality: Mean value >4 in the control
The test is valid.
Standard: >90% control in the standard, compared to the control.
The trial is valid.

3.8 **Study Plan Deviations**
Deviation 1
Study Plan:
Deviation: None
Reason: /
Impact on Study: /
4.0 RESULTS AND DISCUSSION

4.1 Parameter 1: Degree of infestation

<table>
<thead>
<tr>
<th>Nb.</th>
<th>Product</th>
<th>Application</th>
<th>Dose rate %</th>
<th>Total degrees of damage (cumulative)</th>
<th>Abbott (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kobe 1.2 SL Non-organic</td>
<td>Spraying</td>
<td>0,15</td>
<td>3</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>Kobe 1.2 SL Non-organic</td>
<td>Spraying</td>
<td>0,2</td>
<td>1</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>Kobe 1.2 SL Organic</td>
<td>Spraying</td>
<td>0,15</td>
<td>6</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>Kobe 1.2 SL Organic</td>
<td>Spraying</td>
<td>0,2</td>
<td>3</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>Agri 50</td>
<td>Spraying</td>
<td>0,3</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>Spraying</td>
<td>0</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

4.2 Parameter 2: fytotoxicity

<table>
<thead>
<tr>
<th>Plant height</th>
<th>% leaf area chlorosis</th>
<th>% leaf area necrosis</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Kobe 1.2 SL Non-Organic all dose rates</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Kobe 1.2 SL Organic all dose rates</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Agri 50</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

5.0 CONCLUSIONS

- Kobe 1.2 SL Non-Organic and Kobe 1.2 SL Organic at the tested dose rates are very effective against this disease.
- No signs of Phytotoxicity on tomato, var. Moneymaker were observed.
- Agri 50 was highly effective against powdery mildew in this trial.
6.0 **RETENTION OF RECORDS**

For the periods demanded by GLP guidelines and specific country requirements, study documents and materials will be stored in the archives of IPM Impact (Gierkensstraat 21 3511 Hasselt Belgium), including but not limited to:

- study plan;
- any study plan and/or report amendments or addenda or SOP deviations;
- all raw data;
- comments of the sponsor on the draft report;
- one original signed copy of the final report;

Documents and materials are archived according to the principles of Good Laboratory Practice in the organization of the testing facility. If Test Facility name wishes to purge their files of the study records, they will contact the sponsor. Test Facility name must receive written permission from Onze Livre BV to either send these study records to Onze Livre BV for archival or to discard study records.

Copies of the signed original report, study plan, and any study plan amendments were sent to Onze Livre BV upon finalization of the study. These documents are retained in the archives at: Wim Duisenbergplantsoen 29

4/F, Office 04, 6221 SE Maastricht
The Netherlands

7.0 **DISPOSAL OF TEST ITEM**

After issuance of the final report, the remaining test item will be stored at Test Facility name until its expiration date and then destroyed in accordance with local regulations, unless other arrangements are made between the sponsor and the Test Facility name.

8.0 **REFERENCES**


### Appendix 1 Raw Data Assessment

| Nb  | Product                        | Application | Dose rate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total degrees of damage |
|-----|--------------------------------|-------------|-----------|---|---|---|---|---|---|---|--------------------------|
| 1   | Kobe 1.2 SL Non-organic        | Spraying    | 0.15      | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 3                        |
| 2   | Kobe 1.2 SL Non-organic        | Spraying    | 0.2       | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1                        |
| 3   | Kobe 1.2 SL Organic            | Spraying    | 0.15      | 2 | 1 | 0 | 0 | 1 | 2 | 0 | 6                        |
| 4   | Kobe 1.2 SL Organic            | Spraying    | 0.2       | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 3                        |
| 5   | Agri 50                        | Spraying    | 0.3       | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0                        |
| 6   | Control                        | Spraying    | 0         | 4 | 5 | 3 | 4 | 4 | 4 | 5 | 4                        |
|     |                                |             |           |   |   |   |   |   |   |   | 33                       |
Appendix 2  Climatic conditions Linter
APPENDIX  CERTIFICATE OF TESTING FACILITY

To whom it concerns,

IPM Impact is a company specialized in efficacy trials with biological compounds on pests and side-effect trials on beneficial organisms. Although it also carries out tests for registration under GEP and GLP, in cooperation with its partners, IPM Impact is mainly specialized in trials for Integrated Pest Management under practical conditions.

The tests are done under the same stringent conditions that are required for GEP trials.

The results from this research are published on the website that offers the most comprehensive database in the world on effects of pesticides on pollinators (bumblebees), predators and parasitoids.

The company does also a lot of work on the effects of plant protection compounds, mainly fungicides, on entomopathogenic fungi and zoophagous nematodes.

The study director was responsible for doing the efficacy trials for registration of insecticides and acaricides in orchards and soft fruit for the Ministry of Agriculture in Belgium for more than 10 years.

He, was also founding member of the ecotox committee for registration in Belgium.

He was for many years co-convenor of the IOBC working group 'Effects of Pesticides on Beneficial Organisms' where he was responsible for the joint testing programs.

He's an active member of the International commission for plant-bee relationships, bee protection group.

Dr. Guido Sterk

Study Director IPM Impact