

Renewal Assessment Report

***Lecanicillium muscarium* Ve6**

- Mycotal -

Volume 3MP – B.1 Identity

January 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: France

Version history

When	What
January 2018	Initial RAR

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B.1 Identity of the plant protection product

Note to reader:

Information from the original DAR and/or addenda to the DAR is highlighted grey.

B.1.1 Applicant

Name	Koppert B.V.
Address	Veilingweg 14 P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands
Contact person	██████████
Phone	██████████
Fax	██████████
Email	██████████

B.1.2 Manufacturer of the preparation and the micro-organism

Name	Koppert B.V.
Address	Department R&D Microbials and Regulatory affairs, Velingweg 14, 2650 AD Berkel en Rodenrijs
Contact person	██████████
Phone	██████████
Fax	██████████
Email	██████████

B.1.3 Trade name or proposed trade name, and manufacturer's development code number of the preparation if appropriate

Current trade name: MYCOTAL

Former trade names: not applicable

Development code numbers:

19-79, GCRI-79, HRI 19-79 (R. Hall)

Ve 6 (Tate & Lyle, MRL)

KBV 10-88, KBV 88-M01, KV01 3* (Koppert B.V.)

B.1.4 Detailed quantitative and qualitative information on the composition of the preparation

For confidential information, see Volume 4.

Information from the original DAR

Verticillium lecanii Ve6 has been reclassified in 2001:

Kingdom: Fungi

Phylum: Deuteromycotina

Order: Hyphomycetes (syn. Moniliales)

Genus: *Lecanicillium*

Species: *Lecanicillium muscarium*

Strain: Ve6

For more information, please refer to Doc B.1-MA.

Isolate 102071 (MYCOTAL) has been deposited to the CBS Filamentous Fungi database as *Verticillium lecanii* strain 19-79 (= strain Ve6). The species name has been changed to *Lecanicillium muscarium* (Petch) Zare & W. Gams. [<http://www.cbs.knaw.nl/databases/index.htm>, CBS nr. 102071, 31-10-2005.]

The content of the microorganism in the plant protection product

Information from the original DAR

Report: Samson, R.A., Hoekstra, E.S. (2003): Detection, isolation and identification of *Lecanicillium muscarium* in one package MYCOTAL.; Centraalbureau voor Schimmelcultures (Fungal Biodiversity Centre), P.O. Box 85167, 3508 AD Utrecht, The Netherlands; unpublished report no. TM 03.031, 17-03-2003; date of experimental work: March, 2003.

Guideline: -

GLP: No

Materials and Methods:

Test material: MYCOTAL (1.0×10^{10} spores of *Verticillium lecanii* strain Ve 6 per gram product); Batch: 98 MYC 397; Purity: See Document J.

Test material: MYCOTAL (1.0×10^{10} spores of *Verticillium lecanii* strain Ve 6 per gram product); Batch: 98 MYC 399; Purity: See Document J.

Test material: MYCOTAL (1.0×10^{10} spores of *Verticillium lecanii* strain Ve 6 per gram product); Batch: 97 MYC 401; Purity: See Document J

A suspension of each sample of the product was prepared (in duplo) in sterile water (1:10). After stirring thoroughly by using a Vortex, 0.1 mL suspension was plated onto malt extract agar and oatmeal agar. Incubation at 24°C in darkness during 10 days. Identification by Prof Dr. Walter Gams using microscopy (morphology

Findings: The samples of the three batches contained one fungus each, which is identified as *Lecanicillium muscarium* (Petch) Zare & W. Gams. The fungus is also known as *Verticillium lecanii* s. lato. (Table IIM

1.4.4-1).

Table IIM 1.4.4-1 Identification of active ingredient of MYCOTAL

Batch number	Production date	Name fungus	Synonym
98 MYC 397	22-10-2002	<i>Lecanicillium muscarium</i>	<i>Verticillium lecanii</i> s. lato
98 MYC 399	27-12-2002	<i>Lecanicillium muscarium</i>	<i>Verticillium lecanii</i> s. lato
97 MYC 401	31-01-2003	<i>Lecanicillium muscarium</i>	<i>Verticillium lecanii</i> s. lato

Conclusions: The micro-organism has been identified as *Lecanicillium muscarium* (Petch) Zare & W. Gams (synonym: *Verticillium lecanii*).

Report: IIIM 1.7.4/01(3) (Document J). Samson, R.A., Houbaken, J. (2003): Investigation of three samples MYCOTAL.; Centraalbureau voor Schimmelcultures (Fungal Biodiversity Centre), P.O. Box 85167, 3508 AD Utrecht, The Netherlands; unpublished report no. TM 03.078, 2507-2003; dates of experimental work: July, 2003.

Guideline: -

GLP: No

Materials and Methods: Test material: MYCOTAL (1.0×10^{10} spores of *Verticillium lecanii* strain Ve 6 per gram product); Batches: 97 MYC 401, 97 MYC 403, 98 MYC 399; Purity: See Document J.

Spore concentration: determined with Bürker-Türk counting chamber. Three sub samples. Spore concentration of each sub sample determined in eightfold.

Spore germination: dilutions made in sterile demineralised water plated onto Sabaraud Dextrose Agar (with penicillin and streptomycin). Incubation at room temperature for 18 hours.

Findings: The total number of viable (germinating) spores varied between 0.9×10^{10} and 1.0×10^{10} per gram MYCOTAL (Table IIM 1.4.4-2).

Table IIM 1.4.4-2 Spore content and germination of three MYCOTAL batches.

Sample	Number of spores per gram MYCOTAL	Spore germination (%)	Number of germinating spores per gram MYCOTAL
97 MYC 401	1.33×10^{10}	78.2	1.04×10^{10}
97 MYC 403	1.31×10^{10}	77.3	1.0×10^{10}
98 MYC 399	1.20×10^{10}	75.5	0.91×10^{10}

Conclusions: MYCOTAL contains about 1.0×10^{10} germinating spores per gram product.

Content of co-formulants

Confidential information – see Volume 4.

Content of microbial impurities

Information from the original DAR

Contaminants have to be less than 500.000 CFU's per gram of technical spore powder and per gram of the final formulated product. Yeasts and fungi, coliformes, *Staphylococcus aureus* and faecal Streptococcus: < 10.000 per

gram. *Salmonella*: absent in 10 gram.

PRAPeR M3 (22-26 June 2009): Open point open. RMS to transfer the information on the identity and levels of contaminants from Vol. 4 to an addendum Vol. 3 of the DAR.

RMS to move the information on the methods for microbial contamination to a non-confidential addendum and to confirm that methods are ISO methods.

Impurities in final product (Anonymous, 2005)

Contaminants: for the product the contaminants have to be less than 500.000 CFU s per gram of technical spore powder and per gram of the final formulated product. The following maximum levels are set for specific contaminants:

Total plate count: < 500.000 per gram

Yeasts and fungi: < 10.000 per gram

Coliformes: < 10.000 per gram

Staphylococcus aureus: < 10.000 per gram

Faecal *Streptococcus*: < 10.000 per gram

Salmonella: absent in 10 gram

Every batch is tested (the technical spore powders and the final products), an independent laboratory tests the final products. If the product does not meet these criteria the batch is rejected and not sold.

Identification of impurities:

1) Aerobe mesophiles: Plate Count Agar, pour plates, 3 days at 30°C (Mossel & Tamminga, 1980)

2) Yeasts: Oxytetracycline Glucose Yeast extract Agar: pour plates, 3 - 5 days at 22°C (Dutch Food Legislation)

3) Coliformes: Violet Red Bile Lactose Agar: double layer, 20 hours at 30°C (ISO 4832)

4) Faecal Streptococci: Kanamycin Aesculin Azide Agar: brush plate, 3 - 5 days at 37°C (Mossel & Tamminga, 1980)

5) *Staphylococcus aureus*: Baird Parker Agar: brush plate, 48 hours at 37°C. Confirmation by use of coagulase test (Dutch Food Legislation)

6) *Salmonella* (Biochem work prescription): Non-selective accumulation: buffered peptone-water

Selective accumulation: Tetrathionate Brilliant green Gall-broth and Selenite broth, 18 - 24 hours at 42°C Confirm pure culturing Pure culturing: Xylose-Lysine-Deoxycholate-Agar and Bismuth-Sulfide-Agar, 18 - 24 hours at 37°C Confirmation: short polyvalent O-serum, E-tube, Hoffman LaRoche

For the information on the content and functions of co-formulants, please refer to volume 4.

For the information on the production of destruxins, please refer to the information reviewed by (Ben el Hadj et al., 2005, Butt et al., 2004, Koppert Beheer B.V., 2005) in Volume 3MA B.2.

New data 2016

Previously the content of *Lecanicillium muscarium* Ve6 ranges from 9.0×10^9 to 1.2×10^{10} spores/g and 5.0×10^9 to 9.0×10^9 CFU/g, respectively. The nominal content of *L. muscarium* Ve6 is 1×10^{10} spores/g.

The production of MYCOTAL is a continuous process. Therefore a technical material does in fact not exist. Hence, data on the technical material of *L. muscarium* Ve6 are not relevant. Therefore, for the renewal of the active substance, a new 5-batch analysis of the formulated product is submitted. The content of *Lecanicillium muscarium* Ve6 ranges from 9.5×10^9 to 1.2×10^{10} spores/g and 5.3×10^9 to 8.9×10^9 CFU/g, respectively. For content of the microorganism in the current 5-batch data, please refer to the summary of the 5-batch data of the formulated product (Jacobs (2016a-e)), see below.

The nominal content of *L. muscarium* Ve6 is 1×10^{10} spores/g.

According to Skrobek et al., (2005, previously submitted, see for summary B.7.2.1), *L. muscarium* Ve6 does not produce destruxins A, B, or E. No destruxins were detected in spores, mycelium, colonised rice, culture filtrates, or the end-use product.

According to the EFSA conclusion on *L. muscarium* Ve6, the strain does not produce any metabolites of concern.

Results on the analysis relevant impurities on current 5 batches are available (see Kuneman 2016a-e in Volume 4). *Escherichia Coli*, *Salmonella* and *Staphylococcus aureus* are not detected in the batches. Aerobic Plate Count 37°C presented content from 1000 to 53000 CFU/g.

Report: KMP 1.4/01 to KMP 1.4/05: Jacobs, N. (2016a to 2016e)
Certificate of analysis Mycotal - content of active ingredient
Batch numbers: 16M07, 16M15, 16M19, 16M21 and 16M24
Facility: Koppert Laboratory of Microbiology
Non-published report
Dates of analysis: 19.02.2016 - 20.06.2016

Table 1.4-1 Summary of the results of 5 batches of Mycotal (WG formulation)

		Results				
		Batch 16M07	Batch 16M15	Batch 16M19	Batch 16M21	Batch 16M24
<i>Lecanicillium muscarium</i> Ve6	spores/g	1.2×10^{10}	1.1×10^{10}	9.5×10^9	1.2×10^{10}	1.2×10^{10}
	CFU/g	6.8×10^9	5.3×10^9	5.3×10^9	8.9×10^9	7.5×10^9

B.1.5 Physical state and nature of preparation

Information from the original DAR

Original inclusion

Wettable powder [WP].

New data 2016

The nature of the new formulation is Water dispersible granules [WG]

For the details on the compositions of the formulations, please refer to Volume 4.

B.1.6 Function

Biological control of insects.

B.1.7 References relied on

Reference list - data submitted to support the evaluation

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
	Koppert Beheer BV	2005	Identity and content of impurities and contaminating micro-organisms. Koppert Beheer BV, Department R&D Microbials and Regulatory affairs, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer BV - - unpublished statement	Y	KBS
	Ben El Hadj, N., Skrobek, A., Ravensberg, W.J., Wang, C., Lange, C., Vey, A., Butt, T.M.	2005	Destruxin production by the entomopathogenic fungi <i>Lecanicillium muscarium</i> and <i>L. longisporum</i> (Petch) Zare & Gams. Institut National de la Recherche Agronomique (I.N.R.A.), Unité de Recherche de Pathologie Comparée, 30380 St Christol les Alès, France, School of Biological Sciences, University of Wales Swansea, Singleton Park, SA2 8PP, UK, Laboratoire de Spectrométrie de Masse Bio-Organique, CNRS-UMR 6014, Université of Rouen, 76821 Mont-Saint-Aignan cedex, France, Koppert Biological Systems, POB 155, 2650 AD Berkel en Rodenrijs, The Netherlands. - Journal of Natural Products. - Report to be published	N	KBS
	Butt, T.M., Skrobek, A., Wang, C., Shah, F.A., Ben El Hadj, N.	2004	RAFBCA Partner 01, University of Wales, Swansea, UK, Final Report 01.11.01-31.10.04. School of Biological Sciences, University of Wales Swansea, Singleton Park, SA2 8PP, UK Koppert Beheer BV; QLK1-2001-01391 - unpublished report	N	KBS

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
	Koppert Beheer B.V.	2005	Information on the production of metabolites (especially toxins) by <i>Verticillium lecanii</i> strain Ve6. Koppert Beheer B.V., Department R&D Microbials and Regulatory affairs, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer BV unpublished statement	Y	KBS
KMP 1.4/01	Jacobs, N.	2016a	CERTIFICATE OF ANALYSIS Koppert, 16M07 Koppert B.V., Berkel en Rodenrijs, NL GLP/GEP: no Published: no	yes	KBS
KMP 1.4/02	Jacobs, N.	2016b	CERTIFICATE OF ANALYSIS Koppert, 16M15 Koppert B.V., Berkel en Rodenrijs, NL GLP/GEP: no Published: no	yes	KBS
KMP 1.4/03	Delvaux, S.	2016	CERTIFICATE OF ANALYSIS (16M19) Koppert, 16M19 Koppert Biological Systems GLP/GEP: no Published: no	yes	KBS
KMP 1.4/04	Jacobs, N.	2016c	CERTIFICATE OF ANALYSIS Koppert, 16M21 Koppert B.V., Berkel en Rodenrijs, NL GLP/GEP: no Published: no	yes	KBS
KMP 1.4/05	Jacobs, N.	2016d	CERTIFICATE OF ANALYSIS Koppert, 16M24 Koppert B.V., Berkel en Rodenrijs, NL GLP/GEP: no Published: no	yes	KBS
KMP 1.4/06	Kuneman, A.A.	2016a	CERTIFICATE OF ANALYSIS Koppert, AR-16-HE-009176-01 Eurofins Food Netherlands B.V. GLP/GEP: no Published: no	yes	KBS
KMP 1.4/07	Kuneman, A.A.	2016b	CERTIFICATE OF ANALYSIS Koppert, AR-16-HE-018439-01 Eurofins Food Netherlands B.V. GLP/GEP: no Published: no	yes	KBS

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
KMP 1.4/08	Kuneman, A.A.	2016c	CERTIFICATE OF ANALYSIS Koppert, AR-16-HE-024767-01 Eurofins Food Netherlands B.V. GLP/GEP: no Published: no	yes	KBS
KMP 1.4/09	Kuneman, A.A.	2016 d	CERTIFICATE OF ANALYSIS Koppert, AR-16-HE-024767-01 Eurofins Food Netherlands B.V. GLP/GEP: no Published: no	yes	KBS
KMP 1.4/10	Kuneman, A.A.	2016e	CERTIFICATE OF ANALYSIS Koppert, AR-16-HE-027242-01 Eurofins Food Netherlands B.V. GLP/GEP: no Published: no	yes	KBS
KMP 1.4/11	Kuneman, A.A.	2016f	CERTIFICATE OF ANALYSIS Koppert, AR-16-HE-034755-01 Eurofins GLP/GEP: no Published: no	yes	KBS