Renewal Assessment Report

Lecanicillium muscarium Ve6

Volume 3MA – B.9 Effects on non-target organisms

January 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: France

Version history

When	What
January 2018	Initial RAR

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Introduction

The Microbial Pest Control Agent (MPCA) *Lecanicillium muscarium* Ve6 (formerly *Verticillium lecanii* Ve6) is an entomopathogenic fungus that was isolated from the greenhouse whitefly *Trialeurodes vaporariorum*. Based on DNA sequencing data, the species *Verticillium lecanii* was renamed and divided into a number of new taxonomical units, including *Lecanicillium muscarium* (Zare and Gams, 2001).

For the original approval of this MPCA, the notifier has submitted literature that demonstrates the worldwide distribution of *Verticilllium lecanii* (see section B.2.1.2) and the ability of *Verticilllium lecanii* to grow using a broad range of hosts and substrates (e.g. as soil pathogen on other fungi, as a hyperparasite on rust fungi, a as parasite on arthropods and on plant material). The *species Lecanicillium muscarium* also has a worldwide distribution; e.g. see Zare and Gams (2001), in which strains are described from the UK, Italy, but also a location as remote as New Caledonia. In addition, a strain of *Lecanicillium muscarium* has also been isolated from Continental Antarctica (Fenice and Gooday, 2006). No information is provided on the host range for *Lecanicillium muscarium* Ve6 at strain level.

Metabolites/toxins

Lecanicillium muscarium Ve6 has been shown to be able to produce destruxins (see section B.2.7). However, no destruxins were detected in spores, mycelium, colonised rice, filtrates of production-scale cultures, or the end-use product. Destruxins were also not detected in plant material treated with foliar applications of the formulated product Mycotal (at ten times the recommended dose; see reference Butt *et al.*, 2004 in section B.2).

In the DAR (2007), the following was stated in section B.9.1.4.2:

Destruxins A, B and E were detected in culture filtrates of *L. muscarium* (see Vol 3, Annex B.1, B.2.1.8). Also subtilisins and subtilisin-related proteases may be present during growth on insect cuticulas (St Leger *et al.*, 1997). However, there are no indications of any toxicity of such mixtures of toxins, enzymes and other ingredients for birds, although tests with purified toxins or enzymes of *L. muscarium* are not available. Toxins and other metabolites of *L. muscarium* can be considered biodegradable outside micro-organisms. They are effective only during the process of hyphae growth when degrading the host or target.

According to the EFSA conclusion (EFSA Journal 2010; 8(1):1446) on *L. muscarium* Ve6, the strain does not produce any metabolites of concern.

Based on the above, no further risk assessment of metabolites is considered necessary.

B.9.1 Effects on birds

B.9.1.1 Toxicity to birds

Microbial pest control agent (MPCA)

KMA 8.1/01

Previous evaluation:	*DAR 2007*

Reference/notifier	:	[redacted] (1998)	GLP statement	:	yes
Type of study	:	birds, repeated acute oral toxicity, infectivity, pathogenicity	Guideline	:	9-Nousan no. 5090
Year of execution	:	1998	Acceptability	:	acceptable
Test substance	:	*L. muscarium* (Mycotal TGAI), batch no. 020898 79VL133, 6.5×10^{10} CFU/g			

Substance	Species	Route	Duration [d]	Recovery period [d]	Criterion	Value [mg a.s./kg bw/day] or [CFU/kg bw/day]
L. muscarium	*Coturnix coturnix japonica*	repeated oral gavage	5	25	LD_{50}	19 or 1.2×10^{9}

Material and methods:

Micro-organism: *L. muscarium*

Test species: Japanese quail (*Coturnix coturnix japonica*). The male and female quails used in this study were immature (28 days old at start test)

Number of test animals: Ten birds per replicate/cage (five males, five females); three replicates per treatment

	with a.s.; three replicates per control (vehicle control)
Treatments:	In total six cages (three for the vehicle control and three for the treatment) Daily dosage: 19 mg a.s./kg bw (corresponding with 1.2×10^9 CFU/kg bw), for five days. Dosing suspensions in 0.01 M phosphate buffer solution. Oral application with gastric catheter
Duration:	Five days of exposure followed by 25 days of observation
Test conditions:	Target ambient room temperature 22 ± 2.0 ºC; target relative humidity 55 ± 15%; photoperiod 8 h D:16 h L
Deviations from guideline	Only minor guideline deviations, not assumed to have affected the test results (see comments)
Endpoints:	Mortality, clinical signs, infectivity, (histo)pathogenicity
Observations:	A 25-d period of observation followed after five days of exposure. Mortality, clinical signs were recorded (observations once per day) up to 30 days after test initiation. Faeces were collected and plated 1, 2, 5, 7, 14, and 28 after test initiation. All birds were sacrified to plate tissues of the kidneys, brain, liver, lung, spleen and cecum. Blood was sampled and plated as well

Results:

A summary of endpoints is given in the table below. Histopathological analysis of organs after necropsy was not performed as gross observations indicated no abnormalities 30 d after initiation

Table B.9.1.1.a: Toxicity, infectivity and pathogenicity of to birds

Test species	Japanese quail (*Coturnix coturnix japonica*)
Toxicity	No mortalities or signs of toxicity at daily dosages of 19 mg a.s./kg bw, for five days (corresponding with 1.2×10^9 CFU/kg bw). Also no clinical signs. 5-d LD_{50} > 19 mg a.s./kg bw/day (corresponding with > 1.2×10^9 CFU/kg bw/day)
Infectivity, pathogenicity	Tissues, faeces and blood samples showed no occurrence of the a.s. at daily dosages for five days of 19 mg a.s./kg bw (corresponding with 1.2×10^9 CFU/kg bw). Therefore no infectivity was indicated

Comments DAR 2007:

The light strength was not reported. The purity of the test substance was not reported, though probably technical grade. The notifier stated that the quarantine and acclimation period for two of the three replicates were 8 and 9 days rather than the protocolled seven (unverifiable as test protocol was not submitted to the RMS). Contrarily to the author, who stated that the repeated oral dosage was 'high', the evaluator judges the dosage too low to

represent a limit test useful for risk assessment. Therefore, the 5-d LD_{50} of > 19 mg a.s./kg bw/day (corresponding with > 1.2×10^9 CFU/kg bw/day), referring to repeated acute toxicity, infectivity and pathogenicity is less useful for the risk assessment.

RMS comment and conclusion RAR 2017:

According to OPPTS 885.4050 the maximum hazard dose level to be tested should be:

Concentration of MPCA in TGAI x 5 mL/kg bw (x weight of test bird (kg))

When 5 mL is considered ca. roughly equal to 5 g, than this amounts to: 6.5×10^{10} CFU/g x 5 g/kg bw = 3.25×10^{10} CFU/kg bw

Thus, the evaluation from DAR 2007 is valid, however RMS would now prefer to state that the study is 'reliable with remarks'.
Since the endpoint was accepted during Peer review and included without restrictions in the List of Endpoints in the EFSA conclusion (EFSA Journal 2010; 8(1):1446), the endpoint for risk assessment is 5-d LD_{50} > 19 mg a.s./kg bw/day (corresponding with > 1.2×10^9 CFU/kg bw/day).

B.9.1.2 Infectiveness to birds

No signs of infectivity under laboratory conditions at daily dosages of 19 mg a.s./kg bw for five consecutive days (corresponding with 1.2×10^9 CFU/kg bw, see Table B.9.1.1.a).

B.9.1.3 Pathogenicity to birds

No signs of pathogenicity under laboratory conditions at daily dosages of 19 mg a.s./kg bw for five consecutive days (corresponding with 1.2×10^9 CFU/kg bw, see Table B.9.1.1.a).

Other studies on bird toxicity, infectivity and pathogenicity (DAR 2007):

Bird toxicity tests with one of the products with *L. muscarium* under review are not available. Various sources, however, do not indicate adverse toxicological, infective and pathogenic effects on birds following exposure to *L. muscarium* (Burges, 1981; Copping, 2004*). This lack of effects may be due to the intrinsic non-toxic nature of conidia from filamentous fungi as *L. muscarium* and the optimal growth temperature of germinated conidia in hosts below the average bird body temperature (see Vol 3, Annex B.1 Identity). Toxicity of (a mixture of) mycotoxins in actively growing hyphae to birds cannot be excluded. However, there are no reports of incidence cases.

Comment RMS RAR 2017:

*: Since these references concern (chapters of) books, they were not summarised and evaluated. The content of the two following references was checked and confirmed by RMS:

Burges (ed), 1981. Microbial control of pests and plant diseases. Chapter 25: The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales.

Copping (ed.), 2004. The manual of biocontrol agents.

The reference:'Hokkanen and Hajek, 2003. Ecological risk assessment framework for biological control agents', which was also included in the DAR 2007, was removed by RMS since it did not contain information on toxicological, infective and pathogenic effects on non target organisms following exposure to *L. muscarium.*

New data 2017

The submitted literature search (Scholze (2016) – see B.9.8) did not identify any reference reporting effects of *Lecanicillium* sp. on birds.

The applicant submitted a statement on the study by [redacted] (1998, previously submitted), addressing the effects of *L. muscarium* Ve6 on the reproduction of birds (Anonymous, 2006a, KMA 8.1/02; no further summary included in the RAR). There it was stated that: due to the total absence of effects of toxicity, infectivity, pathogenicity and of internal persistency in birds, it has been demonstrated beyond any doubt that *V. lecanii* Ve6 will have no impact on birds, and that consequently there is no reason for a higher tier test to determine the effect of *V. lecanii* strain Ve6 on reproduction of birds. Moreover, according to Commission Regulation (EU) No 283/2013, determination of the effect of *V. lecanii* strain Ve6 on reproduction of birds is not a data requirement for microbial active substances.

RMS agrees that reproduction studies, or any further studies on birds, are not necessary.

B.9.2 Effects on aquatic organisms

B.9.2.1 Effects on fish

B.9.2.2 Toxicity to fish

Microbial pest control agent (MPCA)

KMA 8.2.1/01

Previous evaluation:	*DAR 2007*

Reference/notifier	:	[redacted] (1983)	GLP statement	:	yes
Type of study	:	fish, acute toxicity, infectivity and pathogenicity	Guideline	:	no
Year of execution	:	1983	Acceptability	:	yes, however, the test substance is slightly soluble, so the actual exposure may have been limited

Test substance	:	Unknown product with *L. muscarium*, VE6-58 SSP, white powder, 8.8×10^9 CFU/g

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg a.s./L] or [CFU/L]
Unknown product, see above	*Oncorhynchus mykiss*	acute	14±1.0	7.6	96	LC_{50}	> 97[1] or > 6.2×10^9

1: based on actual concentrations; the nominal 96-h LC_{50} was 1000 mg a.s./L

Material and methods:

Micro-organism	*L. muscarium*
Test species:	*Oncorhynchus mykiss:* juvenile rainbow trouts; average length 47±0.26 mm, average weight 1.5±0.28 g
Number of test animals:	Ten juvenile trouts per replicate; two replicates for the top-dose of 1000 mg a.s./L; one replicate for each lower dose (10 and 100 mg a.s./L) and for the negative control
Treatments:	In total six test tanks with 40 L test solution (one for the control, four for the treatments) and an additional tank for the top-dose without fish. Concentrations were 10, 100 and 1000 mg a.s./L (nominal), corresponding with 6.3×10^8, 6.3×10^9 and 6.3×10^{10} CFU/L, respectively. Concentrations of the a.s. in water were verified by standard plate agar counts 0, 1, 2, 3 and 4 days after application
Duration:	Four days
Test conditions:	Static test. Test water was dechlorinated tap water. During test: dissolved oxygen 8.1-9.8 mg/L. Light conditions: 16 hours light, 8 hours dark. Loading: 0.38 g fish/L. Acclimation to test conditions from six days before treatments
Deviations from guideline	Not relevant
Endpoints:	Mortality, pathogenicity, behaviour
Observations:	Daily observation of the trouts

Results:

A summary of endpoints is given in the table below:

Table B.9.2.1.1.a: Toxicity, infectivity and pathogenicity of *L. muscarium* to fish

Test species	*Oncorhynchus mykiss*
Toxicity	No mortalities in the treated groups and the negative control. No clinical signs, no abnormal behaviour. The following endpoints refer to actual concentrations which were 0.95-11% of the nominal concentrations: 96-h LC_{50} (mortality) is > 97 mg a.s./L, equalling > 6.2 $\times 10^9$ CFU/L (average highest initial concentrations over the two top-dose replicates and the top-dose without fish)
Infectivity/pathogenicity	Gross observations did not show signs of infectivity or pathogenicity

Recoveries were fairly constant per treatment over the five time-points. The recoveries were the highest at the top-dose (10 - 11%). Concomitantly with the analysis of plate counts for the a.s., mesophilic bacteria were measured as well in the test water. The latter increased in time from < 10-65 CFU/L after 0 days up to 1.3×10^8 CFU/L after four days. The mesophilic bacteria were predominantly *Bacillus thuringiensis*

Comments DAR 2007:

In spite of stirring vigorously for c. five minutes, the negative control and all treatments with *L. muscarium* showed residues with granular appearance left on the bottom of the tanks. This slight dispersability probably caused the low recoveries of the a.s. after plating. The actual recoveries in the water may have been even lower than 0.95-11% as the granular residues were apparently vigorously mixed 'to break up cell aggregates' before plating the samples out. As the actual exposure was limited, the test should be considered less reliable. Fish were observed to 'swallow' and then reject the bottom residue. Also, the nature of the test substance was not clearly reported. In view of the reported content of 8.8×10^9 CFU/g, an unknown product with *L. muscarium* is indicated. The less reliable 96-h LC_{50} > 97 mg a.s./L (> 6.2×10^9 CFU/L) is used for the risk assessment.

RMS comment and conclusion RAR 2017:

The evaluation from DAR 2007 is still valid, although it is noted that the endpoint was accepted during Peer review and included without restrictions in the List of Endpoints in the EFSA conclusion (EFSA Journal 2010; 8(1):1446).

It is noted by RMS that the study duration may have been too short to determine pathogenicity/infectivity effects. According to OPPTS 885.4200 the study duration should be 30 days. In the EFSA conclusion (2010) a data gap was set for further information to adress the potential infectivity in fish.

Based on the above, the endpoint 96 h LC_{50} > 97 mg a.s./kg bw/day (corresponding with > 6.2×10^9 CFU/L; based on average highest initial concentrations over the two top-dose replicates and the top-dose without fish) can be used for risk assessment. The conclusions with regard to infectivity and pathogenicity are considered as supporting information for the risk assessment, but are less reliable due to the short study duration.

Other studies on freshwater fish toxicity, infectivity and pathogenicity (DAR 2007):

Various sources do not indicate adverse toxicological, infective and pathogenic effects on freshwater fish following exposure to *L. muscarium* (Burges, 1981; Copping, 2004; see B.9.1.3). Tests with purified toxins, enzymes

or other metabolites of *L. muscarium* are not available. Also, no tests are available with the plant protection product with *L. muscarium* under review.

There is one reported case on swimming-bladder infection of fish in a Finnish fish farm in 1986 allegedly caused by *Lecanicillium* spp. (Aho *et al.*, 1988, cited in Schuler *et al.*, 1991). The fish were Atlantic salmons (*Salmo salar*). Salmons in this case died at rate of 0.1% per fish tank. Schuler *et al.* reported that pathogenicity of fish by *Lecanicillium* spp. had not been confirmed between 1988 and 1991 following Koch's Postulate (*i.e.* to establish a causal relationship between a causative micro-organism and the pathogenicity). Schuler *et al.* therefore concluded that the occurrence of pathogenicity due to *L. muscarium* in fish is rare.

New data 2017

From open literature there is no indication that *L. muscarium* might be pathogenic on fish or any other aquatic organisms. This was confirmed by the submitted literature search (see B.9.8) where no publication was identified reporting any effects on fish.

B.9.2.3 Infectiveness to fish

No signs of infectivity of *L. muscarium* for fish under laboratory conditions after single dosages of ≤ 97 mg a.s./L (equalling ≤ 6.1×10^9 CFU/L; see Table B.9.2.1.1.a). Based on the short observation period, the study is considered as supporting information for the risk assessment.

B.9.2.4 Pathogenicity to fish

No signs of pathogenicity of *L. muscarium* for fish under laboratory conditions after single dosages of ≤ 97 mg a.s./L (equalling ≤ 6.1×10^9 CFU/L; see Table B.9.2.1.1.a). Based on the short observation period, the study is considered as supporting information for the risk assessment.

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B.9.2.5 Effects on freshwater invertebrates

B.9.2.6 Toxicity to freshwater invertebrates

Microbial pest control agent (MPCA)

KMA 8.2.2/01

Previous evaluation:	*DAR 2007*

Reference/notifier	:	Quinlan (1983)	GLP statement	:	no
Type of study	:	*Daphnia*, acute toxicity, infectivity and pathogenicity	Guideline	:	No
Year of execution	:	1983	Acceptability	:	yes, however, test substance is slightly soluble, so the actual exposure to the test substance may have been limited
Test substance	:	Mycotal, as a dry powder with *L. muscarium*, Batch No. V657			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg a.s./L] or [CFU/L]
Mycotal, see above	*Daphnia magna*	static	18-22	6.0	24	EC_{50}	> 6.0[1] > 3.8×10^8

1: based on actual concentrations. The nominal EC_{50} was > 1000 mg a.s./L.

Material and methods:

Micro-organism: *L. muscarium*

Test species: *Daphnia magna* < 24 hours

Number of test animals: Five daphnids per replicate; four replicates per treatment and a (sterile) negative control

Treatments: In total 120 glass beakers were used containing 10 mL of the a.s. in distilled water (20 for the control and 100 for the five treatments). Concentrations were 62, 125, 250, 500 and 1000 mg a.s./L (nominal), corresponding with 3.9×10^9, 7.9×10^9, 1.6×10^{10}, 3.2×10^{10} and 6.3×10^{10} CFU/L, respectively. Concentrations in water were verified by standard malt extract plate agar counts 24 hours after application. Potassium bichromate ($K_2Cr_2O_7$) was used as positive control (62-1000 mg a.s./L, nominally)

Duration: One day

Test conditions: Static test. Distilled ion free water was used. During test: dissolved oxygen 100%. Light conditions: normal daylight

Deviations from guideline	Deviations from test protocol not reported
Endpoints:	Mortality, behaviour. Infectivity was analysed by plate counting of immobilised daphnids
Observations:	Daily observation of daphnids: immobility or clinical signs of toxicity, infectivity or pathogenicity; immobilised daphnids were tested for the presence of CFUs

Results:

A summary of endpoints is given in the Table B.9.2.2.1.a. Actual concentrations of the a.s. were 0.29-0.66% of the nominal and fairly constant per treatment (actual concentrations were 0.42, 0.83, 1.5, 2.1 and 6.0 mg a.s./L at increasing nominal dosages). The immobilised daphnids at 62, 125, 250, 500 and 1000 mg a.s./L (nominal) were 0%, 10%, 5%, 5% and 5%. In the negative control 10% of the daphnids were immobilised. Immobilisation appeared to be due to daphnids trapped in the residual cereal flour used as filler. Immobilised daphnids showed no *L. muscarium* presence. Potassium bichromate showed 95-100% immobilisation

Table B.9.2.2.1.a: Toxicity, infectivity and pathogenicity of *L. muscarium* to freshwater invertebrates

Test species	*Daphnia magna*
Toxicity	The EC_{50} refers to actual concentrations, as these were much lower than the nominal, though consistent per treatment in time. The 24-h EC_{50} is > 6.0 mg a.s./L (> 3.8×10^8 CFU/L), based on actual concentrations
Infectivity/pathogenicity	Immobilised daphnids showed no occurrence of *L. muscarium*. Further gross observations did not show signs of infectivity or pathogenicity at ≤ 6.0 mg a.s./L (≤ 3.8×10^8 CFU/L)

Comments DAR 2007:

A first toxicity test with daphnids showed a negative control with 50% immobilisation within 24 hours. Therefore a new test was run which was acceptable as the negative control immobilisation percentage was then 10%. Although the statement that 'the test substance does not disperse in water' was included, there was no reference in the report to the type and duration of stirring, as would be expected for solving facilitation. The daphnids got caught 'in residual cereal flour', probably a product residual. Slight water dispersability will have caused the low recoveries of 0.29-0.66% of the nominal amounts and may even have been lower as the procedure to verify the actual concentrations in the water was not reported (whether the water samples were *e.g.* filtered and/or remixed or not — in view of the residual cereal flour). The nature of the test substance was not clearly reported. Whereas it was nominated as MYCOTAL, it also contained residual flour, which is not a constituent of the MYCOTAL under review. Taking the probably limited exposure due to the slight dispersability of the test substance into account, the less reliable 24-h EC_{50} of > 6.0 mg a.s./L (> 3.8×10^8 CFU/L), based on actual concentrations is used for the risk assessment.

RMS comment and conclusion RAR 2017:

The endpoint was included in the List of Endpoints in the EFSA conclusion (EFSA Journal 2010; 8(1):1446), with the following remark:

'Study duration considered too short to address the full risk to aquatic invertebrates, given the mode of action. Data gap for study to further address risk to aquatic invertebrates. Due to the low dispersability a chronic study to Chironomus may even be more appropriate.'

According to the OPPTS guideline 885.4240 for invertebrates, study duration should be 21 days.

Based on the above, the endpoint 24-h EC_{50} of > 6.0 mg a.s./L (> 3.8×10^8 CFU/L), based on actual concentrations can be used for risk assessment. The conclusions with regard to infectivity and pathogenicity are considered as supporting information for the risk assessment, but are less reliable due to the short study duration.

KMA 8.2.2/02

Previous evaluation:	*Article from public literature; submitted for the purpose of renewal*

Abstract:

The acute toxicity of five species of entomopathogenic fungi for *Daphnia magna* and *Eisenia foetida*, was investigated in GLP conditions designed in the eco-toxicological facility of RDIPP Bucharest. Tested fungi: *Verticillium lecanii, Metarhizium anisopliae, Beauveria brongniartii, Beauveria bassiana* and *Isaria farinosa.* The entomopathogenic fungi tested to maximum use concentrations showed no acute effects on Daphnids and earthworms. There were no recorded immobilizations and abnormal reactions and it was found that the fungus *Verticillium lecanii, Metarhizium anisopliae* and *Isaria farinosa* had a stimulating action on the *Daphnia magna* reproduction.

Reference: GRADILA, M., HERA, E., SICUIA, O., DINU, M.M., VALIMAREANU, D.G. (2013). EVALUATION OF ACUTE TOXICITY OF THE ENTOMOPATHOGENIC FUNGI ON BIOLOGICAL SYSTEMS
Romanian Journal of Plant Protection, 6, 1-4
Published: yes

Guideline: OECD 202 (Daphnia) and OECD 207 (earthworms)

GLP: no

Material and methods:

Note RMS: The Daphnia assay is further reported below, the earthworm assay is included under section B.9.4.2

Micro-organism:	***Verticillium lecanii**, (Metarhizium anisopliae, Beauveria brongniartii, Beauveria bassiana* and *Isaria farinosa* – not evaluated)
Test species:	*Daphnia magna* (age not reported)
Number of test animals:	5 per replicate
Treatments:	0 and 1.7 x 10^9 UFM/mL (stated to be the maximum use concentration) Reference substance: potassium dichromate (results were not reported)
Duration:	48 hr
Test conditions:	Daphnids were not fed during treatment phase. 2 mL test solution per daphnid.
Deviations from guideline	Not reported, could not be checked due to limited information.
Endpoint:	No effects at tested concentrations. See further comments and conclusions RMS below.
Observations:	Mortality (immobilization), abnormal reactions

Results:

No analytical measurements were reported.

Dissolved oxygen concentration at the end of test was ≥ 3 mg/L in the control and ranging from 6.0 – 6.5 mg/L in treatment vessels.

The following table with biological results is copied from the article:

Table

Daphnia immobilization (%)

Variant	Control		The species of the entomopathogenic fungi / spores concentration (UFM/ml)									
			Verticillium lecanii 1.7 X 10^9		*Metarhizium anisopliae* 7 X 10^7		*Beauveria brongniartiila* 1.9 X 10^8		*Beauveria bassiana* 3.6 X 10^7		*Isaria farinosa* 1.4 X 10^8	
Period -h/ replicative-R	24	48	24	48	24	48	24	48	24	48	24	48
R1-5 *D.magna*	0	0	0	0	0	0	0	0	0	0	0	0
R2-5 *D.magna*	0	0	0	0	0	0	0	0	0	0	0	0
R3-5 *D.magna*	0	0	0	0	0	0	0	0	0	0	0	0
R4- 5 *D.magna*	0	0	0	0	0	0	0	0	0	0	0	0
Total *D.magna* immobilized (48 h)	0		0		0		0		0		0	
Immobilization % (48 h)	0		0		0		0		0		0	
Reactions abnormal	-	-	-	-	-	-	-	-	-	-	-	-

The stimulating effects on reproduction mentioned in the abstract were not reported further in the article.

A summary of endpoints is given in the table below.

Table B.9.2.6.a: Toxicity effects / Infectivity / Pathogenicity of the MPCA to freshwater invertebrates

Test species	*Daphnia magna*
Toxicity	No adverse effects at 1.7 x 10^9 CFU/mL*
Infectivity / Pathogenicity	Not determined

*RMS contacted study author to verify that: UFM/mL = CFU/mL

Comments and conclusion RMS:

The tested strain was not reported.

Validity criteria from OECD 202 were met, however for micro-organisms testing should (also) be performed following OPPTS 885.4240 guideline, meaning a test duration of 21 days and testing at maximum hazard dose. Therefore, the test duration of this study was too short to establish possible infectivity/pathogenicity.

The maximum hazard dose according to OPPTS 885.4240 of at least 10^6 units/mL was tested nominally. However, no analytical confirmation of test concentrations was reported. Since it is an article from the public literature, it cannot be said whether analytical measurements were not performed, or simply not reported.

Based on the above the test is considered reliable with restrictions with regard to results on toxicity, due to the absence of (reporting of) analytical measurements. An 48 h EC50 of > 1.7 x 10^9 CFU/mL, or 1.7x10^6 CFU/L can be used for risk assessment as supportive information.

In the test infectivity and pathogenicity was not investigated and no endpoints for these parameters can be de-

rived for risk assessment.

Other studies on freshwater invertebrates toxicity, infectivity and pathogenicity (DAR 2007):

Various sources do not indicate adverse toxicological, infective and pathogenic effects on freshwater invertebrates following exposure to *L. muscarium* (Burges, 1981; Copping, 2004 (see B.9.1.3)).

A less well documented study report on the effects of *L. muscarium* to the yellow fever mosquito *Aedes aegyptii* was submitted (Quinlan and Chaudhry, year unknown, probably 80s). The effects of test substance 456.82 were studied under laboratory conditions by mixing the product with water at a rate of 0.25 g product/L at 18-22 ºC (air temperature). A control group with attenuated *L. muscarium* was tested as well. No toxic, infective or pathogenic effects were found, neither 5 days after application, nor 28 days after application.

RMS comment RAR 2017:

These less reliable test results are considered as supporting information for risk assessment.

New data 2017

A statement on the previously submitted study by Quinlan (1983) (KMA 8.2.2/01) was submitted by the applicant, addressing the potential effects of *L. muscarium* Ve6 on the reproduction of *Daphnia* as follows (copied from MMA-document section 8):

'Due to the information on the application details that *L. muscarium* Ve6 is only to be applied either in greenhouses or in closed tunnels, the exposure to aquatic invertebrates is very limited.

Additionally, a statement on the previously submitted study by Quinlan (1983) is submitted, addressing the potential effects of *L. muscarium* Ve6 on the reproduction of *Daphnia* (Anonymous, 2006). Here it is stated that the formulated product of *L. muscarium* Ve6 (MYCOTAL) did not cause immobilization or mortality in the studies conducted by Quinlan. This is due to the high specifity *of L. muscarium* as it is only effective to a small and very specific group of insects. Moreover, *L. muscarium* Ve6 has a very limited viability in water, which is shorter than the spray-interval of MYCOTAL. *L. muscarium* is a natural occurring soil organism, and although *L. muscarium* Ve6 is able to enter the surface water under natural conditions, not a single report in public literature is describing effects of *L. muscarium* on aquatic organisms (please refer to the recent literature search on the effects of *Lecanicillium muscarium* Ve6 on freshwater invertebrates, submitted in Point MA 8.1. One article was identified, studying effects of entomopathogenic fungi, including *Verticillium lecanii*, on *Daphnia magna* and *Eisenia foetida* (Gradila et al., 2013). It was shown that the exposition of *D. magna* and *E. foetida* to *V. lecanii* at a concentration of $1.7 \times 10_9$ UFM/mL neither affect aquatic invertebrates nor earthworms. However, identity of the *V. lecanii* strain(s) (or *L. muscarium*, respectively) used is not defined. Nevertheless, it confirms previously findings the on the nontoxicity of *L. muscarium* to aquatic invertebrates.'

B.9.2.7 Infectiveness to freshwater invertebrates

No signs of daphnid infectivity under laboratory conditions at single actual dosages of ≤ 6.0 mg a.s./L (equalling ≤ 3.8×10^8 CFU/L; see study 8.2.2/01, Table B.9.2.2.1.a). Study is considered as supporting information for the risk assessment.

B.9.2.8 Pathogenicity to freshwater invertebrates

No signs of daphnid pathogenicity under laboratory conditions at single actual dosages of ≤ 6.0 mg a.s./L (≤ 3.8×10^8 CFU/L; see study 8.2.2/01, Table B.9.2.2.1.a). Study is considered as supporting information for the risk assessment.

B.9.2.9 Effects on algae growth

Microbial pest control agent (MPCA)

DAR 2007:

Dose-effect study reports on the toxicity of *L. muscarium* for algae have not been submitted. Various sources do not indicate adverse toxic effects on algae following exposure to *L. muscarium* (Burges, 1981; Copping, 2004 (see B.9.1.3)).

A literature report on the toxicity of *L. muscarium* on algae stated that it is considered 'extremely unlikely that [*L. muscarium*] would be toxic to algae' (Verhaar, 2005). This was concluded in view of the narrow host range and the lack of persistence in aqueous environments.

Comment RMS RAR 2017:

The expert statement from Verhaar (2005), an expert from an environmental consultancy in the Netherlands, consists of 2 pages in which various literature references on general biology on *L. muscarium* are presented. The conclusion in the statement is as follows:

'Verticillium lecanii is an insect-specific pathogenic fungal species. Specificity to insects depends on the ability of *V. lecanii* spores to breach (enter and grow through) the chitinous exoskeleton of insects, whereas the basis of the highly insect-species specificity of individual *V. lecanii* strains remains unknown. Other *Verticillium* species, such as *V. dahliae*, can infect plants, usually via root, uptake or lesions. Here also, pathogenicity itself is highly species-specific. Given the fact that *V. lecanii* is not toxic or pathogenic to mammals, including humans, is not phytopathogenic, is not harmful to fish and daphnids, and has a low persistence in water, it is extremely unlikely that *V. lecanii* would be toxic or infective to algae.'

The underlying references were not further checked by RMS, since no relevant specific ecotoxicity data were included. RMS agrees that no further data is required.

New data 2017

No new data was submitted, nor identified in the literature search (B.9.8).

B.9.2.10 Effects on plants other than algae

Microbial pest control agent (MPCA)

DAR 2007:

Studies on aquatic plants on toxicity, infectivity and pathogenicity other than algae are not available.

New data 2017

No new data was submitted, nor identified in the literature search (B.9.8). Considered acceptable by RMS.

B.9.3 Effects on Bees

B.9.3.1 Toxicity to bees

Microbial pest control agent (MPCA)

KMA 8.3/01

Previous evaluation:	*DAR 2007*

Reference/notifier	:	Kling (2000)	GLP statement	:	yes
Type of study	:	honeybees (adult), acute contact toxicity, infectivity, pathogenicity	Guideline	:	EPPO No. 170 (1992)
Year of execution	:	2000	Acceptability	:	acceptable
Test substance	:	*L. muscarium*, Batch No. R 00 M 354, Mycotal technical grade, white powder, actual concentration 9.4×10^{10} CFU/g (c. 98% w/w)			

Substance	Species	Method	Duration [d]	Criterion	Value [µg a.s./bee] or [CFU/bee]
Mycotal	*Apis mellifera*	topical application	4	LD_{50}	> 100 or > 6.3×10^{6}

Material and methods:

Micro-organism	*L. muscarium*
Test species:	Adult honeybees *Apis mellifera carnica* (c. 22 - 32 days), obtained from a healthy colony of a professional beekeeper in Rheinland Pfalz, Germany
Number of test animals:	Ten honeybees per replicate (steel cages). Five replicates per treatment with test substance; five replicates for the negative control and for the positive reference
Treatments:	In total 15 steel cages as test chambers (five for the topical application, five for the negative control and five for the positive reference Perfekthion BAS 152 11I of 0.35 µg a.s./bee). Bees were anaesthetised with CO_2. Four µL of the test substance in water, presoaked for two hours, was pipetted on the ventral side of each bee thorax at a rate of 100 µg a.s./bee (6.3×10^6 CFU/bee)
Duration:	Four days
Test conditions:	Temperature during the test: 24 - 25ºC, relative humidity, 62 - 86%. Constant darkness. Honeybees were fed *ad libitum* with a 50% sucrose mixture in water
Deviations from guideline	Small deviations from the study plan (see comments)
Endpoints:	Mortality, gross pathogenicity and behaviour
Observations:	Observations were made 2, 4, 24, 48, 72 and 96 hours after application. Also, clinical signs of toxicity, pathogenicity and abnormal behaviour were recorded

Results:

A summary of endpoints is given in the table below

Table B.9.3.1.a: Contact toxicity, infectivity and pathogenicity of *L. muscarium* to adult honeybees

Test species	*Apis mellifera*
Toxicity	Mortality in the treatment group was 0, 0, 0, 0, 6 and 12%, at 2, 4, 24, 48, 72 and 96 hours after application, respectively. The concurrent control mortalities were 0, 0, 4, 6, 8 and 14%. No treatment-related mortality was indicated. No clinical signs of toxicity or abnormal behaviour were observed. The positive reference showed 100% mortality at all time-points
Infectivity/pathogenicity	Gross observations did not show signs of infectivity or pathogenicity

Comments DAR 2007:

A pipette was used instead of a micro-applicator (obstruction by small particles). Only one dosage was tested because earlier testing indicated no toxicity or infectivity at ≤ 100 µg a.s./bee. The RH was 62 - 86% instead of 75±5.0%. There was no statistical analysis. However, these aberrations from the study plan are not assumed to

have affected the test results. Under laboratory conditions, no acute toxic, behavioural or pathogenic effects were observed due to topical application of honeybees with *L. muscarium* at a rate of 100 µg a.s./bee (6.3×10^6 CFU/bee). The 96-h LC_{50} of > 100 µg a.s./bee (> 6.3×10^6 CFU/bee) is used for risk assessment. The results that no behavioural or pathogenic effects were observed due to topical application of *L. muscarium* at a rate of 100 µg a.s./bee (6.3×10^6 CFU/bee) are also used for risk assessment.

RMS comment and conclusion RAR 2017:

The study duration according to OPPTS 885.4380 should be 30 days, as in general for microbial a.s. a longer test duration is required in order to establish possible effects. This study was performed according to the chemical EPPO guideline. Therefore, with regard to infectivity and pathogenicity the studies are considered as less reliable, and supporting information only for the risk assessment.

KMA 8.3/02

Previous evaluation:	*DAR 2007*

Reference/notifier	:	Kling (2000)	GLP statement	:	yes
Type of study	:	honeybees (adult), acute oral toxicity, infectivity and pathogenicity	Guideline	:	EPPO No. 170 (1992)
Year of execution	:	2000	Acceptability	:	acceptable
Test substance	:	*L. muscarium*, Batch No. R 00 M 354, Mycotal, technical grade white powder, actual concentration 9.4×10^{10} CFU/g (c. 98% w/w)			

Substance	Species	Method	Duration [d]	Criterion	Value [µg a.s./bee] or [CFU/bee}
L. muscarium	*Apis mellifera*	oral	4	LD_{50}	> 112[1] or > 7.1×10^6

1: intake, based on the actual feed consumption (nominal application rate was 100 µg a.s./bee)

Material and methods:

Micro-organism	*L. muscarium*
Test species:	Adult honeybees *Apis mellifera carnica* (c. 22 - 32 days), obtained from a healthy colony of a professional beekeeper in Rheinland Pfalz, Germany
Number of test animals:	Ten honeybees per replicate (steel cages). Five replicates per treatment with test substance; five replicates for the negative control and for the positive reference
Treatments:	In total 40 steel cages as test chambers (30 for the oral applications, five for the negative control and five for the positive reference Perfekthion BAS 152 11I of 0.17 µg a.s./bee). Actual application rates were 1.3, 2.8, 6.0, 13, 28 and 112 µg a.s./bee. The test substance, pre-soaked for two hours, was mixed with a 50% aqueous sucrose solution at the appropriate rates
Duration:	Four days
Test conditions:	Temperature during the test: 24 - 25ºC, relative humidity, 62 - 86%. Constant darkness. Honeybees were fed *ad libitum* with a 50% sucrose mixture in water. Bees were starved for 130 minutes prior to application. After the a.s. intake, bees were fed *ad libitum* with a 50% sucrose mixture in water only
Deviations from guideline	Small deviations from the study plan (see comments)
Endpoints:	Mortality, gross pathogenicity and behaviour
Observations:	Observations were made 2, 4, 24, 48, 72 and 96 hours after application. Also, clinical signs of toxicity, pathogenicity and abnormal behaviour were recorded

Results:

A summary of endpoints is given in the table below

Table B.9.3.1.b: Oral toxicity, infectivity and pathogenicity of *L. muscarium* to adult honeybees

Test species	*Apis mellifera*
Toxicity	Mortalities after 4 days in the groups treated with the test substance were: 14, 2.0, 24, 20, 12 and 22% at actual intake rates of 1.3, 2.8, 6.0, 13, 28 and 112 µg a.s./bee. The concurrent control showed 14% mortalities after 4 days of exposure. No dose- or treatment related mortality was thus indicated. No clinical signs of toxicity or abnormal behaviour were observed. The positive reference showed a mortality of 92% after 4 days, at an actual intake rate of 0.20 µg a.s./bee
Infectivity/pathogenicity	Gross observations did not show signs of infectivity or pathogenicity

Comments DAR 2007:

The RH was 62 - 80% instead of 75±5.0%. This aberration from the study plan is not assumed to have affected the test results. Under laboratory conditions, the oral ingestion of contaminated feed showed up to 24% mortalities within 96 hours after the test initiation. As mortalities were observed at all application rates, but in particular at the higher rates, these mortalities were apparently not dose-related, whereas treatment-related mortalities could not be excluded (however, no statistical analysis). The results that a 96-h oral LD_{50} was > 112 µg a.s./bee (> 7.1×10^6 CFU/bee) is used for risk assessment. The results that no behavioural or pathogenic effects were observed due to oral application of *L. muscarium* at a rate of 112 µg a.s./bee (7.1×10^6 CFU/bee) are also used for risk assessment.

RMS comment and conclusion RAR 2017:

The study duration according to OPPTS 885.4380 should be 30 days, as in general for microbial a.s. a longer test duration is required in order to establish possible effects. This study was performed according to the chemical EPPO guideline. Therefore, with regard to infectivity and pathogenicity the studies are considered as less reliable, and supporting information only for the risk assessment.

Other studies on bee toxicity, infectivity and pathogenicity (DAR 2007):

Various sources do not indicate adverse toxicological, infective and pathogenic effects on bees following exposure to *L. muscarium* (Burges, 1981; Copping, 2004).

The impact of the product MYCOTAL with *L. muscarium* (10^{10} CFU/g) on adult bumblebees and brood was studied in a possibly less reliable study of van Doorn (1998), under laboratory conditions following (a) the dusting of three bumblebee nestboxes with 1 g MYCOTAL each, and (b) oral exposure for 18 days of the bumblebees of three nestboxes to 1 g MYCOTAL mixed with 80 g pollen, mainly to be consumed by larvae over a period estimated to be c. 18 days. Additionally, three nestboxes were used as negative control. Both the contact dosage and oral dosage were estimated to be 160 mg a.s./nestbox, so c. 8 mg a.s./bee as each nestbox/colony consisted of c. 20 workers of *Bombus terrestris*. Bumblebees were kept at 28ºC and c. 65% RH. The observation period following the dusting was not reported. The effects of oral exposure were monitored up to 18 days after initiation. No treatment-related mortalities of adults, larvae or pupae were observed following both treatments (raw data not reported). 'No obvious impact on the characteristics of colony development' was reported, although both dusting of nestboxes and the oral application with MYCOTAL 'showed some tendency for earlier production of young queens' (no statistical analysis). The biological meaning of this 'tendency' was unclear. In both the contact and oral test, no *L. muscarium* was found in the nestboxes, on brood or on adults. The contact LD_{50} was estimated to be > c. 50 mg product/bee (1 g per nestbox with c. 20 bees $\Rightarrow$ > c. 8.0 mg a.s./bee $\approx$ > c. 5.0×10^8 CFU/bee). This contact LD_{50} > c. 50 mg product/bee, equalling > c. 8.0 mg a.s./bee, is used for risk assessment. The oral dosage was estimated to be c. 50 mg product/bee, so c. 8.0 mg a.s. /bee. Therefore, the oral 18-d NOED based on the endpoints toxicity and reproduction was estimated to be $\geq$ c. 8.0 mg a.s./bee ($\geq$ c. 5.0×10^8 CFU/bee). This oral acute 18-d NOED is used for risk assessment.

RMS comment RAR 2017:

The study report of Van Doorn (1998) is a 2-page document and seems to be an internal document from the applicant company. The study is considered as supporting information.

Whereas the aforementioned studies do not indicate adverse effects to bees, other laboratory studies have indicated the susceptibility of honeybees to high amounts of *Lecanicillium* ssp. via food or via direct spraying (Schuler *et al.*, 1991). MYCOTAL had been tested 'at an application rate ten times higher than recommended', causing a significant treatment-related bee mortality of 15% for both exposure routes compared with the control. Temperature and RH were not reported. In view of the 'maximised' concentrations, and the lack of 'natural' epizootics, Schuler *et al.* concluded that *Lecanicillium* ssp. was probably not hazardous to bees at the recommended application rates.

RMS comment RAR 2017:

The reference from Schuler et al (1991) is a book. The data is considered as supporting information.

In a field test on the possible effects of MYCOTAL with *L. muscarium* on varroa mites in apiculture, no treatment-related bee mortalities were observed (Gerritsen and Cornelissen, 2006). Honeycombs of small Minibeute beeboxes in this field study were sprayed with a spore suspension of 1.5×10^6 CFU/mL. The lack of treatment-related mortalities was probably due to the lack of growth of *L. muscarium* at 35 ºC, the average temperature within beehives. Although RH values during the tests were not reported, the RH was assumed by the authors to be low, thus at least possibly partially explaining the lack of dead honeybees following the treatments. In laboratory experiments by Davidson *et al.* (2003, cited in Gerritsen and Cornelissen, 2006) *L. muscarium* did not grow under laboratory conditions at 35 ºC (0 mm/day on agar), whereas at 30 ºC the growth rate on agar was 20 mm/day.The lack of effects on bumblebees and honeybees following contact may be explained by the difficulties of conidia to pass the arthropod's hairs (pers. comm. M. Bidochka to RIVM).

RMS comment RAR 2017:

The reference from Gerritsen and Cornelissen (2006) appears to be a webpage (http://www.wur.nl/NL/nieuwsagenda/archief/nieuws/2006/Biologische_bestrijding_varroamijt_in_bijenhouderij_is_lastig.htm (in Dutch)), which could not be retrieved anymore. The data is considered as supporting information.

New data 2017

From the recent literature search, no relevant references were identified reporting on effects of *L. muscarium* on bees indicating that bees are affected by *L. muscarium* or that the fungus is known as a bee pathogen. Please refer to the literature search, section B.9.8, for detailed information on the search strategy.

The formulated product *of L. muscarium* Ve6, MYCOTAL, is used in crop systems where there is a common use of plant protection products in combination use with beneficial insects or pollinator bees and bumblebees. In a statement from the applicant (KMA 8.3/01; no further summary incuded in the RAR) it is stated that MYCO-

TAL, the formulated product of *L. muscarium* Ve6, is used more than 15 years in conjunction or simultaneously with natural pollinators (as bumble bees) and various beneficial macroorgansims such as parasitoids and predatory insects and mites, without causing any effects on the organisms.

B.9.3.2 Infectiveness to bees

No signs of infectivity to adult honeybees under laboratory conditions were observed due to topical application of 100 µg a.s./bee (nominal dosage, equalling 6.3×10^6 CFU/bee) (see Table B.9.3.1.a). Also, no signs of infectivity to adult honeybees under laboratory conditions were observed due to oral application of 112 µg a.s./bee (actual dosage, equalling 7.1×10^6 CFU/bee) (see study 8.3/01, Table B.9.3.1.b). Based on the short study duration, the study is considered as less reliable and supporting information only for risk assessment.

B.9.3.3 Pathogenicity to bees

No signs of pathogenicity to adult honeybees under laboratory conditions were observed due to topical application of 100 µg a.s./bee (nominal dosage, equalling 6.3×10^6 CFU/bee) (see Table B.9.3.1.a). Also, no signs of pathogenicity to adult honeybees under laboratory conditions were observed due to oral application of 112 µg a.s./bee (actual dosage, equalling 7.1×10^6 CFU/bee) (see study 8.3/02, Table B.9.3.1.b). Based on the short study duration, the study is considered as less reliable and supporting information only for risk assessment.

B.9.3.4 Effects on arthropods other than bees

B.9.3.5 Toxicity to arthropods other than bees

Other studies on non-target arthropods (bees excl) toxicity, infectivity and pathogenicity (DAR 2007):

Various sources do not indicate adverse toxicological, infective and pathogenic effects on non-target arthropods following exposure to *L. muscarium* (Burges, 1981;Copping, 2004; *see B.9.1.3*).

A less well documented study report on the toxic, infective and pathogenic effects of an unknown product with *L. muscarium* to three terrestrial NTAs was submitted (Quinlan and Chaudhry, year unknown, probably 80s). The effects of a *L. muscarium* strain 456.82 (no further details) to three non-target species (adult *Encarsia formosa*, adult *Phytoseiulus persimilis*, 3rd instar caterpillars *Pieris brassicae*) were studied under laboratory conditions by 'misting' at 18 - 22ºC with a suspension containing 2.5 g product/L. The effects of different RHs were investigated by treating each terrestrial species at 50-70% RH and at 90% RH. Potted dwarf bean plants infested with 2nd instar greenhouse whiteflies (*Trialeurodes vaporariorum*) were 'misted' to test *E. formosa* and *P. persimilis*, whereas butterfly larvae of *P. brassicae* were exposed on cabbage leaves with moist filter paper in Petri dishes. Control groups with attenuated *L. muscarium* were tested as well. The test results showed no toxic effects to adults of *E. formosa*, *P. persimilis* and the catterpillars of *P. brassicae* at RH of 50 - 70%, neither 5 nor 28 days after application. At the higher RH level of 90%, however, the mortalities of *E. formosa* were 12.5%

after 5 and 5% after 28 days (attenuated control no mortalities). The mortalities of *P. persimilis* at this high RH level were lower than for *E. formosa*: 6% (after 5 days), 0.5% (after 28 days) (attenuated control no mortalities). At 90% RH, no catterpillars of *P. brassicae* were killed.

In a review on the effects of microbial plant protection products on non-target arthropods (NTAs), Flexner *et al.* (1986) concluded that the direct mortality of fungi on NTAs was not 'well studied and probably underestimated'. What were the scientific developments in this research field since then, and what conclusions may be drawn from recent research with *L. muscarium* in particular? Flexner *et al.* reported that an investigation in 1982 with '*Verticillium lecanii*' showed no or slight mortality to adult parasitoid *E. formosa* at an application of 3.6×10^7 spores/mL following direct contact. As the target or host was *T. vaporariorum* the tested '*Verticillium*' was likely to be *L. muscarium*. However, further data and the temperature and RH were not reported, so the test methodology and the test results are difficult to verify.

Samson and Rombach (1985) stated that dispersal of conidia of *L. muscarium* in a greenhouse by air movement is unlikely. Dispersion via insects and mites could be more relevant, implying a possibly important role for predatory wasps and mites as used in IPM-programmes. Occasional infection of the parasitoid *E. formosa* has been reported, though the influence of such fungal infections on the parasitoid populations should be considered 'very limited' (Samson and Rombach, 1985).

As both the parasitoid *E. formosa* and the filamentous fungus *L. muscarium* may parasitise whitefly larvae as target organisms, an interaction between the parasitoid and the fungus is of interest. Under 'optimal' — dosage, temperature and RH, however, were not reported — laboratory conditions both adult *E. formosa* and whitefly larvae have been shown to be susceptible to *Lecanicillium* ssp. (Kanagaratnam *et al.* 1981, cited in Schuler *et al.*, 1991). More than 50% of the *E. formosa* parasitised whitefly larvae showed a parasitoid adult emergence when these had been sprayed with *Lecanicillium* ssp. in a relatively late development phase or when sprayed with water only (unfortunately the application rate was not reported). Only 10-20% of the parasitised whitefly larvae showed a proper adult emergence of parasitoids, when sprayed with *Lecanicillium* ssp. in an early phase. Although Pfrommer (1988, cited in Schuler *et al.*, 1991) stated that the susceptibility of *E. formosa* to *Lecanicillium* ssp. was isolate specific, the general conclusion of Schuler *et al.* was that hazards to NTAs as *E. formosa* will not be pre-dominant, but that under particular conditions hazards cannot be excluded. The infrequent occurrence of infectivity to the predatory bug *Nabis alternatus*, an aphid predator, apparently caused Canadian horticulturists to be careful in using *Lecanicillium* ssp. in combination with NTAs in the 1980s (Harper and Huang, 1986, cited in Schuler *et al.*, 1991).

No toxic, infective or pathogenic effects were monitored in laboratory tests in which 15 beneficial non-target arthropod species obtained from fields, gardens and existing cultures of Southampton University were sprayed

with two isolates of *Verticillium lecanii*[1] (Sitch and Jackson, 1997). The tested non-target arthropods were Coleoptera (the ground beetles *Agonum dorsale*, *Bembidion lampros*, *B. obtusum*, *Demetrias atricapillus*, *Harpales rufipes*, *Pterostichus cupreus*, *Trechus quadristriatus*, and the staphylinid beetle *Tachyporus hypnorum*), Collembola (*Folsomia candida*), Hymenoptera (*Lasius niger*), Diptera larvae (*Episyrphus balteatus*), Neuroptera larvae (*Chrysoperla carnea*), Dermaptera (*Forficula auricularia*), a spider *Erigone* spp. and a woodlouse *Oniscus* ssp. The NTAs were placed in groups of 10 - 20 individuals in Petri dishes on wet filter paper and sprayed with spores in sterile water. The suspension contained 1.0×10^7 spores/mL and was sprayed with a Potter Tower. Spore concentrations were adjusted when needed by counting the spore number with an improved Neubauer haemocytometer. All NTAs were kept individually after spraying in perspex containers with damp filter paper and feed supply and the incubation temperature was 25ºC with a photoperiod (16:8 hours L:D). Unfortunately, RH values were not reported. All individual NTAs were observed daily for 7 - 14 days. All the tested pest species, for the largest part aphids, were susceptible to infection of at least one isolate.

No toxic, infective or pathogenic effects to NTAs were observed after the application of the product Micro Germin Plus, containing two *Lecanicillium* isolates: 1 - 72 (aka Ve2; *L. longisporum*) and VT1 (closely resembling *L. muscarium*) (Sterk *et al.*, 1999) (confidential letter of A. Gillespie of the company Chr Hansen, Denmark[2], 2000). In these laboratory tests with *Trichogramma cacoeciae* (dry residue for adults, direct sprays of pupae), *Encarsia formosa* (dry residue for adults, direct spray of pupae on bean leaves), *Aphidius matricariae* (dry residue for adults, direct spray of pupae on paprika leaves), *Phytoseiulus persimilis* (dry residue for larvae on bean leaves; two different laboratories), *Typhlodromus pyri* (dry residue for protonymphs on glass; two different laboratories), *Chrysoperla carnea* (dry residue for larvae on glass), *Forficula auricularia* (dry residue for larvae or adults on glass) and *Semiadalia* 11-*notata* (dry residue for larvae or adults on glass) the application of the product was 'harmless' (< 30% effects). Two semi-field studies with *C. carnea* (dry residue for larvae at bean and barley) and *P. persimilis* (dry residue for adults at bean) showed Micro Germin Plus to be 'harmless' (< 25% effects, see Sterk *et al.*, 1999), whereas Micro Germin Plus was indicated to be 'slightly harmful' to *Typhlodromus pyri* following spraying in an orchard under field conditions (25 - 50% effects). The application rates were 4 kg Micro-Germin Plus/ha. In view of these test results, the IOBC/WPRS Working Group 'Pesticides and beneficial organisms' recommended that microbial insecticides as Micro Germin Plus were 'harmless or 'slightly harmful' to nearly all the beneficial arthropods tested and 'therefore recommended for use in integrated control programmes without any restriction' (Sterk *et al.*, 1999). Unfortunately, environmental test conditions as temperature and RH were not reported.

Beerling and van den Berg (2003) reported in a greenhouse study a synergistic efficacy of the use of MYCOTAL in glasshouse chrysanthemum with the adjuvans Addit in combination with the thrips predator *Amblyseus*

[1] In fact two isolates of the former V. lecanii were used: C3, isolated from the aphid Macrosiphonionella sanborni and 170.76, isolated from Cydia pomonella. Whereas in view of the target arthropod the C3 isolate probably refers to L. longisporum, the muscarium/longisporum option is more difficult to determine for the 170.66 isolate. As both isolates suppressed six hemipteran species, amongst them various aphids, both isolates were probably L. longisporum. Therefore it is difficult to extrapolate these test results to L. muscarium.

[2] 'Test with *Verticillium lecanii* on beneficial insects' (30-08-2000).

cucumeris. This study investigated the effects on western flower thrips (*Frankliniella occidentalis*). Five and nine weekly applications of MYCOTAL at a rate of 10^7 conidia/m^2 and a mixture of plant oils (Addit) caused 38% (significant) and 14% (not significant) less thrips, respectively, when compared with a water control. When the predatory mites were appended as well, the thrips reductions were 62% and 70%, respectively (no details on temperature and RH).

RMS comment RAR 2017:

The study reports/articles from the above references were checked by RMS and no further relevant information than which was summarised in the DAR (2007) could be retrieved, with the exception of Sitch and Jackson (1997), for which some additional relevant information was added. RMS tried to retrieve the content of a.s. for the product Micro Germin (reference Sterk et al., 1999), and the unknown product used in the reference from Quinlan and Chaudry, but this could not be found.

New data 2017

Several articles from the public literature were submitted for the purpose of renewal, which are summarised and evaluated below.

KMA 8.4/01

Previous evaluation:	*Article from public literature; submitted for the purpose of renewal*

Abstract:

The Entomopathogenic fungus, *Lecanicillium lecanii* (Zimm.) Zare and Games is one of the potential microbial biocontrol agents which have wide host range. The present review article contains the information related to its taxonomic position, mode of action, toxins and extracellular enzymes produced by the fungus. The various approaches for mass multiplication, carriers for development of commercial formulations and shelf life of formulated products of the fungus are reviewed and discussed. The factors like genetic variability, tritropic interaction, temperature, humidity, formulation base, inoculum level, isolation host, stage of insect host, compatibility with chemical pesticides which affect the performance of the fungus are presented and discussed. Response of *L. lecanii* to different insect pests, under protected conditions and filed conditions, and its effect on non targeted organisms are reviewed and discussed.

Reference: SHINDE, S.V., PATEL, K.G., PUROHIT, M.S., PANDYA, J.R., SABALPARA, A.N.(2010). LECANICILLIUM LECANII (ZIMM.) ZARE AND GAMES AN IMPORTANT BIOCONTRAL AGENT FOR THE MANAGEMENT OF INSECT PESTS - A REVIEW

Agricultural Review, 31, 235-252

Published: yes

Guideline: n.a.

GLP: no

Comments and conclusion RMS:

The article contained several references to articles in which effects of *V. lecanii* on non-target arthropods were investigated. The underlying articles were not submitted, therefore no evaluation of these results could be performed by RMS. The applicant with regard to this article only states that: 'The species *L. muscarium* is known to parasitize various hosts. However, mainly are sucking insects and pests are described to be parasitized by *L. lecanii'* The article is considered as supporting information.

KMA 8.4/02

Previous evaluation:	*Article from public literature; submitted for the purpose of renewal*

Abstract:
The study was set up to determine side effects of *Lecanillium muscarium* strain V24 on the predatory mite *Phytoseiulus persimilis*. Strain V24 is reported by the study authors to have a high efficacy against sucking insects like whitefly, aphids and thrips (based on several references from the public literature). In two standardised biotests in petri dish and on plants *(P. vulgaris)* individual mites were dipped in suspension or set down on leafs that were sprayed with *L. muscarium* at different spore density. Results indicate infectivity and pathogenicity for the predatory mite in principle, but the dimension of infection risk decreased when conditions approached agricultural practice. The study authors concluded that under practical conditions on plants and under practical relevant concentrations of 10^6 and 10^7 spores/ml a low risk is to be expected.

Reference: DONKA, A., SERMANN, H., BÜTTNER, C. (2008). EFFECT OF THE ENTOMOPATHOGENIC FUNGUS LECANICILLIUM MUSCARIUM ON THE PREDATORY MITE PHYTOSEIULUS PERSIMILIS AS A NON-TARGET ORGANISM
-, not applicable
Comm. Appl. Biol. Sci. Ghent University, 73, 395-403
Published: yes

Guideline: -

GLP: no

Material and methods:

Micro-organism *Lecanicillium muscarium* strain V24

Test species: *Phytoseiulus persimilis* (predatory mite)

Adhesion test

Number of test animals: 20 (dipping) and 3 adults per leaf (indirect contact)

Treatments: Test solutions with $2x10^5$, $2x10^6$ and $2x10^7$ fluorescence marked conidia/mL. Two treatments: dipping of mites in 5 mL test solution, and placing of mites on dried leaves of sprayed bean plants.

Duration: 24 hrs

Test conditions: Laboratory; 20ºC, 95% RH..

Deviations from guideline Not applicable

Endpoint: Number of spores adhering to the mites

Observations: Number of spores adhering to the mites, germination of spores

Results:

The dipping treatment showed a peak number of spores after 3 hrs. After 24 hrs, no difference existed between contact via dipping or indirect contact via oversprayed leaves. The number of spores adhering to the mites after 24 hrs was low and many individuals had not any spores. The figure below is copied from the study report:

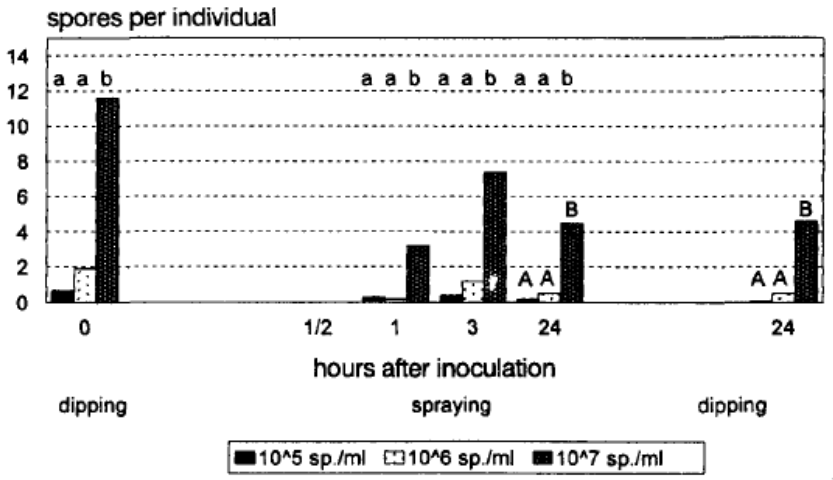

Figure 2. Adhesion of spores of *L. muscarium* on the body of *P. persimilis* after dipping adults in suspension or spraying the leafs at different spore density within 24 hours (20°C, 95% RH)

In their conclusion the study authors report that spore loss on *P. persimilis* (60-80%) is higher than spore loss on target organisms as thrips (30-50%) (Meyer, 2006) and on aphids *{M. persicae* 55%) (Alavo *et at.,* 2001). According to the study authors the results on spore loss could indicate some resistance of P. *persimilis* against L. *muscarium* infection.

Biotest in petri dish

Number of test animals:	2 'dipped' adults per unsprayed bean leaf and 3 'undipped' adults per sprayed bean leaf. 30 food mites per bean leave (*T. urticae*)
Treatments:	Test solutions with 0, $2x10^5$, $2x10^6$, $2x10^7$ and $2x10^8$ conidia/mL. Dipping of mites in all test solution concentrations. Spraying of bean plant leaves with food mites (*T. urticae*) with 1 ml of all test concentrations except $2x10^5$ conidia/mL.
Duration:	7 days
Test conditions:	Laboratory; 20ºC, 95% RH..
Deviations from guideline	Not applicable
Endpoint:	Mortality, infectivity
Observations:	Number of alive, dead and mouldy dead individuals of *P. persimilis*.

Results:

In the dipping treatment the LD50 value was reported to be determined at $2.1x10^7$ spores/mL. (No further results were shown).

Mortality after indirect contact via sprayed leaves and *T. urticae* was lower than in the dipping treatment, but mortality increased after 5 days. Some dead predatory mites in the $2x10^7$ and $2x10^8$ spores/mL treatment were mouldy, indicating infectivity of the fungus.

The figure below is copied from the study report:

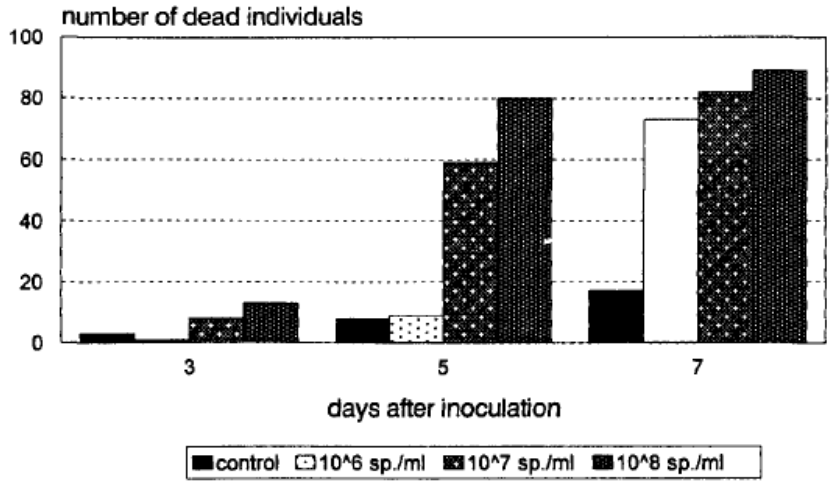

Figure 3. Number of dead individuals of *P. persimilis* after spraying detached leafs of *P. vulgaris* with suspension of *L. muscarium* at different spore density per ml and incubation at 20°C and 95% RH

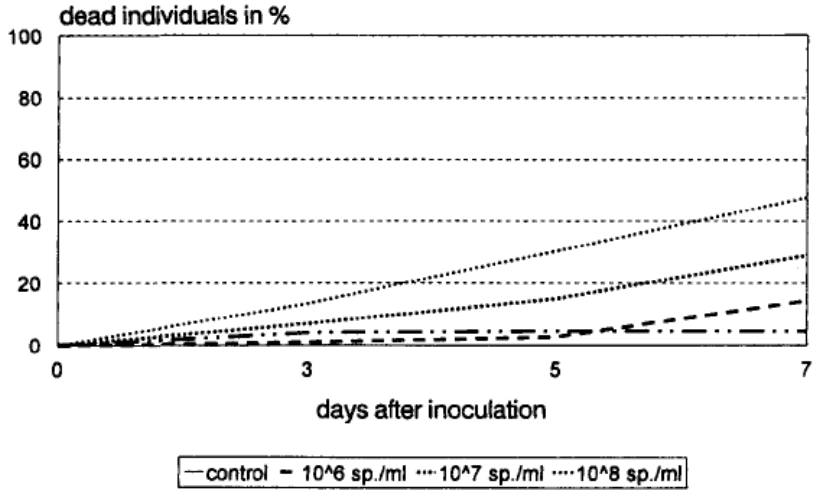

Figure 5. Mortality of *P. persimilis* after spraying detached leafs of *P. vulgaris* with suspension of *L. muscarium* at different spore density per ml and incubation at 20°C and 95% RH

Population growth in the petri dishes was high, due to favaourable climatic conditions and was adversely affected after 5 days in the highest test concentrations (figure copied from study report):

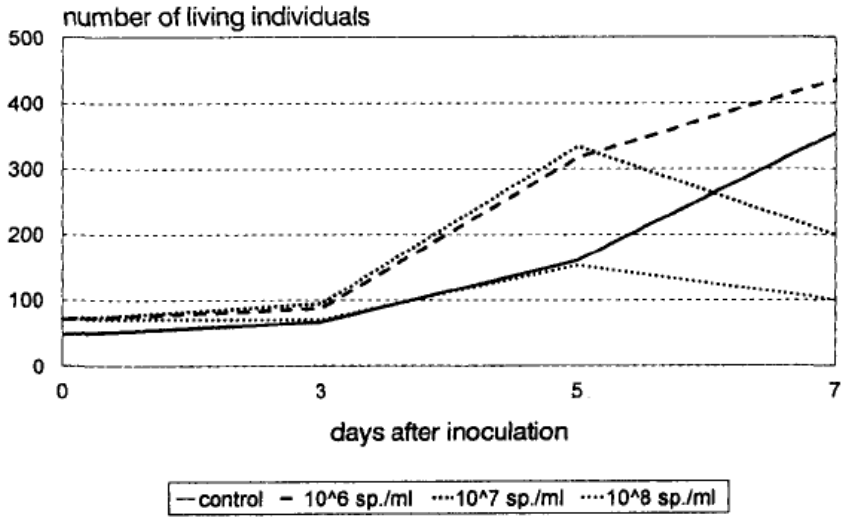

Figure 4. Number of living *P. persimilis* after spraying detached leafs of *P. vulgaris* with suspension of *L. muscarium* at different spore density per ml and incubation at 20°C and 95% RH

Biotest on plants

Number of test animals:	2 'dipped' adults per leaf of unsprayed bean plants and 2 'undipped' adults per leaf of sprayed bean plants. 30 food mites per bean leave (*T. urticae*)
Treatments:	Test solutions with 0, $2x10^6$ and $2x10^7$ conidia/mL. Dipping of mites with all test solution concentrations. Spraying of seedling bean plants with food mites (*T. urticae*) with 3 mL test solution per plant, 1 hr drying before introduction of *P. persimilis*.
Duration:	11 days
Test conditions:	Laboratory; 15 ºC, 65% RH at night, 35 ºC, 35-45% RH at daytime.
Deviations from guideline	Not applicable
Endpoint:	Mortality, infectivity
Observations:	Number of alive, dead and mouldy dead individuals of *P. persimilis* was recorded on

6 leaves

Results:

Mortality was low, both after dipping and after exposure to sprayed leaves and mites. In the $2x10^6$ spores/mL treatment mortality did not differ from the control at any time. In the $2x10^7$ spores/mL treatment, mortality was 75% at the end of the test. The larger part of dead predatory mites in the $2x10^7$ treatment were mouldy, indicating infectivity of the fungus.

The figure below is copied from the study report:

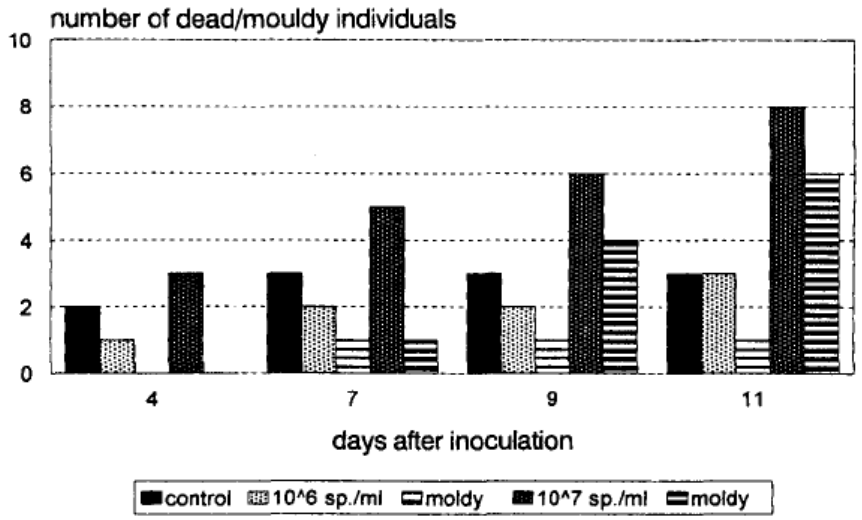

Figure 6. Number of dead and dead mouldy individuals of *P. persimilis* after spraying plants of *P. vulgaris* with suspension of *L. muscarium* at different spore density per ml and incubation at 23°C and x 65% RH

Note RMS: No figure with mortality percentage was given for this assay.

Population growth on the plants was lower than in the petri dishes, which was attributed by the study authors to the lower humidity and changing temperatures.In both treatments there was no indication of an adverse effect on population growth.

The figure below is copied from the study report:

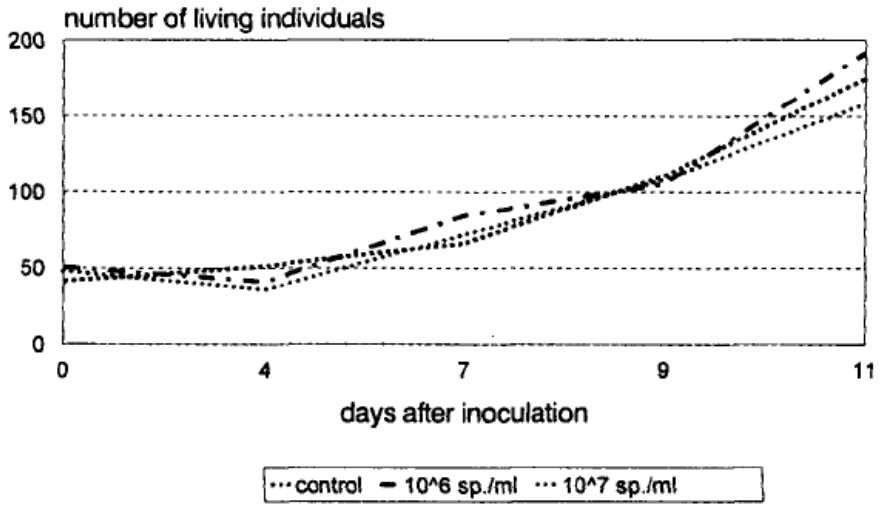

Figure 7. Number of living individuals of *P. persimilis* after spraying plants of *P. vulgaris* with suspension of *L. muscarium* at different spore density per ml and incubation at 23°C and x 65% RH

Summary of mortality results from both biotests:

The results showed a significant higher mortality with higher spore density in the test solution. The high humidity in the petri dishes seemed to stimulate the infection process and led to higher mortality than on the plants. The lower humidity and fluctuating temperatures and the indirect contact decreased the infection success of the fungus.

The table below is copied from the study report:

Table 2. Corrected Mortality of *P. persimilis* afte application of *L. muscarium* V 24 in different spore density and application method

Application method	Biotest	Spore density (sp./ml)	corrected Mortality
dipping	Petri dish	2x10*6	12.2
		2x10*7	67.1
	plant	2x10*6	***8.9***
		2x10*7	***29.8***
spraying	Petri dish	2x10*6	9.8
		2x10*7	38.1
	plant	2x10*6	***4.2***
		2x10*7	***12.7***

The study authors state that the mortality in this study was lower than shown with the same strain V24 for target organisms like white fly (93%), Californian flower thrips (90%), green peach aphid (80%) (Meyer, 2006; Dimitrov, 2005; Alavo *et al.,* 2001, Sermann, Buchner, 1998). (references not available with RMS).

A summary of endpoints is given in the table below.

Table B.9.3.5.a: Toxicity effects/infectivity/pathogenicity of the MPCA to arthropods

Test species	*P. persimilis*
Toxicity	4.4 and 12.7% mortality after exposure to dried spray residues on bean leaves using test solution of resp. $2x10^6$ and $2x10^7$ spores/mL. See also Table 2 in the above.
Infectivity / Pathogenicity	Infectivity shown with test solutions of $2x10^7$ and $2x10^8$ spores/mL

Comments and conclusion RMS:

The spore concentration per cm^2 leaf area was not given and could not be calculated by RMS from the information in the study report. No statistical analysis was reported in the article. The study was not GLP and not performed according to a guideline. Nevertheless, the study seemed well performed and gives relevant information. The study is considered reliable for use in risk assessment.

Based on the study it can be concluded that *L. muscarium* strain V24 – for which the target organisms appear to be comparable with the strain Ve6 in this study – is able to infect and kill the predatory mite *P. persimilis*. However, the mortality decreases with lower spore density and circumstances more resembling agricultural practice. A low risk is indicated for predatory mites exposed to spray residues on leaves using spraying solutions with $2x10^6$ and $2x10^7$ spores/mL

KMA 8.4/03

Previous evaluation:	*Article from public literature; submitted for the purpose of renewal*

Abstract:

The effect of the combined use *of Encarsia formosa or Macrolophus caliginosus* and one of three marketed mycoinsecticides, Mycotal* (*Lecanicillium muscarium*-based), Naturalis-L™ (*Beauveria bassiana*-based) and PreFeRal* (*Isaria fumosorosea*-based), on the control of the whitefly (*Trialeurodes vaporariorum*), was studied under laboratory and greenhouse conditions. The results of both types of tests, the bioassays and the greenhouse trials, for all combinations of E *Formosa* with each of the three mycoinsecticides showed that the total mortality of larval populations of *T. vaporariorum* was not affected. The mortality of *T vaporariorum* larvae treated in the

second instar revealed the capacity for both B. *bassiana*- and *L. muscarium*-based formulations and *E. formosa* to kill the host either separately or in association. Because of its higher pathogenic activity (under these test conditions), *L muscarium* provoked a large proportion of mycoses in larvae exposed to parasitization. In contrast, the efficacy of parasitization was higher in larvae treated with *B. bassiana* and exposed to *E. formosa because* of a lower pathogenic activity of the fungus. Bioassays carried out with third-instar larvae of *T.* vaporariorum showed a low susceptibility to both tested fungi. Consequently, mortalities recorded in larvae subjected to the combined treatments by consecutive exposures or at 2-4 days post-parasitization were mainly caused by the development of the parasitoid. Greenhouse trials showed that fungus-induced mortality of *T. vaporariorum* in plants treated with *L. muscarium, Isaria fumosorosea* and *B. Bassiana* was significant compared to control. *L muscarium, B. bassiana* and /. *fumosorosea* killed young whitefly larvae and limited parasitization to 10% or less. Second-instar larvae of *M. caliginosus* were not susceptible to L *muscarium* and *B. bassiana* formulations with any mode
of contamination: direct spraying of larvae, spraying on the foliar substrate or by contaminated *T. vaporariorum* prey. In greenhouse trials, M. *caliginosus* populations treated with fungi were not significantly affected compared to controls.

Reference:	HAMDI, F., FARGUES, J., RIDRAY, G., JEANNEQUIN, B., BONATO, O. (2011). COMPATIBILITY AMONG ENTOMOPATHOGENIC HYPHOCREALES AND TWO BENEFICIAL INSECTS USED TO CONTROL TRIALEURODES VAPORARIORUM (HEMIPTERA: ALEURODIDAE) IN MEDITERRANEAN GREENHOUSES Journal of invertebrate Pathology, 108, 1-8 Published: yes
Guideline:	n.a.
GLP:	no

Material and methods:

Micro-organism	L. muscarium (product Mycotal)
Test species:	*Macroluphus caliginosus* and *Encarsia formosa*
Number of test animals:	Not reported
Treatments:	Various assays. $3.9x10^4$ CFU/cm^2 sprayed on: In the laboratory:

	- potted whole green bean plants (contaminated with whitefy), or - batches of 20 anaesthetized *M. caliginosus* larvae, or - potted whole tobacco plants In plastic field tunnel 'greenhouse' (located in S-FR): -whole tomato plants, with artificially enhanced whitefly infestation
Duration:	Various bioassays with various duration, ranging from 1 to 19 days
Test conditions:	Laboratory: 20 C and 16:8 hr light period, 80-90 % RH Plastic field tunnels: not reported
Deviations from guideline	n.a.
Endpoint:	Compatibility of *L. muscarium* with two natural enemies used in IPM (*Macroluphus caliginosus* and *Encarsia formosa)*
Observations:	Fungus induced mortality, parasitisation, parasitoid emergence

Results:

In tests with *E. fomosa* in combination with *L. muscarium* it was shown, that parasitation was reduced when *L. muscarium* was applied. However, direct effect of *L. muscarium* on *E. formosa* mortality or reproduction was not tested. Since significantly less whitefly adults emerged when *E. formosa* was used in combination with *L. muscarium*, decrease of parasitization may due to reduction of hosts by *L. muscarium.* Besides this, conducted tests showed that second-instar larvae of *M. caliginosus* were not susceptible to *L. muscarium* Ve6 with any mode of contamination (direct spraying of *M. caliginosus* larvae, spraying of the leave surface or consumptions of infected *T. vaporariorum*). In a greenhouse test it was also shown that *M. caliginosus* populations were not affected by the application of MYCOTAL.

The following tables were copied from the study article, summarizing the results from the various bioassays:

Table 1
Effect of parasitization by *Encarsia formosa* of second-instar *Trialeurodes vaporariorum* in the first 2–4 days after contamination with entomopathogenic fungal inocula: number of whitefly larvae per sampled leaf, emergence rate and mortality due to parasitization, to fungus infection or to other causes.

	No. Whiteflies/sampled leaf live & dead[a]	Emerged Whitefly adults[b]	Whitefly larval mortality[c]			Emerged parasitoids[b]
			Natural mortality	Fungus-induced Mortality	Parasitization (black pupae)	
Control	193.8 ± 20.6[a]	71.8 ± 3.0[a] (90.2%)	18.2 ± 3.0[a] (9.8%)	(0%)	(0%)	(0%)
E. formosa	143.3 ± 2.3[b]	16.4 ± 1.9[c] (8.0%)	8.2 ± 3.5[b] (2.0%)	(0%)	70.7 ± 2.2[a] (89.0%)	61.5 ± 2.6[a] (77.2%)
L. muscarium	161.0 ± 7.8[b]	26.9 ± 6.9[c] (20.5%)	2.7 ± 1.6[b] (0.2%)	62.6 ± 6.7[a] (78.8%)	(0%)	(0%)
E. formosa + *L. muscarium*	194.3 ± 17.1[a]	5.8 ± 3.6 d (1.0%)	1.5 ± 1.5[b] (0.1%)	63.9 ± 3.1[a] (80.7%)	24.4 ± 2.3[c] (17.1%)	21.0 ± 1.7[c] (12.9%)
B. bassiana	239.8 ± 25.7[a]	54.8 ± 3.6[b] (66.8%)	8.4 ± 1.7[b] (2.1%)	33.8 ± 3.1[b] (30.9%)	(0%)	(0%)
E. formosa + *B. bassiana*	213.0 ± 22.5[a]	19.1 ± 3.3[c] (10.7%)	1.6 ± 1.6[b] (0.1%)	39.1 ± 2.3[b] (39.7%)	44.1 ± 2.6[b] (48.4%)	41.4 ± 3.3[b] (43.7%)

[a,b,c] Means within a column followed by the same letter are not significantly different (ANOVA procedure; $\alpha = 0.05$; SNK test).
[a] Mean number of whitefly larvae recorded on each leaf (x ± sem).
[b] Mean survival (x ± SEM), expressed as angular value [arcsin √ (number of emerged adults/initial number of whiteflies)]. Emergence rates (%) in brackets.
[c] Mean larval mortality (x ± SEM), expressed as angular value [arcsin √ (number of dead larvae/initial number of whiteflies)]. Mortality rates (%) in brackets.

Table 2
Effect of parasitization by *Encarsia formosa* of third-instar *Trialeurodes vaporariorum* just after contamination with entomopathogenic fungal inocula: number of whitefly larvae per sampled leaf, emergence rate and mortality due to parasitization, to fungus infection or to other causes.

	No. Whiteflies/sampled leaf live & dead[a]	Emerged Whitefly adults[b]	Whitefly larval mortality[c]			Emerged parasitoids[b]
			Natural mortality	Fungus-induced Mortality	Parasitization (black pupae)	
Control	202.5 ± 36.3[a]	65.6 ± 7.6[a] (83.0%)	24.4 ± 7.6[a] (17.0%)	(0%)	(0%)	(0%)
E. formosa	134.5 ± 20.6[a]	15.5 ± 2.4[b] (7.1%)	14.4 ± 5.3[a] (6.2%)	(0%)	66.6 ± 1.8[a] (84.2%)	56.3 ± 2.2[a] (69.2%)
L. muscarium	203.3 ± 11.2[a]	56.9 ± 6.2[a] (70.2%)	5.3 ± 2.0[c] (0.9%)	32.3 ± 6.2[a] (28.6%)	(0%)	(0%)
E. formosa + *L. muscarium*	168.3 ± 13.1[a]	9.2 ± 9.2[b] (2.5%)	14.1 ± 3.6[a] (6.0%)	30.9 ± 8.0[a] (26.4%)	47.9 ± 4.1[b] (55.0%)	42.5 ± 3.0[b] (45.7%)
B. bassiana	164.0 ± 29.0[a]	59.3 ± 7.8[a] (73.7%)	14.1 ± 8.0[a] (6.0%)	24.3 ± 2.9[a] (17.0%)	(0%)	(0%)
E. formosa + *B. bassiana*	191.5 ± 12.8[a]	12.6 ± 2.0[b] (4.8%)	5.4 ± 5.4[a] (0.9%)	27.8 ± 2.0[a] (21.8%)	56.7 ± 2.8[b] (69.9%)	47.6 ± 1.9[b] (54.6%)

[a,b,c] Means within a column followed by the same letter are not significantly different (ANOVA procedure; $\alpha = 0.05$; SNK test).
[a] Mean number of whitefly larvae recorded on each leaf ($x \pm$ sem).
[b] Mean survival ($x \pm$ SEM), expressed as angular value [arcsin $\sqrt{}$ (number of emerged adults/initial number of whiteflies)]. Emergence rates (%) in brackets.
[c] Mean larval mortality ($x \pm$ SEM), expressed as angular value [arcsin $\sqrt{}$ (number of dead larvae/initial number of whiteflies)]. Mortality rates (%) in brackets.

Table 3
Effect of entomopathogenic fungal contamination of third-instar *Trialeurodes vaporariorum* in the first 2–4 days following parasitization by *Encarsia formosa*: number of whitefly larvae per sampled leaf, emergence rate and mortality due to parasitization, to fungus infection or to other causes.

	No. Whiteflies/sampled leaf live & dead[a]	Emerged White fly adults[b]	Whitefly larval mortality[c]			Emerged parasitoids[b]
			Natural mortality	Fungus-induced Mortality	Parasitization (black pupae)	
Control	168. 0 ± 2.7[b]	78.3 ± 0.5[a] (95.9%)	11.7 ± 0.5[b] (4.1%)	(0%)	(0%)	(0%)
E. formosa	157.3 ± 12.2[b]	14.0 ± 2.4[b] (5.8%)	31.4 ± 3.0[a] (27.1%)	(0%)	54.6 ± 2.9[a] (66.5%)	54.6 ± 2.9[a] (66.5%)
L. muscarium	161.8 ± 20.8[b]	61.5 ± 13.7[a] (77.2%)	(0%)	28.50 ± 13.66[a] (22.8%)	(0%)	(0%)
E. formosa + *L. muscarium*	130.8 ± 2.5[b]	12.3 ± 1.4[b] (4.6%)	14.7 ± 4.0[b] (6.4%)	23.20 ± 10.74[a] (15.5%)	54.3 ± 5.7[a] (66.0%)	53.5 ± 6.2[a] (64.5%)
B. bassiana	228.5 ± 11.2[a]	73.3 ± 2.6[a] (91.8%)	6.7 ± 0.8 (1.4%)	15.10 ± 2.57[a] (6.8%)	(0%)	(0%)
E. formosa + *B. bassiana*	166.3 ± 11.2[b]	16.0 ± 4.2[b] (7.7%)	11.6 ± 0.8[b] (4.0%)	26.62 ± 9.81[a] (20.1%)	53.0 ± 8.8[a] (63.8%)	50.8 ± 7.4[a] (60.1%)

[a,b,c] Means within a column followed by the same letter are not significantly different (ANOVA procedure; $\alpha = 0.05$; SNK test).
[a] Mean number of whitefly larvae recorded on each leaf ($x \pm$ sem).
[b] Mean survival ($x \pm$ SEM), expressed as angular value [arcsin $\sqrt{}$ (number of emerged adults/initial number of whiteflies)]. Emergence rates (%) in brackets.
[c] Mean larval mortality ($x \pm$ SEM), expressed as angular value [arcsin $\sqrt{}$ (number of dead larvae/initial number of whiteflies)]. Mortality rates (%) in brackets.

Table 4
Interaction between entomopathogenic fungi, *Lecanicillium muscarium*, *Beauveria bassiana*, *Isaria fumosorosea*, and natural enemies of *Trialeurodes vaporariorum* in greenhouse tomato crop. Data recorded at day 19: number of whitefly larvae per sampled leaf, emergence rate and mortality due to predation, parasitization, and to fungus infection or to other causes.

	No. whiteflies/sampled leaf live & dead[a]	Emerged Whitefly adults[b]	Whitefly larval mortality[c]		
			Parasitization (black pupae)	Predation	Natural and fungus-induced mortality
Control	1.57 ± 0.13[a] (57.4)	45.10 ± 3.35[a] (51.5%)	33.80 ± 3.88[a] (33.7%)	(0%)	20.97 ± 2.80[c] (14.7%)
L. muscarium as Mycotal	1.53 ± 0.10[a] (45.3)	46.97 ± 4.21[a] (53.8%)	14.69 ± 3.43[b] (9.5%)	(0%)	36.14 ± 3.62[b] (36.6%)
B. bassiana as Naturalis	1.51 ± 0.14[a] (53.6)	28.21 ± 4.43[b] (25.6%)	11.76 ± 3.84[b] (7.9%)	(0%)	54.49 ± 6.20[a] (66.1%)
I. fumosorosea as PreFeRal	1.49 ± 0.10[a] (40.5)	45.33 ± 3.53[a] (50.1%)	15.12 ± 3.32[b] (10.0%)	(0%)	37.54 ± 3.44[b] (39.0%)

[a,b,c] Means within a column followed by the same letter are not significantly different (ANOVA procedure; $\alpha = 0.05$; SNK test).
[a] Mean number of whitefly larvae recorded on each leaf ($x \pm$ sem) expressed as logarithmic value [log ($x + 1$)]. Corresponding number in brackets.
[b] Mean survival ($x \pm$ sem) expressed as angular value (arcsin $\sqrt{}$ [(number of alive larvae +3/8)/total number of whiteflies +3/4)]. Emergence rates (%) in brackets.
[c] Mean larval mortality ($x \pm$ sem) expressed as angular value (arcsin $\sqrt{}$ [(number of parasitized larvae +3/8)/total number of whiteflies +3/4)]. Mortality rates (%) in brackets.

Table 5
Susceptibility of second-instar *Macrolophus caliginosus* to *Lecanicillium muscarium* and *Beauveria bassiana* formulations applied by spraying directly on larvae or by contamination of the leaves as a substratum for larvae.

	Survival of *M. caliginosus* larvae contaminated directly[a]	Survival of *M. caliginosus* larvae exposed to contaminated foliage[a]
Control	68.2 ± 8.6 (86.2%)	70.1 ± 7.2 (88.4%)
L. muscarium	64.8 ± 11.3 (81.9%)	66.3 ± 14.4 (83.8%)
B. bassiana	67.2 ± 1.0 (85.0%)	80.1 ± 12.7 (97.1%)

[a] Means of data (x ± sem), expressed as angular value [arcsin √ (number of emerged adults/initial number of *M. caliginosus* larvae)]. Survival rates (%) in brackets.

Table 6
Susceptibility of second-instar *Macrolophus caliginosus* exposed for 72 h to *Trialeurodes vaporariorum* prey fungus-inoculated 24 h, 72 h, and 5 days before.

	Survival of *M. caliginosus* larvae[a]		
	A: Prey contaminated 24 h prior predation	B: Prey contaminated 72 h prior predation	C: Prey contaminated 5 d prior predation
Control	72.0 ± 12.2 (90.4%)	68.9 ± 6.7 (87.0%)	69.8 ± 5.9 (88.1%)
L. muscarium	73.7 ± 2.8 (93.9%)	66.4 ± 3.9 (84.0%)	72.0 ± 12.2 (90.4%)
B. bassiana	73.2 ± 4.8 (91.7%)	67.2 ± 8.9 (85.0%)	69.8 ± 5.9 (88.1%)

[a] Means of data (x ± SEM) expressed as angular value [arcsin √ (number of emerged adults/initial number of *M. caliginosus* larvae)]. Survival rates (%) in brackets.

Table 7
Effect of two successive applications of *Lecanicillium muscarium*-based products (on April 18 and 26, just after sampling) on *Macrolophus caliginosus* populations to control *Trialeurodes vaporariorum* larvae in tomato crop in climatic regulated greenhouses. Counts of bugs per sampled tomato plant.

No. *M.caliginosus* per tomato plant per sampling date[a]						
	Treatments	11/04[b]	18/04[c]	26/04[d]	02/05[e]	09/05[f]
Greenhouse 1	Control	4.3 ± 0.5	6.3 ± 1.2	5.6 ± 0.7	4.3 ± 0.4	4.0 ± 0.7
	L. muscarium as Mycotal powder	4.4 ± 0.9	7.1 ± 0.8	6.7 ± 0.9	4.0 ± 0.7	4.0 ± 0.9
	L. muscarium as Mycotal oil formulation	4.3 ± 0.7	6.3 ± 1.0	5.8 ± 0.9	4.7 ± 0.7	4.6 ± 0.4
Greenhouse 2	Control	5.3 ± 0.5	7.6 ± 0.7	6.3 ± 0.8	5.8 ± 1.0	7.8 ± 1.2
	L. muscarium as Mycotal powder	5.3 ± 0.8	8.0 ± 1.0	7.3 ± 0.9	5.8 ± 0.8	6.7 ± 1.8
	L. muscarium as Mycotal oil formulation	4.7 ± 0.8	9.0 ± 0.9	7.8 ± 1.1	4.0 ± 0.5	5.8 ± 1.4

[a] Regular distribution of insects (sampling unit: tomato plant).
[b,c,d,e,f] One way ANOVA of counts for each sampling ($Fb = 0.398$; $df = 5.66$; $P = 0.848$; $Fc = 1.185$; $df = 5.66$; $P = 0.326$; $Fd = 0.873$; $df = 5.66$; $P = 0.504$; $Fe = 1.271$; $df = 5.66$; $P = 0.287$; $Ff = 1.701$; $df = 5.66$; $P = 0.147$).

Comments and conclusion RMS:

The study is considered reliable and relevant for risk assessment. Although there may be a slight reduction in parasitisation efficacy by *E. formosa*, the study indicates that *L. muscarium* (product Mycotal) poses a low risk for natural enemies (bugs (*M. caliginosus*) and parasitic wasps (*E. formosa*)) at the recommended dose rate (0.1%, corr. to $3.9x10^4$ CFU/cm^2, corr. to $3.9x10^{12}$ CFU/ha).

KMA 8.4/04

Previous evaluation:	*Internal report from Koppert B.V. ; submitted for the purpose of renewal*

Abstract: Since the introduction of Addit as a spray adjuvant, the efficacy of MYCOTAL against white flies, thrips and also spider mites was largely improved. To see if there also is a negative effect on beneficials, this combination was tested on *Encarsia formosa, Macrolophus caliginosus* and *Phytoseiulus persimilis* on plants in cages under semi-field conditions in a greenhouse.

Reference: Anonymous. (2006c), Side effect of MYCOTAL & Addit on *Encarsia Formosa, Macrolophus caliginosus* and *Phytoseiulus persimilis* unpublished report, Koppert BV (31 January 2006)

Guideline: n.a.

GLP: no

Material and methods:

Micro-organism: L. muscarium (product Mycotal and adjuvant Addit (emulsifiable vegetable oil)

Test species: *Encarsia formosa, Macrolophus caliginosus* and *Phytoseiulus persimilis*

Number of test animals: Not reported

Treatments: Spider mites and white flies were introduced on the plants (resp. 7 and 18 days) before the beneficials were put in to build up a good population.
MYCOTAL & Addit was applied at the recommended dosages: 1 g/L and 0.25%, respectively three times with an interval of a week.

Duration: Total mortality was assessed before every application and one week after the last

	application.
Test conditions:	Conditions in the greenhouse were kept constantly at an average of 75% RH and 21°C.
Deviations from guideline	n.a.
Endpoint:	Side effects on *Encarsia formosa, Macrolophus caliginosus* and *Phytoseiulus persimilis:* mortality, parasitisation
Observations:	Fungus induced mortality, parasitisation, parasitoid emergence

Results:

Tables are copied from study report.

Side effects on Encarsia formosa:

Application of MYCOTAL & Addit resulted in less larvae parasitized by *E. formosa*, but total whitefly control was higher after application of MYCOTAL & Addit than when untreated. Direct spray with a handheld pressurised sprayer on EN-STRIP cards (containing pupae with *E. formosa*) on 5/3 and 20/3 gave a decline in fly emerge from 90 to 50% compared to unsprayed cards. A control treatment in which cards were sprayed with water only was not included.

Table 1: Effect of treatment with MYCOTAL & ADDIT on whitefly parasitation by *Encarsia Formosa*.

	Untreated	MYCOTAL & ADDIT
Parasitized pupae (%)	80.6	30.9
Emerged parasitized pupae (%)	94.2	89.9
Living whitefly pupae (%)	19.4	0
Dead whitefly pupae with external fungal growth (%)	0	67.3
Dead parasitized pupae with external fungal growth (%)	0	1.8

Side effects on *Phytoseiulus persimilis*:

Table 2: Effect of treatment with MYCOTAL & ADDIT on *Phytoseiulus persimilis*.

	Untreated	MYCOTAL & ADDIT
Mortality *P. persimilis* 21/03/2000 (%)	1.0	7.5
Mortality *P. persimilis* 27/03/2000 (%)	3.0	9.8
Mortality *P. persimilis* 06/04/2000 (%)	3.1	14.3

Side effects on *Macrolophus caliginosus*:

After treatment with MYCOTAL & Addit almost no living white fly were observed anymore due to *M. caliginosus* but also due to the application of MYCOTAL & Addit. The corrected mortality of *M. caliginosus* was 13.3% (of which 61.3% were starved adults and 38.6% nymphs killed by MYCOTAL & Addit).

Table 3: Effect of treatment with MYCOTAL & ADDIT on *M.caliginosus*.

	Untreated	MYCOTAL & ADDIT
Mortality *M.caliginosus* 03/05/2000 (%)	3.3	11.2
Mortality *M.caliginosus* 10/05/2000 (%)	5.8	19.6
Mortality *M.caliginosus* 17/05/2000 (%)	9.2	21.3

Comments and conclusion RMS:

According to the applicant/Koppert, the combined use of MYCOTAL & Addit can be classified as harmless (<25% adverse effect) for the tested organisms, and can be integrated without any problems in the biological control of white fly and spider mite.

RMS notes that some adverse side effects do occur on the tested beneficial insects, with the most pronounced effect on parasitisation by *E. formosa*. The reduction in parasitisation by *E. formosa* seems to be >50%. However, the report from Koppert is very concise and not according to guideline or GLP. Therefore, no firm conclusions can be drawn from this data, although it is clear that the company would not benefit if their product would adversely affect beneficials whilst stating that this is not the case to the users. The report is considered as supporting information.

KMA 8.4/05

Previous evaluation:	*Statement; submitted for the purpose of renewal*

Report KMA 8.4/05 – Kamilova, F. (2016), Declaration MYCOTAL
Not published

Abstract: Declaration that MYCOTAL is used more than 15 years in conjunction or simultaneously with natural pollinators (as bumble bees) and various beneficial macroorgansims such as parasitoids and predatory insects and mites. No complains on side effects on MYCOTAL were received to the company. Also submitted under KMA 8.3/01.

KMA 8.4/06

Previous evaluation:	*Article from public literature; submitted for the purpose of renewal*

Abstract: Entomopathogenic fungi such as *Verticillium lecanii* (Z.) (MYCOTAL®) are used for pest control as an alternative to chemical control. In this study, the effect of *V. lecanii* on cereal aphids is assessed. In addition, an investigation is carried out to determine whether the use of *V. lecanii* affects the performance of two natural enemies of aphids, the predator *Harmonia axyridis* (P.) and the parasitoid *Aphidius colemani* (V.), in no-choice experiments under laboratory conditions.**Results:** The number of *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.) killed was increased by increasing the concentration of *V. lecanii*. The timing of application of fungus to aphids affected the efficacy of other biocontrol agents, a parasitoid and a predator. Parasitation by *A. colemani* (V.) in both cereal aphids (*S. avenae* and *R. padi*) was not affected by *V. lecanii* when aphids were first treated with *V. lecanii* and then exposed to *A. colemani*. The emergence of adults from parasitised mummies was, however, lower in infected aphids than in uninfected aphids when the aphids were first exposed to the parasi-

toids and then treated with fungus. The female sex ratio in the emerging adults was lower in *V. lecanii*-treated aphids in both species. When aphids were first treated with *V. lecanii*, 72 h before predation, fewer aphids of both species were consumed by *H. axyridis* (P.).**Conclusion:** Use of entomopathogenic fungus as a biological control agent could be a complementary strategy in an integrated pest management program against cereal aphids, but it can reduce the efficiency of other biocontrol agents (parasitoids and predators) when applied simultaneously.

Reference: Aqueel, M.A., Leather, S.R. (2013). Virulence of *Verticillium lecanii* (Z.) against cereal aphids; does timing of infection affect the performance of parasitoids and predators

Pest Management Science, 69: 493-498

Published: yes

Guideline: n.a.

GLP: no

Material and methods:

Micro-organism: L. muscarium (product Mycotal)

Test species: Beneficials: the predator *Harmonia axyridis* (P.) and the parasitoid *Aphidius colemani*. Aphids; *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.)

Number of test animals: Not reported

Treatments: The active substance *L. muscarium* Ve6 was gained from the formulation MYCOTAL and prepared as a stock solution by mixing 10 g WP in 50 mL deionised water. *L. muscarium* was than applied at a dose rate of 0.0, 2.5×10^{10}, 5×10^{10} and 10×10^{10} conidia/L. The experiment was conducted on plants sown individually in pots in walk-in growth chambers. Plants were sprayed once.

To measure the aphid LC50, one adult aphid was placed on one plant and was allowed to reproduce overnight. Before the application (two weeks after reproduction), the number of mixed –instar larvae was counted (31.1 ± 1.51 for S. avenae and 39.7 ± 1.92 for *R. padi*). New produced nymphs were removed daily. Seven days after application, the number of dead aphids, showing symptoms of fungal attack, were considered to be killed by *L. muscarium.*

The effect of *L. muscarium* Ve6 treatment on parasitism, emergens and sex ratio of parasitioids was tested with female parasitoids on treated (72 h before contact) aphids. Parasitoids were removed the following day and the newborn nymphs were

removed daily. To measure successful parasitism, the numbers of mummies formed were counted and the percentage parasitism was calculated for *L. muscarium*-infected and uninfected aphids. In the second part of this experiment, parasitioids were released first onto the aphids and then the aphids were treated 72 h after parasitoid contact to determine the percentage emergence and female sex ratio of parasitoids. Aphids were checked daily until mummy formation. Sec ratio of parasitoids was determined under binocular and was expressed in the proportion of females and compared to uninfected aphids.

To study the relationship between the ladybird *Harmonia axyridis* and *L. muscarium*, Aphids were treated with *L. muscarium* Ve6 and were fed to 10 third-instar larvae of *H. axyridis* when aphids began to show symptoms of fungus infection (pale green and swollen). 10 additional *H. axyridis* larvae were fed to uninfected aphids. The number of aphids consumed by each larva within a 24 period was recorded. Forth-instar larvae and adults were provided with 90 aphids, and the same procedure was repeated to compare the predation rates.

Duration:	Various bioassays with various duration, 1-7 d; see above.
Test conditions:	Walk in growth chambers: 20-22 C and 16:8 hr light period, 60-65 % RH
Deviations from guideline	n.a.
Endpoint:	Compatibility of *L. muscarium* with two natural enemies used in IPM (*Aphidius colemani* and *Harmonia axyridis)*
Observations:	Fungus induced mortality, parasitisation, predation

Results:

It was shown, that *L. muscarium* was effective against the pests *R. padi* and *S. avenae*. The mortality was increased with the population density of *L. muscarium*. The LC_{50} of *L. muscarium* was determined as 4.8×10^{10} CFU/L ($4.38\text{-}5.14 \times 10^{10}$ CFU/L) and 5.3×10^{10} CFU/L ($4.82\text{-}5.81 \times 10^{10}$ CFU/L) for *R. padi* and *S. avenae*, respectively.

When aphids were first treated with *L. muscarium* and then exposed to *A. colemani*, the number of parasitized aphids was not significantly affected by *L. muscarium*.

When aphids were first exposed to the parasitoids and than treated with *L. muscarium*, the number of parasitized mummies was lower in infected aphids than in uninfected aphids (see figure below).

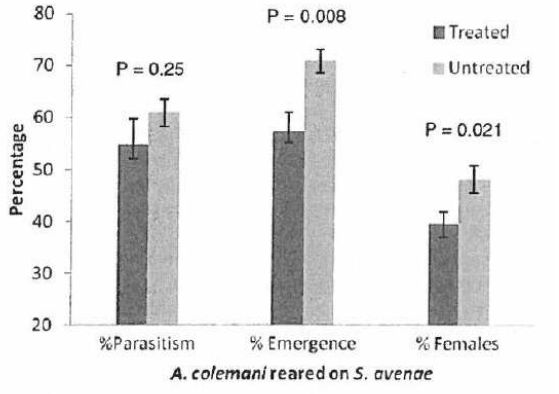

Figure 1. Percentage parasitism of *A. colemani* on *V. lecanii*-treated (72 h before parasitism) and untreated *S. avenae*. Percentage emergence and female sex ratio of *A. colemani* when reared on *V. lecanii*-treated (72 h after parasitism) versus untreated *S. avenae* (mean ± SEM, $n = 10$).

Although fewer aphids were consumed by *H. axyridis* when treated with *L. muscarium* Ve6, no significant differences were observed on any stage of *H. axyridis* exept for *H. axyridis* adults feeding on *S. avenae*.

Comments and conclusion RMS:

The authors conclude that, based on the reduced reproductive succes of *A. colemani*, the use of entomopathogenic fungus as a biological control agent could be a complementary strategy in an integrated pest management program against cereal aphids, but it can reduce the efficiency of other biocontrol agents (parasitoids and predators) when applied simultaneously.

It is noted by RMS that it was unclear from the presented results in the article, including Fig 1 above, for which treatment rates the results were presented (test dose rates were 0.0, 2.5×10^{10}, 5×10^{10} and 10×10^{10} conidia/L). The study is considered reliable and relevant for risk assessment, however no firm conclusion can be drawn in relation to the tested rates.

B.9.3.6 Infectiveness to arthropods other than bees

See B.9.3.5, in which also results on infectivity and pathogenicity are reported.

B.9.3.7 Pathogenicity to arthropods other than bees

See B.9.3.5, in which also results on infectivity and pathogenicity are reported.

B.9.4 Effects on earthworms

B.9.4.1 Toxicity to earthworms

KMA 8.5/01

Previous evaluation:	*DAR 2007*

Reference/notifier	:	Wachter (2000)	GLP statement	:	yes
Type of study	:	earthworm, acute toxicity	Guideline	:	OECD 207
Year of execution	:	2000	Acceptability	:	acceptable
Test substance	:	Mycotal Technical grade *L. muscarium*, Batch No. R 00 M 354, white powder, actual concentration 9.5 ×10^{10} CFU/g (c. 98% w/w)			

Substance	Species	Soil type	OM [%]	pH	T [°C]	Duration [d]	Criterion	Value [mg a.s./kg soil (dwt)] or [CFU/kg soil dwt]
L. muscarium	*Eisenia fetida*	OECD artificial	10	6.0±0.5	20±2.0	14	LC_{50}	1000 or 6.3×10^{10}

Material and methods:

Micro-organism *L. muscarium*

Test species: *Eisenia fetida* with clitellum

Number of test animals: Forty earthworms per treatment with test substance: four replicates (test containers) per treatment, each replicate with ten earthworms; four replicates for the negative control. For the toxic reference: twenty earthworms per treatment, each treatment

	two replicates with ten earthworms per replicate
Treatments:	In total 24 test containers for the treatments with the test substance and 10 test containers for the toxic reference 2-chloroacetamide. Each test container with 500 gram (dwt) of OECD artificial soil supplemented with 150 g deionised water. Treatments consisted of artificial soil homogeneously mixed with the test substance. The nominal concentrations were 100, 178, 316, 562 and 1000 mg a.s./kg soil (dwt). These corresponded with 6.3×10^9, 1.1×10^{10}, 2.0×10^{10}, 3.5×10^{10}, 6.3×10^{10} CFU/kg soil (dwt), respectively. The toxic reference concentrations were 10, 18, 32, 56 and 100 mg/kg soil (dwt). Concentrations in the soil were not verified
Duration:	Fourteen days
Test conditions:	During test: continuous lightness, 400 - 800 lux; moisture content soil, 35%. Worms were not fed during the experiment
Deviations from guideline	Study performed according to study plan
Endpoints:	Mortality, body weight, behaviour
Observations:	Mortalities and behaviour were assessed 7 and 14 days after application. Body weights were assessed immediately before and 14 days after application.

Results:

A summary of endpoints is given in the table below.

Table B.9.5.1.a: Effects of *L. muscarium* on earthworms (study report)

Test species	*Eisenia fetida*
Toxicity	Mortalities after 14 days were 0, 0, 2.5%, 2.5%, 0% and 2.5% at 0, 100, 178, 316, 562 and 1000 mg a.s./kg soil (dwt), respectively. No dosis-effect relation was indicated and the mortalities were not significantly different from the controls either. No signs of clinical toxicity or abnormal behaviour.
Infectivity/pathogenicity	Gross observations did not show signs of infectivity or pathogenicity
Body weight	Average body weight as % of initial weight in the treatment groups ranged from 82.6% (178 mg/kg) to 86.5% (562 mg/kg); in the control group this was 86.2%

Comments DAR 2007:

The result that no adverse toxic effects were monitored at ≤ 1000 mg a.s./kg soil (dwt) (equalling ≤ 6.3×10^{10} CFU/kg soil dwt) is used for risk assessment.

RMS comment and conclusion RAR 2017:

No additional comments.

Other studies on earthworms toxicity, infectivity and pathogenicity (DAR 2007):

Various sources do not indicate adverse toxicological, infective and pathogenic effects on earthworms following exposure to *L. muscarium* (Burges, 1981; Copping, 2004; *see B.9.1.3*).

New data 2017

In the literature search on the effects of *Lecanicillium muscarium* Ve6 (see B.9.8), no additional references were identified, reporting on effects of the genus *Lecanicillium* on earthworms. One article was identified (Gradila et al., 2013), studying effects of entomopathogenic fungi, including *Verticillium lecanii*, on *Daphnia magna* and *Eisenia foetida*. This study is summarised and evaluated below.

KMA 8.5/02 (submitted under KMA 8.2.2/02)

Previous evaluation:	*Article from public literature; submitted for the purpose of renewal*

Abstract: For the selection of bacterial and fungal strains of biotechnological interest on biological compatibility criteria and for inclusion in integrated control schemes of diseases and pests frequently in vegetable crops protected, the acute toxicity of five species of entomopathogenic fungi at *Daphnia magna* and *Eisenia foetida*, was tested in GLP conditions designed in the eco-toxicological facility of RDIPP Bucharest. Tested species: *Verticillium lecanii,Metarhizium anisopliae,Beauveria brongniartii, Beauveria bassiana* and *Isaria farinosa*. The entomopathogenic fungi tested to maximum use concentrations showed no acute effects on Daphnids and earthworms. There were no recorded immobilizations and abnormal reactions and it was found that the fungus *Verticillium lecanii, Metarhizium anisopliae* and *Isaria farinosa* had a stimulating action on the *Daphnia magna* reproduction.

Reference: GRADILA, M., HERA, E., SICUIA, O., DINU, M.M., VALIMAREANU, D.G. (2013). EVALUATION OF ACUTE TOXICITY OF THE ENTOMOPATHOGENIC FUNGI ON BIOLOGICAL SYSTEMS
Romanian Journal of Plant Protection, 6, 1-4
Published: yes

Guideline: OECD 202 (Daphnia) and OECD 207 (earthworms)

GLP:	no
Material and methods:	The earthworm assay is further reported below, the daphnia assay is included under section B.9.2.6
Daphnia assay:	
Micro-organism:	***Verticillium lecanii****, (Metarhizium anisopliae, Beauveria brongniartii, Beauveria bassiana* and *Isaria farinosa* – not evaluated)
Test species:	*Eisenia foetida* (aged at least 2 months)
Number of test animals:	10 per replicate
Treatments:	Stated to be the maximum use concentration, therefore RMS assumed they are the same concentrations as in the Daphnia assay (see B.9.2.6), i.e. 0 and 1.7 x 10^9 UFM/mL (UFM = CFU). This concentration was mixed through 2500 g artificial soil, however the volume of test solution was not reported. Therefore the concentration in the test soil cannot be determined. Reference substance: chloroacetamide (results were not reported)
Duration:	15 d
Test conditions:	Testing in glass vessels of 1 L, temeperature of 200 ºC, but probably a typo (should be 20 ºC), continuous light of 400-800 lux.
Deviations from guideline	Not reported, could not be checked due to limited information.
Endpoint:	No effects at tested concentrations. See further comments and conclusions RMS below.
Observations:	Mortality

Results:

The following table with biological results is copied from the article:

Table 2

Earthworms mortality after 15 days

The fungus tested	No. of earthworms	Lot 1	Lot 2	Lot 3	Lot 4	Total
		Mortality (no. of earthworms)				
C1 *B. bassiana*	10	0	0	0	0	0
C2 -*B.brongniartii*	10	0	0	0	0	0
C3 –*V. lecanii*	10	0	0	0	0	0
C4 – *I. farinosa*	10	0	0	0	0	0
C5 –*M. anisopliae*	10	0	0	0	0	0
Control	10	0	0	0	0	0

A summary of endpoints is given in the table below.

Table B.9.4.1.a: Toxicity effects / Infectivity / Pathogenicity of the MPCA to earthworms

Test species	*Eisenia foetida*
Toxicity	No adverse effects at 1.7 x 10^9 UFM/mL dispersed in artificial soil
Infectivity / Pathogenicity	Not determined

Comments and conclusion RMS:

The tested strain was not reported.

Validity criterium from OECD 207 was met (< 10% control mortality). No OPPTS guideline is available for earthworms.

The test concentration in soil could not be derived from the article, since the volume of test solution dispersed in the test soil was not reported.

Based on the above the test is considered only useful as indicative information in a weight of evidence approach with regard to results on toxicity, due to the unknown test concentration in the soil.

The test is considered not useful with regard to infectivity and pathogenicity, since it was not investigated.

B.9.4.2 Infectiveness to earthworms

No signs of infectivity to earthworms under laboratory conditions were observed following an acute exposure of 14 days of ≤ 1000 mg a.s./kg soil dwt (equals ≤ 6.3×10^{10} CFU/kg soil dwt) (see study 8.5/01, Table 9.5.1.a).

B.9.4.3 Pathogenicity to earthworms

No signs of pathogenicity to earthworms under laboratory conditions were observed following an acute exposure of 14 days of ≤ 1000 mg a.s./kg soil dwt (equals ≤ 6.3×10^{10} CFU/kg soil dwt) (see study 8.5/01, Table 9.5.1.a).

B.9.5 Effects on non-target soil micro-organisms

DAR 2007:

No data available.

New data 2017

From the literature search not any reference was identified, reporting on effects of *Lecanicillium* sp. on soil microorganisms. Please refer to the literature search, section B.9.8, for detailed information on the search strategy.

B.9.6 Effects on terrestrial plants

No information required as not a data requirement for micro-organisms.

B.9.7 Additional studies

Not available.

B.9.8 References relied on

Literature search

A literature search on the effects of *L. muscarium* Ve6 on non-target organisms was conducted (Scholze, 2016). The review was made in order to identify scientific peer-reviewed open literature on the active substance *Lecanicillium muscarium* Ve6, which may affect the assessment on non-target organisms. The literature research was conducted on the DIMDI database provided by the German Institute of Medical Documentation and comprised searches in MEDLINE; BIOSIS, CAB Abstracts and SCISEARCH databases. Search strategy aimed to find all recent (from 2006 onwards) references that are of ecotoxicological relevance, regarding possible effects on non-target organisms. Obtained references were first subjected to a *rapid assessment* based on title and the abstract. Summary records that appeared to be relevant passed to a second step in which a detailed assessment of full text documents was conducted. The reliability assessment for relevant studies was done according to the recommendations of the EFSA (2011)[3]. In total 76 references were evaluated on their relevance basing on title and abstracts. Of those, 4 references were assessed in detail by full text evaluation, resulting in three relevant articles for Section 8.

Below the relevance and reliability criteria that were used by the applicant are reported (copied by RMS from Literature review report (Scholze, 2016)):

[3] Guidance of EFSA: Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092

Table 3.2-1 Criteria for relevance and reliability used in the review

Relevance criteria
• Property investigated was relevant for data requirements of Regulation (EC) No 1107/2009? • Subject relevant for ecotoxicological considerations? • Test species/system relevant to the ecotoxicological assessment? • Non-target organism not known as beneficial organism or described as pest? • Non-target organism relevant in the geographical location of intended use? • Route of administration / exposure relevant for assessment? • Endpoint relevant for the assessment? • Is the test substance relevant for the assessment? • Is the effect relevant from the species and up to the population level? • In the case of reports on known *Lecanicillium muscarium* pathogens in a certain non-target organism, is there any relevance for *Lecanicillium muscarium* Ve6?
Reliability criteria
• Minimum information reported e.g.: • Test item or related compound • Test species relevant • Clear and comprehensive description of material and methods, incl. duration, replicates, test conditions • Definition of endpoints • Presentation of result • Guideline compliance

The following search terms were used (copied by RMS from table 4-1 from Literature review report (Scholze, 2016)):

Search strategy	Search term 1:	((Lecanicillium OR Verticillium lecanii OR Mycotal) AND (bird? OR Aves))
	Search term 2:	((Lecanicillium OR Verticillium lecanii OR Mycotal) AND (fish? OR daphnid? OR daphnia OR (aquatic invertebrate?) OR alga? OR (aquatic plant?)))
	Search term 3:	((Lecanicillium muscarium OR Verticillium lecanii OR Mycotal) AND

		(phytotox? OR phytopathogen?) NOT (efficacy OR bioefficacy OR strateg? OR management))
	Search term 4:	((Lecanicillium OR Verticillium lecanii OR Mycotal) AND (bee OR bees OR honeybee OR honeybees OR bumblebee OR bumblebees))
	Search term 5:	((Lecanicillium muscarium OR Mycotal) AND (arthropod? OR insect?) AND (tox? OR pathogen?) NOT (bee OR bees OR whitefly OR whiteflies OR thrips))
	Search term 6:	((Lecanicillium OR Verticillium lecanii OR Mycotal) AND earthworm?)
	Search term 7:	((Lecanicillium OR Verticillium lecanii OR Mycotal) AND soil microorganism?)

* Use of „?“ at the end of keyword will lead to an expansion of the search criteria at DIMDI database

The overall results of the literature research are presented in the following table (copied by RMS from table 5-1 from Literature review report (Scholze, 2016)):

Data requirement capture in the search:	**n**
Total number of summary records retrieved after all searches of peer-reviewed literature	76
Number of summary records excluded from the search after rapid assessment for relevance	72
Total number of full-text documents assessed in detail	4
Number of studies excluded from further consideration after detailed assessment of relevance	1
Number of studies not excluded for relevance after detailed assessment	3

According to RMS the literature search and selection was performed in an acceptable manner, but notes that metabolites were not included in the search terms. Therefore, in line with the question posed in MA Vol. 3, section B.6, a comprehensive search of the published literature will be requested with the aim to find all references regarding the production of toxins or metabolites of ecotoxicological concern.

The 3 relevant articles are included under the relevant sections in this Volume 3 B.9.

Reference lists

New data submitted for the purpose of renewal:

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMA 8.1/01	Scholze, I.	2016	LITERATURE REVIEW ON LECANICILLIUM MUSCARIUM VE6 (19-79): EFFECTS ON NON-TARGET ORGANISMS Koppert, 2191392-MA-08-01 GAB Consulting GmbH, Heidelberg, Germany GLP/GEP: no Published: no	no	yes	New data for active ingredient, not previously submitted nor evaluated	KBS
KMA 8.1/02	Anonymous	2006a	EFFECTS OF VERTICILLIUM LECANII STRAIN VE6 ON REPRODUCTION OF BIRDS. Koppert, not stated Koppert Biological Systems GLP/GEP: no Published: no	no	yes	New data for existing formulation, not previously submitted nor evaluated	KBS
KMA 8.2.2/01	Anonymous	2006 b	EFFECTS OF VERTICILLIUM LECANII STRAIN VE6 ON REPRODUCTION OF DAPHNIA. Koppert, not stated Koppert Biological Systems GLP/GEP: no Published: no	no	yes	New data for existing formulation, not previously submitted nor evaluated	KBS
KMA 8.2.2/02	Gradila, M., Hera, E., Sicuia, O., Dinu, M.M., Valimareanu, D.G.	2013	EVALUATION OF ACUTE TOXICITY OF THE ENTOMOPATHOGENIC FUNGI ON BIOLOGICAL SYSTEMS , not applicable Romanian Journal of Plant Protection, 6, 1-4 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.3/01	Kamilova, F.	2016	DECLARATION MYCOTAL Koppert, not stated Koppert B.V., Berkel en Rodenrijs, NL GLP/GEP: no Published: no	no	yes	New data for active ingredient, not previously submitted nor evaluated	KBS

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMA 8.4/01	Shinde, S.V., Patel, K.G., Purohit, M.S., Pandya, J.R., Sabalpara, A.N.	2010	LECANICILLIUM LECANII (ZIMM.) ZARE AND GAMES AN IMPORTANT BIOCONTRAL AGENT FOR THE MANAGEMENT OF INSECT PESTS - A REVIEW , not applicable Agri. Review, 31, 235-252 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/02	Donka, A., Sermann, H., Büttner, C.	2008	EFFECT OF THE ENTOMOPATHOGENIC FUNGUS LECANICILLIUM MUSCARIUM ON THE PREDATORY MITE PHYTOSEIULUS PERSIMILIS AS A NON-TARGET ORGANISM , not applicable Comm. Appl. Biol. Sci. Ghent University, 73, 395-403 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/03	Hamdi, F., Fargues, J., Ridray, G., Jeannequin, B., Bonato, O.	2011	COMPATIBILITY AMONG ENTOMOPATHOGENIC HYPHOCREALES AND TWO BENEFICIAL INSECTS USED TO CONTROL TRIALEURODES VAPORARIORUM (HEMIPTERA: ALEURODIDAE) IN MEDITERRANEAN GREENHOUSES , not applicable J Invertebr Pathol, 108, 1-8 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/04	Anonymous	2006c	SIDE EFFECT OF MYCOTAL & ADDIT ON ENCARSIA FORMOSA, MACROLOPHUS CALIGINOSUS AND PHYTOSEIULUS PERSIMILIS Koppert, not stated Koppert Biological Systems GLP/GEP: no Published: no	no	yes	New data for active ingredient, not previously submitted nor evaluated	KBS

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMA 8.4/05	Kamilova, F.	2016	DECLARATION MYCOTAL Koppert, not stated Koppert B.V., Berkel en Rodenrijs, NL GLP/GEP: no Published: no **Submitted in: KMA 8.3/01**	no	yes	New data for active ingredient, not previously submitted nor evaluated	KBS
KMA 8.4/06	Aqueel, M.A., Leather, S.R.	2013	VIRULENCE OF VERTICILLIUM LECANII (Z.) AGAINST CEREAL APHIDS; DOES TIMING OF INFECTION AFFECT THE PERFORMANCE OF PARASITOIDS AND PREDATORS? , not applicable Pest Management Science, 69, 493-498 GLP/GEP: no Published: yes	no	no	not protected	-

Data from previous evaluation (DAR 2007):

Annex IIM Data and Information

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIM 8.1	Benkerroum S Tantaoui-Elaraki A	2001	Study of toxigenic moulds and mycotoxins in poultry feeds. Revue Méd. Vét. **152** (4): 335-342	N	-
IIM 8.1	[redacted]	1998	An acute toxicity study of Mycotal TGAI administered orally to Japanese quails. [redacted] Report No.7L785	Y	KBS
IIM 8.1-8.8; IIM 8.9.1	Burges HD (ed)	1981	Microbial control of pests and plant diseases, 1970-1980. Academic Press, London, New York, Toronto	N	-

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIM 8.1-8.8; IIM 8.9.1	Copping LG (ed)	2004	The Manual of Biocontrol Agents. A world compendium. rev. 3rd edition. The British Crop Protection Council. Surrey, UK	N	-
IIM 8.1-8.8; IIM 8.9.1	Hokkanen HMT Hajek AE (eds)	2003	Environmental impacts of microbial insecticides. Needs and methods for risk assessment. Kluwer Academic Publishers, Dordrecht/Boston/London	N	-
IIM 8.1-8.8; IIM 8.9.1	OECD	2005	Directory of microbial pesticides for agricultural crops in OECD countries Revised version, Kabaluk T. and Gazdik K. (eds) Agriculture and Agri-Food Canada	N	-
IIM 8.2	[redacted]	1983	The acute toxicity of Ve6-58 SSP to rainbow trout (*Salmo gairdneri*); [redacted] Report No. TTL 3/83415	Y	KBS
IIM 8.2-8.6	Skrobek A Butt T	2005	Toxicity testing of destruxins and crude extracts from the insect-pathogenic fungus *Metarhizium anisopliae*. FEMS Microbiology Letters **251**: 23–28	N	-
IIM 8.3	Quinlan RJ	1983	*Verticillium lecanii* acute immobilisation of *Daphnia* test. University of Reading, England. Date: 02-03-1983	Y	KBS
IIM 8.4	Verhaar HJM	2005	*Verticillium lecanii*. Toxicity to algae. ENVIRON Netherlands B.V. Doc No 77KO-MYCST-20050211	Y	KBS
IIM 8.7	Gerritsen L Cornelissen B	2006	Biologische bestrijding van varroa met behulp van schimmels. PPO Bijen http://www.wur.nl/NL/nieuwsagenda/archief/nieuws/2006/Biologische_bestrijding_varroamijt_in_bijenhouderij_is_lastig.htm (in Dutch)	N	-
IIM 8.7	Kling A	2000	Assessment of side-effects of *Verticillium lecanii* to the honeybee, *Apis mellifera* L. in the laboratory. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. Report No. 20001292/01-BLUE	Y	KBS
IIM 8.7	van Doorn A	1998	Impact of the fungi *Verticillium* and *Trichoderma* on adult bumblebees and bumblebee brood. Research Report R&D Natupol. Koppert Biological Systems. Report No. Pr980701	Y	KBS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIM 8.7; IIM 8.8	Schuler T Hommes M Plate HP Zimmermann G	1991	*Verticillium lecanii* (Zimmermann) Viégas (Hyphomycetales: Moniliaceae): Geschichte, systematik, Verbreitung, Biologie und Anwendung im Pflanzenschutz. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Heft 269. Berlin, Dahlem.	N	-
IIM 8.8	Beerling EAM van den Berg D	2003	36th Annual Meeting Program and Abstracts Society for Invertebrate Pathology 26-30 July 2003 Burlington, Vermont, US	N	-
IIM 8.8	Flexner JL Lighthart B Croft BA	1986	The effects of microbial pesticides on non-target beneficial arthropods. Agric Ecosystems Environ **16**: 203-254	N	-
IIM 8.8	Quinlan RJ Chaudhry MA	unknown	Unpublished study report: non-target insect tests for toxicity/pathogenicity. University of Reading, UK	Y	KBS
IIM 8.8	Samson RA Rombach MC	1985	Biology of the fungi *Verticillium* and *Aschersonia.* In: Biological control. The glasshouse experience. Hussey NW and Scopes N (eds). Blenford press, Dorset, UK , p. 34-42	N	-
IIM 8.8	Sitch JC Jackson CW	1997	Pre-penetration effects affecting host specificity of *Verticillium lecanii.* Mycol Res **101** (5): 535-541	N	-
IIM 8.8	St Leger RJ Joshi L Roberts DW	1997	Adaptation of proteases and carbohydrases of saprophytic, phytopathogenic and entomopathogenic fungi to the requirements of their ecological niches. Microbiology **143**: 1983-1992	N	-
IIM 8.8	Sterk, G. et al.	1999	Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS working group "Pesticides and Beneficial Organisms". BioControl (formerly Entomophaga) **44**: 99-117	N	-
IIM 8.9.1	Wachter S	2000	Acute toxicity of *Verticillium lecanii* on earthworms, *Eisenia foetida* using an artificial soil test. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. Report No. 20001292/01-NLEf	Y	-

* Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted in any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used in any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization, of a plant protection product before the date of submission of the dossier to Rapporteur Member State.

** Owners' code identifications and names (Code identification: KBS Name: Koppert Beheer Systems)

Annex IIIM Data and Information

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIIM 10.1	Benkerroum S Tantaoui-Elaraki A	2001	Study of toxigenic moulds and mycotoxins in poultry feeds. Revue Méd. Vét. **152** (4): 335-342	N	-
IIIM 10.1	[redacted]	1998	An acute toxicity study of Mycotal TGAI administered orally to Japanese quails. [redacted] Report No.7L785	Y	KBS
IIIM 10.1; IIIM 10.2; IIIM 10.4; IIIM 10.5; IIIM 10.7	Burges HD (ed)	1981	Microbial control of pests and plant diseases, 1970-1980. Academic Press, London, New York, Toronto	N	-
IIIM 10.1; IIIM 10.2; ;IIIM 10.3; IIIM 10.4; IIIM 10.5; IIIM 10.7	Hokkanen HMT Hajek AE (eds)	2003	Environmental impacts of microbial insecticides. Needs and methods for risk assessment. Kluwer Academic Publishers, Dordrecht/Boston/London	N	-
IIIM 10.1; IIIM 10.2; IIIM 10.3; IIIM 10.4; IIIM 10.5; IIIM 10.7	Copping LG (ed)	2004	The Manual of Biocontrol Agents. A world compendium. rev. 3rd edition. The British Crop Protection Council. Surrey, UK	N	-
IIIM 10.1; IIIM 10.2; IIIM 10.3; IIIM 10.4; IIIM 10.5; IIIM 10.7	OECD	2005	Directory of microbial pesticides for agricultural crops in OECD countries Revised version, Kabaluk T. and Gazdik K. (eds) Agriculture and Agri-Food Canada	N	-
IIIM 10.2	[redacted]	1983	The acute toxicity of Ve6-58 SSP to rainbow trout (*Salmo gairdneri*); [redacted] Report No. TTL 3/83415	Y	KBS
IIIM 10.2	Quinlan RJ	1983	*Verticillium lecanii* acute immobilisation of *Daphnia* test. University of Reading, England. Date: 02-03-1983	Y	KBS
IIIM 10.2	Skrobek A Butt T	2005	Toxicity testing of destruxins and crude extracts from the insect-pathogenic fungus *Metarhizium anisopliae*. FEMS Microbiology Letters **251**: 23–28	N	-
IIIM 10.2	Verhaar HJM	2005	*Verticillium lecanii*. Toxicity to algae. ENVIRON Netherlands B.V. Doc No 77KO-MYCST-20050211	Y	KBS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIIM 10.3	Gerritsen L Cornelissen B	2006	Biologische bestrijding van varroa met behulp van schimmels. PPO Bijen http://www.wur.nl/NL/nieuwsagenda/archief/nieuws/2006/Biologische_bestrijding_varroamijt_in_bijenhouderij_is_lastig.htm (in Dutch)	N	-
IIIM 10.3	Kling A	2000	Assessment of side-effects of *Verticillium lecanii* to the honeybee, *Apis mellifera* L. in the laboratory. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. Report No. 20001292/01-BLUE	Y	KBS
IIIM 10.3	van Doorn A	1998	Impact of the fungi *Verticillium* and *Trichoderma* on adult bumblebees and bumblebee brood. Research Report R&D Natupol. Koppert Biological Systems. Report No. Pr980701	Y	KBS
IIIM 10.3; IIIM 10.4	Schuler T Hommes M Plate HP Zimmermann G	1991	*Verticillium lecanii* (Zimmermann) Viégas (Hyphomycetales: Moniliaceae): Geschichte, systematik, Verbreitung, Biologie und Anwendung im Pflanzenschutz. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Heft 269. Berlin, Dahlem.	N	-
IIIM 10.4	Beerling EAM van den Berg D	2003	36th Annual Meeting Program and Abstracts Society for Invertebrate Pathology 26-30 July 2003 Burlington, Vermont, US	N	-
IIIM 10.4	Flexner JL Lighthart B Croft BA	1986	The effects of microbial pesticides on non-target beneficial arthropods. Agric Ecosystems Environ **16**: 203-254	N	-
IIIM 10.4	Quinlan RJ Chaudhry MA	unknown	Unpublished study report: non-target insect tests for toxicity/pathogenicity. University of Reading, UK	Y	KBS
IIIM 10.4	Samson RA Rombach MC	1985	Biology of the fungi *Verticillium* and *Aschersonia.* In: Biological control. The glasshouse experience. Hussey NW and Scopes N (eds). Blenford press, Dorset, UK , p. 34-42	N	-
IIIM 10.4	Sitch JC Jackson CW	1997	Pre-penetration effects affecting host specificity of *Verticillium lecanii.* Mycol Res **101** (5): 535-541	N	-
IIIM 10.4	St Leger RJ Joshi L Roberts DW	1997	Adaptation of proteases and carbohydrases of saprophytic, phytopathogenic and entomopathogenic fungi to the requirements of their ecological niches. Microbiology **143**: 1983-1992	N	-

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIIM 10.4	Sterk G. et al.	1999	Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS working group "Pesticides and Beneficial Organisms". BioControl (formerly Entomophaga) **44**: 99-117	N	-
IIIM 10.5	Wachter S	2000	Acute toxicity of *Verticillium lecanii* on earthworms, *Eisenia foetida* using an artificial soil test. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. Report No. 20001292/01-NLEf	Y	-

*: Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization, of a plant protection product before the date of submission of the dossier to Rapporteur Member State.

**: Owners' code identifications and names (Code identification: KBS Name: Koppert Beheer Systems)