Purpureocillium lilacinum 251

Microbial pest control agent against plant parasitic nematodes

Dossier according to OECD guidance for industry data submissions for microbial pest control products and their microbial pest control agents – August 2006

Summary documentation, Tier II

Annex IIM, Section 6

Point IIM 8: Effects on non-target organisms

Date: January 2016

Applicant

Bayer CropScience AG

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Introduction

The company Bayer CropScience AG is submitting a dossier for the re-approval of the microorganism *Purpureocillium lilacinum* 251 as an active substance under regulation (EC) 1107/2009.

The Microbial Pest Control Agent *Paecilomyces lilacinus* strain 251 was included into Annex I of Directive 91/414/EEC on 01/08/2008 (Commission Directive 2008/44/EC) and then approved according to the Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011, implementing Regulation (EC) No 1107/2009 of the European Parliament [1)]. *P. lilacinus* strain 251 was notified and defended by Prophyta GmbH. The active ingredient has been evaluated in Belgium according to Uniform Principles. The representative formulated product for the initial evaluation was the experimental formulation PBP-01001-I, containing 2×10^9 spores/g. PBP-01001-I, is comparable to the commercial formulation BioAct WG, containing 1×10^{10} spores/g, and the only changes between both formulations were slight adjustments of the content of two co-formulants, without any impact on the performance or physical properties of the formulated product. The recommended rate in terms of spores per hectare remained exactly the same. The data on PBP-01001-I can therefore be extrapolated to the formulated product BioAct WG, a wettable granule formulation (WG), the representative formulation in the present application for the renewal.

In 2013 Bayer CropScience AG acquired Prophyta Biologischer Pflanzenschutz GmbH, now named Bayer CropScience Biologics GmbH. Bayer CropScience AG is the notifier for the renewal of *P. lilacinus* strain 251 in the procedure of AIR 3.

The microorganism has been previously classified as *Paecilomyces lilacinus* until 18S rRNA gene internal transcribed spacer (ITS) and partial translation elongation factor 1-α (TEF) sequencing revealed that *P. lilacinus* is not related to *Paecilomyces.* The new genus name *Purpureocillium* has been proposed for *P. lilacinus* and the new species name was assigned: *Purpureocillium lilacinum*. Therefore the strain is now identified as *Purpureocillium lilacinum*. In this dossier *Paecilomyces lilacinus* 251 and *Purpureocillium lilacinum* 251 are used as synonyms: *Paecilomyces lilacinus* = *Purpureocillium lilacinum*.

It has to be taken into account that data on *Paecilomyces lilacinus* from the open literature stated before 2011 may not necessarily provide reliable information due to insufficient classification methods used in these studies, especially, if the strain identification is not provided and/or identification methods used were based solely on morphological characteristics. However, they may provide relevant information transferrable to *Purpureocillium lilacinum*.

Purpureocillium lilacinum 251 is a ubiquitous, saprobic filamentous fungus commonly isolated from soil, decaying vegetation, insects and nematodes. Strains of *P. lilacinum* are used in plant protection products due to their nematicide activity. The mode of action against plant pathogenic nematodes of *P. lilacinum* strain 251 is principally based upon parasitism of nematode eggs as well as the vermiform stages of the nematodes, leading eventually to their death. With regard to the results of toxicity and ecotoxicity studies of the active substance *P. lilacinum* strain 251, it can be concluded that *P. lilacinum* strain 251 shows no risk for exposed humans, animals and environment.

P. lilacinum 251 is intended to be used in plant protection products to control plant pathogenic nematodes. The representative use presented in this dossier comprises applications of the formulation BioAct WG in protected and non-protected vegetable crops to control root know nematode, *Meloidogyne* spp.

Here we submit data that were previously evaluated by RMS Belgium as well as new data and information based on literature searches and studies.

[1] OJEU L94/13 Commission Directive 2008/44/EC of 4 April 2008 amending Council Directive 91/414/EEC to include benthiavalicarb, boscalid, carvone, fluoxastrobin, *Paecilomyces lilacinus* and prothioconazole as active substances

IIM 8 Effects on non-target organisms

General remark: eco-toxicological studies performed with the formulated product are considered applicable and relevant with regard to the evaluation of the active substance, and vice versa, since > 99 % of the formulated product are natural organic food additives and the TGAI and impose no health or environmental risk (see Doc. J, Safety Data Sheets for all inert ingredients). The ingredients also serve as nutrients for the fungus, supporting its growth, and therefore testing of the formulated product presents worst case exposure conditions. Correspondingly, studies on the preparation have been submitted for following Points: IIM 8.1 Effects on birds, IIM 8.2 Effects on fish, IIM 8.3 Effects on aquatic invertebrates, IIM 8.4 Effects on algae growth, IIM 8.10 Effects on soil micro-organisms. (EU-Dossier: IIB, 8.2.1; IIB, 8.2.2, IIB, 8.2.3, and IIB, 8.6 respectively). In these eco-toxicological studies the employed batch of PBP-01001-I contained more than the minimum certified CFU content of 2×10^9 CFU/g, i.e. 4.53×10^9 CFU/g, thus presenting worst case conditions of exposure. Within this dossier for registration of *P. lilacinus* strain 251 all data on eco-toxicology have been included that appeared relevant for assessing this fungus, i.e. published literature and supplemental data on strain 251 derived from a thesis, partly performed with a comparable formulation of this fungus, tested for supporting registration in Australia.

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New Data 2015

The current formulation is BioAct WG. However, the experimental formulation PBP-01001-I, containing 2.7×10^9 CFU/g, that it's very similar to the commercial formulation BioAct WG, containing 1×10^{10} CFU/g. The only changes between both formulations, apart of the content of active substance, were slight adjustments of the content of co-formulants, without any impact on the performance or physical properties of the formulated product. Therefore, it could be considered valid the extrapolation of these data to the formulated product BioAct WG

A literature search was conducted in order to identify scientific peer-reviewed open literature on the active substance *Purpureocillium lilacinum* 251 which may affect the assessment on non-target organisms. The search was performed by use of the STN database and comprised searches in Agricola, BIOSIS, MEDLINE, CAB Abstracts, SCISEARCH and Chemmical Abstracts, DRUGU, EMBASE, Esbiobase, IPA, Pascal, POSciTech, Toxcenter and FSTA databases. Keywords considered in the search were *Paecilomyces lilacinus*, *Penicillium lilacinum*, *Purpureocillium lilacinum*, bird, aves, fish, pisces, daphnid?[2], *Daphnia*, alga, water fleas, *Glaucophytoa*, *Haptophyta*, *Cryptista*, *Eugleonphyta Dinoflagellaten*, *Rhaphidophyceae*, *Chlorarachniophyta*, *Xanthophyceae*, *Chrysophyta*, Diatomeen, *Phaeophyta*, *Rhodophyta*, *Chlorophyta*, *Chloromonadophyta*, *Heterokontophyta*, adverse effect? tox?, aquatic?, phytotox?, phytopathogen?, plant, bee?, *Apis mellifera*, insect?, arthropod?, earthworm, *Pheretima sieboldi*, *Metaphire sieboldi*, soil microorg?. Search warrant „?" was used to consider also related search terms. In total 132 references were evaluated basing on their title and abstracts, whether they contain relevant information. Of these, 12 references were evaluated in detail, basing on their full texts, revealing 10 relative and supportive references to be considered for the dossier, Section 6 ([redacted], 2015 M-542804-01-1).

Cited references (abstracts):

Report: KIIM 801 – [redacted], I. (2015) M-542804-01-1, Literature review on *Purpureocillium lilacinum* strain 251 and metabolites - Section 6: Effects on non-target organisms

Published report.

Abstract: The review was made in order to identify scientific peer-reviewed open literature on the active substance *Purpureocillium lilacinum* 251 which may affect the assessment on non-target organisms. The literature research was conducted on the STN database and comprised searches in Agricola, BIOSIS, MEDLINE, CAB Abstracts, SCISEARCH and Chemmical Abstracts, DRUGU, EMBASE, Esbiobase, IPA, Pascal, POSciTech, Toxcenter and FSTA databases. Search strategy aimed to find all recent (from 2005 onwards) references that are of ecotoxicological relevance,

[2] Use of „?" at the end of keyword will lead to an expansion of the search criteria at DIMDI database

regarding possible effects on non-target organisms. The criteria for relevance and reliability used were:

- Property investigated was relevant for data requirements of Regulation (EC) 1107/2009
- Subject relevant for ecotoxicological considerations?
- Test species/system relevant to the ecotoxicological assessment?
- 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 *Paecilomyces lilacinus*/*Purpureocillium lilacinum* pathogens in a certain non-target organism, is there any relevance for *Paecilomyces lilacinus*/*Purpureocillium lilacinum?*

Paecilomyces lilacinus, *Penicillium lilacinum*, *Purpureocillium lilacinum*, bird, aves, fish, pisces, daphnid?[3], Daphnia, alga, water fleas, Glaucophytoa, Haptophyta, Cryptista, Eugleonphyta Dinoflagellaten, Rhaphidophyceae, Chlorarachniophyta, Xanthophyceae, Chrysophyta, Diatomeen, Phaeophyta, Rhodophyta, Chlorophyta, Chloromonadophyta, Heterokontophyta, adverse effect? tox?, aquatic?, phytotox?, phytopathogen?, plant, bee?, *Apis mellifera*, insect?, arthropod?, earthworm, *Pheretima sieboldi*, *Metaphire sieboldi* soil microorg?

In total, 132 reports were retrieved after all searches or peer-reviewed literature and checked for relevance basing on their title and abstracts. Of these, 12 references were assessed in detail, and 10 references were identified as relevant for Section 6.

IIM 8.1 Effects on birds

EU-Dossier: Doc M-IIB, Point 8.1

Following Good Agricultural Practise (see Doc. D 1) the water dispersible granules of the preparation PBP-01001-I will be dispersed in water and sprayed directly onto the soil surface as pre-planting soil treatment with subsequent incorporation into the soil, or as transplant or soil drench. Therefore the risk for exposure of birds to the active substance or the formulated product is ruled out. In addition, this strain of *P. lilacinus* is not pathogenic or infectious for vertebrates, as indicated by the submitted toxicological studies (see Annex II, Doc IIM, Section 3). In conclusion studies on toxicity, pathogenicity and infectivity towards non-exposed vertebrates, such as birds, are not required.

New Data 2015

An acute oral toxicity study on birds with *P. lilacinum* 251 was conducted and the results are summarized in the table below. Reasoning for providing this study in document MII section 8.1 is provided within the study report and also in the summary below.

Table 8.1-1 Ecotoxicological endpoints for birds

Study type	Test substance	Species	Endpoint		Reference
Acute oral	*P. lilacinum* 251 (1.21 x 10^{10} spores/g prod.)	Bobwhite quail (*Colinus virginianus*)	LD50	>2000 mg test item/kg bw >3.38 x 10^{10} spores/kg bw.	[redacted]s; [redacted]; 2015; M-534859-01-1

Report:	KIIM 8.1/01; [redacted], J. and [redacted]-[redacted], M., 2015
Title:	Acute oral LIMIT-test toxicity of *Purpureocillium lilacinum* to Bobwhite quail (*Colinus virginianus*)
Report No:	M2902363-5
Document No:	M-534859-01-1
Guidelines:	OECD Guideline 223 US EPA OCSPP 850
GLP:	Yes

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

Materials and Methods:

The study was conducted during the period 26.05.2015 to 02.07.2015, by the facility Environmental Safety - Testing of Bayer CropScience AG, Development, [redacted], Germany.

Native spores of *Purpureocillium lilacinum* are extremely small, light-weight and electrostatically charged and thus cannot be handled in an open system. This makes weighing the spores into gelatine capsules in the testing facility technically impossible. Therefore for the purpose of application a vehicle was used consisting to 99.8% of easily digestibly carbohydrates, proteins and lipids. The test item (vehicle plus spores denominated as "*Purpureocillium lilacinum* 251 WG 6 (6 %)"; TOX20047-00; Supplier Batch ID: EBMX000282; Specification no.: 102000028478) contained 1.69 x 10^{10} total spores /g (1.21 x 10^{10} viable spores/g).

As test animals adult female and male Bobwhite quails (*Colinus virginianus*) were used. The birds were housed individually and acclimated to laboratory conditions for 21 days. After this period they were orally dosed one-time with gelatine capsules filled with the test item. The limit dose group of 5 quails was dosed with 2000 mg test item per kg body weight. Additionally, 10 control quails were administered with capsules containing the vehicle only at the same amount per unit body weight that was given to the birds dosed with the test item. After dosing, all quails were continuously observed for a time period of 14 days. All quails were identified by numbered and coloured leg bands. Each cage was identified by the study number, cage no. and test concentration. The individual test item amounts were calculated based on the body weights of the quails, one day prior to dosing (day -1). The quails were starved for 16 hours prior to dosing. Afterwards they had free access to feed. During the whole test period, the control quails were held under the same conditions as the dosed quails. The test units were maintained at a mean temperature of 21.8 °C, a mean relative humidity of 52.3 % and a 8 hour light/16 hour dark cycle. Mortality and signs of intoxication were observed continuously during the first two hours and hourly on the day of dosing and at least once daily throughout the 14 days observation period. Body weights were recorded at day -1 (one day before dosing), on study days 3 and 7, and on day 14 (termination of the study). Feed consumption was measured daily until day 3 after dosing and afterwards for the time periods of days 3 – 7 and 7 – 14. On study days 1, 2, 3, 7 and 14 all remaining feed was replaced by fresh feed after cleaning of the feeding container. At the end of the study all surviving quails were sacrificed by CO2 asphyxiation and afterwards gross necropsies were carried out on all the sacrificed quails.

Findings:

No mortality was observed. During the whole experimental phase (0-14 days), all quails showed a good and healthy condition. No symptoms were visible, only one bird had signs of transient (1h) diarrhea after 4 hours after application of the test item. Throughout the study conduct feed consumption was similar between dosed and control birds. There was no considerable difference in body weights during the course of the study between dosed and control quails. Neither in the quails administered with the spores nor in the control quails signs of intoxication were found.

Conclusions:

The acute LD50 for Bobwhite quail, orally dosed with Purpureocillium lilacinum, was >2000 mg test item/kg bw equivalent to > 3.38 x 10^{10} total spores/kg bw.

The non-lethal dose (NLD) accounted for ≥ 2000 mg test item /kg bw equivalent to > 3.38 x 10^{10} total spores/kg bw.

IIM 8.2 Effects on fish

EU-Dossier: Doc M-IIB, Point 8.2.1

Report: KIIM 8.2/01 [redacted], A. (2001) M-467660-01-1: Acute toxicity testing of PDP-01001-1 (*Paecilomyces lilacinus*, strain 251, formulated as WG) in rainbow trout (*Oncorhynchus mykiss*) (Teleostei, *Salmonidae*)
[redacted], Germany – published: no, report No. 20011290/01-AAOm (Dates of work: Aug. 1, 2001 to Sept. 14, 2001)
Guidelines: OECD 203; EEC C.1

Deviations: combined range finding and limit test: a range of 6 concentrations up to the limit concentration of 100 mg/L was tested, employing the suggested 10 animals/concentration to achieve a 99.9% probability level. This deviation has no impact on the validity of this study

GLP: Yes

Material and methods: *P. lilacinum* 251 formulated as WG (code: PBP-1001-I); purity (nominal) 2×10^9 active conidia/g; batch no.: 201062701; solid granules, lilac tan
Rainbow trout (*Oncorhynchus mykiss*), from [redacted], Germany, size 5 ±1 cm: 10 fish per test concentration were exposed to PBP-01001 at 0.001, 0.01, 0.1, 1, 10 and 100 mg/L (equivalent to nominal 2×10^3 to 2×10^8 CFU/L), control to the test was as negative control, respectively, under static conditions for 96h.
Mortality and clinical signs were assessed at 3, 6, 24, 48, 72 and 96h after test start to determine the median effective concentration (EC_{50}).
Temperature, pH value and dissolved oxygen concentration were monitored daily, at 24h intervals, throughout the study performance.

Findings: All fish survived the 96h exposure to the test substance up to 100 mg/L, and no fish exhibited signs of toxicity or behavioural changes during the course of this study (data on mortality see table 8.2.1-1, summary of clinical signs see table 8.2.1-2). Therefore, the NOEC (No Observable Effect Concentration) for PBP-01001-I was 100 mg/L, and the 96h EC_{50} was estimated to exceed the maximum concentration tested, i.e. 100 mg/L of test substance, with a probability of 99.9%.

Table 8.2.1-1: Mortality (%) of fish exposed to PBP-01001-I (*P. lilacinum* 251, formulated as WG) under static conditions for 96h

Time of exposure [h]	Nominal concentrations of PBP-01001-I [mg/L]						
	Control	0.001	0.01	0.1	1	10	100
3	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0

Table 8.2.1-2: Summary of clinical observations for fish toxicity of PBP-01001-I (*P. lilacinum* 251, formulated as WG); number of fish affected / total number of fish; symbol for clinical sign[1])

Time of exposure [h]	Nominal concentrations of PBP-01001-I [mg/L]					
	Control	0.001	0.01	0.1	1	10
3	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**
6	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**
24	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**
48	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**
72	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**
96	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**	10/10 **0**

0= no clinical signs; U= unusual behaviour (reduced activity); * difficulties with maintenance of equilibrium; #= fish upside down with loss of equilibrium; †= no sign of life

Body size and body weight were not adversely affected by exposure to the test substance.

All criteria for validity were met in this test, i.e. mortality in control was <10%, and measured values for physical-chemical parameters varied within acceptable limits.

Conclusion: NOEC = 100 mg/L
96h EC_{50} > 100 mg/L PBP 01001-I, equivalent to nominal 2×10^8 cfu of *P. lilacinum*251 and

actual 4.5×10^8 (according to analytical certificate). PBP-01001-I is not toxic to rainbow trout up to a concentration of 100 mg/L. Therefore, no labelling is required according to EU labelling regulations.

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New Data 2015

From literature search, no new reference relevant for risk assessment was identified describing the effect of *P. lilacinus* on fish. For more information on the literature search, please refer to the submitted document under Point IIM 8 ([redacted], 2015 M-542804-01-1).

IIM 8.3 Effects on aquatic invertebrates

EU-Dossier: Doc M-IIB, Point 8.2.2

Report : IIM 8.3/01 [redacted], A.(2001) M-467656-01-1: *Assessment of toxic effects of PBP-01001-I (Paecilomyces lilacinus, strain 251, formulated as WG) on Daphnia magna using the 48h acute immobilization test*
Arbeitsgemeinschaft [redacted]
Germany – published no, report No. 20011290/01-AADm (Dates of work: Aug. 1, 2001 to Oct 24, 2001)

Guideline: OECD 202; EEC C.2
Deviations: combined range finding and limit test: a range of 6 concentrations up to the limit concentration of 100 mg/L was tested under conditions of a limit test design. This deviation has no impact on the validity of this study

GLP: Yes
Materials and Methods: *P. lilacinum*251 formulated as WG (code: PBP-1001-I); purity (nominal) 2×10^9 active conidia/g; batch no. 20100701; solid granules, light tan

Daphnia magna STRAUS, clone 5, freshly hatched: for 48h under static conditions 4 × 5 daphnids per treatment group were exposed to PBP-01001 at 0.001, 0.01, 0.1, 1.0, 10 and 100 mg/L (equivalent to nominal 2×10^3 to 2×10^8 cfu/L), and to a blank consisting of the test medium water, and two concentrations of reference substance potassium dichromate (0.9 or 1.9 mg/L), respectively.
Immobilized daphnids were enumerated at 24 and 48 h following study start to determine the median effective concentration (EC_{50}).
Temperature, pH value and dissolved oxygen concentration were monitored initially and at 24 and 48 h of the study performance.
Findings: No mortalities or effects were observed in the test substance groups up to a concentration of 100 mg/L, which therefore represents the NOEC (No Observable Effect Concentration). The EC_{50} was estimated to exceed the tested maximum concentration of 100 mg/L with a probability of 95%. Results of the immobilization test are summarized in table 8.2.2-1

Table 8.3-1: Results of the immobilization test after 24h and 48h of exposure to PBP-01001-I (*P. lilacinum* 251 formulated as WG) to the reference substance K2CR2O7

24h of exposure								
Parameter	Nominal concentrations of PBP-01001-I [mg/L]						$K_2CR_2O_7$ [mg/L]	
	0.001	0.01	0.1	1	10	100	0.9	1.9
No. affected	0	0	0	0	0	0	0	11
% immobilized	0	0	0	0	0	0	0	55
48h of exposure								
	Nominal concentrations of PBP-01001-I [mg/L]						$K_2CR_2O_7$ [mg/L]	
	0.001	0.01	0.1	1	10	100	0.9	1.9
No. affected	0	0	0	0	0	0	9	20
% immobilized	0	0	0	0	0	0	45	100

Observations: All criteria for validity were met in this test, i.e. mortality in control was <10%, and measured values for physical-chemical parameters varied within acceptable limits. The results with the positive control potassium dichromate confirmed the validity of this test.

Conclusions: NOEC = 100 mg/L

48 h EC_{50} > 100 mg/L PBP 01001-I, equivalent to nominal 2×10^8 CFU of *P. lilacinum* strain 251 and actual 4.5×10^8 CFU (according to analytical certificate). PBP-01001-I is not toxic to Daphnids up to a concentration of 100 mg/L. Therefore, no labelling is required according to EU labelling regulations.

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Further data on acute toxicity to aquatic invertebrates are available from [redacted] (2000 M-490114-01-1), who tested *P. lilacinum* 251 towards brine shrimp (Class *Branchiopoda*, Order *Anostraca*) within the scope of her thesis (chapter 6.5).

Test design: for 15 days brine shrimp were exposed 8.5×10^4, 8.5×10^6 and 8.5×10^8 spores in 200 mL beakers, in 3 replicates per treatment group. The control group with no spores was run separately for each treatment group due to different starting times. At study termination water samples of the high dose were analysed for *P. lilacinum* spores, and some dead shrimps of high dose and control groups were incubated on semi-selective agar for recovery of *P. lilacinum*.

Findings: There were no significant differences in mortality among treated and untreated brine shrimp at study termination (survival data of final assessment, see table 8.2.2-2). Upon incubation on semi-selective agar plates, *P. lilacinum* was recovered from dead shrimp in treatment groups but initially dead animals showed no signs of fungal colonization. *P. lilacinum* also was not seen to colonize live brine shrimp. Dead test animals from untreated containers grew either bacterial colonies or an unidentified white fungus. The settled detritus from the highest dose container produced lawns of *P. lilacinum* on potato-dextrose agar, indicating that fungal spores survived in sea water. But germination was not seen in seawater alone.

Table 8.3-2: Survival of brine shrimp exposed to *P. lilacinum* 251 at three doses for 15 days (#survived/total #) (1 dose and 1 control = 1 experiment)

Treatment:	Control	8.5×10^4 spores	Control	8.5×10^6 spores	Control	8.5×10^8 spores
Replicate 1	12/ 20	9/20	5/20	9/20	0/20	8/20
Replicate 2	11/ 20	9/20	7/20	5/20	9/20	9/20
Replicate 3	13/20	12/20	7/20	3/20	6/20	6/20
AVERAGE	11.7/20	10/20	6.3/20	5.6/20	5/20	7.6/20

Conclusions: Recovery of *P. lilacinum* spores from dead shrimp was concluded to be most likely due to ingestion of spores which had settled among the detritus. Brine shrimp often were observed to swim across this detritus and stir it up. Ingested spores apparently had passed the gut without germinating and growing to colonies, but merely were present in the gut at time of death, and therefore germinated under optimum conditions of incubation on agar plates.

According to [redacted] (2000, M-490114-01-1) it can be safely concluded that brine shrimp are not adversely affected by spores of *P. lilacinum* 251.

New Data 2015

From literature search, no new relevant reference was identified (see [redacted], 2015 M-542804-01-1).

IIM 8.4 Effects on algal growth and growth rate

EU-Dossier: Doc M-IIB, Point 8.2.3

Report : IIM 8.4/01 [redacted], D.(2001) M-467680-01-1: Testing of toxic effects of PBP-01001-I (*Paecilomyces lilacinus* strain 251 formulated as WG) on the single cell green algae *Desmodesmus subspicatus*
Arbeitsgemeinschaft [redacted] Germany –published: no; report No. 20011290/01-AADs (Dates of work: Aug. 1, 2001 to Oct. 19, 2001)

Guideline: OECD 201; EEC C.3
Deviations: none

GLP: Yes

Materials and Methods: *P. lilacinum* 251, formulated as WG (code: PBP-01001-I), purity (nominal) 2 × 10^9 active conidia/g; batch no. 20100701; solid granules, lilac tan.

Exponentially growing cultures of the single cell green alga *Desmodesmus subspicatus* CHODAT, strain no. SAG 86.81, were exposed to 6 concentrations of test substance under defined conditions in a synthetic growth medium for several generations. According to results of a range finding test employing concentrations from 0.01 to 100 mg/L (spaced by a factor of 10) test concentrations were set as 10 to 141.99 mg/L, differing by a geometric factor of 1.4.
The cell growth was measured 24, 48 and 72 hours after initiation of the test.
The inhibition of growth was determined by calculating the E_rC, E_bC, LOEC, and NOEC (EC = effective concentration; indices r and b refer to "growth rate" and "biomass", respectively). The EbC and ErC values were calculated by log-linear regression, both based on the nominal concentrations of *P. lilacinum* 251 formulated as WG at t = 72 h.
Findings: Range finding test: Inhibitory effects of the test substance were observed at 100 mg/L (12.2% for the growth rate and 34.5 % for biomass integral). These results were taken to select the concentration range for the main test.
Results of the range finding test are summarized in table 8.2.3-1.

Table 8.2.3-1: Results of the range finding test (72 h)

Paecilomyces lilacinus [mg/L]	Conidia/L	Cells/mL · 10^{-4} *
0	0	203.65
0.01	$2 \cdot 10^4$	218.75
0.1	$2 \cdot 10^5$	209.38
1.0	$2 \cdot 10^6$	195.31
10	$2 \cdot 10^7$	228.13
100	$2 \cdot 10^8$	106.51

* Algal counts are divided by 10000. At the start, the cell density was adjusted to 10^4 cells/mL

Main test: Significant inhibitory effects were observed from 49.13 to 141.99 mg/L after 72 h for the biomass integral and for the growth rate (calculated by Dunnett's-Test).

Table 8.2.3-2 summarizes the percentage inhibition of *Desmodesmus subspicatus* biomass integral and growth rates. % inhibition of biomass integral and % inhibition of growth rates are also presented in figures 8.2.3-1 and 8.2.3-2, respectively.

Table 8.4-2: Percentage inhibition of *Desmodesmus subspicatus* biomass integral and growth rates (0 - 72 h)

Paecilomyces lilacinus [mg/L]	Conidia per L	Inhibition [%] of biomass integral	Inhibition [%] of growth rate
0.0	0	0.0	0.0
10	$2 \cdot 10^7$	-3.8	0.4
17	$3.40 \cdot 10^7$	4.1	2.8
28.9	$5.78 \cdot 10^7$	-7.0	2.9
49.13	$9.83 \cdot 10^7$	43.7	20.4
83.52	$1.67 \cdot 10^8$	60.2	25.0
141.99	$2.84 \cdot 10^8$	64.9	28.0

Fig. 8.2.3-1: % Inhibition of biomass integral of *Desmodesmus subspicatus* at different concentrations of *P. lilacinum* 251 formulated as WG

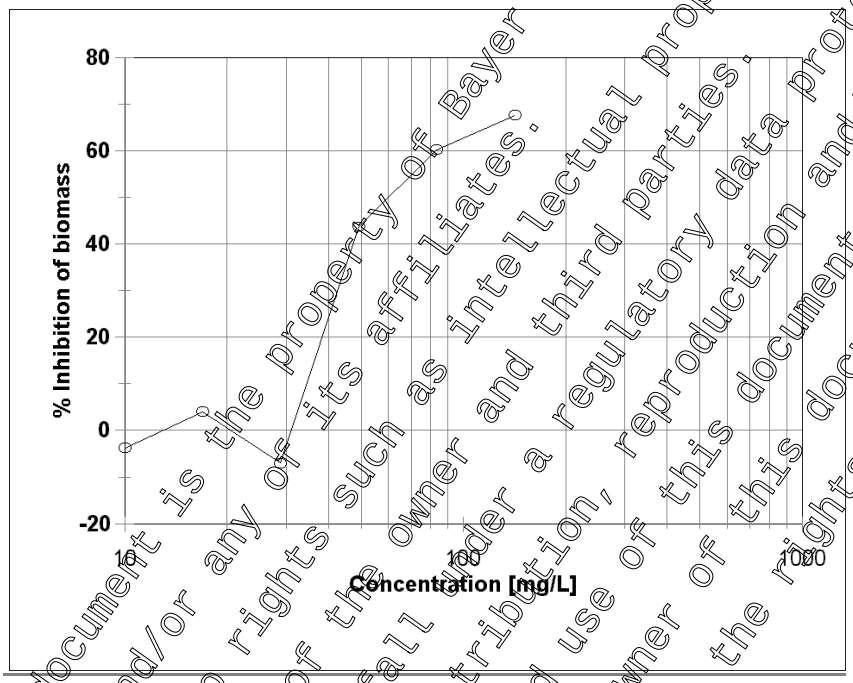

Table 8.4-3: Summary table of inhibitory concentrations of PBP 010841 for algae growth

72h EC value	*P. lilacinum* [mg/L]	Conidia/L[1] -nominal-	Conidia/L[2] -analytical-
EbC_{50}	71.7	1.44×10^8	3.2×10^8
ErC_{50}	256.8	5.13×10^8	1.2×10^9
LOEC	49.13	9.83×10^7	2.2×10^8
NOEC	28.90	5.78×10^7	1.3×10^8

[1] based on a nominal content of 2×10^9 conidia per gram product
[2] based on the determined content of 4.53×10^9 conidia per gram product (see analytical certificate)

Observations: Variation in test conditions, i.e. temperature and pH remained in acceptable limits. The increase of the cell concentration (cell multiplication factor) for the control cultures between 0 and 72 h was 19.6. The test, therefore, fulfils the validity criteria.
During the test at the three highest test substance concentrations, active growth of *Paecilomyces lilacinus* (spore germination and mycelial growth) was observed.

Conclusions: Under the conditions of this test the effective concentration to reduce the growth rate by 50% was determined as 256.43 mg/L of PBP-01001-I, and reduction of biomass by 50% required a concentration of 71.77 mg/L.
The inhibitory effects reflect the nutrient competition between the test organism and the green alga *Desmodesmus subspicatus,* considering that fungal growth was observed at concentrations of 50 mg/L test substance and higher, and that the employed conditions were supportive for growth of saprophytic micro-organisms. Under the conditions of this test, where conidia of *P. lilacinum* were incubated at 23 to 24 °C in a nutrient solution on a rotary shaker, additionally offering organic substrate in the form of algal debris this growth is a natural consequence. Under growth limiting conditions prevailing in natural waters the alga is more competitive and spores will be subject to sedimentation.

IIM 8.5 Effects on aquatic plants

EU-Dossier: Doc M-IIB, Point 8.5

No adverse effects on plants, to the contrary beneficial effects on plant growth and yield are known from published literature (see Annex II, Doc IIM, Section 1, Point IIM 2.4; EU-Dossier: Doc M-IIB, Section 1, Point 2.3), as anticipated due to nematode control. Regarding aquatic plants there is no exposure to this fungus, since the intended use excludes direct application on waters.

New Data 2015

From literature search, no new relevant reference was identified (see [redacted], 2015 M-542804-01-1).

IIM 8.6 Effects on terrestrial plants

Please refer to Point IIM 8.5

IIM 8.7 Effects on bees

EU-Dossier: Doc M-IIB, Point 8.3

Following Good Agricultural Practise (see Doc. D-1) the water dispersible granules of the preparation PBP-01001-I will be dispersed in water and sprayed directly onto the soil surface as pre-planting soil treatment with subsequent incorporation into the soil or as transplant or soil drench. Therefore exposure of honeybees to the active substance or the formulated product is ruled out. Therefore the honey bee is not an appropriate non-target organism and no studies are required. In addition, this strain of *P. lilacinum* is not pathogenic or infectious for non-target arthropods, as indicated by the following toxicological studies (see Point IIM 8.8).

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New Data 2015

There were only slight changes of the content of co-formulants between the old formulation PBP-01001-I and the new formulation BioAct WG, apart from the content of active substance. However, Following Good Agricultural Practice (see Doc. D-1) *P. lilacinum* 251 will be applied directly onto the soil surface by soil irrigation (drip or drench) or by tray drench/dipping, with subsequent incorporation into the soil by watering. Therefore, exposure of honeybees to the active substance or the formulated product is ruled out and studies on honey bees are not required. In addition, this strain of *P. lilacinum* is not pathogenic or infectious for non-target arthropods, as indicated by the following toxicological studies (please refer to Point IIM 8.8).
Moreover, no adverse effects of *P. lilacinum* on bees were reported in the peer reviewed open literature (see [redacted]
2015 M-542804-01-1). Effects on bees due to an application of BioAct WG are therefore unlikely.

IIM 8.8 Effects on terrestrial arthropods other than bees

Effects on foliage dwelling non-target arthropods

Following Good Agricultural Practice (see Doc. D-1) *P. lilacinum* 251 will be applied directly onto the soil surface by soil irrigation (drip or drench) or by tray drench/dipping, with subsequent incorporation into the soil by watering. No means of spraying are allowed thereby preventing any exposure of above ground and leaf dwelling non-target arthropods-to BioAct WG.

EU-Dossier: Doc M-IIB, Point 8.4

General remark: two studies have been performed with the preparation PBP-01001-I in compliance with GLP. In addition a non-GLP test with this strain has been performed on beneficial nematodes. These studies are presented under Point IIIM 8.9 (Anonymous, 1992, M-489347-01-1; [redacted] (2000, M-490114-01-1)).

Report : **IIM 8.8/01** [redacted], M.(2001), M-467682-01-1, PBO 01001-I: Acute Toxicity to the Aphid Parasitoid Aphidius rhopalosiphi (Hymenoptera, Braconidae) in the Laboratory, Arbeitsgemeinschaft [redacted], Germany – published: no, report No. 20011290/01-NLAp (Dates of work: July 18, 2001 to Aug. 13, 2001)

Guideline: [redacted] (1988), [redacted] (1992), Escort Guidance Document (Barrett et al. 1994) and the guideline of the ring testing group (Mead-Briggs et al. 2000).
Deviations: none

GLP: Yes

Materials and Methods: *P. lilacinum* 251 formulated as WG (code: PBP-I001-I); content of a.i. (nominal) 2×10^9 active conidia/g; batch no. 101062001; solid granules, lilac tan. Toxic standard: Perfekthion (BAS 15[illegible]11 I); batch no. 99-19; Active ingredient: Dimethoate 400 g/l a.i. The aphid parasitoid, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) was obtained from the company [redacted], Germany. The test was carried out with three treatments:
1. Test substance – 30 kg product/ha at water rate of 2000 L/ha, equivalent to 3.0 kg/ha in 200 L/ha (=1.5 %), 2. Toxic standard – 0.3 mL/ha at water rate of 200 L/ha, and 3. Control, treated with deionized water at rate of 200 L/ha.
Each treatment group included 4 replicates containing 10 adults (five male and five females), less than 48 hours old. The test organisms were introduced into exposure units with an aspirator, and were exposed to the test substance on glass plates, which were assembled to the exposure unit after the test substance had been sprayed and allowed to dry. At ½, 2, 24 and 48 hours of exposure mortality was assessed and 15 surviving females of the treatment group and of the control group were transferred to individual fertility cages to assess fecundity. After 24 hours the females were removed and their condition (alive or dead) was recorded. The plants bearing the aphids were maintained at test conditions for 11 additional days after which the number of parasitized aphids was counted, as a parameter for fecundity.
Findings: Mortality: After 48 hours the mortality in the group exposed to PBP01001-I was 37.5 % compared to 0 % in the control, and 100 % in the group exposed to the toxic standard. The mortality in the toxic standard and in the test substance group were statistically significantly increased compared to the control. The mortality values needed not be corrected for mortality in control, since there was 0% mortality in untreated.
Fecundity: The total number of mummies developed within 11 days was 10^7 in the control group, corresponding to 7.13 mummies per female. In the test substance group a total of 35 mummies were produced, corresponding to 2.92 mummies per female. These values are statistically significantly different from the numbers found in the control group. The resultant reduction in reproduction was calculated to 59.05 %. The reproduction factor was calculated to be 0.41 compared to the control.

The results are summarized in tables 8.8/01-1 and 8.8/01-2.

Table 8.8/01-1: Mortality of *A. rhopalosiphi* after 48h exposure to glass plates treated with PBP-01001-I and a toxic standard, respectively

Treatment [product in g/ha, in 200 L water/ha]	Mortality after exposure [%]				Mcorr. at 48h post-exposure [%]
	½ h	2h	24h	48h	
Control	0.00	0.00	0.00	0.00	---
PBP-01001-I [3000g]	0.00	0.00	10.00	37.50[1]	37.50
Perfekthion [0.3mL]	0.00	0.00	92.50	100.00[1]	100.00

[1] significantly different from control (Fisher's exact test, $p \leq 0.05$)

Table 8.8/01-2: Reproduction rate of *A. rhopalosiphi* after 48h exposure to PBP-01001-I

Treatment:	**PBP-01001-I [3000 g/ha in 200L/ha]**	**Control**
No. females	12	15
No. mummies	35	107
Mummies per female	2.92	7.13
Reproduction factor	0.41	---
Reduction in reproduction	159.05	

Observations: Mortality in the control group was below 13%, the toxic standard gave consistent results (mortality>50%), the minimum control parasitation of 5 mummies per surviving female was met, no more than two females failed in producing mummies in the control group. The test, therefore, fulfils the validity criteria.

Conclusions: In adults of *A. rhopalosiphi* PBP-01001-I applied at a dose corresponding to 30 kg/ha caused 37.50% mortality, which is below the applicable trigger value of 50%, as suggested within recent discussions in the ESCORT II working group. In the fertility test a reduction in reproduction rate was determined (reproduction factor 0.41), but there was high variation among the 15 individual females in both control and test substance group, ranging from 0 to 23 in control and 0 to 19 in treated animals. Compared to previous control data both results were regarded to be in the range of normal variability as found in the test system. Investigations of the Expert's group of *Aphidius rhopalosiphi* showed that a 50% treatment effect on fecundity of treated insects can only be determined with at least 80% confidence.

Considering the current discussions within the Expert's group, it is concluded that PBP-01001-I will cause no detrimental effects on the mortality of *A. rhopalosiphi*, even when applied at a rate of 30 kg/ha in 200 L water/ha (1.5x).

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Report : **IIM8.8/0[redacted]**H. (2001) M-467670-01-1: PBP-01001-I: Toxicity to the predatory mite, Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the laboratory
Arbeitsgemeinschaft [redacted]
Germany – published: no, report No. 20011290/01-NLTp
(Dates of work: July 30, 2001 to Aug 9, 2001)

Guideline: Lewis and Lefer (1995) based on Overmeer (1988), improvements of the ring test group (Blümel et al. 2000) and Guidance Document for Regulatory Testing Procedures for Pesticides with Non-Target Arthropods (Barrett et al. 1994)
Deviations: none

GLP: Yes

Materials and Methods: *P. lilacinum* 251 formulated as WG (code: PBP-1001-I); content of a.i. (nominal) 2×10^9 active conidia/g; batch no.: 201062701; solid granules, lilac tan
Toxic standard: Perfekthion (BAS 152 11 I), Batch no. 99-1. Active ingredient: Dimethoate, 400 g/L a.i.
The aphid parasitoid, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) was obtained from the company '[redacted]', Germany. The test was carried out with three treatments:
1. Test substance – 30 kg product/ha at water rate of 2000 L/ha, equivalent to 3.0 kg/ha in 200 L/ha (=1.5%)
2. Toxic standard – 15 mL/ha at water rate of 200 L/ha, and
3. Control, treated with deionized water at rate of 200 L/ha
Each variant included 5 replicates with 20 mites each. Protonymphs were exposed to a freshly applied dry layer of the test substance on glass cover slides for 7 days. Mortality was assessed after 3 and 7 days. The fecundity of treated and control mites was assessed at day 11, 13 and 14 following exposure, by enumerating eggs and juveniles and determining the cumulative number of eggs per female
Findings: Mortality: The mean mortality of *Typhlodromus pyri* after 7 days exposure to glass plates treated with PBP-01001-I was 6.0 % compared to 0.0 % in the control group and 85% in the group exposed to the toxic standard. The corrected mortality for the PBP-01001-I treated and toxic standard group were the same, since no mortality was observed in the untreated group. Significant effects on the mortality of *T. pyri* were observed in the toxic standard treatment (Fisher's Exact Test, $p \leq 0.05$).
The mortality results are summarized in table 8.8-3.

Table 8.8-3: Mortality of *T. pyri* after 7 days exposure to PBP-01001-I or Perfekthion (toxic standard), compared to deionized water treated control (# affected/total #)

Treatment:	Control	PBP-01001-I [3000 g/ha in 200 L/ha]	Toxic Standard* [15 mL/ha]
Vital mites	100/100	94/100	15/100
Missing mites	0/100	3/100	9/100
Dead mites	0/100	3/100	76/100
Mortality [%], ± SD[1]	0.0	6 ± 4.2	85.0[2] ± 6.1
Corrected mortality [%]	---	6	85.0

[1] SD: standard deviation
[2] significantly different from control (Fisher's exact test, $p \leq 0.05$)

Fecundity: During the 7 day egg-laying period the mean cumulative number of offspring per female in the PBP-01001-I treated group was 10.8 compared to 10.7 in the control group. The reproduction of mites exposed to the test substance was not reduced. Results of the fecundity test are summarized in Table 8.8-4.

Table 8.8-4: Fecundity effects on *T. pyri* after 7 days exposure to PBP-01001-I

Treatment:	Control	PBP-01001-I [3000 g/ha in 200 L/ha]
Mean cumulative offspring per female	10.7	10.8
SD	1.2	1.2
Reduction in reproduction [%]	---	-0.9

Observations: Mortality in the control group was below 20%, and the mean mortality in the toxic standard group ranged between 50-100% at the final assessment. The cumulative offspring per female was >4 eggs in the control group. The test, therefore, fulfils the validity criteria.

Conclusions: Under the simulated worst case exposure conditions dried residues of PBP-01001-I did not cause adverse effects on survival or reproduction of the test species and can be regarded to be non harmful to *T. pyri* up to a dose rate of 30 kg/ha applied in 2000 L/ha (=1.5%).

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Under worst case conditions of exposure to PBP-01001-I testing of *Aphidius rhopalosiphi* and *Typhlodromus pyri,* as sensitive representative species for beneficial arthropods, did not indicate an unacceptable impact under conditions of field use. Therefore, no further studies regarding assessment of side-effects on non-target arthropods are required for *P. lilacinum* 251. Still, additionally available information on this fungus is being submitted.

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Further information on effects of *P. lilacinum* 251 on other non-target arthropods is provided by [redacted] (2000, thesis), who tested six different Families or species of insects, which may be exposed to the fungus *P. lilacinum* 251 under field use conditions, and are relevant for Europe, at least on the Family level:

Order, sub-order	Family/ species tested
1. Collembola, Arthropleona:	mixed natural population, mainly of the Family Brachystomellidae; in addition mites were collectively assessed, without further determination
2. Blattodea:	*Blattodea germanicus*; common name: cockroach
3. Coleoptera:	*Tenebrio molitor* (larvae); common name: mealworm
4. Diptera:	*Drosophila melanogaster*
5. Hymenoptera:	Fam. Vespidae, sub-Family Polistinae; common name: paper-nest wasps
6. Hymenoptera:	Family Formicidae (ants), species *Lamprotus atrepidus;* common name: sugar ant

Table 8.8-6 summarizes information on the trial design and findings of the relevant studies.

Table 8.8-6: Summary of trials on non-target insects exposed to *P. lilacinus* strain 251 ([redacted], 2000b)

Family or species (Order)	Trial design, no. of specimen/ replicate	No. replicates	Treatments (dose of *P. lilacinus* conidia)	Exposure period	Findings/ observations
Mainly Brachystomelli-dae (Collembola), also mites	Exposure of natural population in compost, 100g/container, replicate (no initial counting). Enumeration of collembola & mites after exposure	5	- untreated - 0.25 g/L - 25 g/L with 3 × 10^9 viable spores/g (~10× max. field dose*)	2 weeks	Collembola and mites survived in compost treated with *P. lilacinus* conidia at both doses. High variability in animal counts among replicates of all treatments. Numbers in control < numbers in treated containers, but no statistically significant difference in no. of animals among treatments
Blattella germanicus (Coleoptera) – common name: cockroach	11 or 12 specimen/ container, replicate exposed in the dark. assessment of mortality after 1 and 2 weeks	5	- untreated - 0.1 g - 10 g with 3 × 10^8 viable spores/g	2 weeks, interim assessment after 1 week	No significant differences in survival rate among treatments after 1 and 2 weeks. At study end numerous young 1st instar larvae were found, recently hatched, indicating that *P. lilacinus* had not infected the egg cases. Dead cockroaches did not grow *P. lilacinus* colonies after incubation.
Tenebrio molitor (larvae) (Coleoptera) – common name: mealworm	Substrate: sandy loam mixed with bran. 10 mealworms/ container, replicate. Spore suspension added to substrate. Exposure at [illegible] °C. assessment of mortality after 1 and 2 weeks	3	- untreated - 2.5 mL - 5 mL of a 10^8 spores/mL suspension	2 weeks, interim assessment after 1 week	No significant differences in survival rate among treatments after 1 and 2 weeks. None of the dead mealworms had signs of fungal growth on the cuticle. In all treatments a few mealworms were pupating.

* For European conditions, according to Doc. D-1 the maximum applied a.s. dose per ha of PBP-01001-I dispersion can be calculated as 1.3 × 12 (125 mL), with 2 × 10^8 viable spores/g.

Continued:

Table 8.8-5: Summary of trials on non-target insects exposed to *P. lilacinus* strain 251 (HOLLAND 2000b)

Family or species (Order)	Trial design, no. of specimen/ replicate	No. replicates	Treatments (dose of *P. lilacinus* conidia)	Exposure period	Findings/ observations
Drosophila melanogaster (Diptera) T96 wild type	Culture method complying with standard laboratory method, in Erlenmeyer flasks with potato-based medium. Exposure of developing pupae to spore suspension Assessment of non-eclosed flies after 3 to 5 days	3	1 mL of a 10^8 spores/mL spore suspension/ flask, sprayed directly onto the pupae	3 days	Total no. of pupae per flask: 350 to 400, no. of not eclosed flies was very low in all treatments (6-7 after 3 days and ~2 after 5 days of study start). There was no treatment-related difference in numbers not eclosed. The fungus apparently did not infect the pupae of *Drosophila melanogaster*.
Fam. Vespidae, sub-Family Polistinae, common name: paper-nest wasps (Hymenoptera)	Outdoor application of spores sprayed into naturally occurring nests. Assessment of fungal infections in adults, pupae, larvae and eggs collected after 8 days	2 nests treated (dia-meter 6 cm and 10 cm	No. 7 untreated control. Total amount of spores /nest 5×10^7	8 days	In both nests all life stages were present, and appeared healthy with no overt sign of fungal colonization. When incubated for 6 days on agar plates for recovery of fungal colonies many different fungi grew from all stages but only in 1 of 3 adult wasps and 1 of 3 pieces of the paper nest *P. lilacinus* was grown. The 2 incidences can be attributed to attached spores, germinating only on the agar plates.
Family Formicidae (ants), species *Camponotus intrepidus* (Hymenoptera) common name: sugar ant	Cultured on plaster dishes with ventilated lid, ~10-11 ants/container, replicate (with 2 or 3 major workers and the rest minor workers) % survival assessed after 1and 2 weeks	4	- untreated - 0.1 g - 10 g with 3.4×10^9 viable spores/g	2 weeks, interim assessment after 1 week	No deaths occurred in the1st week, but after 2 weeks survival in the high dose group was 78% compared to 100% in control. This significant reduction of survival is attributable to the extremely high dose of fungus ($3.4x10^{10}$ conidia /10-11 ants), which created a film of dust on their bodies. Dead ants placed onto water agar supported conidiophores of *P. lilacinus* after 1 week, mainly arising from limb joints, from the mouthparts and joints between thoratic segments. At the exoskeletal joints the cuticle is thinner and easier to penetrate. Here, spores may have been lodged, as indicated by the colonies evolving, and were not accessible for cleaning procedures.

[redacted] (2000) concludes that *P. lilacinum* 251 does not present a risk for survival or reproduction of the tested insects, including the ants, and gives an explanation for the observed fungal colonization of dead wasps, following incubation on agar plates: naturally occurring fungal spores of many species may come in contact to wasps (or other insects) without causing infections, until the wasp is dead and the insect cuticle presents no barrier any more. For ants the risk of exposure to high doses of spores is not given under field use conditions, due to the overall lower exposure level and incorporation of spores into the soil. These findings suggest that insects of different orders will not be adversely affected under the extent of exposure resulting from field use of PCI-010001 according to Good Agricultural Practise (see Doc. D-1 and Annex II, Doc IIM, Point 9; EU-Dossier: Doc. M-IIB, Point 9).

1st additional Submission (Sept. 2004)

Report: IIM 8.8/04 [redacted] J. (2004a) M-467505-01-1: BioAct® WG toxicity to larvae of the ground beetle, *poecilus cupreus* l. (coleoptera, *carabidae*) in the laboratory [redacted], Germany – published no, report No. 20041022/01-NEPc (Dates of work: 30/05/2004 to 17/06/2004)

Guideline: Heimbach. 1995 and Heimbach *et al.* 2002
Deviations: none

GLP: Yes

Materials and Methods: BioAct® WG, active ingredient *Paecilomyces lilacinus* Strain 251, batch: 1303202111, purity: at least 1.0×10^{10} viable spores/g (nominal); 1.0×10^{9} viable spores/g (analysed)

The effect of BioAct® WG, active ingredient *Paecilomyces lilacinus*, Strain 251, to the ground beetle Poecilus cupreus was investigated during a six to seven week exposure study. Larvae of P. cupreus, 1 to 48 hours old, were exposed in a test unit to a dose of 400 mg BioAct® WG per kg dry soil, corresponding to 6.0×10^{9} viable spores (analysed) of *Paecilomyces lilacinus* per kg dry soil. Each test unit consisted of a glass tube filled with 25 g dry weight) of LUFA 2.1 standard soil. Several days prior to the start of the test, the soil was heated at 80 °C for two hours. The next day, soil moisture was adjusted to 20 % of its maximum water holding capacity.

On the day of application, the test substance was introduced into the test containers with enough water to adjust the soil moisture to 35 % of the maximum water holding capacity. *Poecilus* larvae were introduced 30 to 60 minutes after mixing the test substance into the soil. Sixty replicate test units, each containing one larva, were used. A toxic reference item, Perfekthion, 400 g dimethoate/L and a tap water control were run in parallel, each comprising sixty replicates.

The test units were kept in the dark at 20 ± 2 °C. Soil was remoistened where necessary during the third week after application and in each following week. Pupae of *Calliphora* spp. were provided as food three times per week during the first two weeks after application, twice per week during the next two weeks, and once per week where necessary during the following weeks. At each feeding time old food was removed and observations for larvae and any effects were made. 28 days after application, test units where no larvae or moulting holes could be observed during the previous two to three weeks were examined and searched for *Poecilus* larva or pupa. Where none were found, the introduced larva was counted as dead. One week after the first pupa in the whole experiment was observed, the hatching of adults was checked daily and undeformed hatched beetles were weighed.

Findings: No significant difference between the test substance and control treatment groups was observed in terms of mortality, days until hatching or weight of hatched beetles. These parameters in the toxic reference item were statistically significantly different to the control. Biological results are presented below in **Table 8.8/04-1.**

Table 8.8/04-1: Mortality, time until hatching and hatching weight of *Poecilus cupreus* after an exposure to 400 mg BioAct® WG per kg dry soil, corresponding to 6.0 x 10^9 viable spores (analysed) of *Paecilomyces lilacinus*, strain 251, per kg dry soil

Parameter	Water Control [2]	BioAct® WG	Toxic Reference Item
Mortality	18.6 %	25.0 %	61.7 %
Corrected Mortality [1]	-	7.9 %	57.9 % *
Days until Hatching	37.2	37.5	39.5 *
Hatching Weight	66.4 mg	67.5 mg	57.3 mg *

1) according to the formula of Abbott (1925), modified by Schneider-Orelli (1947)
2) one larva was damaged during an assessment. Therefore, for the control, 59 larva were considered
* statistically significantly different to the control

Conclusions: No adverse effects of BioAct® WG, active ingredient *Paecilomyces lilacinus*, strain 251, to the ground beetle *Poecilus cupreus* were observed at an application rate of 400 mg per kg dry soil, corresponding to 6.0× 10^9 viable spores (analysed) of *Paecilomyces lilacinus* per kg dry soil.

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Report: **IIM 8.8/05** [redacted],; 2004; M-467517-01

: BioAct® WG: An Extended Laboratory Study Conducted on Natural Soil to Evaluate the Effects on the Rove Beetle, *Aleochara bilineata* Gyll. (*Coleoptera, Staphilinidae*)

[redacted], Germany – published: no, report No. 20041022/01-NEAb (Dates of work: 05/03/2004 to 17/05/2004)

Guideline: IOBC/WPRS Grimm *et al.* (2000); Escort I & II; Guidance Document for Regulatory Testing Procedures for Pesticides with Non-Target Arthropods (Barrett *et al.* 1994)

Deviations: none

GLP: Yes

Materials and Methods: BioAct® WG, active ingredient *Paecilomyces lilacinus* Strain 251, batch: 1303202111, purity: at least 1.0×10^{10} viable spores/g (nominal); 1.5×10^{10} viable spores/g (analysed)

The toxicity of BioAct® WG to the rove beetle *Aleochara bilineata* was investigated during a 28-day exposure study in treated LUFA 2.1 standard soil. Five to three days before exposure, copulating beetle pairs (2 - 6 days old) were collected and placed on moist sand in a plastic beaker. They were kept under test conditions and fed with thawed *Chironomus* larvae. The test vessels contained approx. 800 g of soil that had been heated for two hours at 80 °C. On the day of application, the water content of the soil was adjusted to 70 % of its maximum water holding capacity with the incorporation of the test substance at a rate of 400 mg BioAct® WG per kg dry soil, corresponding to 6.0×10^9 viable spores (analysed) of *Paecilomyces lilacinus* per kg dry soil. Immediately afterwards, ten pairs (ten males and ten females) of beetles were released into the test vessels. An untreated water control and a toxic reference item, Dursban 480 480g chlorpyrifos/L, were run in parallel. Each treatment group was replicated four times.

During the exposure period, the test vessels were maintained under a 16 hour light per day photoperiod. Test vessels were weighed after application and one day after application, water lost via evaporation was replenished as required. This was repeated every one to three days. Approx. one hour after application, beetles were fed with thawed *Chironomus* larvae and thereafter every working day. Approx. 500 pupae of *Delia* spec. per replicate were incorporated into the soil 7, 14 and 21 days after application to provide hosts for *Aleochara* larvae.

28 days after application, all beetles were removed from the test vessels. The vessels were kept under test conditions for one further week at which time the fly pupae were removed from the soil and the number of parasitised pupae and hatched *Aleochara* were recorded for a further approx. 35 days.

Findings: Test conditions during exposure and hatching were maintained at 20 to 21 °C, a relative humidity of 60 to 80 % and 16 hours light per day at 500 to 600 lux.

Reproduction of *Aleochara bilineata* in the test substance treatment group was reduced by 10.4 % compared to the control. Reproduction in the toxic reference item was reduced by 99.8 %. Results are presented below in **Table 8.8/05-1:**

Table 8.8/05-1: Reproduction of *Aleochara bilineata* after 28 days exposure to 400 mg BioAct® WG per kg dry soil, corresponding to 6.0 x 109 viable spores (analysed) of Paecilomyces lilacinus Strain 251, per kg dry soil

Treatment	Mean Number of Emerged Beetles per Replicate ± SD	Reduction of Reproduction Capacity
Water Control	754.0 ± 114.1	-
BioAct® WG	675.8 ± 43.8	10.4 %
Toxic Reference Item	1.8 * ± 1.0	99.8 %

SD Standard Deviation

* statistically significantly different to the control

Conclusions: No reduction in parasitic capacity of *Aleochara bilineata* compared to the control was observed after exposure to BioAct® WG, active ingredient *Paecilomyces lilacinus*, Strain 251, at an application rate of 400 mg per kg dry soil, corresponding to 6.0 x 10^9 viable spores (analysed) of *Paecilomyces lilacinus* per kg dry soil.

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2nd additional Submission (Nov. 2004)

The findings from studies conducted on non-target arthropods demonstrate that *Paecilomyces lilacinus* is not harmful to *Typhlodromus pyri* and *Aphidius rhopalosiphi*, considered the most sensitive species for testing of pesticides [redacted],; 2003; M-542628-01

. The reproduction effects on *A. rhopalosiphi* are lower compared to the values stated in the monograph by the Rapporteur. Soil dwelling species are not affected by the micro-organism. The micro-organism does not produce any harmful toxins. Thus, the possible effects from *P. lilacinus* on non-target arthropods other than bees are sufficiently reported in the dossier by the present statement. There were no harmful effects found and there is no need to conduct any additional extended laboratory or semi-field studies on other non-target arthropod species.

The Rapporteur claimed that *P. lilacinus* was reported to be pathogenic to several insects. Pathogenicity might be caused by toxins produced from the fungi, mainly paecilotoxin.

A recent study by [redacted],; [redacted]; 2003; M-542637-01 corroborated previous results that *P. lilacinum* 251 does not produce detectable levels of paecilotoxin or other toxins with antimicrobial activity. In order to evaluate potential toxin production, culture extract and concentrated culture supernatant of *P. lilacinum* 251 were tested against Gram-negative and Gram-positive bacteria. High-performance liquid chromatography analysis was carried out to compare the chromatograms of *P. lilacinum* 251 with the chromatogram of known paecilotoxin.

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New Data 2015

A reproduction study in artificial soil on the collembolan *Folsomia candida* with *P. lilacinum* 251 was conducted and the results are summarized in the table below. Reasoning for providing this study in Annex II, Doc IIM, Section 6, Point IIM 8.8 is provided within the study report and also in the summary below. Further, a literature search was conducted to identify the risk of *P. lilacinum* 251 on non-target organisms. Please refer to the literature search by [redacted] (2015) M-542804-01-1 submitted in Point IIM 8. No articles were identified, presenting data on toxicity on non-target arthropods. Some articles report the effect of *P. lilacinum* on other pests, when evaluating alternative biological control agents, or to identify biological agents against mosquitos.

Table 8.8-1 Ecotoxicological endpoints for soil dwelling arthropods

Test item	Endpoint	Test species	Reference
P. lilacinum 251 (1.21 x 10^{10} spores/g prod.)	NOEC 562 mg/kg soil corresponding to 6.8 x 10^9 spores/kg d.w. soil	*Folsomia candida*	[redacted], S., 2015, M-542556-01-1

Report:	KIIM 8.8; [redacted], S., 2015
Title:	*Purpureocillium lilacinum*: Effects on the reproduction of the collembolan *Folsomia candida* in artificial soil
Report No:	15 10 48 255 S
Document No:	M-542556-01-1
Guidelines:	OECD Guideline 232
GLP:	Yes

Material and methods

The influence on the reproduction of the collembolan species *Folsomia candida* of the test item BioAct WG was tested in artificial soil ([redacted], S.; 2015; M-542556-01-1). 10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated) and vehicle control, 18 - 32 - 56 - 100 - 178 – 316 – 562 - 1000 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 – 800 lux, 16 h light : 8 h dark. Native spores of *Purpureocillium lilacinum* are extremely small, light-weight and electrostatically charged and thus cannot be handled in an open system. This makes weighing the spores and dissolving them in water technically impossible. Therefore, for the purpose of application a vehicle was used consisting to 99.8% of easily digestibly carbohydrates, proteins and lipids. The test item (spores plus vehicle) is denominated as "*Purpureocillium lilacinum* 251 WG 6 W.

During the study, they were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days. The tested reference item was 44, 67, 100, 150 and 225 mg Boric acid/kg d.w. artificial soil. The study was valid.

Findings

Significant differences were measured between the control and the treatment regarding mortality and reproduction at the dose of 1000 mg test item/kg d.w. artificial soil. Thus, the No-Observed-Effect-Concentration (NOEC) for mortality and reproduction is 562 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 1000 mg test item/kg artificial soil dry weight.

Summary

Effects on reproduction and mortality to the soil arthropod Folsomia candida were assessed during a study with the formulation BioAct WG. The study of [redacted] (2015; M-542556-01-1) revealed a No-Observed-Effect-Concentration (NOEC) for mortality and reproduction of 562 mg test item/kg artificial soil dry weight.

[redacted] et al. (2012, M-534518-01-1) tested various alternative methods to control the pest cotton thrips (*Thrips tabaci* Lind.). Therefore *Paecilomyces lilacinus* (or *P. lilacinum*, basing on current information on taxonomy) was tested alone and in combination with other non-conventional agents PyriSec® and neem extract. For detached leaf-disc bioassays, cotton leaf disks were dipped for 5 seconds in *P. lilacinus* conidial suspension, containing (2.3 × 10^9 conidia/mL). Additionally, studies under semi-natural conditions on greenhouse potted plants were carried out by spaying conidial suspension on both sides of the leaves. Leaf-disc bioassays showed an increase of mortality with the exposure time, while the highest thrips mortality with *P. lilacinus* was observed after 10 days exposure period (58.58%). Under greenhouse conditions, similar results were shown. However, mortality was slightly lower after 10 days exposure (53.65%). Nevertheless, higher effects were shown when *P. lilacinus* was blended with PyriSec® or neem extract.

Against the cotton aphid *Aphis gossypii* Glover, *Beauveria bassiana* and *P. lilacinum* were tested, to find alternative control mechanisms against this cotton pest ([redacted] et al., 2014, M-534749-01-1). In this study greenhouse and field trials were carried out. In greenhouse experiments, reproduction tests were conducted on cotton plants. Seeds of cotton plants were inoculated with sterile water (control), 1 × 10^6 spores/mL (treatment 1) and 1 × 10^7 spores/mL (treatment 2). Field trials were carried out twice over two years (2012, 2013). Therefore, five seed inoculation treatments were tested: T1: Control, T2: *B. bassiana* 1 × 10^6 spores/mL, T3: *B. bassiana* 1 × 10^7

spores/mL, T4: *P. lilacinum* 1 × 10^6 spores/mL and T5: *P. lilacinum* 1 × 10^7 spores/mL. Regarding greenhouse tests, aphid reproduction was shown to be reduced on plants treated with *P. lilacinum*. However, this effect was not significant. Although a significant effect of the endophyte treatment was shown for the field trials, *P. lilacinum* did not significantly reduce aphid populations in comparison to the control.

Additionally, a fungal pathogenicity experiment was carried out to assess pathogenicity of *P. lilacinum* on *A. gossypii*. Application rates as described above were used in field and greenhouse trials to treat cotton leaves, placed in petri dishes. In each petri dish, 10 aphids were placed in three replicates and checked for mortality. Dead aphids were placed on PDA medium to confirm emergence of the entomopathogenic fungi. *P. lilacinum* was shown to significantly affect the survival of aphids (60% mortality) in comparison to the control treatment (10% mortality). In conclusion, although both, the greenhouse and the field trials, showed not significant effects of *P. lilacinum* on the pest *A. gossypii*, mortality of *P. lilacinum* on *A. gossypii* was clearly demonstrated in a cotton aphid survival experiment.

Against the urban vector of dengue and yellow fever, the mosquito *Aedes aegypti*, ovicidal activity of 21 fungal species was tested ([redacted] et al., 2007, M-534363-01-1). Eggs were treated topically with 50 µL of suspended conidia at a final density of 5 × 10^6 conidia/cm^2. *P. lilacinus* showed ovicidal activity with 76%, 94% and 86% egg hatching after 5, 10 and 15 days of incubation, respectively. However, egg hatching decreased strongly at 25 days (21%). It was therefore concluded, that *inter alia Paecilomyces* spp. has the potential to be used as for control of *A. aegypti*.

It was also shown, that naturally occurring *P. lilacinum* strains were active against the tick species *Amblyomma cajennense* and *Rhipicephalus sanguineus* (D'Alessandro et al., 2012, M-534519-01-1). *A. cajennense* is a heteroxenic ectoparasite common on horses, which is one of the main vectors of *Riccettsia rickettsi*, the causal agent of Rocky Mountain spotted fever. *R. sanguineus* is another potential vector for *R. rickettsia* in the neotropics, mainly attacking dogs but can also affect humans.

Cited references (abstracts):

Report: KIIM 8.8/01 - [redacted], W.; [redacted], M.U.; [redacted], Y.L.; [redacted]-[redacted]-[redacted]; [redacted], K. (2012), Toxicity of Paecilomyces lilacinus blended with non-conventional agents to control cotton thrips (*Thrips tabaci* Lind.) (Insecta: *Thysanoptera: Thripidae*).
Published report. African Journal of Microbiology Research, 6, 526-533

Abstract: The entomopathogenic fungus *Paecilomyces lilacinus* (2.3 x 10(9) conidia ml(-1)) was blended with other non-conventional agents like *Azadirachta indica* (10 ml L-1) and diatomaceous earth formulation PyriSec (3 g L-1) for the control of cotton thrips (*Thrips tabaci* Lind.) (Insecta: *Thysanoptera*: *Thripidae*) using leaf detached bioassay and under semi-natural conditions. The bioassays were set at 25 +/- 1 degrees C and greater than 70 percent relative humidity at 16L/8D photoperiod and the data for mortality was taken after 2, 4, 6, 8 and 10-d. All the treatments showed significant control of thrips population on cotton. Over all, the application of *P. lilacinus* blended with *A. indica* exhibited higher mortality compared with its combination with PyriSec against *T. tabaci*. The results of the present study showed that *P. lilacinus* may provide effective control of the insect pest when blended with other non-conventional safer control agents.

Report: KIIM 8.8/01 - [redacted], D.C.; [redacted]-[redacted], K.; [redacted]-[redacted], M.J.; [redacted], G.A. (2014), The entomopathogenic fungal endophytes *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) and *Beauveria bassiana* negatively affect cotton aphid reproduction under both greenhouse and field conditions.
Published report. PLoS ONE (2014) , 9, Number 8, e103891 p. 71

Abstract: The effects of two entomopathogenic fungal endophytes, Beauveria bassiana and Purpureocillium lilacinum (formerly *Paecilomyces lilacinus*), were assessed on the reproduction of cotton aphid, Aphis gossypii Glover (*Homoptera: Aphididae*), through in planta feeding trials. In replicate greenhouse and field trials, cotton plants (*Gossypium hirsutum*) were inoculated as seed treatments with two concentrations of *B. bassiana* or *P. lilacinum* conidia. Positive colonization of cotton by the endophytes was confirmed through potato dextrose agar (PDA) media plating and PCR analysis. Inoculation and colonization of cotton by either B. bassiana or *P. lilacinum* negatively affected aphid reproduction over periods of seven and 14 days in a series of greenhouse

trials. Field trials were conducted in the summers of 2012 and 2013 in which cotton plants inoculated as seed treatments with *B. bassiana* and *P. lilacinum* were exposed to cotton aphids for 14 days. There was a significant overall effect of endophyte treatment on the number of cotton aphids per plant. Plants inoculated with *B. bassiana* had significantly lower numbers of aphids across both years. The number of aphids on plants inoculated with *P. lilacinum* exhibited a similar, but non-significant, reduction in numbers relative to control plants. We also tested the pathogenicity of both *P. lilacinum* and *B. bassiana* strains used in the experiments against cotton aphids in a survival experiment where 60 percent and 57 percent of treated aphids, respectively, died from infection over seven days versus 10 percent mortality among control insects. Our results demonstrate (i) the successful establishment of *P. lilacinum* and *B. bassiana* as endophytes in cotton via seed inoculation, (ii) subsequent negative effects of the presence of both target endophytes on cotton aphid reproduction using whole plant assays, and (iii) that the *P. lilacinum* strain used is both endophytic and pathogenic to cotton aphids. Our results illustrate the potential of using these endophytes for the biological control of aphids and other herbivores under greenhouse and field conditions.

Report: KIIM 8.8/01 – [redacted] C.; [redacted], M.H.H.; [redacted], A.H.; [redacted], L.F.N.; [redacted], D.A.S; [redacted], H.H.G. (2007), Ovicidal Activity of Entomopathogenic Hyphomycetes on *Aedes aegypti* (Diptera: Culicidae) Under Laboratory Conditions
Published report.Journal of medical entomology, 44, 799-804

Abstract: Summary: The ovicidal activity of 21 hyphomycete fungi species against *Aedes aegypti* (L.) (*Diptera*: *Culicidae*) was tested. Fungi with ovicidal activity developed on high numbers of eggs (70 percent) during 25 d of exposure. A clear ovicidal activity with low values of hatch (1.3-40 percent) was observed after 25 d of incubation with *Isaria farinosa* (Holm: Fries) Fries, *Paecilomyces carneus* (Duche and Heim) Brown and Smith, *Paecilomyces marquandii* (Massee) Hughes, *Isaria fumosorosea* (Wize), *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Penicillium* sp., *Paecilomyces lilacinus* (Thom) Samson, *Beauveria bassiana* (Balsamo) Vuillemin, and *Evlachovaea kintrischica* Borisov and Tarasov. More than 63 percent of eggs hatched after 25-d exposures to 11 other fungi species deemed as ineffective. These are the first results to show the effects of entomopathogenic fungi against eggs of *Ae. aegypti,* and they suggest their potential as control agents of this vector.

Report: KIIM 8.8/01 – [redacted], W.B; [redacted], R.A.; [redacted] C. (2012), Occurrence of pathogenic fungi to *Amblyomma cajennense* in a rural area of Central Brazil and their activities against vectors of Rocky Mountain spotted fever. Published report.
Veterinary parasitology, 188, 156-159

Abstract: Summary: Two isolates of *Beauveria bassiana* and one *of Purpureocillium lilacinum* (equals *Paecilomyces lilacinus*) were found infecting *Amblyomma cajennense* engorged females collected on horses (0.15 percent infection rate from a total of 1982 specimens) and another two isolates of P. lilacinum and one *Metarhizium anisopliae* detected in soils (2.1 percent from 144 samples) collected in typical pasture habitats of this tick in Central Brazil from October 2009 to March 2011. Fungi were isolated from soils with *Rhipicephalus sanguineus* as surrogate baits. No fungi were found in ticks or soils during the driest months (May to August). Testing pathogenicity of fungi all *R. sanguineus* females were killed regardless of the isolate and fungi sporulated abundantly on the cadavers. *A. cajennense* was less susceptible to infection with *P. lilacinum* within 20 days than *R. sanguineus.* All three fungal species probably act as natural antagonists of *A. cajennense* particularly in the rainy season and have interest for integrate control of vectors of Rocky Mountain spotted fever.

IIM 8.9 Effects on other terrestrial invertebrates

IIM 8.9.1 Effects on earthworms

P. lilacinum was not known to be toxic on earthworms. Moreover, a study on earthworms was conducted with *P. lilacinum* 251 ([redacted], 1998). The results showed that *P. lilacinum* 251 did not affect earthworms.

EU-Dossier: Doc M-IIB, Point 8.5

Report : IIM 8.9.1/01 [redacted]; 1998; M-492004-01): Results of an experiment to test the effect of *Paecilomyces lilacinus* on earthworms

Macquarie University, Sydney, Australia – published: no, report No. 98-1 (Dates of work: not stated)

Guideline: not specified
Deviations: not applicable

GLP: No

Materials and Methods: *P. lilacinum* 251

Earthworms, sized 3 to 15cm, collected from a compost heap.

24 earthworms per treatment were exposed to 2 dose rates of *P. lilacinum*: 0.5 and 5g per 2 litres, and a control comprised 24 earthworms in 2 L soil. All treatment groups were incubated at 21°C for 2 weeks.

At study end, and after 5 and 9 weeks mortality was assessed by counting, and infectivity towards adults and eggs was determined by visual inspection.

Findings: There was no mortality, but reproduction of earthworms in all treatments and at all assessment dates, as indicated by the recovery of additional very small (<1cm) earthworms. No infected worms were found. Eggs appeared not to be infected either, since earthworms continued to hatch within the 7 weeks post-exposure period. The number of hatched earthworms was even higher in the test substance groups. The results for the different assessment dates are summarized in **Table 8.9.1/01-1**.

Table 8.9.1/01-1: Recoveries of earthworms exposed to *P. lilacinum* 251 for 2 weeks

Treatment	2 weeks (exposure end)		5 weeks		9 weeks	
	No. recovered	Change +/- to initial no.	No. recovered	Change +/- to initial no.	No. recovered	Change +/- to initial no.
Untreated	26	+2	30	+6	37	+13
0.5 g */ 2L	29	+5	34	+10	48	+24
5 g */ 2L	30	+6	35	+11	48	+24

*relating to the product "Paecil" (a.s.= *P. lilacinum* at 3 × 10⁹ cfu/g), according to [redacted], 2000

Conclusions: *P. lilacinum* 251 did not cause infection or mortality in earthworms exposed to a high dose of conidia. Although direct exposure of skin and digestive tract via ingestion of spores is likely to have occurred.

This study has not been performed in compliance with directive OECD 207, e.g. the species were not identified and not obtained from cultures out of natural origin, the soil substrate was not specified and there were no replicates. Still, the design of this study is appropriate to conclude that this strain lacks an infection potential towards earthworms and will not be a risk for natural populations of earthworms. The NOEL (no observed effect level) can be assumed to be 2.5g product/L soil.

1st additional Submission (Sept. 2004)

Report: IIM 8.9.1/02 [redacted]; 2004; M-467522-01: Bioact® WG: sublethal toxicity to the earthworm *Eisenia fetida* in artificial soil

[redacted], Germany – published: no, report No. 20041022701-NRH (Dates of work: 19/02/2004 to 16/04/2004)

Guideline: OECD 222
Deviations: At the end of the study, the soil water content of one of the control replicates differed by 23% from its value at the start of the test. It should not differ by more than 10%. This is not considered to have had an effect on the outcome of the study.

GLP: Yes

Materials and Methods: BioAct® WG, active ingredient *Paecilomyces lilacinus*, Strain 251, batch: 1303202111, purity: at least 1.0×10^{10} viable spores/g (nominal); 1.5 x 10^{10} viable spores/g (analysed)

The sublethal toxicity of BioAct® WG to the earthworm *Eisenia foetida* was evaluated during an eight week exposure period. Approx. 600 g (dry weight) artificial soil (10% sphagnum peat, 20% Kaolin clay, 69 % sand, 1 % $CaCO_3$,) was prepared and moistened. On the day of application, the water content of the soil was adjusted to 50 % of the soil water holding capacity with incorporation and mixing in of the test substance. Two treatment rates of 133 and 400 mg BioAct® WG per kg dry soil were tested, corresponding to 2.0×10^9 and 6.0×10^9 viable spores (analysed) of *Paecilomyces lilacinus* per kg dry soil. Ten adult earthworms (between two and twelve months old with clitellum) that had been acclimatised for one day in test soil were rinsed, blotted dry, weighed and placed onto the soil within half an hour of application. A water control group was tested in parallel with four replicates of each treatment group.

Feeding was on a weekly basis for the first four weeks of the study with dry ground manure added to the soil surface and moistened. On day 1 8,5 g of food was mixed into the soil and no more feed was provided for the next 28 days of the study. Test units were maintained under a 16 hour light per day photoperiod at 500 to 660 lux. Four weeks after application, surviving adult worms were removed, counted and weighed and remaining offspring were left for a further four weeks of exposure after which survivors were counted.

In a separate study using identical procedures, a toxic standard Decosal flüssig, 37.49% carbendazim, was tested at a rate of 12.6 mg per kg soil dry weight.

Findings: During the study, the temperature ranged from 20 to 21 °C, soil pH from 6.0 to 6.3 and soil water content from 47.9 to 49.8 %. The effect of the toxic standard on reproduction after 8 weeks was significant, with 4 juveniles recorded as compared to 114 in the control.

In the BioAct® WG treatment groups, observed changes in mortality, weight change and reproduction capacity are presented below in **Table 8.9.1/02-1**.

Following the exposure to BioAct® WG, no mortality was recorded in any of the treatment groups. In the test substance treatment groups, weight change was similar compared to the control and the number of juveniles produced after eight weeks was not significantly different compared to the control.

Table 8.9.1/02-1: Mortality, weight change and reproduction capacity of Eisenia foetida after a 4 to 8 week exposure to 133 and 400 mg BioAct® WG per kg dry soil, corresponding to 2.0 and 6.0 × 10^9 viable spores (analysed) of *P. lilacinum* 251, per kg dry soil, respectively

Treatment (mg/kg soil dry weight) BioAct® WG	Mortality after 4 Weeks (%)	Weight Change after 4 Weeks (%)	Number of Juveniles after 8 Weeks & SD
0 (control)	0	119.3	114 ± 32
133	0	120.9	93 ± 17
400	0	124.5	98 ± 21

Values represent the mean of four replicates with earthworms per replicate

SD Standard Deviation

Conclusions: Following the exposure to BioAct® WG, active ingredient *Paecilomyces lilacinus*, Strain 251 at application rates of 133 and 400 mg per kg dry soil, corresponding to 2.0 and 6.0 × 10^9 viable spores (analysed) of *Paecilomyces lilacinus* per kg dry soil, no effects on mortality, mean body weight change and reproduction were observed.

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New Data 2015

A literature search was conducted to identify the risk of *P. lilacinum* 251 on earthworms (please refer to the literature review report submitted under Point IIM 8). No scientific papers were

identified, presenting any toxic effects of *P. lilacinum* on earthworms. One article was identified studying the effect of *P. lilacinus* soil application on earthworms ([redacted] et al., 2010, M-534380-01-1). *P. lilacinus* was shown not to affect earthworms. However, identification of the strain used was not sufficient, since it was based on morphological characterization only, and the strain ID is not stated. Moreover, the application rate is not described.

Cited references (abstracts):

Report: KIIM 8.9.1/03 – [redacted], R.K.; [redacted], P.K.; [redacted], N.K.; [redacted], A.K.; [redacted], A.K.; [redacted], S.; [redacted], S.C. (2010), Short-term impact of egg parasitic fungi *Paecilomyces lilacinus* and *Verticillium chlamydosporium* on earthworms and mice .
Published report. Proceedings of the Indian National Science Academy Part B Biological Sciences, 80, No. Part 1, pp. 42-46.
Abstract: Studies were conducted on earthworms and mice fed on *Paecilomyces lilacinus* and *Verticillium chlamydosporium* to know their short-term environmental impact. Vermicultures of earthworms with or without *Paecilomyces lilacinus* and *Verticillium chlamydosporium* revealed no adverse impact as evidenced by their growth and absence of external and internal mycosis through gross studies and histopathology. Similarly, experiments with mice fed or unfed on millet grain cultures of *P lilacinus* and *V chlamydosporium* revealed no apparent evidence of growth anomaly and external mycosis. Histopathology of visceral organs of fungus fed and unfed mice revealed no significant differences in cellular details of small intestine, liver, lungs, kidney and spleen.

IIM 8.9.2 Effects on other terrestrial invertebrates

P. lilacinum 251 is intended for the use against plant pathogenic nematodes. Due to its mode of action, various pathogenic nematode species may be affected. To exclude possible effects on beneficial nematode species, two studies were carried out.

EU-Dossier: Doc M-IIB, Point 8.4

General remark: Two studies have been performed with the preparation PBP-01001-I, in compliance with GLP. In addition a non-GLP test with this strain has been performed on beneficial nematodes ([redacted]; 1992; M-489347-01, not published). Further data are available from [redacted]; 2000; M-490114-01, who tested *P. lilacinum* 251 towards several arthropod species within the scope of her thesis.

Report : IIM 8.9.2/1 [redacted]; 1992; M-489347-01): Tests of the active ingredient of the nematicide Nemacheck on some beneficial nematodes
ATIC Pty. Limited; 99 St Johns Road; Glebe, NSW, Australia and School of Biological Sciences, Macquarie University, Sydney, NSW, Australia – published: no, report No. not stated (Dates of work: not stated)
Guideline: not specified (currently no guideline is available for this test)
Deviations: not applicable
GLP: no
Materials and Methods *P. lilacinum* 251
Test species were entomopathogenic nematodes of following species (as representatives for potential biological insecticides): *Heterorhabditis bacteriophora* strain C1 [= *H. heliothidis*]; *Steinernema feltiae* (Filipjev) [= *Neoaplectana bibionis* Bovien]; *S. carpocapseae* (Weiser); *S. glaseri* (Steiner) all employed as unsheathed third stage juveniles, and the common free living nematode *Caenorhabditis elegans*, employed as a mixed population of juveniles and adults.

The test design comprised of 4 replicates per species and 4 for the untreated control. Approximately 200 juvenile nematodes per species were suspended in 80 µL of water and placed at the centre of a 5cm Petri dish containing 2.2% w/w water agar. *P. lilacinum* conidia were harvested from sporulating cultures and transferred to the Petri dishes using a needle to coat the drop of water containing the nematodes with ~5 × 10^7 conidia per treatment. Within 8 h the conidia were absorbed by the agar and came into contact with the nematodes. Treated and untreated dishes were incubated in the dark for an exposure period of 3 days. At 26±1°C. After 3 days mortality was assessed. Dead nematodes were examined under the light microscope (200x) for evidence of fungal growth.

Findings: After 3 days recovery of nematodes was only about 50% of the initially applied number for most species, except *S. carpocapsae*, for all treatments including the control. This high loss can be due to either escaping of nematodes or by nematodes having entered the agar. The percentage of dead nematodes was <3% in any treatment. There was no difference between test substance treated and control nematodes for both, the % recovery and % mortality, as shown in **Table 8.9.2/01-1**.

Examination of all 53 found dead nematodes revealed no colonization or fungal growth in 51 nematodes, and fungal hyphae protruding from the carcasses of two individuals belonging to the species *H. bacteriophora*.

Table 8.9.2/01-1: Mortality of entomopathogenic and free living nematodes exposed to *P. lilacinum* 251 for 3 days (# = numbers as mean of 4 replicates, ± standard deviation, initial number of nematodes: 200)

Nematode species	Treated		Untreated	
	# Live nematodes	# Dead nematodes	# Live nematodes	# Dead nematodes
C. elegans	110± 13	0	97± 7	0
H. bacteriophora	84± 13	3± 0.7*	107± 12	3± 0.8
S. feltiae	98± 7	0.5± 0.6	131± 28	0.5± 0.4
S. carpocapsae	202± 22	3± 0.4	190± 9	2.5± 0.9
S. glaseri	61± 8	0.5± 0.2	72± 6	0.5± 0.2

*two carcasses contained fungal mycelia

Observations: In all treatments the nematodes migrated from the initial site of application, thereby spreading the fungal conidia (if present). Micro-colonies of *P. lilacinum* were observed on all test plates. Most nematodes of the species *C. elegans* and *S. carpocapsae* remained on the agar, while numerous nematodes of the other species had left the agar, partly being found on the lid of the Petri dish.

Conclusions: Mortality under the test conditions employed was very low and not related to the treatment, but to the test species, with some mortality among *H. bacteriophora* and *S. carpocapsae*. The observed fungal growth on 2 of 53 individuals that were found dead do not indicate a potential to parasitize the relevant species, since these 2 individual nematodes represent less than 1% of the specimens exposed to the fungus with 4 × 200 at study start and 1350 nematodes in the test substance group at study termination, due to overall loss.

This positive test result is regarded as worst-case relative to field applications, due to the employed exposure conditions with unrealistically high numbers of conidia coated directly on the nematode cuticle. Further, for entomopathogenic nematodes the tested juvenile stage 3 is an appropriate stage to test side-effects since this is the only stage found outside the insect host, which could be exposed to the fungus.

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New Data 2015

To identify the risk of toxicity of *P. lilacinum* 251 on other non-target organisms, a literature search was conducted (please refer to the literature search submitted in Point IIM 8 or the literature review report ([redacted], 2015 M-542804-01-1).

P. lilacinum has a wide range of activity against nematodes. Thus it has been identified as possible biocontrol agent against other plant pathogen nematodes. *P. lilacinum* was detected to be associated with and to be active against *Criconemoides* sp., a phytoparasitic nematode on sugar cane ([redacted] et al., 2014, M-534524-01-1), or the citrus nematode *Tylenchulus semipenetrans* ([redacted] et al., 2009, M-534377-01-1).

P. lilacinus also has been reported for the dog parasite *Toxocara canis* ([redacted] et al., 2010, M-534376-01-1). *in-vitro* studies with *P. lilacinus* showed 12-20% ovicidal activity with lytic effects accompanied by morphological changes in the embryo and eggshell, with hyphal penetration and internal egg colonization by use of chitinases.

Cited references (abstracts):

Report: KIIM 8.9.2/03 – [redacted]-[redacted], D.; [redacted], G.; [redacted]-[redacted], A. E. (2014), Isolation of fungi associated with *Criconemoides* sp. and their potential use in the biological control of ectoparasitic and semiendoparasitic nematodes in sugar cane.
Published report. Australian Journal of Crop Science, 8, . 389-396

Abstract: Phytoparasitic nematodes are the important pests of sugar cane and controlled with the application of highly toxic chemicals. This study isolated fungi from the sugar cane phytoparasitic nematode *Criconemoides* sp. and tested the pathogenicity of one of these isolates on the nematode community of the sugar cane producing region of [redacted], Mexico. One fungus was selected in order to monitor the in vitro infection process of *Criconemoides* sp. and the effect of this fungus on the density of nematodes associated with sugar cane in greenhouse was evaluated, using naturally infested soil and plants. Two treatments were established: biocontrol, applied with a spore suspension of fungus, and control treatment. A total of 42 fungal isolates were obtained, including Purpureocillium lilacinum, which was selected for use in the in vitro and greenhouse experiments. From 48 h after in vitro infection, blastospores and mycelia were observed within the body of the nematode. The most abundant phytoparasitic nematodes found in samples of the greenhouse experiment were *Criconemoides* sp. and *Helicotylenchus* sp. The initial phytoparasitic nematode populations in biocontrol and control treatments were 253±98 and 287±164 100 mL soilsup^{-1}, respectively. Ten days following application of the fungus, the population of phytoparasitic nematodes was significantly (p less than 0.01) lower in the biocontrol (91±26) than in the control (230±5) treatment. The fungus used in the experiment efficiently reduced the population of ectoparasitic and semiendoparasitic nematodes. We recommend field-testing of this fungus in order to determine its potential effectiveness under field crop conditions.

Report: KIIM 8.9.2/04 – [redacted]-[redacted], S.; [redacted], A.; [redacted], A.M.; [redacted], F.J. (2009), Screening culture filtrates of fungi for activity against *Tylenchulus semipenetrans*.
Published report. Spanish Journal of Agricultural Research, 7, 896-904

Abstract: Culture filtrates of 20 fungi isolated from citrus soils were screened for their activity against *Tylenchulus semipenetrans* in both in vitro and greenhouse tests. The filtrates of *Talaromyces cyanescens* (isolates 2-4 and 2-5), *Paecilomyces lilacinus* , *Chaetomium robustum*, *Acremonium strictum*, *Engyodontium album*, *Myrothecium verrucaria*, *Emericella rugulosa*, and *Tarracomyces gigaspora* consistently inhibited the motility of second-stage juveniles at various concentrations of the filtrate. Dose-response models were used to determine the filtrate concentration required to inhibit the motility of 50 percent of the juveniles (CI50). The culture filtrate of *P. lilacinus* showed the highest activity with a CI50 value of 58 percent that differed from that of *C. robustion* (CI50 equals 68 percent), and *A. strictum* CI50 equals 82 percent. The culture filtrates of *P. lilacinus*, *E. album*, and *T. cyanescens* 2-5 maintained their activity when autoclaved at 120 degrees C for 20 min. The autoclaved filtrate of *T cyanescens* 2-4 was more effective at inhibiting juvenile motility (CI50 equals 28 percent) than that of *T. cyaneseens* 2-5 (CI50 equals 80 percent), *C. robustum* (CI50 equals 72 percent) and *P. lilacinus* (CI50 equals 72 percent). The culture filtrate of *T. cyanescens* 2-4 also inhibited egg hatching. Nematode reproduction on *Cleopatra mandarin* and *Carrizo citrange* were respectively reduced by the culture filtrate of *P. lilacinus* and the autoclaved filtrate of *T. cyanescens* 2-4. These results support the hypothesis that soil fungi may contribute to regulate nematode densities by the production of secondary metabolites with nematicidal activity.

Report: KIIM 8.9.2/05 – [redacted], R.O.; [redacted], J.V.; [redacted], F.R.; [redacted], J.M.; [redacted], C.D.F. (2010), Ovicidal activity of *Pochonia chlamydosporia* and *Paecilomyces lilacinus* on *Toxocara canis* eggs. Published report.
Veterinary parasitology, 169, 123-127

Abstract: Summary: An assessment was made of the ovicidal activity of egg-parasitizing fungi *Pochonia chlamydosporia* (isolates VC1 and VC4) and *Paecilomyces lilacinus* on *Toxocara canis* eggs in vitro. The fungal isolates were inoculated onto Petri dishes with 2 percent water-agar (2 percent WA) and stored at 25.degree.C for 10 days in an incubator, in the dark. The control group was comprised of Petri dishes without fungi, containing the 2 percentWA medium only. Later, 1000 embryonated eggs were placed on the surface of the plates with fungal isolates and also on the control plates, and were then incubated at 25.degree.C for 7, 14 and 21 days. At these intervals, the eggs were retrieved and underwent percentage assessment according to the following parameters: no changes; type 1 effect, physiological and biochemical effect without morphological damage to eggshell, with visualization of hyphae adhered to eggshell; type 2 effect, lytic effect with morphological changes in embryo and eggshell, without hyphal penetration through the

eggshell; type 3 effect, lytic effect with morphological changes in embryo and eggshell, with hyphal penetration and internal egg colonization. All the fungal isolates showed ovicidal activity (type 3 effect) on *T. canis* eggs, with 13.8 percent, 20.5 percent and 20.3 percent of ovicidal activity using *P. chlamydosporia* isolate VC1 after 7, 14 and 21 days, whereas isolate VC4 showed 15.2 percent, 19.0 percent and 21.7 percent of ovicidal activity at the same time intervals. *P. lilacinus* showed ovicidal activity of 12.3 percent, 18.8 percent and 20.0 percent after 7, 14 and 21 days. *P. chlamydosporia* and *P. lilacinus* were effective in vitro on *T. canis* eggs and can be considered a potential candidate to biological controller of those nematodes.

IIM 8.10 Effects on soil micro-organisms

EU-Dossier: Doc M-IIB, Point 8.5

Report : IIM 8.10/01 [redacted], U. (2002) M-467720-01-1. Assessment of the side effects of PBO-01001-I (Paecilomyces lilacinus strain 251 formulated as WG) on the activity of the soil microflora
Arbeitsgemeinschaft [redacted]
Germany – published, no. report No. 20011290/01-ABMF (Dates of work: Aug. 1, 2001 to Sept. 19, 2001)

Guideline: SETAC guideline (March 1995)
Deviations: none

GLP: Yes

Materials and Methods: *P. lilacinum* 251 formulated as WG (code: PBP-10015); purity (nominal) 2×10^9 active conidia/g; batch no.: 20106270; solid granules; lilac in
Soil of stated origin, low in organic carbon and high in sand content (loamy sand) was employed as worst case, with maximum availability of active substance. Soil characteristics were determined, and the soil was sieved to <2mm particle size.
Trial design: A dose rate of 2 × the maximum field dose rate of PBP-01001-I = 60 kg/ha was applied as a stock solution to 6 kg of test soil resulting in calculated 10.0 mg product/kg soil. PBP-01001-I treated and deionized water treated control received Lucerne flour in addition. Soils were thoroughly mixed and subdivided into 3 replicates a 2 kg soil each, placed in 2 L glass bottles for incubation at 20 ± 2°C in the dark under constant humidity conditions.
Samples were taken after 6 h, 14 days and 28 days to determine soil dry weight, pH, ammonium-N, nitrate-N, nitrite-N (changes in the content of different nitrogen forms indicate the nitrogen turnover). In addition a test for short-term respiration was performed on 200 g sub-samples according to the OxiTop®System®, to assess the carbon mineralization capacity.

Findings: Nitrogen transformation: The incubation of soils was terminated at day 28, since the deviation in nitrogen mineralization of control soil and test substance treated soil did not reach the trigger value of 25% defined by the SETAC guideline. The deviation in the nitrate content of PBP-01001-I treated soil compared to control was -16.74%. Results are presented in **Table 8.10/01-1**.

Table 8.10/01-1: Content of mineral N forms in soil treated with PBP-01001-I at the 2 × field dose rate, compared to untreated control soil [mg/100 g dry weight]

Mineral N-form	Treatments and sampling dates						Deviation from the control [%]		
	Control			PBP-01001-I					
	6h	14d	28d	6h	14d	28d	6h	14d	28d
NH4+-N	b.q.[1]	0.25	b.q.	b.q.	0.26	b.q.	---	4.0	---
NO3--N	0.88	1.33	2.15	0.82	0.95	1.79	-6.82	-28.57	-16.74
NO2--N	b.q.	b.q.	b.q.	b.q.	b.q.	b.q.	---	---	---
N sum[2]	0.88	1.58	2.15	0.82	1.21	1.79	-6.82	-23.42	-16.74

[1] b.q.= below the limit of quantification
[2] min = sum of NH4+-N, NO3-- N, NO2--N

Carbon mineralization: There was no significant deviation in short-term respiration among the different treatments at study end, 28 days after treatment. The observed difference of +6.49% for the PBP-01001-I treated soil is in the range of normal variability. Data of this test are summarized in **Table 8.10/01-2**.

Table 8.10/01-2: Results of the short-term respiration test on soil treated with PBP-01001-I at the 2× field dose rate, compared to untreated control soil [mg CO_2/h/100 g dry weight]

sampling dates	Treatments		Deviation from the control [%]
	Control	PBP-01001-I	
6h	1.02	1.25	22.55
14d	0.90	0.97	7.78
28d	0.77	0.82	6.49

Observations: Validity criteria for this study were met, since the pH values of control and test substance treated soil did not vary significantly.

Conclusions: The effects of *P. lilacinum* 251 formulated as WP on the nitrogen turnover and the impact on soil respiration are considered to be negligible.

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Additional information on side-effects of *P. lilacinum* applications on soil micro-organisms can be derived from published references:

[redacted].; 1995; M-489342-01 reports on a greenhouse experiment designed to test the efficacy of *P. lilacinus* in reducing *Meloidogyne javanica* infestation in tomato in presence and absence of mycorrhizal colonization of tomato roots, and to assess the protective effect of mycorrhizal colonization alone. Side-effects on root and plant growth and health were assessed in parallel.

Root systems inoculated with *Glomus mosseae* alone or combined with *P. lilacinus* showed a similar incidence and intensity of mycorrhizal colonization (20-30% incidence and 3-5% intensity). Efficacy of *P. lilacinus* in controlling *M. javanica* was not affected by the combined application with *G. mosseae*. Synergistic effects of *P. lilacinus* and *G. mosseae* were observed for root growth of tomato plants.

A comparable approach is reported for *P. lilacinus* and *B. subtilis* applications, tested singly or in combination, for control of *Meloidogyne incognita* and *Macrophomina phaseolina* on chickpea ([redacted].; [redacted]; 1993; M-489315-01). Although *B. subtilis* was not the target organism to be monitored, indirect conclusions can be drawn based on the improved efficacy for combined applications. Highest reductions in nematode population and galling were achieved when *P. lilacinus* and *B. subtilis* were used together, followed by *P. lilacinus* alone. *B.* subtilis treatment alone was least effective in reducing nematodes and galling. This implies that *B. subtilis* was active and contributed to the efficacy of the increased combined application.

Remarkably there also was a synergistic effect of combined application of *P. lilacinus* and *B. subtilis* on dry shoot weight of nematode infested chickpea.

These results indicate that *P. lilacinum* has no adverse effects on the beneficial mycorrhizal fungi or members of the ubiquitous saprophytic soil bacilli.

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New Data 2015

From the latest literature search (please refer to the literature review report submitted in Point IIM 8), one report was identified studying the effects of *P. lilacinum* 251 on soil microorganisms.

[redacted] et al. (2013, M-534140-01-1) studied the effect of different bio-pesticides as BIOACT® (*P. lilacinum* 251), and QLAgri® (*Quillajia saponaria* plant extract), as well as of the synthetic nematicides oxamyl and fluensulfone on ammonia oxidizing microorganisms (AOM). Moreover, the

effect of the formulation components of BIOACT® on the abundance and function of AOM was determined. It was shown, that QLAgri® and the synthetic nematicides did not influence the microbial community. In contrast, the application of BIOACT® significantly increased the total amount of phospholipid fatty acids (PLFA), which are linked to the fast-growing bacteria and fungi. BIOACT® was also shown to induce transient inhibitory effects on the abundance, diversity and function of ammonium oxidizing bacteria and archaea. However, studies with the BIOACT® components indicated clearly, that effects were not caused by the active substance *P. lilacinum* 251. These effects were expected to be caused indirectly by the co-formulants due to a competitive exclusion by copiotrophic microorganisms feeding on co-formulants

Cited references (abstracts):

Report: KIIM 8.10/04 – [redacted], C.; [redacted], E. S.; [redacted], M.; [redacted], I. O.; [redacted], B. K.; [redacted]-[redacted], U.; [redacted], D. G. 2013). Bio-pesticides: Harmful or harmless to ammonia oxidizing microorganisms? The case of a Paecilomyces lilacinus -based nematicide

Published report. Soil Biology and Biochemistry, 67, 98-105

Abstract: Bio-pesticides are considered as low-risk compds., a belief mainly based on their natural origin rather than on exptl. evidence. Thus there is a need to explore the ecotoxicity of biopesticides and mostly their impact on soil microbes which is largely unknown. The effect of Quillajia saponaria plant ext. (QLAgri) and Paecilomyces lilacinus strain 251 (PL251, BIOACT) on the microbial community was investigated comparatively to the synthetic nematicides oxamyl and fluensulfone. Particular attention was given to potential effects on ammonia-oxidizing bacteria (AOB) and archaea (AOA). No effect of QLAgri, oxamyl and fluensulfone on the soil microbial community and AOB/AOA was obsd. In contrast BIOACT stimulated the growth of copiotrophic Gram neg. bacteria and fungi as detd. by phospholipid fatty acids (PLFA) anal. Terminal restriction fragments length polymorphism (TRFLP) and qPCR anal. of the amoA gene showed a significant time-dependent inhibitory effect of BIOACT on the abundance of AOB/AOA up to 20 days post application. Further qPCR anal. indicated that PL251 did not proliferate in soil. These results suggested the establishment of complex interactions between BIOACT and AOB/AOA which were further explored. In a follow-up study BIOACT and its co-formulants, both induced a transient inhibitory effect on potential nitrification and abundance of AOB/AOA, whereas no effect was seen when PL251 spores were used. Overall, our data suggest that the transient effect of BIOACT on nitrifiers was the result of a competitive exclusion by copiotrophic microorganisms feeding on co-formulants rather than a direct toxicity effect.

IIM 8.11 Other/special studies

New Data 2015

From literature search one new article were identified, which report effects of *P. lilacinus* on vertebrates. However, it has to be considered that all identified reports lack sufficient information in the used identification methods and are therefore reliable only with restrictions.

[redacted] et al. (2005, M-534535-01-1) evaluated the pathogenicity of *P. lilacinus* LPL-01 in rats via several routes of exposure: Oral (1×10^8 CFU/rat), pulmonary (5×10^7 CFU/rat) and intravenous (2.25×10^7 CFU/rat). Clinical examinations were performed daily after administration, and body weight gain of animals was evaluated. Additionally, the authors estimated clearance of the fungus in feces and examination of lungs and blood, depending on the route used, and infectiveness was evaluated by enumerating microorganisms from organs and corporal fluids in animals sacrifice at intervals. A gross necropsy of all animals was performed at interim or final sacrifice. No mortality, and no pathogenicity or toxicity was observed when *P. lilacinus* was administered orally or pulmonary. Some pathogenic effects were observed (anatomical changes in liver and spleen) when intravenous injection was performed. However, this is not a route of exposure for humans and other non-target organisms when the product will be applied according to GAP table. Therefore, a risk can be excluded.

Cited references (abstracts):

Report: KIIM 8.11/01 – [redacted], A. (Correspondence); [redacted], F.; [redacted], B.; [redacted], L.; [redacted], S.; [redacted], A.M.; [redacted], L.; [redacted], Y.; [redacted], M.E.; [redacted], D. (2005), [Pathogenicity evaluation of *Paecilomyces lilacinus* to rats]. Evaluacion de la patogenicidad en ratas del *Paecilomyces lilacinus* LPL-01 utilizando vias diferentes de exposicion.
Published report. Revista de Toxicologia, 22, 185-190

Abstract: Plant parasitic nematodes have been recognized as agricultural pests in Europe as early as the late 19th century. It has been estimated that plant parasitic nematodes cause crop yield losses of nearly 9 percent in the developed world, and over 14 percent in developing countries. The Paecilomyces lilacinus is a parasitic fungi attacking sedentary stages of nematodes, e.g. eggs. Evaluation of this fungus as a microbial control agent, must include an evaluation of its virulence towards non - target organisms, especially vertebrates, with consideration given to potential human exposure scenarios. With the aim of assessing the pathogenicity in rats of the strain LPL-01 of *Paecilomyces lilacinus*, this fungus was given using several routes of exposure (oral, pulmonary and intravenous route). In all of the assays, clinical examinations were performed daily after administration, and body weight gain of animals was evaluated. Clearance was estimated by means of collection of feces and examination of lungs and blood, depending on the route used, and ineffectiveness was evaluated by enumerating microorganisms from organs and corporal fluids in animals sacrifice at intervals. A gross necropsy of all animals was performed at interim or final sacrifice. There were no mortalities, and no evidence of pathogenicity or treatment-related toxicity either in oral or pulmonary toxicity /pathogenicity tests, without significant infection of test animals. In the intravenous toxicity /pathogenicity test, *P. lilacinus* caused anatomopathological changes in liver and spleen at the same period when higher infectivity was achieved. It was concluded that *P. lilacinus* is not pathogenic by oral and pulmonary route, but has some pathogenic effects when intravenous injection is performed.

References

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 8 /01	[redacted], I.	2015	Literature review on Purpureocillium lilacinum strain 251 and metabolites: Section 6: Effects on non-target organisms [redacted], Germany Bayer CropScience, Report No.: M-542804-01-1, Edition Number: M-542804-01-1 Date: 2015-11-23 GLP/GEP: n.a., unpublished **...also filed: KIIM 8.2 /02** **...also filed: KIIM 8.3 /03** **...also filed: KIIM 8.5 /01** **...also filed: KIIM 8.7 /01** **...also filed: KIIM 8.8 /09** **...also filed: KIIM 8.9.2 /03**	Yes	Bayer CropScience
KIIM 8.1 /01	[redacted] J.; [redacted], M.	2015	Acute oral LIMIT-test toxicity of of Purpureocillium lilacinum to bobwhite quail (Colinus virginianus) in accordance with OECD 223 and US EPA OCSPP 850 Bayer CropScience, Report No.: M2902363-5, Edition Number: M-534859-01-1 Date: 2015-09-21 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.2 /01	[redacted] A.	2001	Acute toxicity testing of PBP-01001-1 (Paecilomyces lilacinus, Strain 251, formulated as WG) in rainbow trout (Oncorhynchus mykiss) (Teleostei, Salmonidae) [redacted], Germany Bayer CropScience, Report No.: 20011290/01-AAOm, Edition Number: M-467660-01-1 Date: 2001-12-18 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 8.2 /02	[redacted], I.	2015	Literature review on Purpureocillium lilacinum strain 251 and metabolites: Section 6: Effects on non-target organisms [redacted] Germany Bayer CropScience, Report No.: M-542804-01-1, Edition Number: M-542804-01-1 Date: 2015-11-23 GLP/GEP: n.a., unpublished **...also filed: KIIM 8 /01** **...also filed: KIIM 8.3 /03** **...also filed: KIIM 8.5 /01** **...also filed: KIIM 8.7 /01** **...also filed: KIIM 8.8 /09** **...also filed: KIIM 8.9.2 /03**	Yes	Bayer CropScience
KIIM 8.3 /01	[redacted], A.	2001	Assessment of toxic effects of PBP-01001-I (Paecilomyces lilacinus, Strain 251, formulated as WG) on Daphnia magnus using the 48 h acute immobilisation test [redacted], Germany Bayer CropScience, Report No.: 20011290/01-AADm, Edition Number: M-467656-01-1 Date: 2001-12-18 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.3 /02	[redacted], R. G.	2000	Paecilomyces lilacinus as a biocontrol agent - Chapter 6 - The effect of Paecilomyces lilacinus on non-target invertebrates Report No.: M-490114-01-1, Edition Number: M-490114-01-1 GLP/GEP: n.a., published **...also filed: KIIM 7.1.2 /01** **...also filed: KIIM 8.8 /03** **...also filed: KIIM 8.9.2 /02**	No	

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 8.3 /03	[redacted], I.	2015	Literature review on Purpureocillium lilacinum strain 251 and metabolites: Section 6: Effects on non-target organisms [redacted] Bayer CropScience, Report No.: M-542804-01-1, Edition Number: M-542804-01-1 Date: 2015-11-23 GLP/GEP: n.a., unpublished **...also filed: KIIM 8 /01** **...also filed: KIIM 8.2 /02** **...also filed: KIIM 8.5 /01** **...also filed: KIIM 8.7 /01** **...also filed: KIIM 8.8 /09** **...also filed: KIIM 8.9.2 /03**	Yes	Bayer CropScience
KIIM 8.4 /01	[redacted], D.	2001	Testing of toxic effects of PBP-2001-1 (*Paecilomyces lilacinus* Strain 251, formulated as WG) on the Single Cell Green Alga *Desmodesmus subspicatus* [redacted] Germany Bayer CropScience, Report No.: 2001290/01-AADs, Edition Number: M-467680-01-1 Date: 2001-11-12 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.5 /01	[redacted], I.	2015	Literature review on Purpureocillium lilacinum strain 251 and metabolites: Section 6: Effects on non-target organisms [redacted] Germany Bayer CropScience, Report No.: M-542804-01-1, Edition Number: M-542804-01-1 Date: 2015-11-23 GLP/GEP: n.a., unpublished **...also filed: KIIM 8 /01** **...also filed: KIIM 8.2 /02** **...also filed: KIIM 8.3 /03** **...also filed: KIIM 8.7 /01** **...also filed: KIIM 8.8 /09** **...also filed: KIIM 8.9.2 /03**	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 8.7 /01	[redacted], I.	2015	Literature review on Purpureocillium lilacinum strain 251 and metabolites: Section 6: Effects on non-target organisms [redacted] Germany Bayer CropScience Report No.: M-542804-01-1, Edition Number: M-542804-01-1 Date: 2015-11-23 GLP/GEP: n.a., unpublished **...also filed: KIIM 8 /01** **...also filed: KIIM 8.2 /02** **...also filed: KIIM 8.3 /03** **...also filed: KIIM 8.5 /01** **...also filed: KIIM 8.8 /09** **...also filed: KIIM 8.9.2 /03**	Yes	Bayer CropScience
KIIM 8.8 /01	[redacted], H.	2001	PBP-01001-l: Toxicity to the predatory Mite, Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the laboratory [redacted], Germany Bayer CropScience Report No.: 2001/290/01-NLTp, Edition Number: M-467670-01-1 Date: 2001-11-26 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.8 /02	[redacted]	1992	Tests of the active ingredient of the bionematicide nemacheck on some beneficial nematodes Year:1992, Report No.: M-489347-01-1, Edition Number: M-489347-01-1 GLP/GEP: n.a., published **...also filed: KIIM 8.9.2 /01**	No	
KIIM 8.8 /03	[redacted], R.J.	2000	Paecilomyces lilacinus as a biocontrol agent - Chapter 6 - The effect of Paecilomyces lilacinus on non-target invertebrates Report No.: M-490114-01-1, Edition Number: M-490114-01-1 GLP/GEP: n.a., published **...also filed: KIIM 7.1.2 /01** **...also filed: KIIM 8.3 /02** **...also filed: KIIM 8.9.2 /02**	No	

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 8.8 /04	[redacted], M.	2001	PBP-01001-l: Acute toxicity to the Aphid Parasitoid, Aphidius rhopalosiphi (Hymenoptera, Braconidae) in the laboratory Arbeitsgemeinschaft GAP [redacted], Germany Bayer CropScience, Report No.: 20011290/01-LAp, Edition Number: M-467682-01-1 Date: 2001-10-10 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.8 /05	[redacted], J.	2004	Toxicity to larvae of the ground beetle, Poecilus cupreus L. (Coleoptera, Carabidae) in the laboratory - Bio Act WG [redacted], Germany Bayer CropScience, Report No.: 20041022/01-NEAc, Edition Number: M-467505-01-1 Date: 2004-08-12 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.8 /06	[redacted], J.	2004	An extended laboratory study conducted on natural sil to evaluate the effects on the rove beetle, Aleochara bilineata Gyll. (Coleoptera, Staphylinidae) - Bio Act WG [redacted], Germany Bayer CropScience, Report No.: 20041022/01-NEAb, Edition Number: M-467517-01-1 Date: 2004-06-07 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.8 /07	[redacted], W.	2003	Paecilomyces lilacinus strain 251 - PBP-01001-I - Annex IIB, point 8 resp. annex HIB, point 10: Effects on non-target organisms - Statement to the draft monograph (DAR) [redacted], Germany Bayer CropScience, Report No.: M-542628-01-1, Edition Number: M-542628-01-1 Date: 2003-11-01 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 8.8 /08	[redacted], A.; [redacted], K.; [redacted], H.	2003	Testing the nematophagous biological control strain Paecilomyces lilacinus 251 for paecilotoxin production Publisher:Elsevier, Journal:FEMS Microbiology Letters, Volume:227, Pages:107-111, Year:2003, Report No.: M-542637-01-1, Edition Number: M-542637-01-1 GLP/GEP: n.a., published	No	
KIIM 8.8 /09	[redacted], I.	2015	Literature review on Purpureocillium lilacinum strain 251 and metabolites; Section 6: Effects on non-target organisms [redacted] Germany Bayer CropScience, Report No.: M-542804-01-1, Edition Number: M-542804-01-1 Date: 2015-11-23 GLP/GEP: n.a., unpublished **...also filed: KIIM 8 /01** **...also filed: KIIM 8.2 /02** **...also filed: KIIM 8.3 /03** **...also filed: KIIM 8.5 /01** **...also filed: KIIM 8.7 /01** **...also filed: KIIM 8.9.2 /03**	Yes	Bayer CropScience
KIIM 8.8 /10	[redacted] S.	2015	Purpureocillium lilacinum: Effects on the reproduction of the collembolan Folsomia candida in artificial soil [redacted] Germany Report No.: 15 10 48 255 S, Edition Number: M-542556-01-1 Date: 2015-12-15 GLP/GEP: yes, unpublished	Yes	
KIIM 8.8 /11	[redacted], W. J. A.; [redacted] M. U.; [redacted], Y. J.; [redacted], K.	2012	Toxicity of Paecilomyces lilacinus blended with non-conventional agents to control cotton thrips (Thrips tabaci Lind.) (Insecta: Thysanoptera: Thripidae). Year:2012, Report No.: M-534518-01-1, Edition Number: M-534518-01-1 Date: 2012-12-31 GLP/GEP: no, published	No	

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KIIM 8.8 /12	[redacted], D. [. A.; [redacted], K.; [redacted], M.; [redacted], G. A.	2014	The Entomopathogenic Fungal Endophytes Purpureocillium lilacinum (Formerly Paecilomyces lilacinus) and Beauveria bassiana Negatively Affect Cotton Aphid Reproduction under Both Greenhouse and Field Conditions. Year: 2014, Report No.: M-534749-01-1, Edition Number: M-534749-01-1 Date: 2014-12-31 GLP/GEP: no, published	No	
KIIM 8.8 /13	[redacted], C.; [redacted], M. H. H.; [redacted], A. H.; [redacted], L. F. N.; [redacted], D. A. S.; [redacted], H. H. G.	2007	Ovicidal Activity of Entomopathogenic Hyphomycetes on Aedes aegypti (Diptera: Culicidae) Under Laboratory Conditions Journal:Journal of medical entomology (2007) , Year:2007, Report No.: M-534363-01-1, Edition Number: M-534363-01-1 Date: 2007-12-31 GLP/GEP: no, published	No	
KIIM 8.8 /14	[redacted], W. B.; [redacted], R. A.; [redacted], C.	2012	Occurrence of pathogenic fungi to Amblyomma cajennense in a rural area of Central Brazil and their activities against vectors of Rocky Mountain spotted fever Journal:Veterinary parasitology (2012) , Year:2012, Report No.: M-534519-01-1, Edition Number: M-534519-01-1 Date: 2012-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /29**	No	
KIIM 8.9.1 /01	[redacted] R.	1998	Results of an experiment to test the effect of paecilomyces lilacinus on earthworms Report No.: M-492004-01-1, Edition Number: M-492004-01-1 GLP/GEP: n.a., published	No	

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KIIM 8.9.1 /02	[redacted], J.	2004	BioAct WG: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil [redacted], Germany Bayer CropScience, Report No.: 20041032/01-NREf, Edition Number: M-467522-01-1 Date: 2004-06-04 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.9.1 /03	[redacted], R. K. [. A.; [redacted], P. K.; [redacted], N. K.; [redacted], A. K.; [redacted], A. K.; [redacted], S.; [redacted] S. C.	2010	Short-term impact of egg parasitic fungi Paecilomyces lilacinus and Verticillium chlamydosporium on earthworms and mice . Year:2010, Report No.: M-534380-01-1, Edition Number: M-534380-01-1 Date: 2010-12-31 GLP/GEP: no, published	No	
KIIM 8.9.2 /01	[redacted]	1992	Tests of the active ingredient of the biomaticide nematode on some beneficial nematodes Year: 1992, Report No.: M-489347-01-1, Edition Number: M-489347-01-1 GLP/GEP: n.a., published **...also filed: KIIM 8.8 /02**	No	
KIIM 8.9.2 /02	[redacted], R.A.	2000	Paecilomyces lilacinus as a biocontrol agent - Chapter 6 - The effect of Paecilomyces lilacinus on non-target invertebrates Report No.: M-490114-01-1, Edition Number: M-490114-01-1 GLP/GEP: n.a., published **...also filed: KIIM 7.1.2 /01** **...also filed: KIIM 8.3 /02** **...also filed: KIIM 8.8 /03**	No	

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KIIM 8.9.2 /03	[redacted], I.	2015	Literature review on Purpureocillium lilacinum strain 251 and metabolites: Section 6: Effects on non-target organisms [redacted] Germany Bayer CropScience, Report No.: M-542804-01-1, Edition Number: M-542804-01-1 Date: 2015-11-23 GLP/GEP: n.a., unpublished **...also filed: KIIM 8 /01** **...also filed: KIIM 8.2 /02** **...also filed: KIIM 8.3 /03** **...also filed: KIIM 8.5 /01** **...also filed: KIIM 8.7 /01** **...also filed: KIIM 8.8 /09**	Yes	Bayer CropScience
KIIM 8.9.2 /04	[redacted], D.; [redacted], G.; [redacted], A. E.	2014	Isolation of fungi associated with Criconemoides sp. and their potential use in the biological control of ectoparasitic and semiendoparasitic nematodes in sugar cane. Journal Australian Journal of Crop Science (2014) , Year:2014, Report No.: M-534524-01-1, Edition Number: M-534524-01-1 Date: 2014-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /24**	No	
KIIM 8.9.2 /05	[redacted], [redacted], [redacted], A.; [redacted], A. M.; [redacted], F. J.	2009	Screening culture filtrates of fungi for activity against Tylenchulus semipenetrans. Year: 2009, Report No.: M-534377-01-1, Edition Number: M-534377-01-1 Date: 2009-12-31 GLP/GEP: no, published **...also filed: KIIM 2.3.2 /21** **...also filed: KIIM 2.4 /26**	No	

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KIIM 8.9.2 /06	[redacted]	2010	Ovicidal activity of Pochonia chlamydosporia and Paecilomyces lilacinus on Toxocara canis eggs Journal:Veterinary parasitology (2010) , Year:2010, Report No.: M-534376-01-1, Edition Number: M-534376-01-1 Date: 2010-12-31 GLP/GEP: no, published	No	
KIIM 8.10 /01	[redacted]	2002	Assessment of the side effects of PBR1001 (Paecilomyces lilacinus strain 251 formulated as WG) on the activity of the Soil Microflora Arbeitsgemeinschaft [redacted] Germany, Bayer CropScience, Report No.: 2001/990/01/ABMF, Edition Number: M-467720-01-1 Date: 2002-01-09 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIM 8.10 /02	[redacted]	1995	Interaction of glomus mosseae and paecilomyces lilacinus on meloidogyne javanica of tomato Publisher:Springer-Verlag, Journal:Mycorrhiza, Pages:233-236, Year:1995, Report No.: M-489342-01-1, Edition Number: M-489342-01-1 GLP/GEP: n.a., published **...also filed: KIIM 2.4 /21** **...also filed: KIIM 2.7.1 /01**	No	
KIIM 8.10 /03	[redacted]	1993	Biological control of meloidogyne incognita race 3 and macrophomina phaseolina by paecilomyces lilacinus and bacillus subtilis alone and in combination on chickpea Publisher:Gauthier-Villars - Ostrom, Journal:Fundam. appl. Nematol., Pages:215-218, Year:1993, Report No.: M-489515-01-1, Edition Number: M-489515-01-1 GLP/GEP: n.a., published	No	

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KIIM 8.10 /04	[redacted]	2013	Bio-pesticides: Harmful or harmless to ammonia oxidizing microorganisms? The case of a Paecilomyces lilacinus -based nematicide Year:2013, Report No.: M-534140-01-1, Edition Number: M-534140-01-1 Date: 2013-12-31 GLP/GEP: no, published	No	
KIIM 8.11 /01	[redacted]	2005	[Pathogenicity evaluation of Paecilomyces lilacinus to rats]. Evaluacion de la patogenicidad en ratas del Paecilomyces lilacinus LPL-01 utilizando vias diferentes de exposicion. Year:2005, Report No.: M-534535-01-1, Edition Number: M-534535-01-1 Date: 2005-12-31 GLP/GEP: no, published **...also filed: KIIM 2.4 /40**	No	