FACULTY OF BIOSCIENCE ENGINEERING

FOOD SAFETY EVALUATION OF THE USE OF *B. THURINGIENSIS* AS A BIOLOGICAL CONTROL AGENT IN PRIMARY PRODUCTION OF LETTUCE

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Background and research objective

Bacillus thuringiensis (Bt):

1) A soil-dwelling bacterium closely related to the human pathogen *B. cereus*
2) An often used microbial control agent

Does the use of Bt-containing Plant Protection Products (PPPs) lead to an increased risk for food intoxications when plant becomes food?

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Need to perform a risk analysis

Hazard identification

- *B. cereus:* emetic and diarrhoeal syndrome.
- Classical cultural detection, nor 16S rDNA sequencing can differentiate *B. cereus* and Bt.
- Two papers report foodborne outbreaks related to Bt.
- One specific outbreak related to biocontrol strain.

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Bt established as microbial hazard
Needs further study to assess the risk

Hazard characterization

Based on literature (refer to De Bock et al., 2019):

- *B. cereus* enterotoxin genes: also in Bt.
- Enterotoxin production in vitro: also in Bt.
- Cytotoxic effects in Vero cell assays: also in Bt.
- In vivo experiments & GI tract simulations: contradictory results on Bt spores germination and subsequent enterotoxin production.

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No evidence to conclude that Bt has a higher infective dose compared to *B. cereus*

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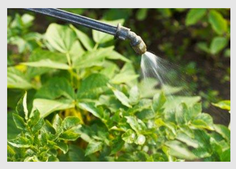
Suggested: maximum 10^5 CFU/g as safety limit

Exposure assessment

PLANTING

Expected
10^2 CFU/g
(naturally present by transfer from soil)

Observed
≤ 10^2 CFU/g
(non-treated plants)

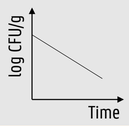


SPRAYING

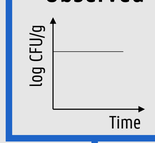
Calculated
10^5 CFU/cm²
(based on maximum dosage of 1 kg/ha)

PLANT GROWTH

Expected



Observed

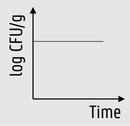


HARVEST

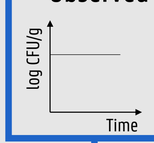
Calculated
Maximum
10^4 – 10^6 CFU/g
(worst case)

DISTRIBUTION

Expected



Observed



CONSUMER

Calculated
Maximum
10^4 – 10^6 CFU/g
(worst case)

In practice
≤ 10^4 CFU/g
(butterhead lettuce in supermarkets)

Risk characterization

IN THEORY
Maximum concentration > safety limit

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Increased risk for foodborne disease

IN PRACTICE
No strong epidemiological evidence
+
No elevated numbers of presumptive *B. cereus* found on lettuce in retail market

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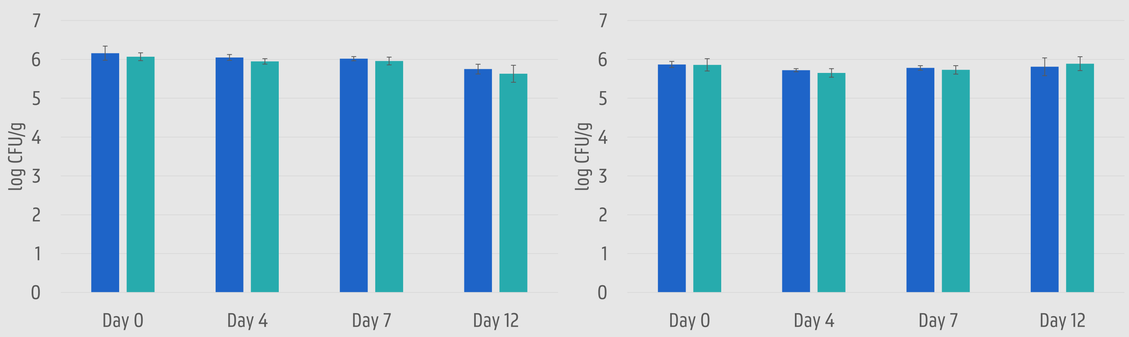
The food safety risk is assumed to be low

Pre-harvest experimental data

Methods

LETTUCE PLANTS → **Inoculation** Spray inoculation with Bt and *B. cereus* spores → **Plant growth** Open to air, in front of window, RT (22°C) → **Analysis** Presumptive *B. cereus* (total and spores) using MYP agar

Results



Pre-harvest persistence of *B. thuringiensis* (left) and *B. cereus* (right) spores during plant growth of 12 days. Blue bars show total presumptive *B. cereus*, green bars show presumptive *B. cereus* spores.

Post-harvest experimental data

Methods

LETTUCE LEAVES → **Inoculation** Spray inoculation with Bt and *B. cereus* spores and vegetative cells → **Storage** Packed in stomacher bags, sealed and stored at 12°C → **Analysis** Presumptive *B. cereus* (total and spores) using MYP agar

Results

Post-harvest persistence of *B. thuringiensis* and *B. cereus* spores and vegetative cells during cold storage (12°C) of 7 days. PBC = presumptive *B. cereus*, Bt = *B. thuringiensis*, Bc = *B. cereus*.

Inoculum	Day 0		Day 7	
	Total PBC (log CFU/g)	PBC spores (log CFU/g)	Total PBC (log CFU/g)	PBC spores (log CFU/g)
Bt (XenTari®) spores	5.82 ± 0.04	5.84 ± 0.02	6.02 ± 0.17	5.94 ± 0.09
Bt (XenTari®) spores	3.03 ± 0.10	3.00 ± 0.00	3.04 ± 0.07	3.02 ± 0.11
Bt (XenTari®) vegetative cells	3.38 ± 0.07	<2.00 ± 0.00	2.16 ± 0.28	<2.00 ± 0.00
Bc (FMFP 311) spores	3.78 ± 0.14	3.73 ± 0.04	3.64 ± 0.17	3.68 ± 0.09
Bc (FMFP 311) vegetative cells	3.46 ± 0.17	<2.00 ± 0.00	<2.00 ± 0.00	<2.00 ± 0.00

Risk management options

Risk interpreted as intolerable

- Maximum concentration may exceed the safety limit, product should be banned.
- Shifts the pesticide usage back to chemical products.

Risk interpreted as tolerable

- Lowering the allowed dose in primary production, lowers the maximum theoretical concentration as well.
- More research needed: possible to lower the dose without lowering the plant protection capacity?

Risk interpreted as acceptable

- Risk is assumed to be low and can therefore be accepted.
- Communication to vulnerable groups not to consume these Bt-treated products.

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