From: Maxson-Rushton Kimberly <KRUSHTON@cooperlevenson.com>
Sent: Monday, February 22, 2021 8:34 PM
To: Amber Virkler; Tyler Klimas
Cc: GTerry@radsource.com; Will Hartman (whartman@radsource.com)
Subject: RAD Source Technologies - CAPH X-ray Study
Attachments: x-ray remediation results 12_21_2020.pptx

Importance: High

Dear Chairman Douglas, Members of the CCB and Ex. Dir. Klimas,

Please find attached hereto a study conducted in January 2021 by the Cannabis Testing Section, Food and Drug Laboratory Branch, California Department of Public Health on the effectiveness of X-ray radiation in remediation of *Aspergillus in* contaminated cannabis flower. The objective of the study was to determine if X-ray irradiation is a feasible remediation for cannabis flowers that are contaminated with any of the four pathogenic species of *Aspergillus (A. niger, A. terreus, A. flavus, and A. fumigatus)*.

On behalf of RAD Source Technologies, we'd respectfully request that the study be made a part of the "public record" specific to the **Petition to Repeal CCB Regulation 12.065**. Additionally, RAD would request that the study be identified during "Public Comment" at the regularly scheduled February CCB Meeting (*in the interest of time the CCB may consider noting that a study was submitted by RAD Source Technologies in support of the Petition to Repeal Reg. 12.065 then reading into the record slides 1, 2, and 15, which describes the entity performing the study, the objective of the study and findings).

Thank you for your consideration of this matter.

Warmly,
Kimberly

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Use of X-ray radiation to remediate Aspergillus contaminated cannabis flower

Cannabis Testing Section

Food and Drug Laboratory Branch

California Department of Public Health

Objective:

- Determine whether x-ray irradiation can be a feasible method of remediating cannabis flower contaminated with any of the 4 pathogenic species of Aspergillus (*A. niger*, *terreus*, *flavus* and *fumigatus*).

Aspergillus strain type	Cannabis flower weight (grams)	Initial irradiation dosage (grays)	Aspergillus detection via PCR of spiked Cannabis flower treated with 2000 grays of x-rays	Aspergillus viability/growth on PDA plates post enrichment (2000 grays)	Aspergillus detection via PCR of naturally contaminated Cannabis flower treated with 2500 grays of x-rays	Aspergillus viability/growth on PDA plates post enrichment (2500 grays)	Aspergillus detection via PCR of spiked Cannabis flower treated with 5000 grays of x-rays	Aspergillus viability/growth on PDA plates post enrichment (5000 grays)
Aspergillus niger	1 g	2000, 2500 and 5000 grays	Detected	Not Detected	Not Detected	Not Detected	Detected	Not Detected
Aspergillus flavus	1 g	2000, 2500 and 5000 grays	Detected	Detected	Not Detected	Not Detected	Detected	Not Detected
Aspergillus terreus	1g	2000, 2500 and 5000 grays	Detected	Not detected	Not Detected	Not Detected	Detected	Not Detected
Aspergillus fumigatus	1 g	2000, 2500 and 5000 grays	Detected	Detected	Not Detected	Not Detected	Detected	Not Detected

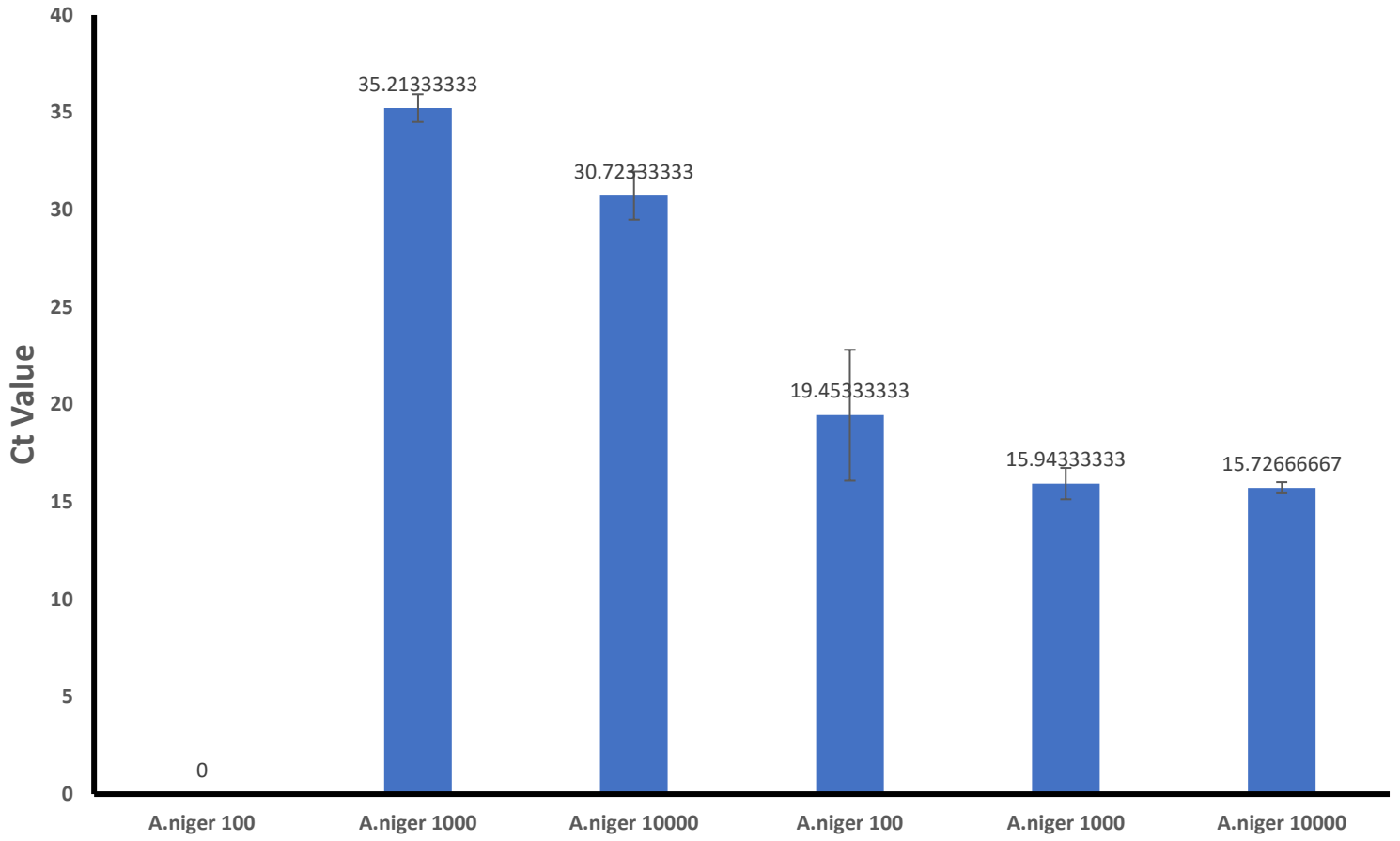
Experimental Design

Contaminated cannabis flower was weighed out (1g/sample) and pretreated with 2.5kGy of X-rays

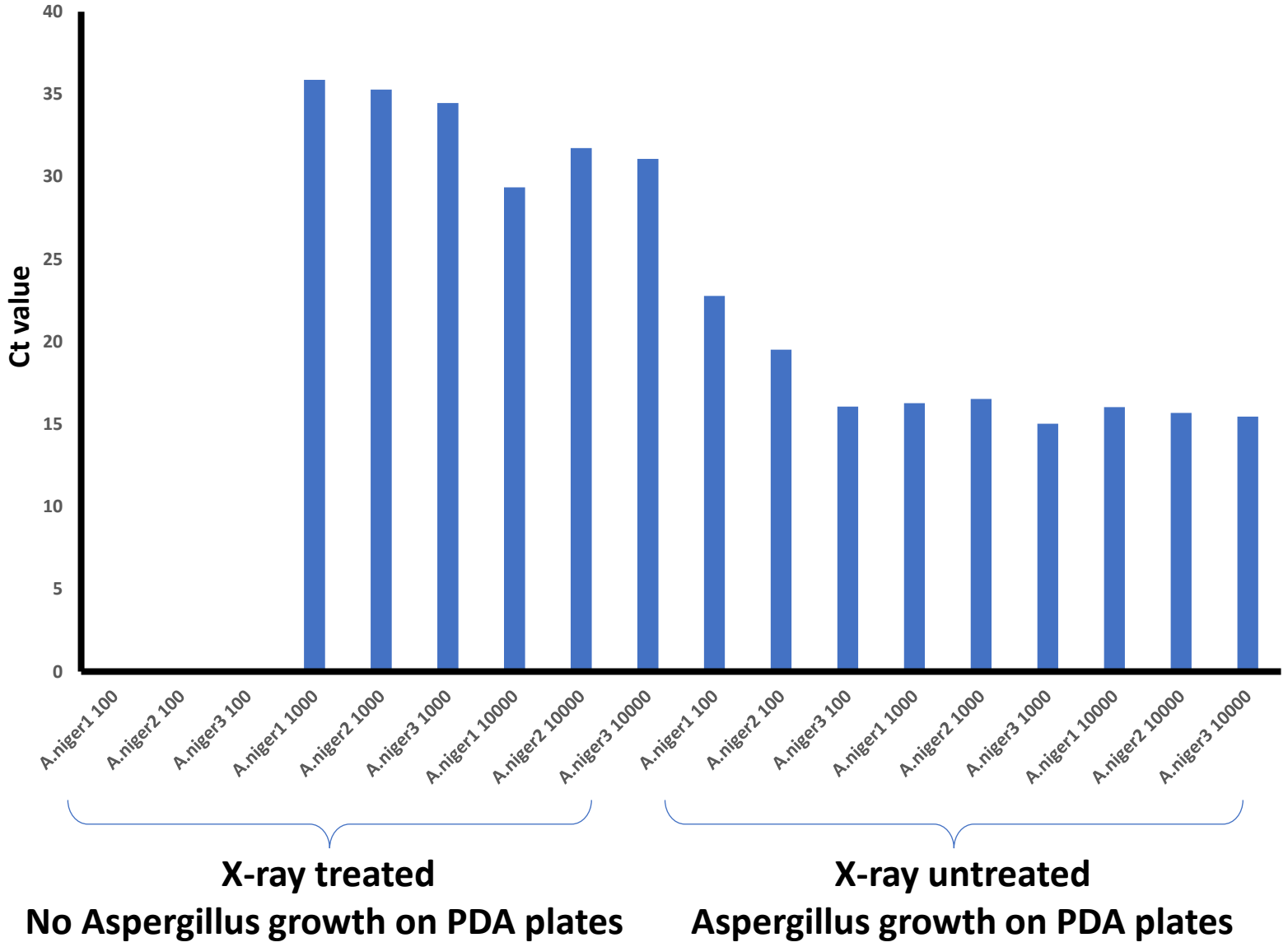
Samples were inoculated with known amount of Aspergillus spores 100, 1000 or 10,000 (3x). One set of samples was then treated with x-rays again while the second set served as untreated controls.

All samples were then processed according to CTS Aspergillus in flower detection method. Enrichments were also plated on PDA plates to determine viability.

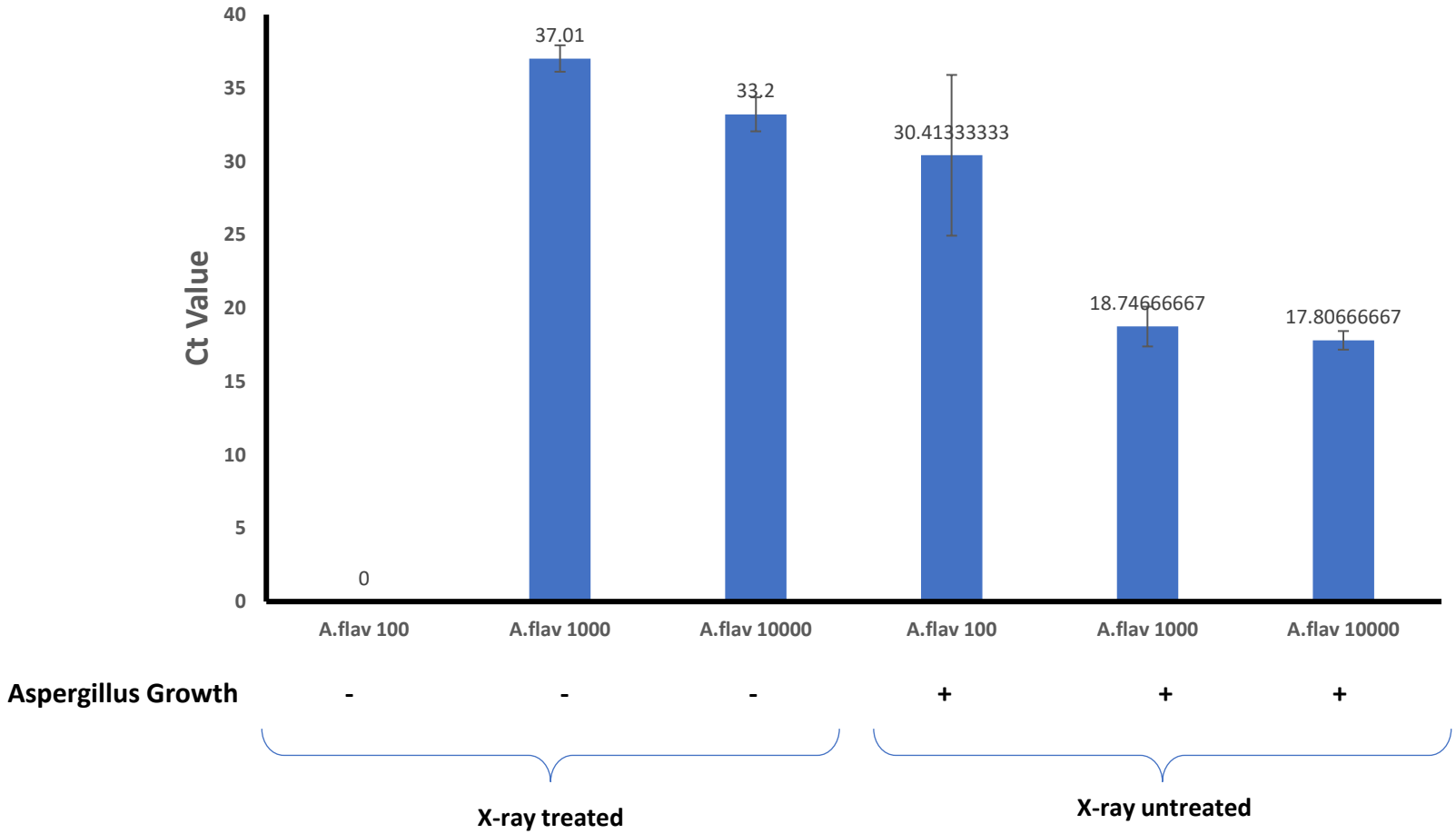
Aspergillus niger



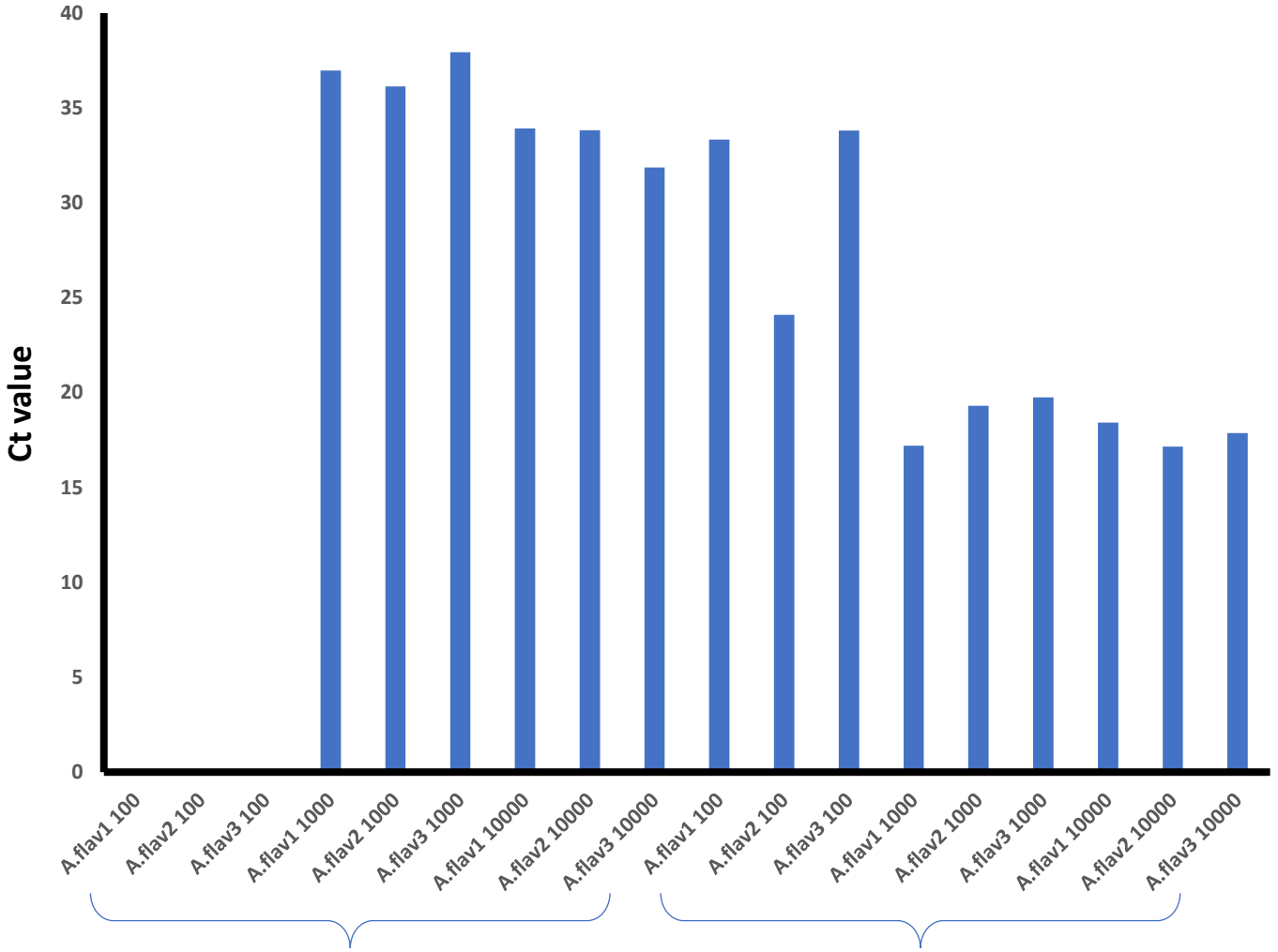
Aspergillus niger



Aspergillus flavus



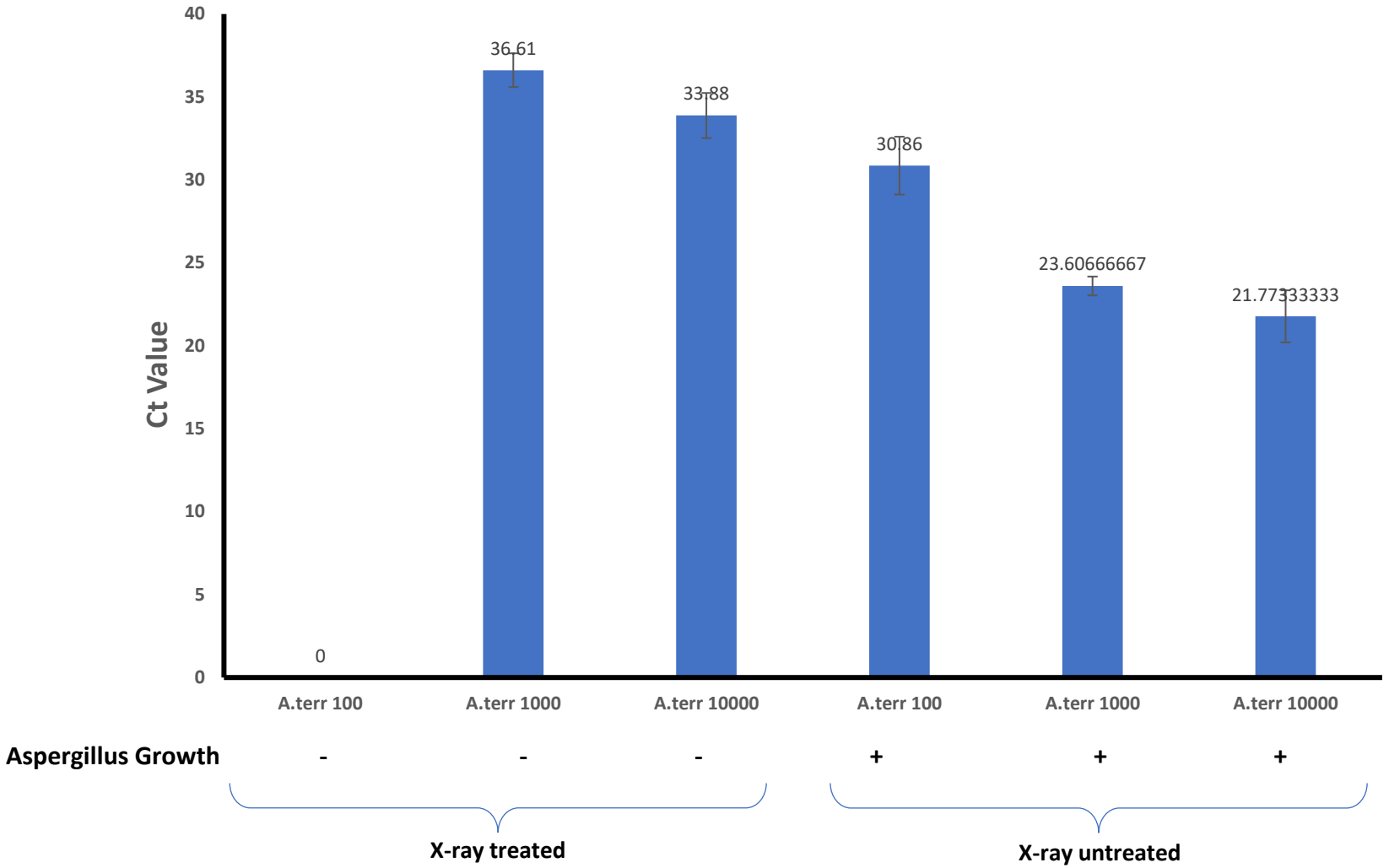
Aspergillus flavus



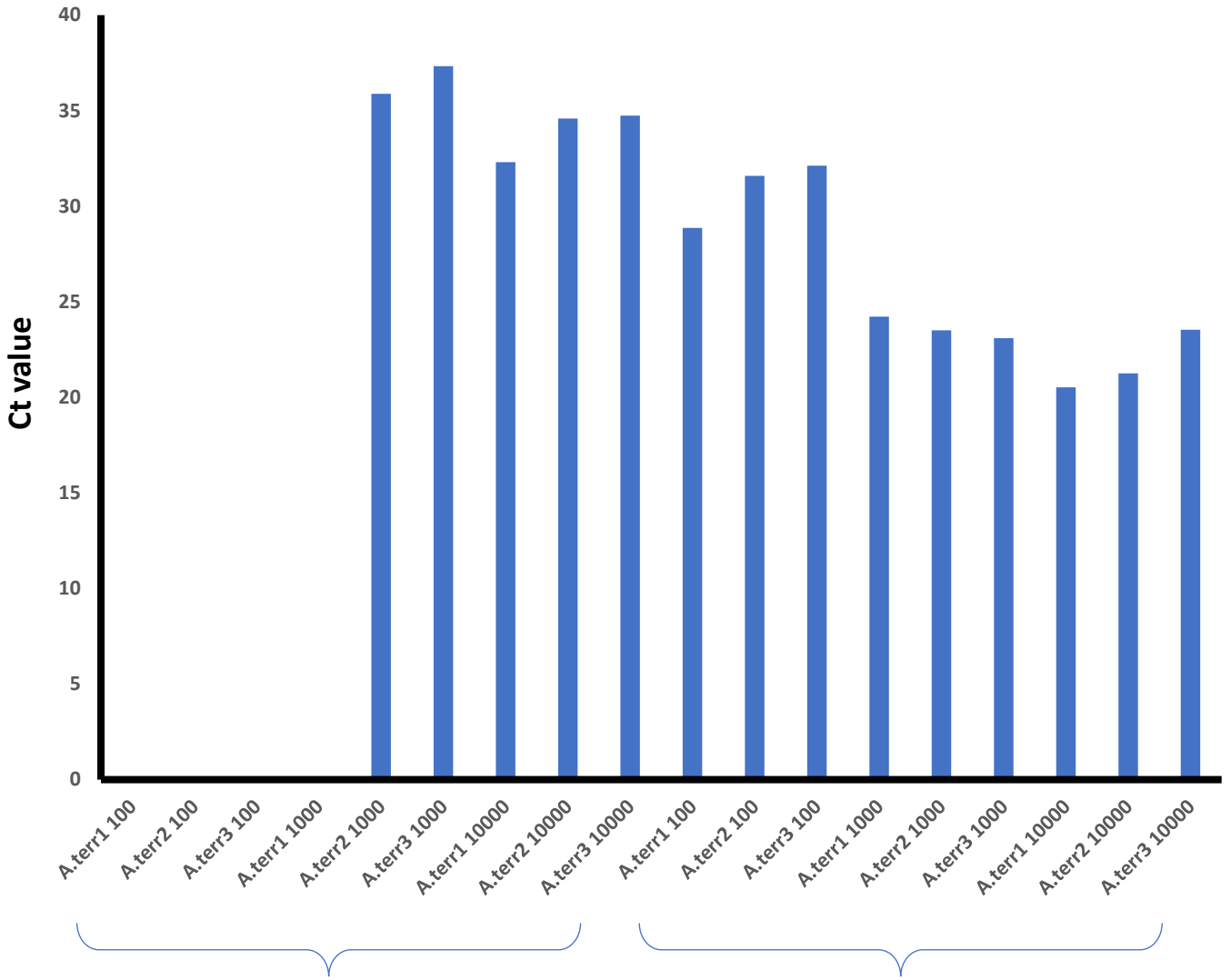
X-ray treated
No Aspergillus growth on PDA plates

X-ray untreated
Aspergillus growth on PDA plates

Aspergillus terreus



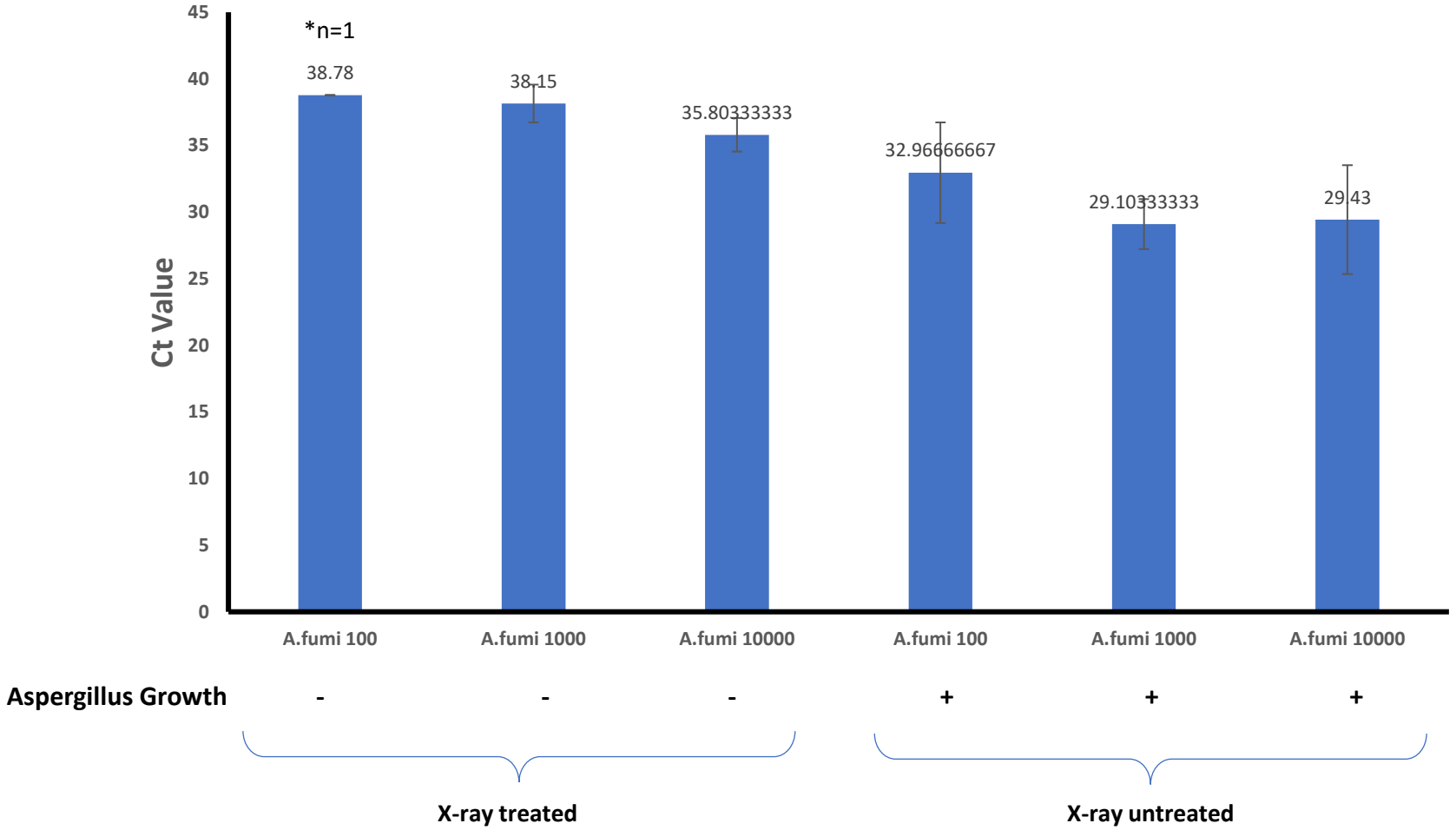
Aspergillus terreus

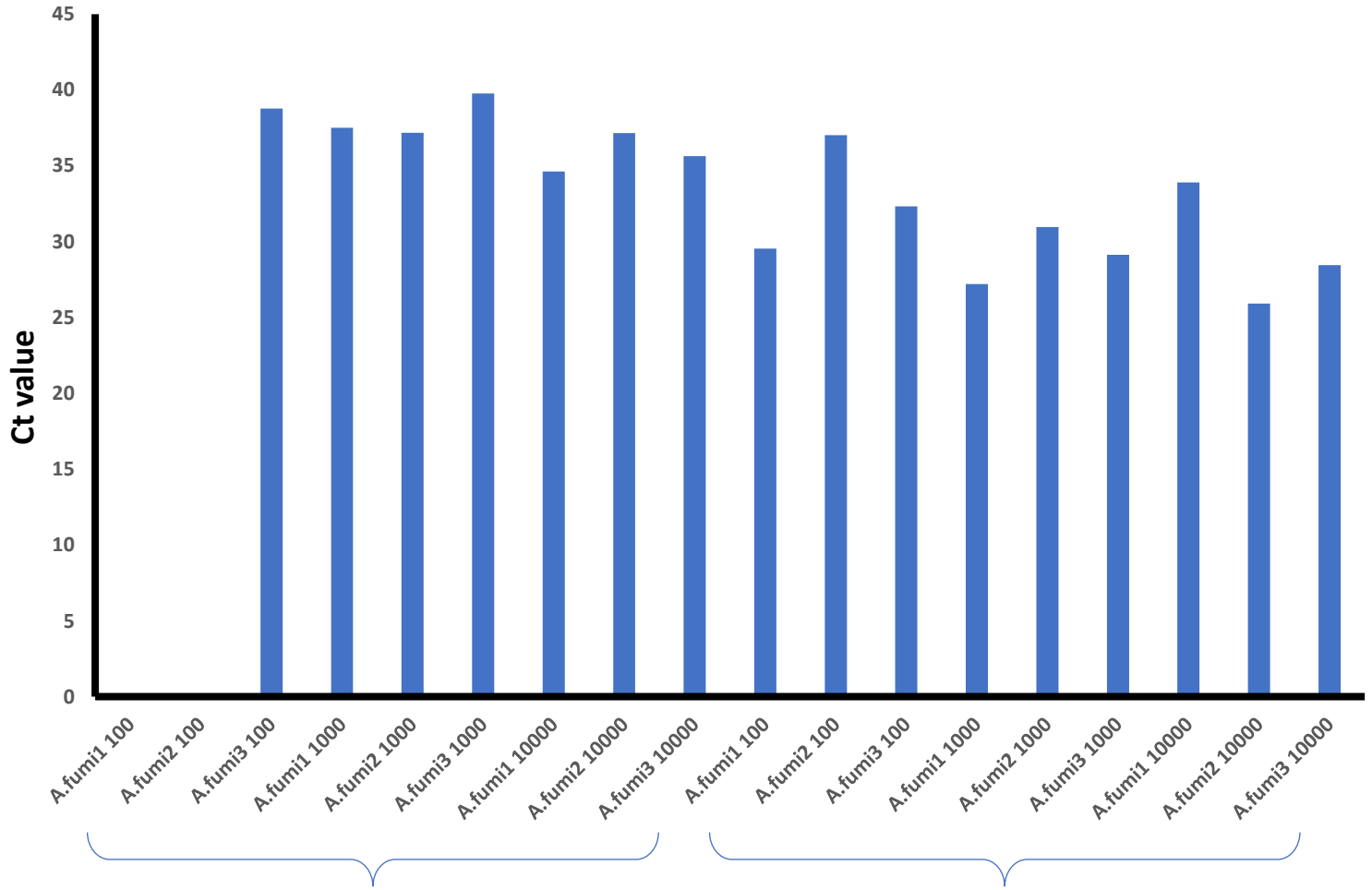


X-ray treated
No Aspergillus growth on PDA plates

X-ray untreated
Aspergillus growth on PDA plates

Aspergillus fumigatus




Ct value
45
40
35
30
25
20
15
10
5
0
A.fumi1 100
A.fumi2 100
A.fumi3 100
A.fumi1 1000
A.fumi2 1000
A.fumi3 1000
A.fumi1 10000
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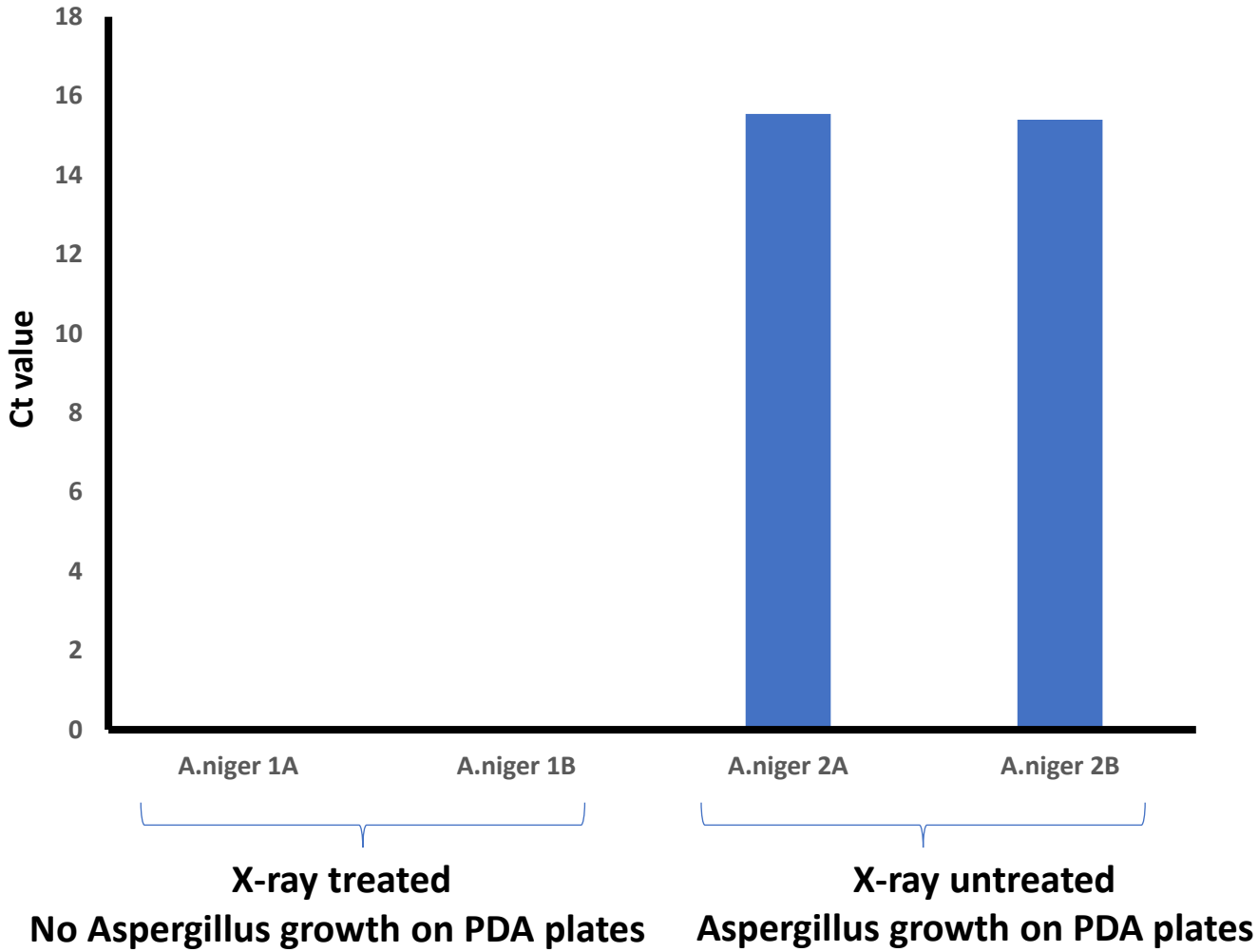
A.fumi3 1000
A.fumi1 10000
A.fumi2 10000
A.fumi3 10000
X-ray treated
No Aspergillus growth on PDA plates
X-ray untreated
Aspergillus growth on PDA plates

Scale up study: Experimental Design

Contaminated cannabis flower was weighed out (100g/sample) and pretreated with 3.5kGy of X-rays

Samples were inoculated with known amount of Aspergillus spores 1,000,000/sample (10,000 spores/gram). One set of samples was then treated with x-rays again while the second set served as untreated controls.

Aspergillus niger



Conclusion

- X-ray treatment at 2.5kGy seems sufficient to render the Aspergillus spores nonviable.
- X-ray treatment of 2.5kGy does not destroy fungal DNA to insignificant levels.
- PCR detection method needs to be accompanied by an Aspergillus specific viability assay in order to determine the effectiveness of irradiation treatment.
- Chemical profiling of cannabis flower treated with x-rays need to supplement this data in order to determine the effects of x-rays on chemical composition of cannabis flower.