

Using *Rhizopus* sp. against the growth of *Aspergillus carbonarius* as a potential biological control method of ochratoxin A contamination in raw cocoa beans



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Ochratoxin A (OTA) is a carcinogenic and nephrotoxic mycotoxin produced by fungal species belonging to *Aspergillus* and *Penicillium* genus. OTA occurrence in foodstuffs depends on the climate during harvest and on the post-harvest storage conditions. Among known ochratoxins, OTA currently contaminates crops. Due to its prevalence and toxicity, OTA occurrence development conditions in raw cocoa beans were previously studied during post-harvest treatments. This research aimed to investigate on the biological control of OTA excretion in cocoa.

Material and Methods

Cocoa

Cocoa pods used were the variety of clone CLM 99 harvested at Trinidad Tobago in 2013.

Analyzes

• Cocoa pod inoculation by *Rhizopus*

Rhizopus sp. isolated from rotten cocoa pods opened after 7 days delay and *A. carbonarius* isolated from coffee cherries were separately grew on PDA agar for 5 days at 25°C (Figure 1). Different conidia inocula were prepared from calibrated suspensions at 10², 10⁴ and 10⁶ conidia per millileter. Then 1 mL of each prepared inoculum was aseptically injected using a syringe inside health cocoa pods. Inoculated cocoa pods were incubated in a climatic chamber at 30°C, 60% relative humidity for 4 or 8 days.

• Ochratoxin A content measurement

OTA was extracted from cocoa beans sourced from inoculated pods and then separated by immuno-affinity, identified by HPLC and quantified by spectro-fluorescence detector.



Figure 1. Cultures of *Aspergillus carbonarius* [A] and *Rhizopus* sp. [B] on PDA agar used for cocoa beans inoculation inside pods

Results

Table I presents the photographs of beans inside cocoa pods after inoculation with various concentrations of *A. carbonarius* and *Rhizopus* sp. conidia suspensions.

Figure 2 indicates OTA content for cocoa beans sourced from:

- Pods inoculated with 10⁴ conidia per mL of *A. carbonarius* and 10⁴ conidia per mL of *Rhizopus* sp .
 - incubated for 4 days: no OTA production was observed
 - incubated for 8 days: OTA production of 37 µg.kg⁻¹ was highlighted when control presented no OTA production.
- Pods inoculated with 10⁴ conidia per mL of *A. carbonarius* and 10⁶ conidia per mL of *Rhizopus* sp.
 - incubated for 4 days: no OTA production was noticed
 - incubated for 8 days: OTA production of 5.6 µg.kg⁻¹ was obtained against 0 µg per kg for the control.
- Pods inoculated with 10⁴ conidia per mL of *Rhizopus* sp. and 10⁶ conidia per mL of *A. carbonarius*
 - incubated for 4 days: 36.3 µg.kg⁻¹ of OTA was recorded
 - incubated for 8 days: 6.2 µg.kg⁻¹ of OTA was observed

Table I. Photographs of beans inside cocoa pods inoculated with suspensions of various concentrations in *A. carbonarius* and *Rhizopus* sp. conidia

Incubation time (days)	Concentration of fungal conidia (conidia.mL ⁻¹)							
	<i>A.carbonarius</i>	<i>Rhizopus</i> sp	<i>A.carbonarius</i>	<i>Rhizopus</i> sp	<i>A.carbonarius</i>	<i>Rhizopus</i> sp	<i>A.carbonarius</i>	<i>Rhizopus</i> sp
	0	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶	10 ⁶
4								
8								

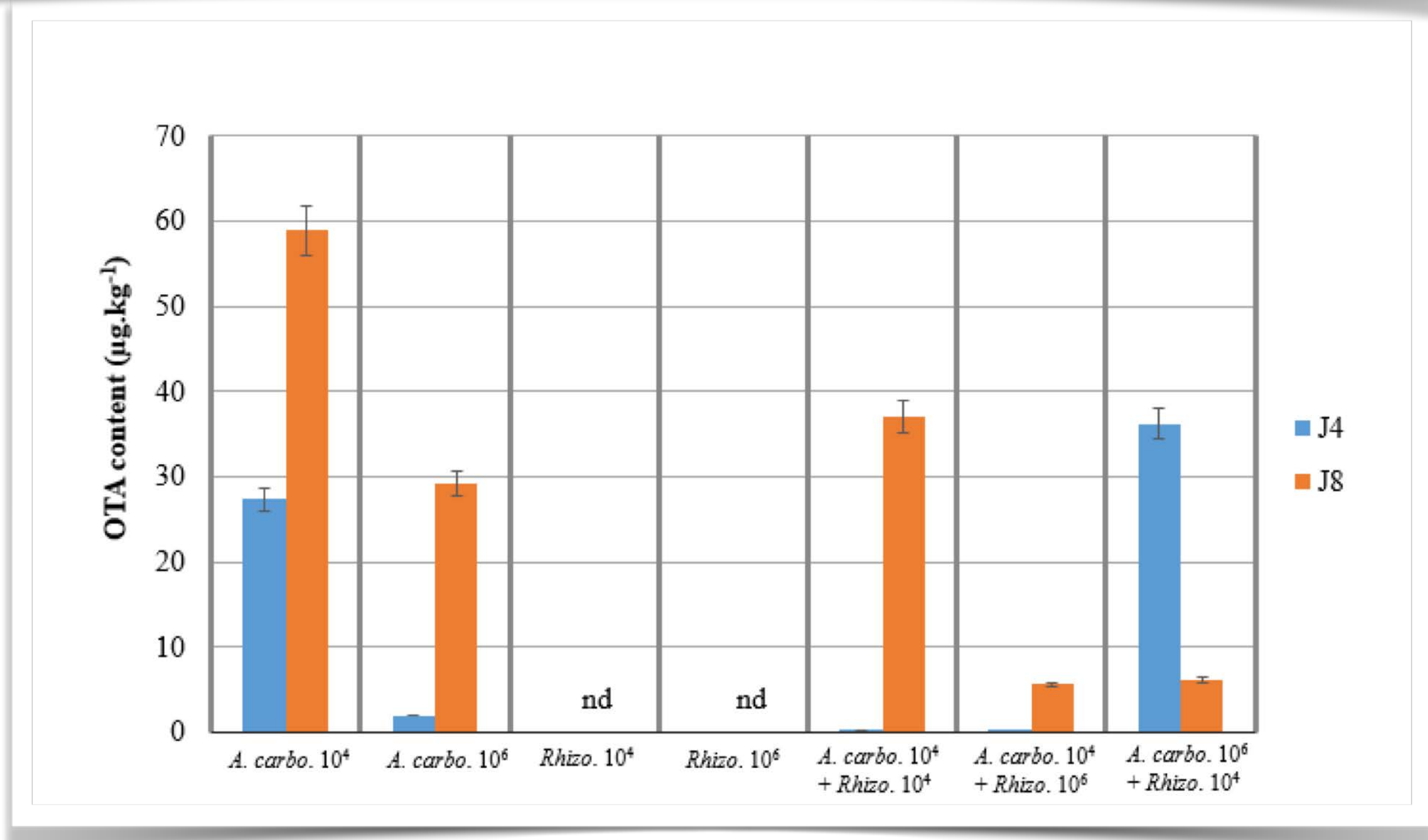


Figure 2. OTA content of cocoa beans from pods inoculated with various concentrations of *A. carbonarius* and *Rhizopus* sp conidia suspensions of

Conclusion

Rhizopus sp appeared as a promising biological control fungal agent for OTA production in raw cocoa beans. As the fungal growth in raw cocoa beans constituted a serious qualitative default, further investigation must be done on the identification of the metabolites leading to the control of OTA excretion in cocoa.

References

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