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Anti-mycotoxigenic efficacy of redox-active natural compounds and derivatives

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Background

- Crops such as tree nuts, peanuts, etc., are susceptible to infestation by the mold ***Aspergillus flavus*** and ***Aspergillus parasiticus*** and subsequent **aflatoxin (AF)** contamination

- AF is a serious threat to **food safety**; many importing countries imposing limits as low as 4 ppb
- The EU's **Rapid Alert System for Food and Feed** (RASFF; https://food.ec.europa.eu/safety/rasff_en) continually issued border rejection on the AF-contaminated food products imported
- Although the exporting countries set strict standards to ensure the food products maintain high level of quality, it is often difficult to determine the **root causes of AF contamination**

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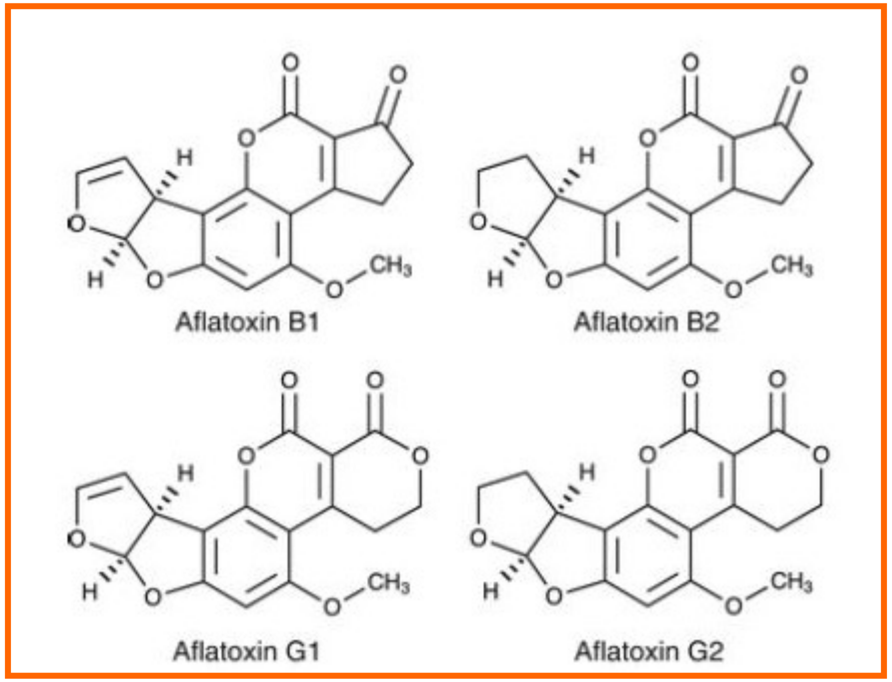
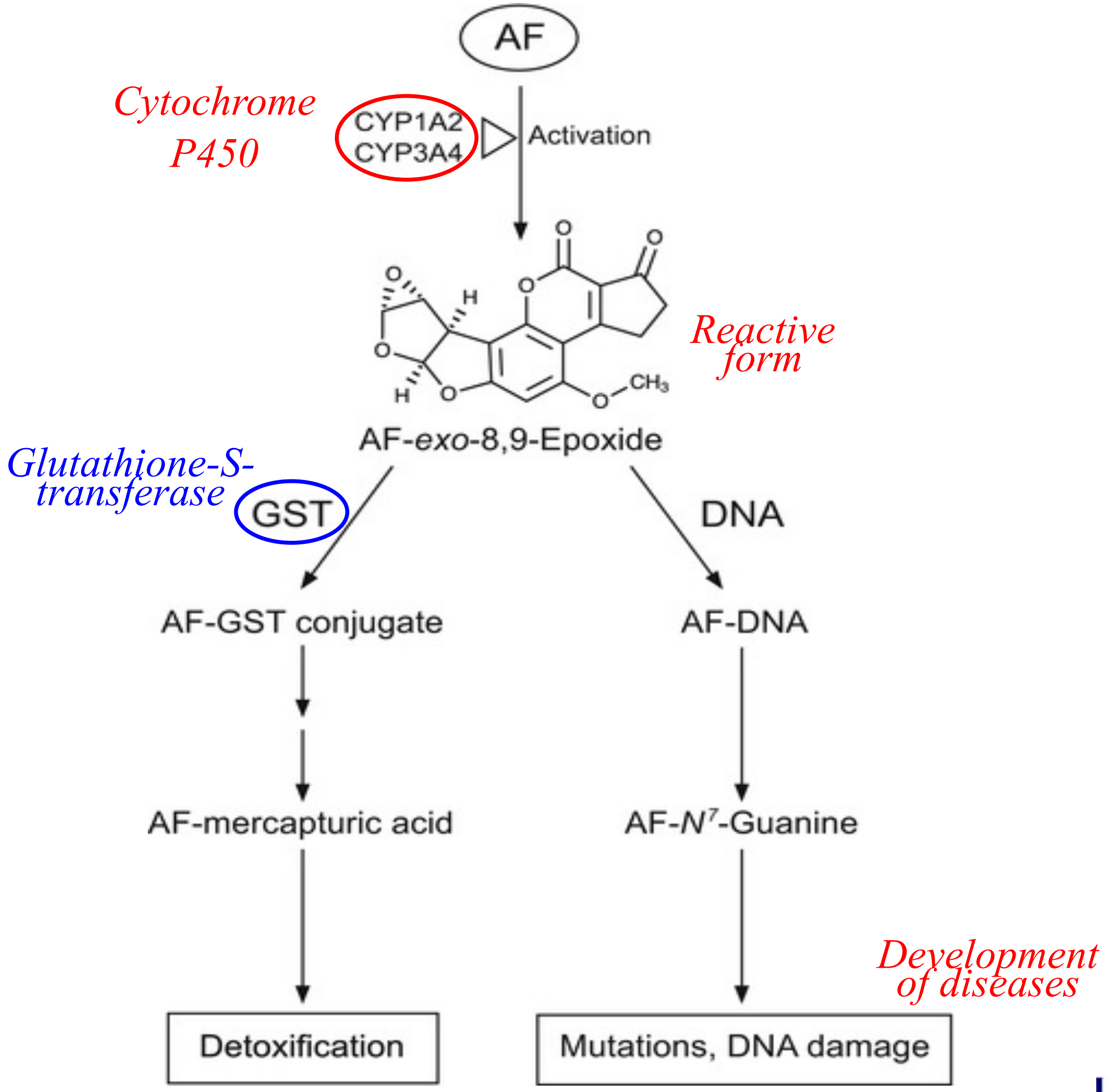
Producers and toxicity of AFs

Producers:

Aspergillus flavus: B_1, B_2

Aspergillus parasiticus: B_1, B_2, G_1, G_2





Fungicide resistance and mycotoxins

- Traditional control of *A. flavus* and *A. parasiticus* has been through the application of fungicides; **fungicide resistance** have been correlated with increased mycotoxin production

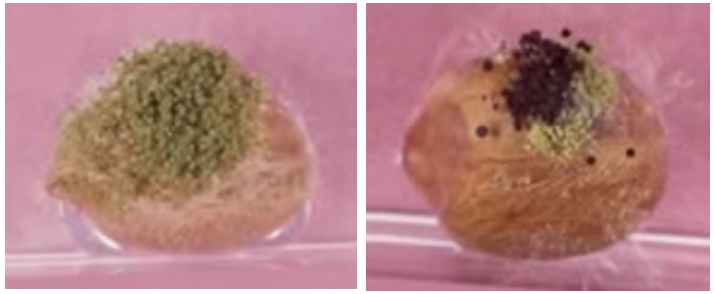
Examples of fungicide-potentiation of mycotoxin production in resistant pathogens

(Adapted from Kim et al. 2015. Outlooks on Pest Management. 26:172-176)

Fungi	**Fungicides**	**Mycotoxins**
Aspergillus parasiticus	Anilinopyrimidine	Aflatoxin
Fusarium graminearum	Benzimidazole	Citrinin
Fusarium culmorum	Carbendazim	Patulin
Fusarium sporotrichioides	Fludioxonil	Trichothecene
Penicillium expansum	Flusilazole	
Penicillium verrucosum	Iprodione	
	Phenylpyrrole	
	Strobilurins	
	Tebuconazole	

Example: Prevalence of fungicide resistant *A. flavus* (*Ali et al. 2021. J of Fungi. 7: 284*)

- In 2019: High frequency of **AF contamination** in peanuts (USA)
- In 2020: **Poor seed quality**; peanut seeds had lower than expected germination and a high frequency of *A. flavus* contamination
- **Mitochondrial *cytochrome b* gene mutations**: These *A. flavus* had a single nucleotide mutations at CytB G143A (36.8% isolates) and at CytB F129L (15.8% isolates), resulting in fungicide resistance
- Ineffectiveness of current seed treatments: Thus, **needs for new fungicides** to avoid AF contamination



Aspergillus-contaminated peanut seeds (Courtesy: USDA)

Rationale

- **Natural compounds** that do not have any significant environmental impact are a potential source of antimycotic agents, either in their nascent form or as lead structures for more effective derivatives
- Especially, **natural phenolic compounds** can serve as potent **redox cyclers** that inhibit microbial growth through destabilization of cellular **redox homeostasis** and/or **antioxidation systems**
- However, as determined in commercial fungicides, if **natural phenolic compounds** are applied at **suboptimal concentrations** than that required for fungal control, the compounds would **potentiate mycotoxin production** by *A. flavus* and *A. parasiticus*
- In this study, two sub-inhibitory concentrations of **natural phenolic compounds/derivatives** were tested against *A. flavus* and *A. parasiticus* for determining their anti-mycotoxigenecity & potentiation of mycotoxin production (**Risk assessment**)

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Materials and Methods

- **AF determination/analysis:**

  Aflatoxin determination was performed (w/ or w/o treatments of natural phenolic compounds) using an Agilent 1260 system (Palo Alto, CA, USA) with fluorescence detection at 365 nm excitation and 455 nm emission, as previously described Hua et al. 2019. *Mycotoxin Res*. 35: 381-389. https://doi.org/10.1007/s12550-019-00364-w. Analysis was performed using OpenLAB CDS Chemstation Edition for LC & LC/MS Systems (Rev. C.01.08) (Agilent Technologies, Palo Alto, CA, USA).

- **Natural phenolic compounds/derivatives examined:**

Compounds	Low concentration (mM)	High concentration (mM)
2,3-Dihydroxybenzaldehyde (2,3-D)	0.1	0.2
2,4-Dihydroxybenzaldehyde (2,4-D)	0.5	1.0
2,5-dihydroxybenzaldehyde (2,5-D)	0.5	1.0
3,5-Dimethoxybenzaldehyde (3,5-D)	0.5	0.75
2-Hydroxy-4-methoxybenzaldehyde (2H4M)	0.1	0.2
2-Hydroxy-5-methoxybenzaldehyde (2H5M)	0.1	0.2
Salicylaldehyde (SLAD)	0.5	1.0
Salicylic acid (SACID)	0.5	1.0
4-Isopropyl-3-methylphenol (4I3M)	0.2	0.4
Thymol (2-Isopropyl-5-methylphenol; THY)	0.2	0.4
Carvacrol (5-Isopropyl-2-methylphenol; CARV)	0.2	0.4
4-Methylcinnamic acid (4MEC)	0.5	1.0
4-Methoxycinnamic acid (4MOC)	0.5	1.0

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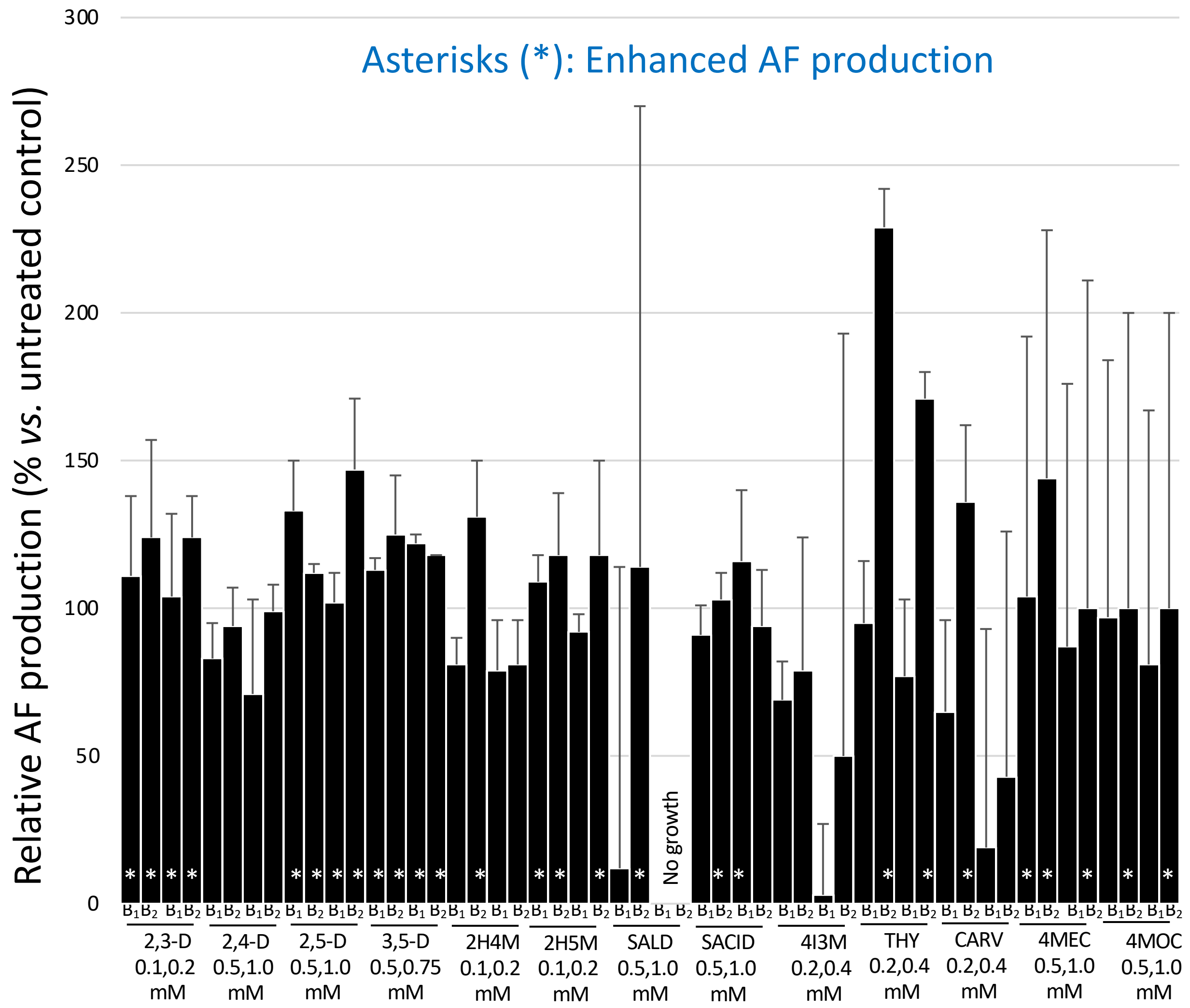
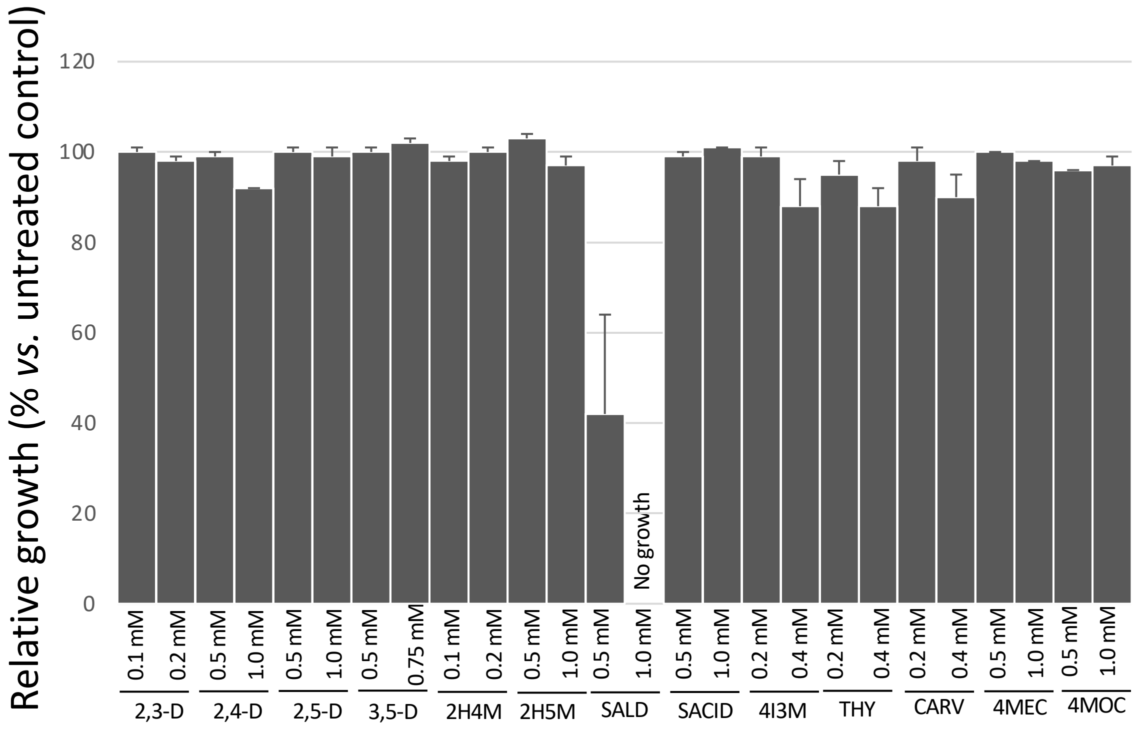
Results: AF production in *A. flavus*



KEYS:

- Twenty-seven (marked as "asterisks") out of fifty-two concentrations of test compounds exhibited enhanced AF (B_1, B_2) production compared to the untreated control
- 4I3M and CARV at 0.4 mM showed the most potent anti-mycotoxigenic activity whereas THY at 0.2 mM exhibited the highest mycotoxin enhancement
- 4I3M and 2,4-Dihydroxybenzaldehyde (2,4-D) did not enhance AF production at all concentrations (0.5, 1.0 mM) tested; viz., **no risks identified in *A. flavus***
- AFB_1 and AFB_2 production seems to be **differentially** influenced by test compounds at different concentrations

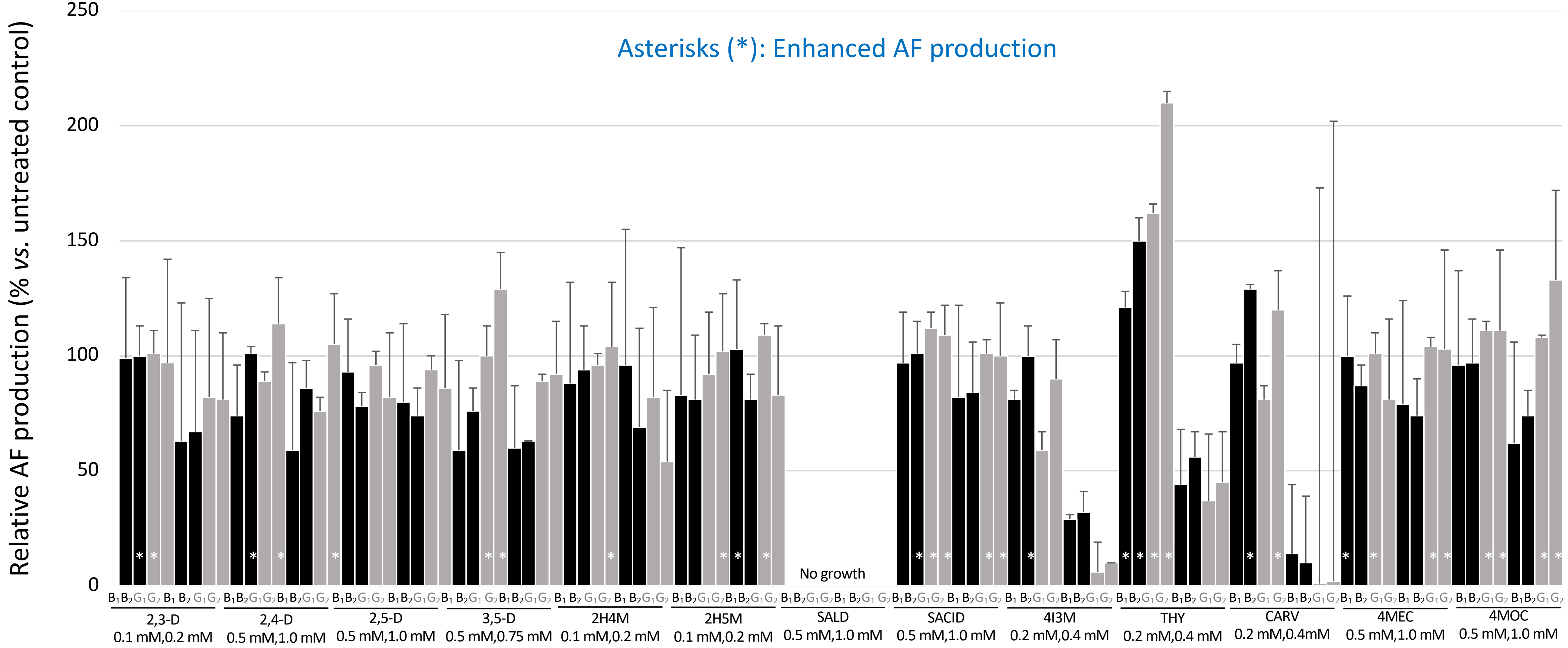
Results: *A. flavus* growth



KEYS:

- Except salicylaldehyde (SALD), the concentrations of most compounds **marginally** affected the growth of *A. flavus*

Results: AF production in *A. parasiticus*

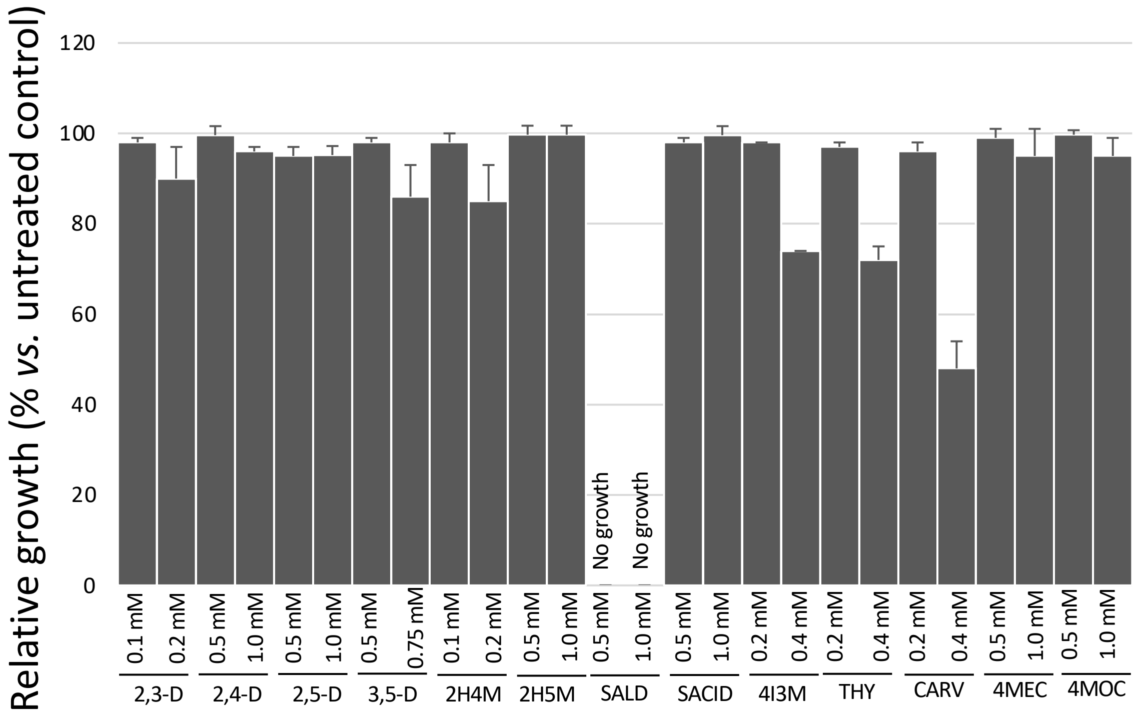


Results (continued): AF production in *A. parasiticus*

KEYS:

- Thirty-one (marked as "asterisks") out of fifty-two concentrations of test compounds exhibited enhanced AF (B_1, B_2, G_1, G_2) production compared to untreated control
- As determined in *A. flavus*, 4I3M and CARV at 0.4 mM showed the most potent anti-mycotoxigenic activity while THY at 0.2 mM exhibited the highest mycotoxin enhancement
- Except AFB_1 at 0.5 mM, 2,5-Dihydroxybenzaldehyde (2,5-D) did not enhance AF production at all concentrations (0.5, 1.0 mM) tested
- As determined in *A. flavus*, AFB_1, AFB_2, AFG_1 and AFG_2 production seems to be differentially influenced by test compounds at different concentrations

Results: *A. parasiticus* growth



KEYS:

- Except salicylaldehyde (SALD; 0.5 & 1.0 mM), 4I3M (0.4 mM), THY (0.4 mM) and CARV (0.4 mM), the concentrations of most compounds tested **marginally affected the growth** of *A. parasiticus*

Summary

- In both *A. flavus* and *A. parasiticus*, **4I3M** and **CARV** at 0.4 mM showed the **most potent** anti-mycotoxigenic activity while **THY** at 0.2 mM exhibited the highest **mycotoxin enhancement**
- **Twenty-seven** (***A. flavus***) to **thirty-one** (***A. parasiticus***) out of fifty-two concentrations of natural phenolic compounds tested exhibited **enhanced AF (B_1, B_2, G_1, G_2) production** compared to the untreated control
- **2,4-Dihydroxybenzaldehyde (2,4-D)** or **2,5-Dihydroxybenzaldehyde (2,5-D)** did not enhance AF production at all concentrations (0.5, 1.0 mM) tested in *A. flavus* or *A. parasiticus*, respectively (except AFB_1 at 0.5 mM in *A. parasiticus*)
- AFB_1, AFB_2, AFG_1 and AFG_2 production were **differentially influenced/regulated** by test compounds at different concentrations
- In conclusion, **natural phenolic agents** could be applied as anti-mycotoxigenic agents but **should be used at optimum concentrations**, thus preventing the enhanced AF production in *A. flavus* and *A. parasiticus*

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