

# MARINE BIOPROSPECTING FOR AGRICULTURE

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# INTRODUCTION

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- Marine bioprospecting: Mining the untapped potential of living marine resources.
- Oceans harbor myriad organic molecules with utility for the development of pharmaceuticals, nutraceuticals and agrochemicals.
- Objective: Searching for anti fungal agent against *Fusarium* spp.

# *Fusarium* spp.

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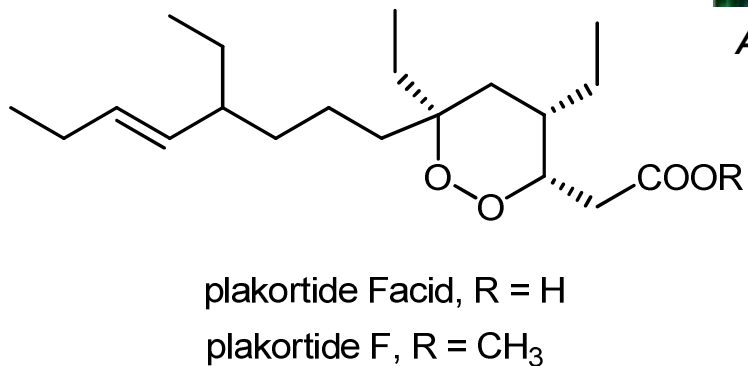
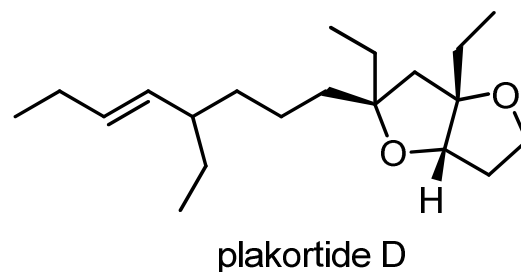
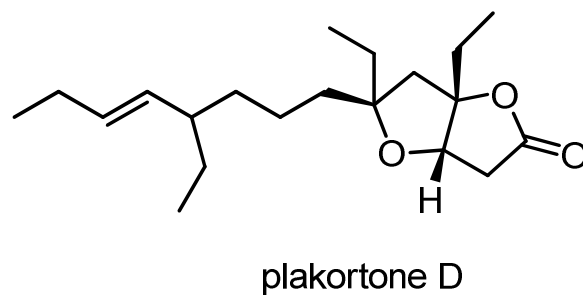
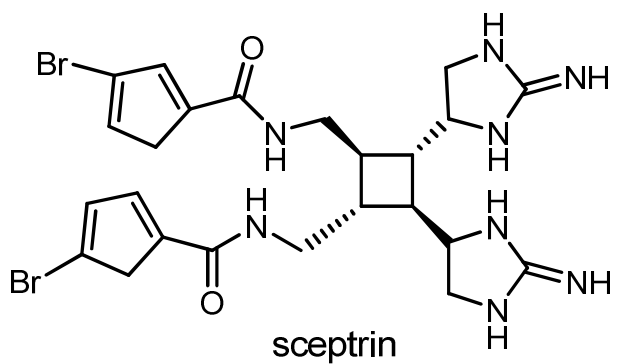
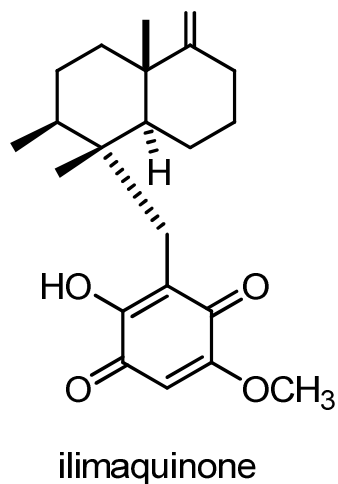
- ❑ Common soil saprophyte and plant pathogen
- ❑ Problem in agriculture and human health: mycotoxins
- ❑ Potential cause of severe opportunistic infections in human immunocompromised patients
- ❑ *F. solani*, *F. oxysporium*, *F. moniliforme*, *F. verticilloides* and *F. proliferatum*
- ❑ Limited number of antifungal that are effective
- ❑ Resistance to the available antifungal agents

**Guarro, J and Gene, J. *Eur. J. Clin. Microbiol.Infect. Dis.* (1995), 14: 741-745**

**Martino, P., et al. *J. of Infections* (suppl). 1 (1994), 28: 7-15.**

# MARINE NATURAL PRODUCTS

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*Plakortis* sp.

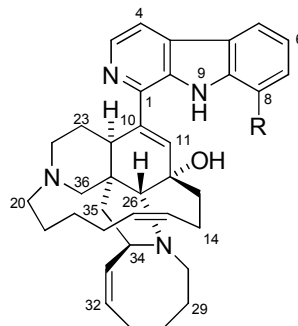


*Agelas* sp.

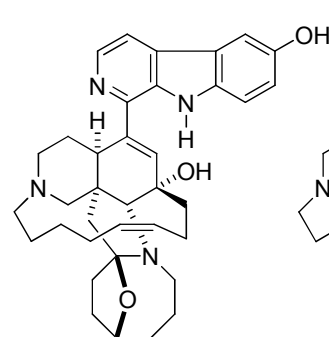
# Manzamine alkaloids



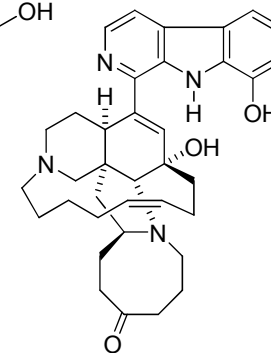
*Acanthostrongylophora* sp.  
From Manado, Indonesia



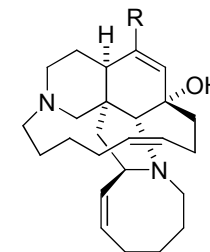
manzamine A (1), R = H  
8-hydroxymanzamine A (2), R = OH



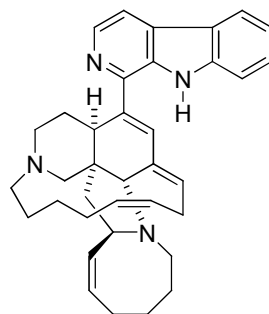
manzamine X



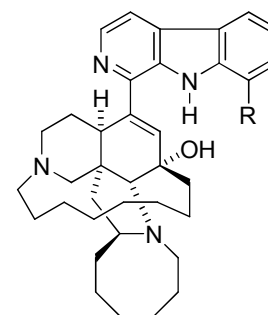
manzamine F (5)



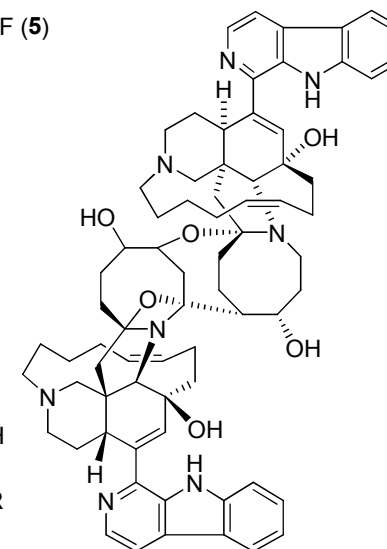
ircinal A (3), R = CHO  
ircinol A (4), R = CH<sub>2</sub>OH



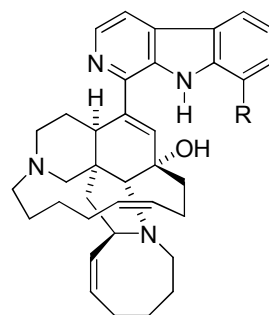
dehydromanzamine A



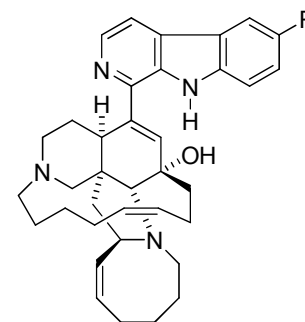
tetrahydromanzamine A, R = H  
tetrahydro-8-hydroxymanzamine A, R = OH



neo-kaulamine



8-methoxymanzamine A, R = OMe  
8-acetoxymanzamine A, R = OAc



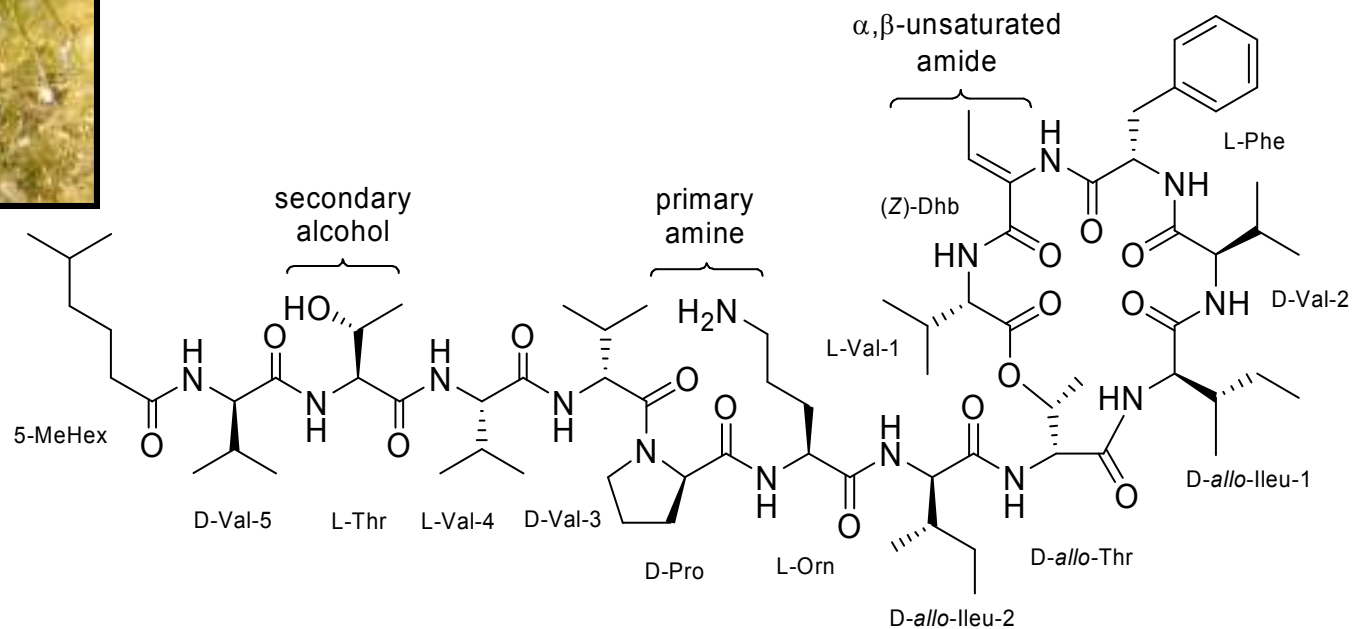
6-hydroxymanzamine A, R = OH  
6-aminomanzamine A, R = NH<sub>2</sub>  
6-methoxymanzamine A, R = OMe  
6-*n*-propoxymanzamine A, R = O<sup>n</sup>Pro  
6-(2-ethylhexyloxy)manzamine A, R = O-(2-Et-Hex)

# Kahalalide F

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*Elysia rufescens*  
*Bryopsis* sp.



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# METHODS

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- **One dimensional bioautography** is technique in pre-screening large numbers of extracts, fractions or compounds.
- **Microbioassay** in a 96-well, micro-dilution broth format was used to evaluate pure compounds identified by bioautography

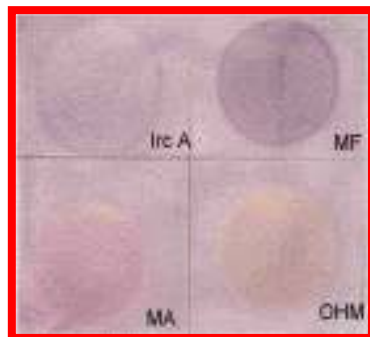
# One dimensional bioautography

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- ❖ *F. solani*, *F. proliferatum* and *F. oxysporium*
- ❖  $3 \times 10^5$  conidia/mL
- ❖ Spray: 0.25% MTT in buffer pH 7
- ❖ Standards:
  - Amphotericin B
  - Captan
  - Azoxystrobin
  - Benomyl
  - Cyprodinil



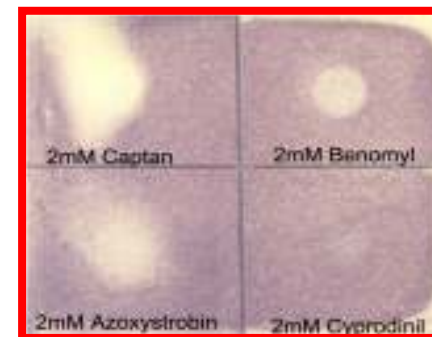
## Manzamines-*Fusarium*



*F. oxysporum*

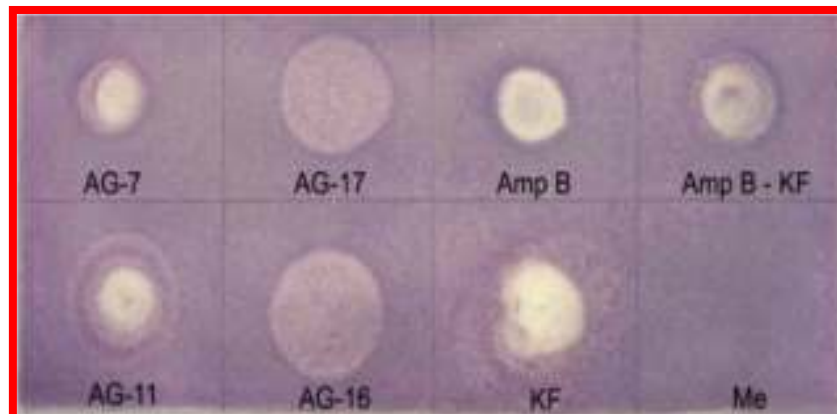


*F. proliferatum*



*F. oxysporum*

## Kahalalides-*F. proliferatum*



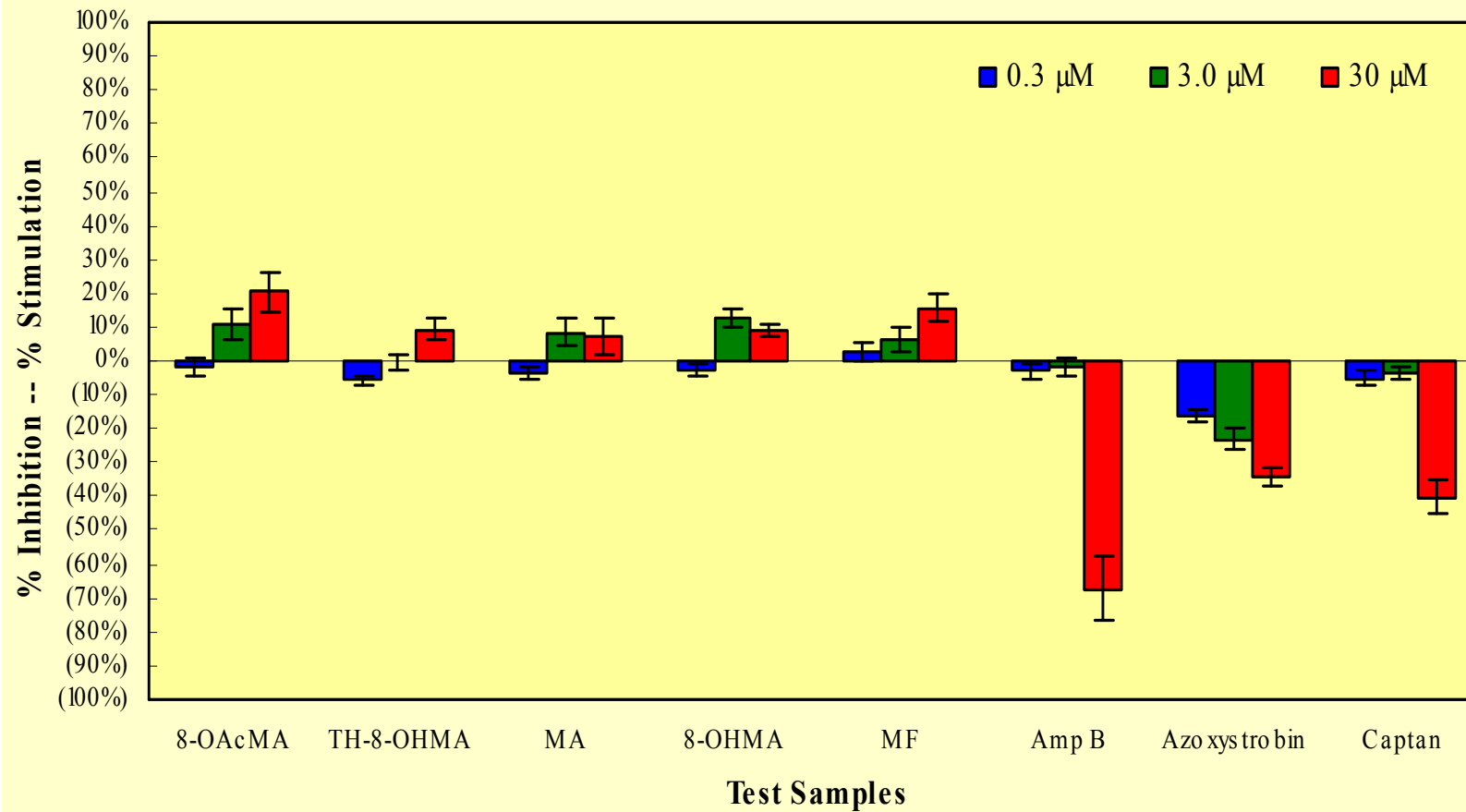
# Microtiter bioassay

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- 96-well format
- Determine the sensitivity of fungi to compounds
- Dose-response: 0.3, 3.0 and 30  $\mu\text{M}$
- Antifungal action: spore germination or mycelial growth

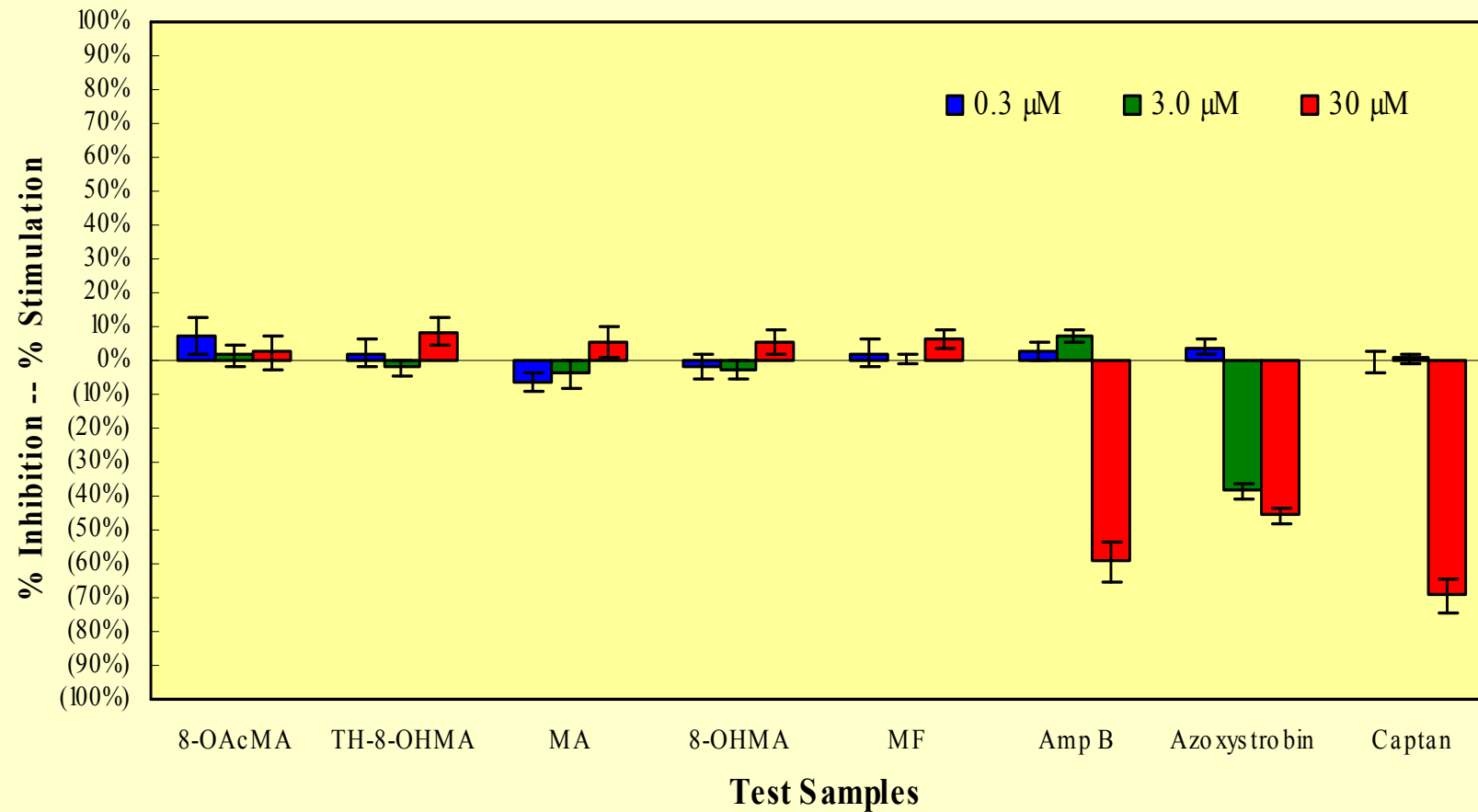
# The growth response of *F. oxysporum* to manzamine alkaloids

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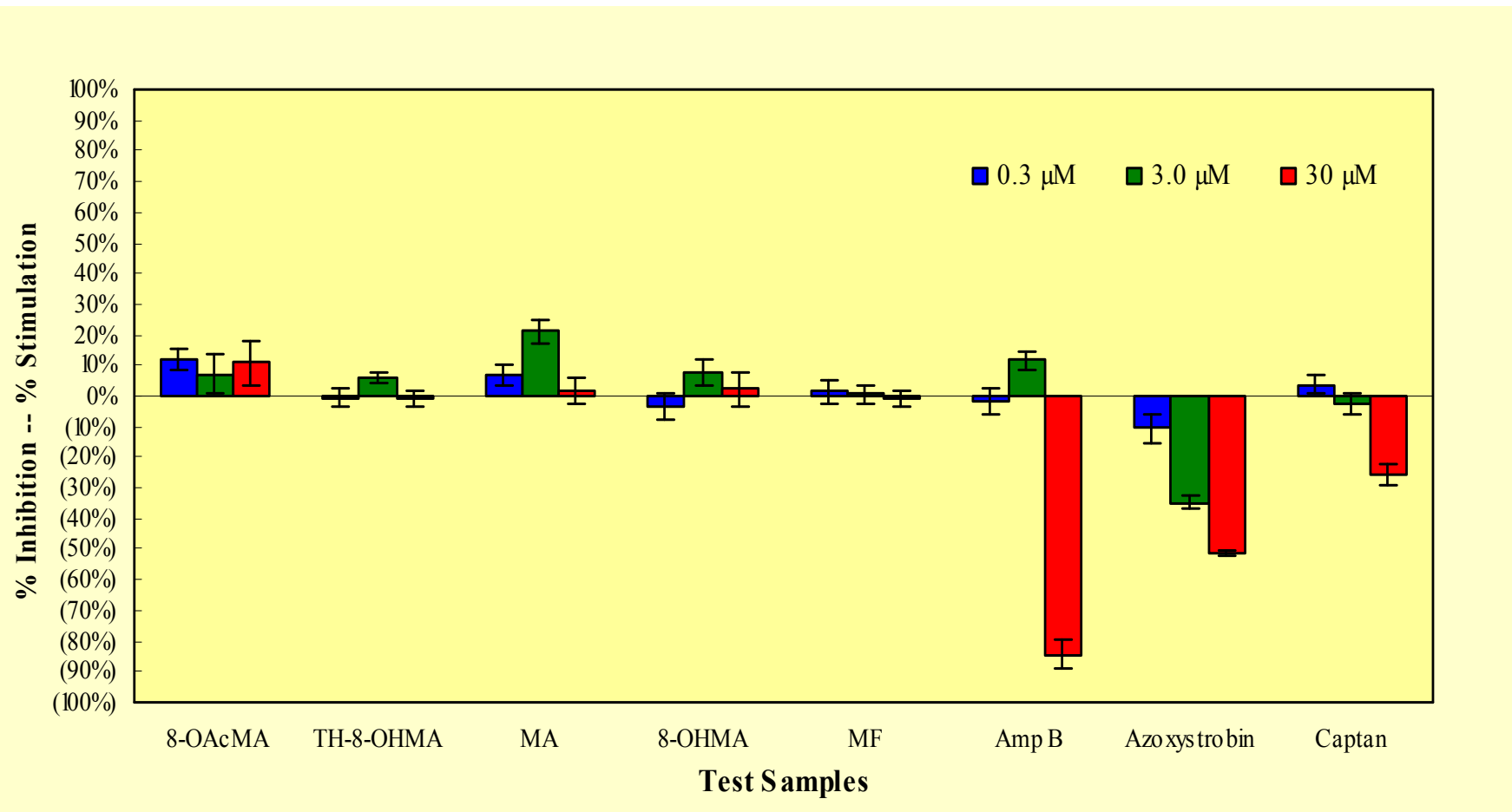
# The growth response response of *F. proliferatum* to manzamine alkaloids

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# The growth response response of *F. solani* to manzamine alkaloids

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# MECHANISMS OF ANTIFUNGAL RESISTANCE

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## Antifungal resistance:

Failure of fungal infection to respond to antifungal

## Types of resistance:

- Primary resistance (Intrinsic resistance)  
Zygomycetes: *Rhizopus arrhizus*, *Scedosporium* spp.,  
*Acremonium* spp., *Fusarium* spp.
- Secondary resistance

Kontoyiannis, D and Lewis, R.E. 2002. *The Lancet*. 359: 1135-44  
Perea, S and Patterson, T.F. 2002. *Clin. Infect. Dis.* 35: 1073-80.

# MECHANISMS OF ANTIFUNGAL RESISTANCE

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- Alteration of target
- Alteration of enzyme (biosynthesis or metabolism)
- Efflux pumps
- Decrease in drug accumulation
- **Modification or degradation of compound**

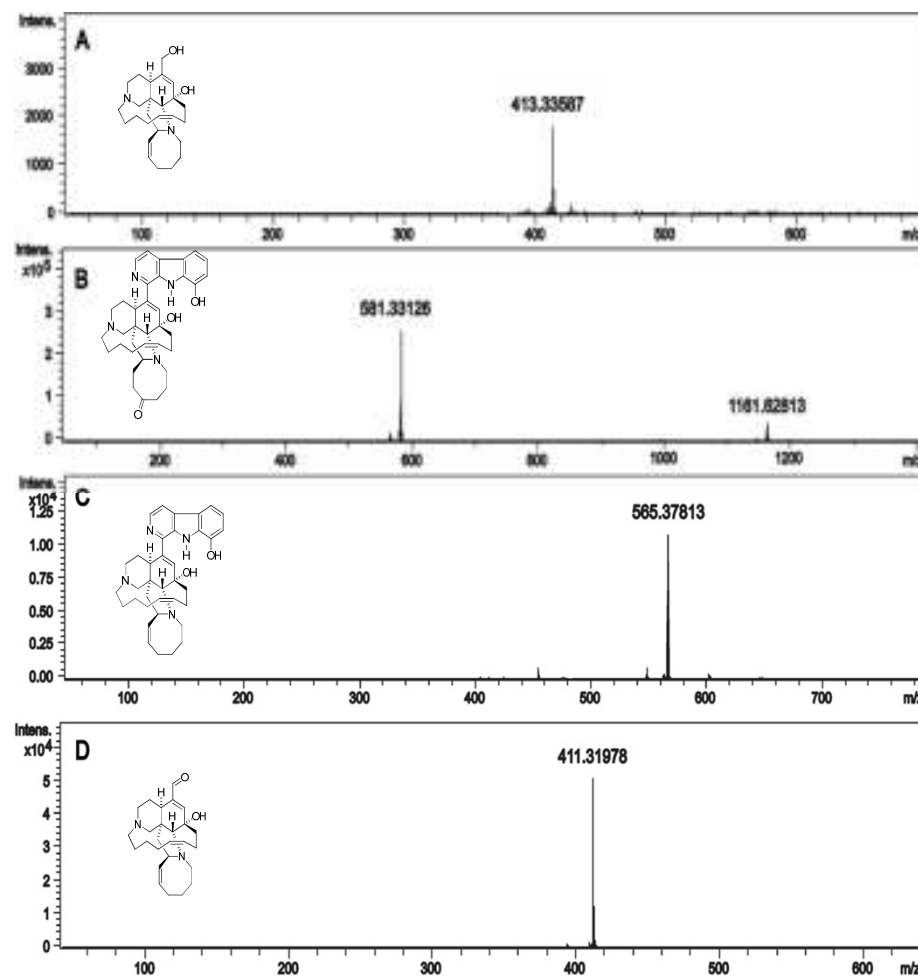
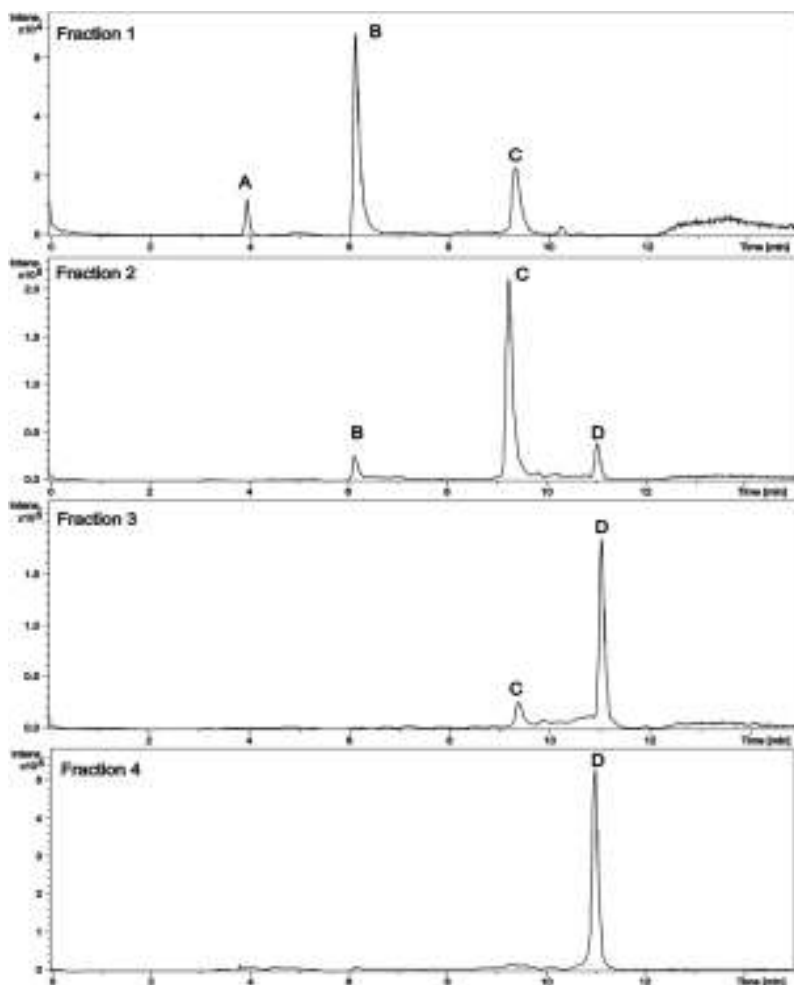
White, T.C., *et al.* 1998. *Clin. Microbiol. Rev.* 11: 382-402

Loeffler, J and Stevens, D.A. 2003. *Clin. Infect. Dis.* 36 (Suppl): S31-S41

Morrissey, J.P and Osbourn, A.E. 1999. *Microbiol. Mol. Biol. Rev.* 63: 708-724.

# Biodegradation of manzamine alkaloids

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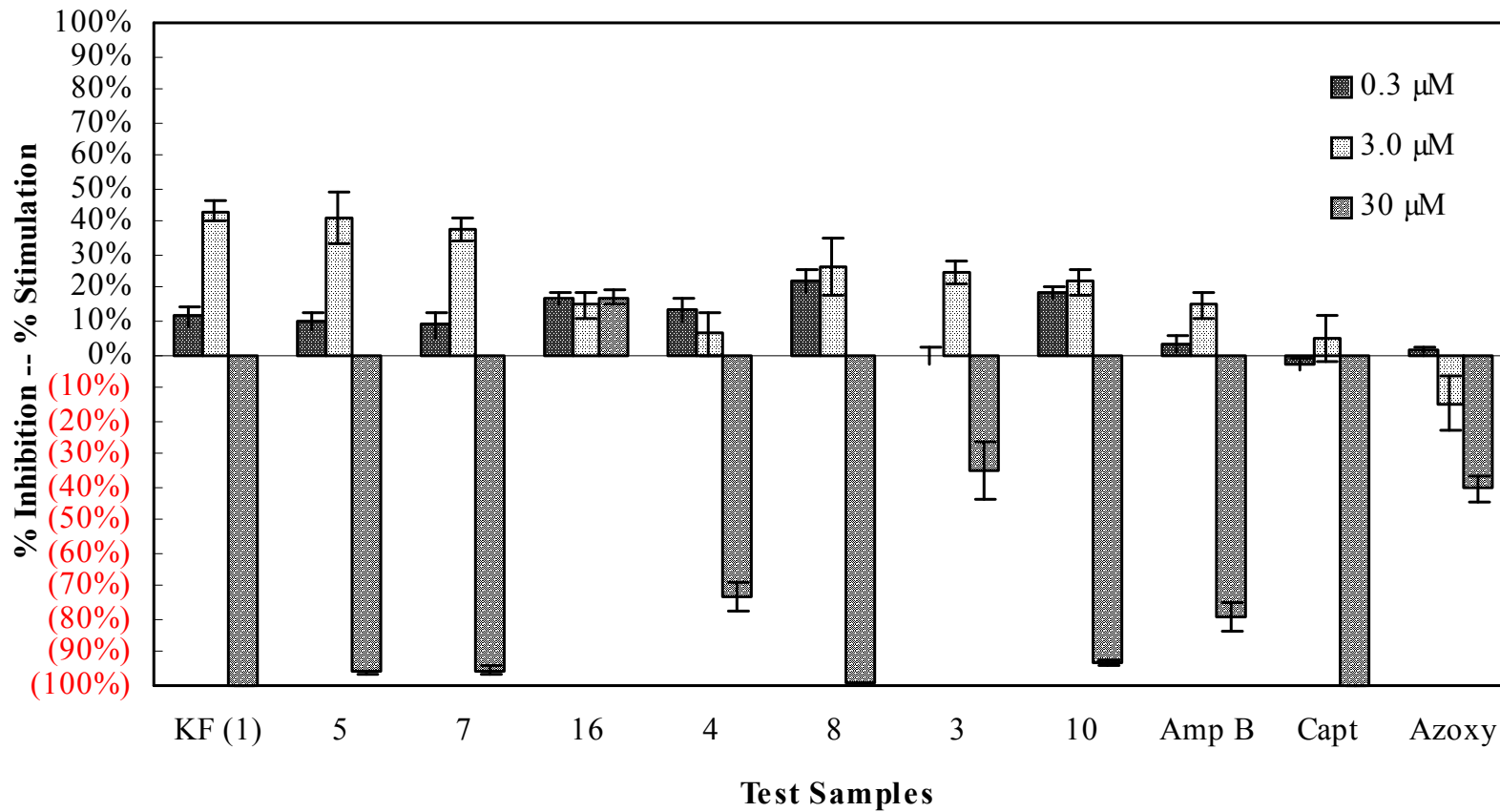


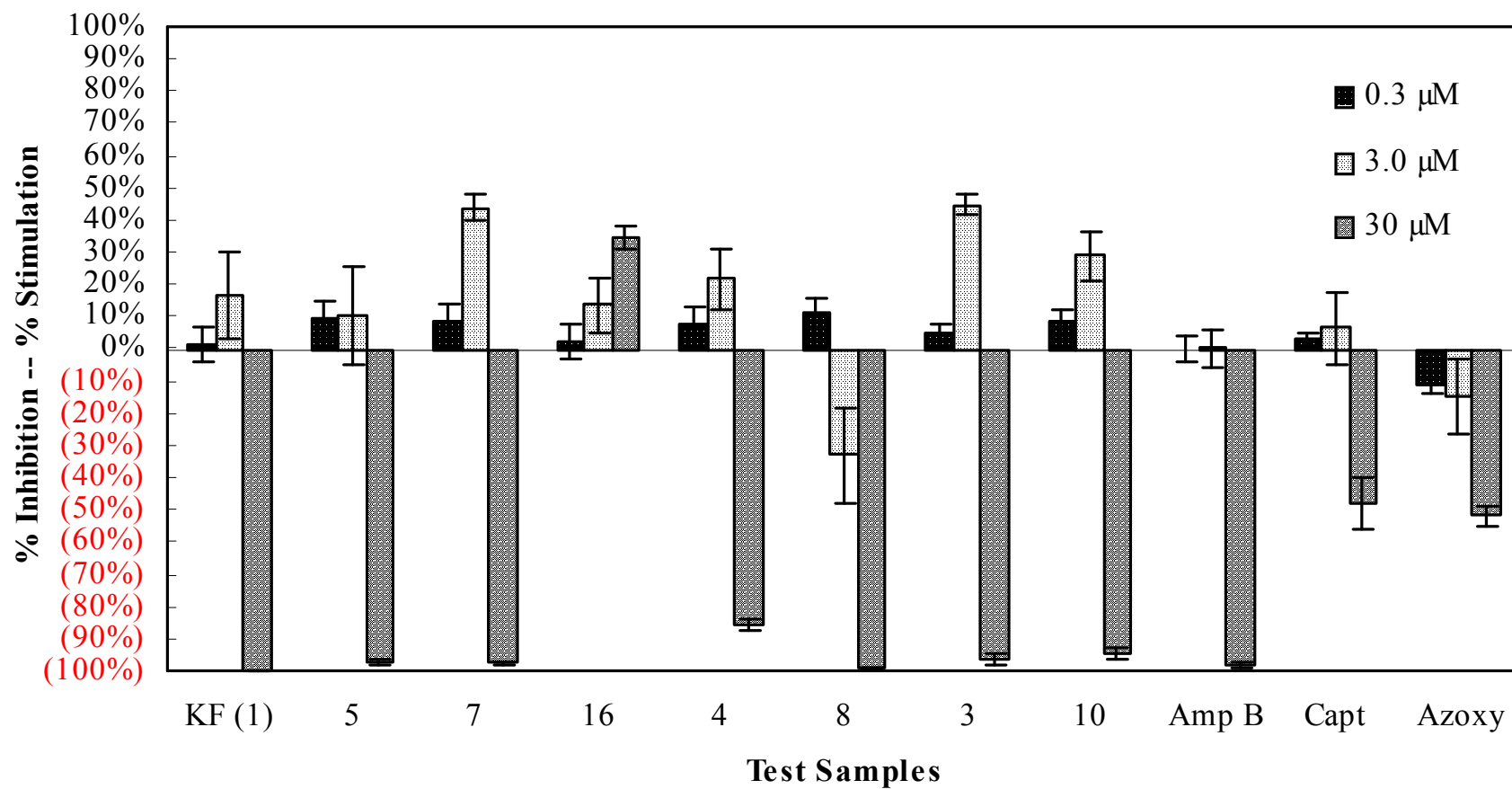


# Kahalalide F and its derivatives

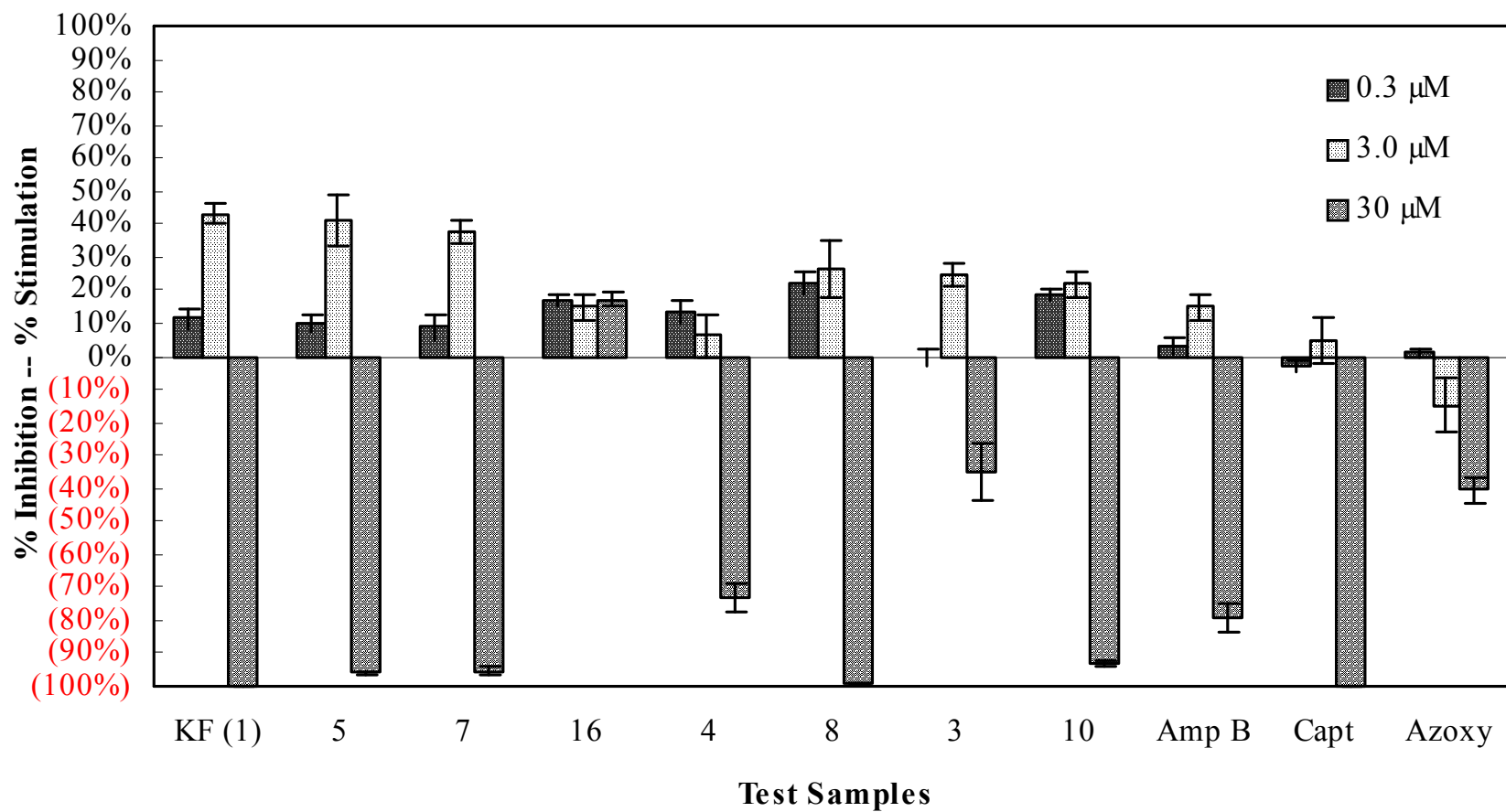
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*F. proliferatum* Growth Response to Samples at 72 hrs.



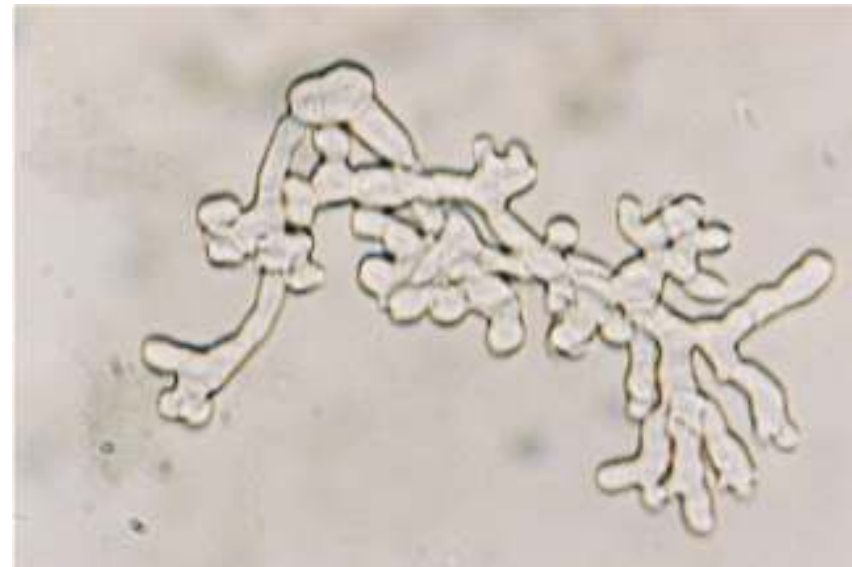
*F. solani* Growth Response to Samples at 72 hrs.

*F. proliferatum* Growth Response to Samples at 72 hrs.





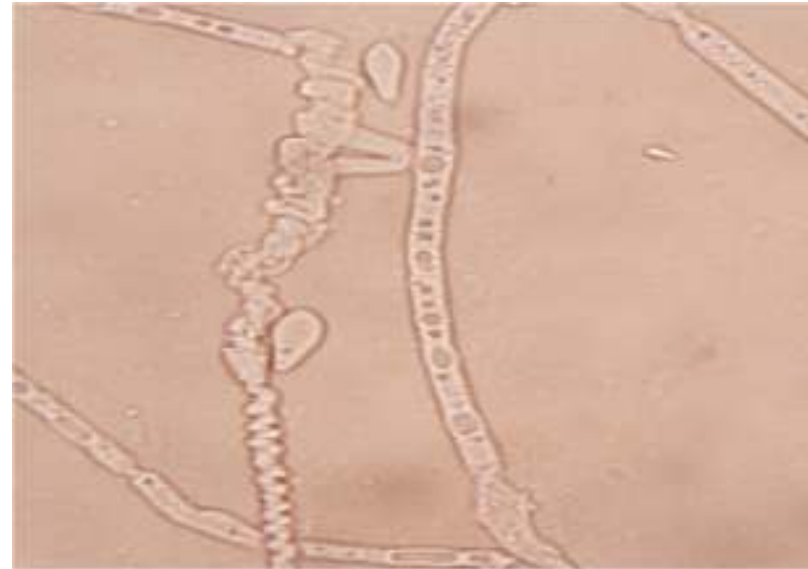
**Growing hyphae and mycelia of *F. oxysporum* in medium PDB without KF.**



**The hyphae of *F. oxysporum* exposed to KF at concentration 5  $\mu\text{M}$  after germination**



**The hyphae of *F. proliferatum* exposed to KF at concentration 5  $\mu$ M after germination**



**Twisted and destruction hyphae of *F. oxysporum* due to long exposure of KF (after 7 days)**



**Mycelia of *F. proliferatum* after 3 days exposure with fluorescent**

# CONCLUSIONS

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- ❑ Scepterin, ilimaquinon and Plakortides are inactive against *Fusarium* spp.
- ❑ *Fusarium* spp are resistance to manzamina alkaloid through mechanism modification or degradation of compounds
- ❑ Kahalalide F is active against *Fusarium* spp, interfere growing hyphae and damage the mycelia
- ❑ Marine bioprospecting for new agrochemical compounds is complicated by the nature of compounds: complex structure, low yield, physicochemical properties

# Acknowledgments

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- Dr. Abbas S. Gholipour
- Dr. Anna Kochanowska
- Dr. Rabab Muhammad
- Lorei Lucas, Pharm.D



THANK YOU

