SUPPLEMENTARY MATERIALS

Table S1. Utility of natamycin (NAT) in food and agricultural industries.

Test system	Fungi targeted	Effects	References
Anti-mycotoxigenic:			
Whole olives, olive paste samples	*Aspergillus flavus*	The growth level was parallel to the temperatures of incubation. The aflatoxin B_1 production in all samples at all levels of preservatives were negative.	[1]
Grape juice-based medium	*Aspergillus carbonarius*	Natamycin appears to be a very effective for controlling growth and ohcratoxin production by strains of *A. carbonarius.*	[2]
A glucose-yeast extract-salt medium	*Aspergillus parasiticus*	Natamycin (Pimaricin), with a low pH, low temperature or 4% or 6% NaCl, could initially slow mycelial growth and inhibit toxin production; the mold overcame the inhibitory effects.	[3]
Olive paste	*Aspergillus ochraceus*	Natamycin delayed growth and sporulation by *A. ochraceus*. Production of penicillic acid was also decreased.	[4]
Antifungal synergism:			
Apple juice	*Candida tropicalis, Candida krusei, Candida kefyr, Rhodotorula mucilaginosa*	The farnesol (yeast quorum sensing molecule) enhanced natamycin efficacy; reduced biofilm development from juice on stainless steel surfaces.	[5]
Blackberry fruits	*Aspergillus japonicus, Gilbertella persicaria*	Synergistic antifungal activity of ferulic acid and natamycin.	[6]
Phyllo pastry	Mesophilic total viable counts, yeasts and molds, psychrotrophic bacteria, lactic acid bacteria, Enterobacteriaceae, enterococci	The combination of chitosan and natamycin can delay the spoilage of phyllo pastry.	[7]
Galotyri cheese	Yeasts, molds	Natamycin, added alone or in combination with nisin, suppressed fungal growth.	[8]
Shiitake mushroom (*Lentinus edodes*)	Yeasts, molds	Mushroom coated with gum arabic + natamycin maintained tissue firmness and showed reduction in fungal counts.	[9]
Concord and Niagara grape juices	Yeast cocktail of *Dekkera*, *Kluveromyces*, *Brettanomyces*, and *Zygosaccharomyces*	Dimethyldicarbonate and natamycin combination treatments in still Niagara juice and in carbonated Concord and Niagara juices exhibited promising results.	[10]
Button mushroom (*Agaricus bisporus*)	Yeasts, molds	Treatment with natamycin + pure oxygen maintained tissue firmness, inhibited increase of respiration rate, delayed browning and cap opening, and reduced yeasts and molds.	[11]
Aqueous solutions	N/A	The natamycin:beta-cyclodextrin complex and natamycin:gamma-cyclodextrin complex	[12]

		were significantly more stable ($p < 0.05$) than natamycin in its free state; aqueous solutions stored in darkness at 4 °C.	
Formulated blend	N/A	Stability study of a nisin/natamycin blend; after 1 year, 50% of nisin and 90% of natamycin remained in the formulation. pH 5-6.	[13]
Natamycin sensitivity to low pHs & light:			
Agriculture and food. foster: Review	*Candida* spp.	Acidic pH natamycin degradation. The use of any anti-infective agent as a food preservative to be limited to an absolute minimum.	[14]
Wine	N/A	Detection method optimization; Instability in wine; light sensitivity.	[15]
Cow milk	N/A	Photoprotective effect of mycosporine-like amino acids for natamycin stability; protect cow milk exposed to light in refrigerated glass containers.	[16]
Effect of ingredients on natamycin efficacy:			
Simulated acid sauces	*Zygosaccharomyces bailii*	Efficacy of natamycin as a function of xanthan gum and sodium chloride concentrations.	[17]
Cheese-mimicking matrix	*Galactomyces geotrichum*, *Mucor racemosus*, *Penicillium commune*, *Phoma pinodella*, *Candida parapsilosis*, *Meyerozyma guilliermondii*, *Rhodotorula mucilaginosa*, *Trichosporon asahii*, *Yarrowia lipolytica*	The effect of the screening medium on natamycin minimum inhibitory concentration.	[18]
Table olive	*Saccharomyces cerevisiae*, *Wickerhamomyces anomalus*, *Candida boidinii*	Antagonistic effects between natamycin and citric acid in table olive packaging.	[19]
Induced tolerance to natamycin:			
Agar medium	*Saccharomyces cerevisiae*, *Candida parapsilosis*, *Candida albicans*, *Candida krusei*, *Rhodotorula mucilaginosa*, *Trichosporon asahii*, *Geotrichum candidum*, *Fusarium solani*, *Aspergillus terreus*, *Aspergillus fumigatus*,	Natamycin-exposure caused an increase of minimum inhibitory concentrations; a continuous and prolonged increasing selection pressure induced natamycin tolerance in fungi.	[20,21]

	Cladophialophora potulentorum, *Neosartorya spinosa*, *Penicillium discolor*, *Mucor plumbeus*, *Aspergillus ochraceus*, *Verticillium fungicola*, *Colletotrichum musae*, *Fusarium oxysporum*, *Trichoderma aggressivum*		
Natamycin-containing films/coatings:			
Cherry tomato	*Botrytis cinerea*	Natamycin/methyl-β-cyclodextrin coatings; inhibit fruit decay in tomato fruit during storage.	[22]
Cheese	*Penicillium* spp.	Corn starch-based coating with 0.1 % natamycin; mold control on semi-hard cheese during ripening.	[23]
Agar medium	*Aspergillus niger*	Natamycin-containing thermoplastic films; heat-pressed films had higher inhibition zone diameters than blown films.	[24]
Kashar cheese	Molds	Casein/natamycin edible films for mold control.	[25]
Commercial semi-soft cheese; food packaging	Molds	Natamycin based sol-gel antimicrobial coatings on polylactic acid films.	[26]
Tapioca starch edible films	N/A	Effect of natamycin on the physicochemical properties of edible films.	[27]
Gliadin films	*Penicillium* sp., *Alternaria solani*, *Colletotrichum acutatum*	Incorporation of natamycin to films resulted in greater water uptake, weight loss/diameter gain, higher water vapor and oxygen permeabilities; a looser packing of the protein chains by natamycin.	[28]
Cheeses	Yeasts, molds	Antimicrobial edible coatings inhibited the growth of pathogenic or contaminant microorganisms (yeasts and molds).	[29]
Natamycin modes of action in foods:			
Malt extract	*Penicillium discolor*	Unlike other polyene drugs such as nystatin and filipin, natamycin cannot permeabilize germinating conidia, but disrupts endocytosis.	[30]
Commercially manufactured poultry feed	*Aspergillus fumigatus*, *Aspergillus parasiticus*, *Aspergillus flavus*, *Paecilomyces spp.*, *Rhizopus spp.*, *Fusarium moniliforme*, *Penicillium rubrum*	The growth inhibition by natamycin was more pronounced compared with the sporicidal activity.	[31]
Mandarin fruit	*Botrytis cinerea*	Postharvest disease control; management of pathogens resistant to fungicides with different modes of action.	[32]
Others:			

Morus notabilis Schneid. Daye	Fungal mixture	Mulberry fruit postharvest decay control.	[**33**]
Strawberry crown Rot	QoI-Resistant *Colletotrichum acutatum*	Disease severity and plant mortality in field studies were reduced; Fruit yield was significantly increased.	[34]
Livestock food systems: Review	*Aspergillus flavus*, *Aspergillus paraciticus*	Natamycin (and nisin): the only natural preservatives being regulated.	[35]
Food-producing industry	*Candida* spp., *Aspergillus fumigatus*	Natamycin has to be used cautiously by the food industry and that exposure of the human resident flora should be minimized extensively in order to maintain the life-saving potential of polyenes.	[36,37]
Yogurt	N/A	Natamycin content and quality of yogurt.	[38]
Artificial diet	*Aspergillus niger*	Natamycin for the rearing of rice leaf folder.	[39]
Greek traditional deli salad "Tzatziki"	Yeasts, molds	The shelf-life of Tzatziki was extended by ca. 5-6 days (natamycin, citrus and citrus plus natamycin).	[40]
Iranian Yogurt Drink	N/A	Natamaycin was detected in 10.25% of the samples.	[41]
South African fruit juices	N/A	Detection of natamycin above the legal limits in some samples.	[42]
Natural black olive fermentation	Yeasts, molds	Natamycin inhibited the growth of fungi.	[43]
Orange juice	*Pichia fermentans*	The effect of natamycin-pulsed electric fields (PEF) combination against *P. fermentans* was not significantly different to that of PEF alone.	[44]
Soil of a vegetable field	*Fusarium oxysporum*, *Botrytis cinerea*, *Monilinia laxa*	Application as a biological control agent for fungal plant diseases.	[45]
Shredded Cheddar cheese	Molds	The semisynthetic natamycin derivative is less effective than the parent natamycin.	[46]
Serum dextrose agar With *Brucella* selective supplement	*Brucella abortus*, *Brucella melitensis*	Natamycin would be an alternative to cycloheximide.	[47]
Argentinian cheese factory	*Phoma glomerata*	0.5% (w/v) Natamycin was effective to treat *P. glomerata.*	[48]
Agar medium	*Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Penicillium* sp. M46, *Penicillium patulum*, *Penicillium roquefortii*, *Penicillium citrinum*	Antimycotic effect of natamycin.	[49,50]
Italian dry sausage	Molds	Antimycotic activity.	[51]
Cheese Warehouses	*Acremomium*, *sclerotigenum*, *Aspergillus versicolor*, *Beauverla alba*, *Cladosporium cladosporioides*, *Penicillium brevicompactum*,	Antimycotic activity.	[52]

	Penicillium cf. *lividum*, *Penicillium chrysogenum*, *Penicillium nigricans*, *Penicillium citreoviride*, *Penicillium roseopurpureum*, *Penicillium verrucosum*, *Penicillium viridicatum*, *Scopulariopsis asperula*		
Cottage cheese	*Aspergillus niger*, *Saccharomyces cerevisiae*	Antimycotic activity.	[53]
Natamycin detection methods in foods:			
Dairy products (Cheese, yogurt)	N/A	Determination of preservatives in dairy Products by HPLC and Chemometric analysis.	[54]
Cheeses	N/A	A liquid chromatography-tandem mass spectrometric with electrospray ionization method for detecting preservatives (benzoic acid, citric acid, hexamethylenetetramine, lysozyme, natamycin, nisin and sorbic acid) in cheese.	[55]
Wines	N/A	A novel method for determination of natamycin in wines; ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry.	[56]
Dulce de leche, a traditional South American product	N/A	Liquid chromatography-tandem mass spectrometry method for the simultaneous determination of sorbic acid, natamycin and tylosin.	[57]
Hard and pasta filata cheeses	N/A	RP-HPLC with UV detection of natamycin.	[58]
Cheese, cheese rind	N/A	A rapid spectrophotometric method for determining natamycin. Quantitation and detection-limits were estimated at 0.5 and 0.25 mg/kg, respectively.	[59]
Cheese, cheese rind	N/A	Spectrometric method gave good results; liquid chromatographic method with ultraviolet detection gave reasonable results.	[60,61]
Cheese	N/A	A high-performance liquid chromatographic method for natamycin detection.	[62]

Table S2. Examples of differential antifungal efficacy of drugs/preservatives affected by different pHs.

Drug/compound tested	Drug/compound characteristics	Fungi	Low pH	High pH	Mechanisms	References
5-Flucytosine (5-FC)	Derivative of the nucleobase cytosine	*Aspergillus fumigatus*	High efficacy of 5-FC at pH 5	Low efficacy of 5-FC at pH 7	Down regulation of *fcyB* gene encoding a purine-cytosine permease (orthologous to 5-FC importers) at pH 7	[63]
Naftifine	Allylamine derivative	*Trichophyton mentagrophytes* var. *quinckeanum*, *Microsporum racemosum*	Low efficacy at pH 4	High efficacy at pH 8	-	[64]
Methotrexate (MTX)	Antifolate	*Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*	High efficacy at pH 5	Low efficacy at pH 7	The pH-dependent antifungal activity of MTX is via differential cellular uptake or permeability and is species specific. *Candida glabrata* was insensitive at either pH.	[65]
Valproic acid (VPA)	Antipsychotic drug	*C. albicans*	High efficacy at acidic pH (pH 4.5)	Low efficacy at pH 8.0	VPA alters vacuole integrity. VPA synergizes with the terbinafine (allylamine antifungal).	[66]
Dimethylaminododecyl methacrylate (DMADDM),	Added to acrylic resin (oral) as a non-releasing antifungal agent	*C. albicans* (planktonic and biofilm)	Low efficacy at pH 4	High efficacy at alkaline pH (pH 7 to 9)	The pH values and pH-regulated genes affect the zeta potential of fungal cells, thus the binding between DMADDM and cells.	[67]
Potassium sorbate	Food preservative	*Aspergillus flavus*	High efficacy at pH 3.0	Low efficacy at pH 4.5	-	[68]
Sodium sulfite, Sodium propionate	Food preservatives	*A. flavus*, *Aspergillus parasiticus*	High efficacy at pH 3.0	Low efficacy at pH 5.0 to 7.0	-	[69]

Chloroquine (CQ)	Antimalarial drug	*A. fumigatus, Aspergillus nidulans*	No significant difference in antifungal efficacy against *A. fumigatus* versus *A. nidulans* at pH 6	*A. fumigatus* is more susceptible to CQ than *A. nidulans* at pH 8	A higher extracellular pH enabled increased diffusion rate of CQ into the fungal organisms.	[70]

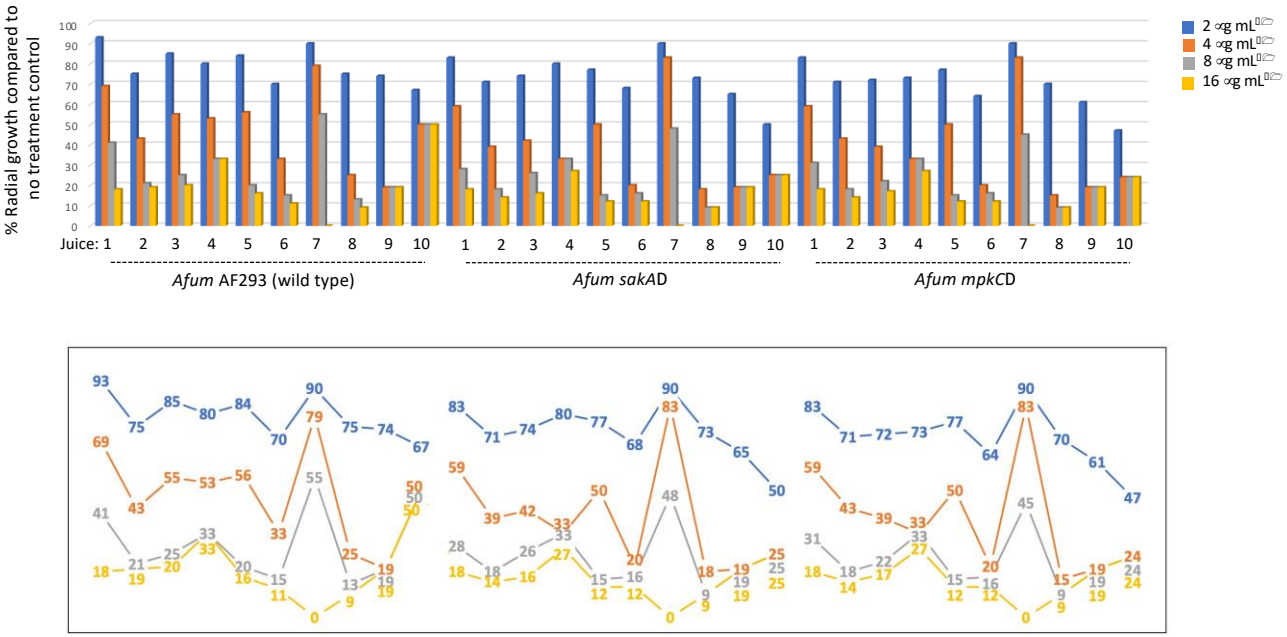


Figure S1. Susceptibility of *A. fumigatus* AF293 (wild type) and MAPK mutants (*sakA* , *mpkC*) to natamycin (NAT) (2, 4, 8, 16 g mL^{-1}) tested in the commercial, organic fruit juices (triplicate) (SD < 2%). Lower panel: Line graph showing numerical values of % radial growth at each test condition.

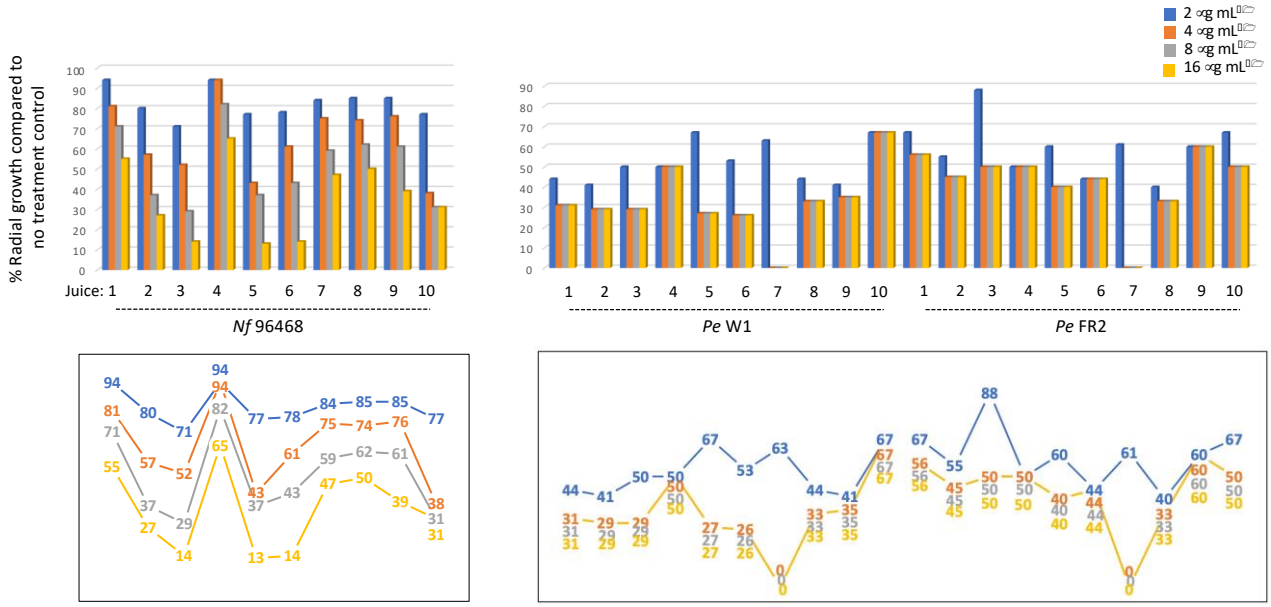


Figure S2. Susceptibility of foodborne fungal contaminants (*Pe, P. expansum*; *Nf, N. fischeri*) to natamycin (NAT) (2, 4, 8, 16 g mL^{-1}) tested in the commercial, organic fruit juices (triplicate) (SD < 2%). Lower panel: Line graph showing numerical values of % radial growth at each test condition.

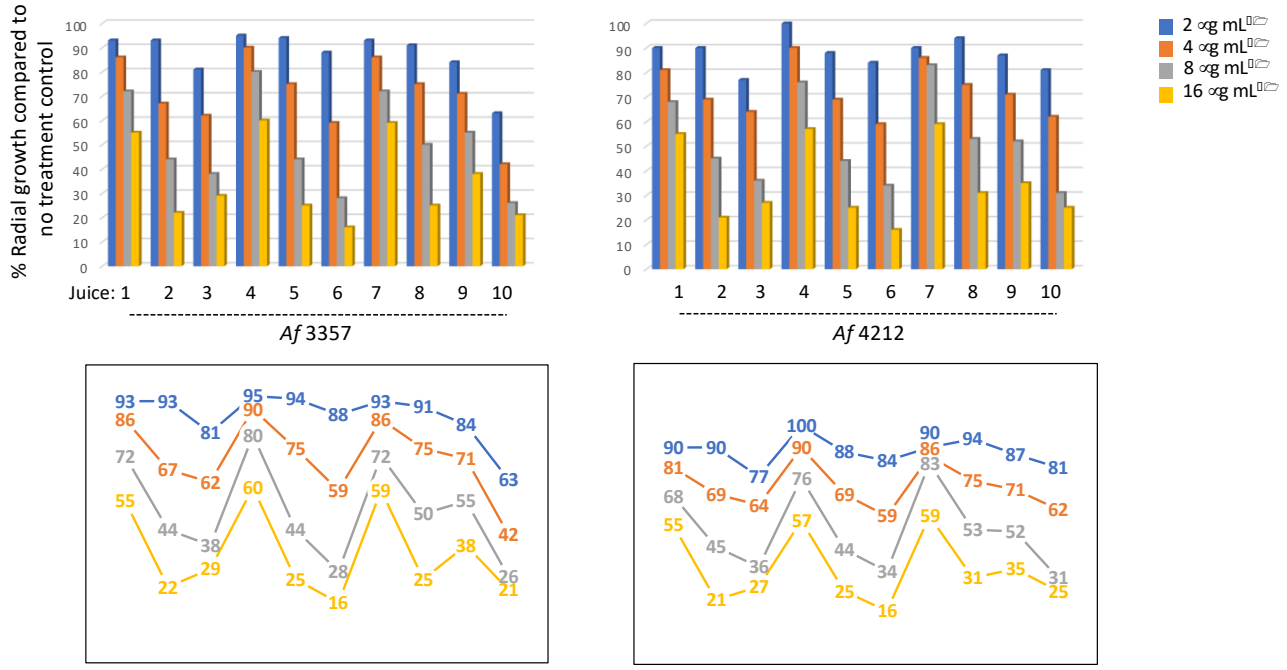


Figure S3. Susceptibility of foodborne fungal contaminants (*A. flavus* NRRL 3357, NRRL 4212) to natamycin (NAT) (2, 4, 8, 16 g mL^{-1}) tested in the commercial, organic fruit juices (triplicate) (SD < 2%). Lower panel: Line graph showing numerical values of % radial growth at each test condition.

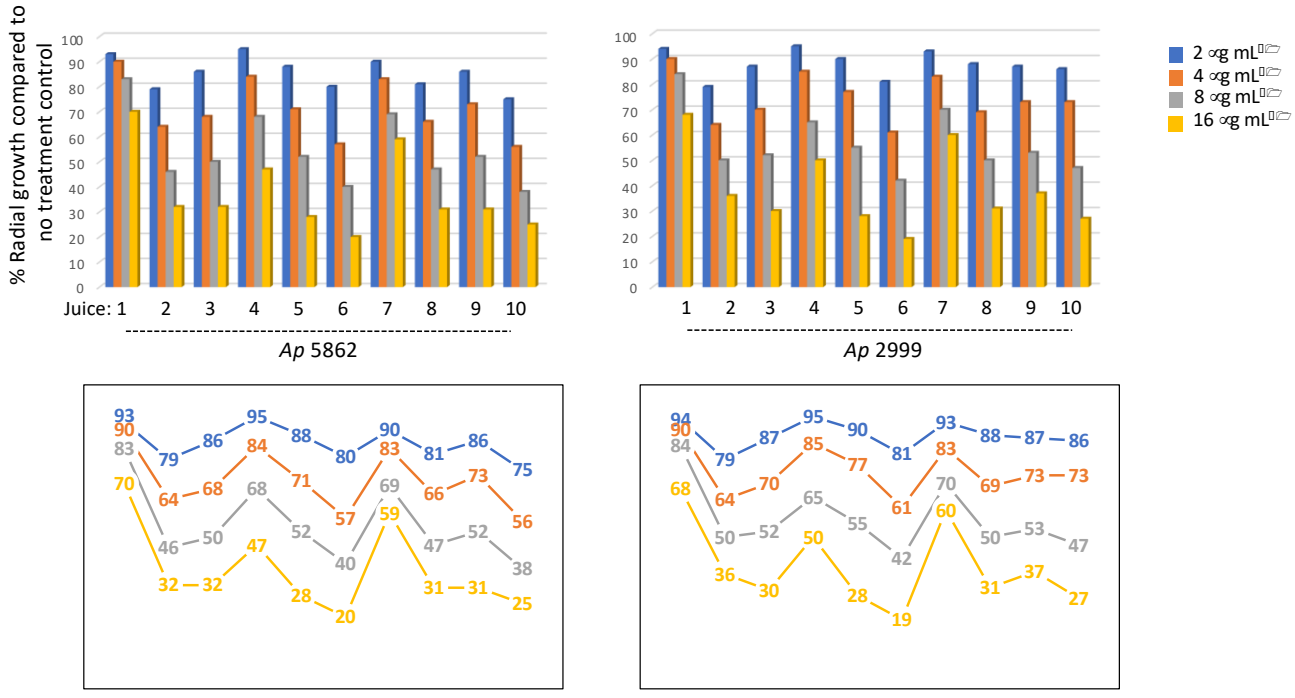


Figure S4. Susceptibility of foodborne fungal contaminants (*A. parasiticus* NRRL 2999, NRRL 5862) to natamycin (NAT) (2, 4, 8, 16 g mL^{-1}) tested in the commercial, organic fruit juices (triplicate) (SD < 2%). Lower panel: Line graph showing numerical values of % radial growth at each test condition.

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